UNIVERSITÉ DU QUÉBEC À MONTRÉAL

LE MÉCANISME DE DISSIPATION NON-PHOTOCHIMIQUE ET SON RÔLE SOUS FORTE INTENSITÉ LUMINEUSE ET FORTES CONCENTRATIONS DE ZINC CHEZ LES CYANOBACTÉRIES

THÈSE

PRÉSENTÉE

COMME EXIGENCE PARTIELLE

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PREFACE

I discovered an interest in phycology at the first year of my Master study. My decision to continue to this area research brought me to UQAM, to the laboratory of Philippe Juneau. During my first year of my Master in 2007, I did my first experiment about the high light acclimation ability among diatom, green algae and cyanobacteria. I found that cyanobacteria presented lower ability to acclimate high light compared to green algae and diatom, which was contradictory with my hypothesis. These strange results lead me to read many articles about the physiology of cyanobacteria. As I understood more about that, I learned that a lot of species of cyanobacteria can form serious and harmful blooms, and that the acclimation mechanisms to stress conditions were much less clear compared to green algae and diatoms. Consequently, it is harder to understand the growth and therefore avoid the cyanobacterial bloom formation.

I started my PhD study in September 2010, and few months later, in order to understand the regulation mechanism of cyanobacteria under high light stress condition, I started to investigate the mechanism of state transition in cyanobacteria, since it plays an important role to balance energy between the two photosystems (Chapter I).

For more than 40 years, the state transition mechanism under high light stress conditions was not clearly understood in cyanobacteria. When I found that one phosphatase inhibitor, the sodium fluoride, can inhibit the state 2 to state 1 transition induced by far red light and dark-high light shift, an opportunity to continue this research about the role of state transition under high light stress in cyanobacteria opened to me. Therefore, I performed experiments to investigate the state 2 to state 1 transition role under high light stress conditions (Chapter II).

Later during my PhD, I have continued to investigate the main energy dissipating mechanisms under high light condition in cyanobacteria. According to these experiments, I proposed a response model to high light stress in cyanobacteria (Chapter III).

After obtaining very interesting results for the first part of my thesis, I was wondering about the role of high light response mechanisms for other stress conditions. Due to the increased human activities worldwide, metal pollution can be found in many freshwater ecosystems. Zinc is one of the most common pollutants found in these ecosystems. Therefore, I studied the response mechanisms under zinc stress conditions and the interaction of light and zinc on photosynthesis and physiology of cyanobacteria (Chapter IV). I have also investigated the effect of zinc on high light regulation processes in cyanobacteria (Chapter V).

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77K	low temperature fluorescence measurement
APC	allophycocyanin
BBM	bold basal media
Car	Carotenoid
Chl *a*	Chlorophyll *a*
CO_2	Dioxide carbone
Cyb b_6f	Cytochrome b_6f
Cyt c_{553}	cytochrome c_{553}
DCMU	Diuron
F'_M	Maximal fluorescence yield under light condition
F_0	Minimal fluorescence yield
F693	The fluorescence emission at 693 nm under 77K measurement
F729	The fluorescence emission at 729 nm under 77K measurement
F_M	Maximal fluorescence yield
FRP	fluorescence recovery protein
Fs	steady-state fluorescence level
LHCII	light-harvesting complex II
MT	multiple-turnover flash
$NADP^+$	Nicotinamide adenine dinucleotide phosphate
NADPH	reduced form of $NADP^+$
NaF	Sodium fluoride

NPQ	Non-photochemical quenching
OCP	orange carotene protein
OCP^o	orange form OCP
OCP^r	red form OCP
PBS	Phycobilisome
PC	Phycocyanine
PE	Phycoerytrine
P_M	maximum change of P700 signal in dark adapted state
P_M'	maximum change of P700 signal in light adapted state
PO	minimal level of P700 transmittance change measured after the saturating pulse, when P700 is reduced by electrons from PSII
PQ	plastoquinone
PS (I or II)	photosystem I or II
Psbs	an intrinsic chlorophyll-binding protein of PSII protein
Q_A and Q_B	quinone a and quinone b
qE	a fast phase of NPQ
qI	a slow phase of NPQ
qT	a medium phase of NPQ
RubisoCO	ribulose-1,5-bisphosphate carboxylase/oxygenase
Y[I]	PSI quantum yield
Φ'_M	operational PSII quantum yield
Φ_M	maximal PSII quantum yield

RÉSUMÉ GÉNÉRAL

Les cas de floraisons de cyanobactéries, aussi appelées «fleurs d'eau», ont lieu fréquemment à travers le monde. Ainsi, la compréhension des réponses photosynthétiques et physiologiques des cyanobactéries face aux diverses conditions environnementales est importante afin de contrôler ces floraisons. Une intensité lumineuse élevée est un stress fréquent pour les cyanobactéries puisque ces organismes peuvent former des «fleurs d'eau» à la surface des plans d'eau. De plus, dues à l'augmentation de l'activité humaine, de fortes concentrations en zinc sont retrouvées dans de nombreux écosystèmes d'eau douce et constituent également un stress pour ce phytoplankton. L'un des mécanismes les plus importants pour faire face à ces conditions de stress est le quenching non-photochimique (NPQ). Cependant, le fonctionnement des deux principaux mécanismes de photo-protection du NPQ, soit l'*orange carotenoid protein* (OCP) et les états de transition, sont encore loin d'être entièrement compris pour les cyanobactéries. Au cours de mon doctorat, j'ai donc étudié le fonctionnement et les mécanismes de résistance du NPQ en réponse aux conditions de stress lumineux élevé et d'exposition au zinc chez les cyanobactéries.

Les résultats ont été obtenus à partir d'expériences *in vitro* en utilisant plusieurs espèces de cyanobactéries. Initialement, une espèce de cyanobactérie (*Synechocystis* sp.) a été utilisée pour étudier le mécanisme d'état de transition. *Synechocystis* sp. a été pré- acclimatée avec un inhibiteur de phosphatase (NaF) à 40 mM pendant 50 minutes, puis la lumière rouge lointain ou le DCMU ont été utilisés pour induire la transition de l'état 2 à l'état 1. Les résultats ont montré que le NaF peut inhiber partiellement la transition de l'état 2 à l'état 1 induite par la lumière rouge lointain mais pas par le DCMU. Ces résultats suggèrent que la déphosphorylation est impliquée dans la transition de l'état 2 à l'état 1 induite par la lumière rouge lointain.

Plus tard, cette espèce a également été utilisée pour mesurer le rôle de la transition de l'état 2 à l'état 1 induite par l'exposition à une intensité lumineuse élevée chez une cyanobactérie adaptée au noir. Le NaF (80 mM) a été ajouté et l'état 1 ou 2 a été induit par une pré-acclimatation sous une lumière bleue ou au noir pendant 50 minutes, respectivement. Après 50 minutes de pré-acclimatation, les cellules de cyanobactéries sous l'état 1 ou 2 ont été exposés à une intensité lumineuse élevée (1700 µmol photons·m^{-2}·s^{-1}) pendant 12 minutes, puis elles ont été mises au noir pendant 40 minutes pour une période de récupération. Nous avons démontré que le NaF a augmenté la sensibilité à la lumière élevée lorsque les cyanobactéries étaient à l'état 2, puisque la transition de l'état 2 à l'état 1 a été partiellement inhibée. Ceci suggère que la transition de l'état 2 à l'état 1 joue un rôle dans la régulation à la lumière de haute intensité.

De plus, nous avons étudié les effets de l'interaction entre l'OCP et les états de transition sous haute lumière chez *Synechocystis* sp. et *Microcystis aeruginosa*. Ces deux espèces ont été exposées à une forte lumière (1700 µmol photons·m^{-2}·s^{-1}) pendant 12 minutes et ont ensuite été mises à l'obscurité pendant 40 minutes. Les résultats ont démontré que *M. aeruginosa* avait une plus grande quantité d'OCP, une transition inférieure de l'état 1 à l'état 2, mais une capacité de régulation supérieure à l'intensité lumineuse élevée par rapport à *Synechocystis* sp.. Ces résultats suggèrent que l'OCP est plus efficace que la transition de l'état 1 à l'état 2 pour les espèces étudiées.

Suite aux résultats obtenus dans la première partie de ma thèse, j'ai étudié les mécanismes de réponse à court terme à une exposition au zinc pour *Synechocystis* sp. et une souche toxique et une non-toxique de *M. aeruginosa*. Ces trois souches de cyanobactéries ont été exposées à trois concentrations de zinc pendant 5 heures, puis la photosynthèse ainsi que les paramètres physiologiques ont été mesurés. Les trois souches ont démontré des sensibilités différentes au zinc, et nous avons trouvé que la teneur interne en zinc, les concentrations en pigments et les données NPQ expliquent ces différentes sensibilités. Enfin, les deux souches de *M. aeruginosa* ont été choisies pour une étude à long terme sur les mécanismes de réponse suivant un traitement au zinc et de lumière. Les cyanobactéries ont été cultivées sous deux intensités lumineuses (50 µmol photons·m^{-2}·s^{-1} et 500 µmol photons·m^{-2}·s^{-1}) et deux concentrations de zinc ($7{,}8 \times 10^{-7}$ M et $7{,}8 \times 10^{-6}$ M). Dans leur phase exponentielle, les cellules de cyanobactéries ont été exposées à une intensité de lumière élevée et mises au noir pour la période de récupération. Ces deux souches ont démontré des sensibilités différentes à la lumière élevée à différentes conditions de lumière de croissance et de zinc. Les résultats de pigments, de l'état de transition et le contenu en microcystines pourraient expliquer ces différences.

Mots clé: *Synechocystis* sp., *Microcystis aeruginosa*, quenching non-photochimique, transition d'état, déphosphorylation, lumière, zinc et NaF

ABSTRACT

As cyanobacterial blooms occurs frequently worldwide, the understanding of photosynthetic and physiological responses to variety of environmental conditions in these organisms is important for controlling and avoiding these blooms. High light is a frequent stress condition for cyanobacteria since these phytoplankton cells may form blooms at the surface of waterbodies. Besides high light, as the human activity increased, high zinc concentration may also be found in many freshwater ecosystems throughout the world. However, the non-photochemical quenching (NPQ), as one of the most important mechanisms to cope with stress conditions, and the two major components of NPQ (Orange Carotenoid protein (OCP)-related quenching and state transition), are still far to be clearly understood in cyanobacteria. In this research, I am interested to investigate the NPQ mechanism, and the high light and zinc resistance mechanisms in relation to NPQ in cyanobacteria.

The results were obtained from *in vitro* experiments by using several species of cyanobacteria. Initially, one species of cyanobacteria (*Synechocystis* sp.) was used to investigate the mechanism of state transition. *Synechocystis* sp. was pre-acclimated in one phosphatase inhibiter NaF (40 mM) for 50 min, then far red light or DCMU was used to induce the state 2 to state 1 transition. The results showed that NaF can partly inhibit the state 2 to state 1 transition induced by far red light but not by DCMU. These results suggested that dephosphorylation is involved in state 2 to state 1 transition induced by far red light.

Later, this species was also used to measure the role of state 2 to state 1 transition induced by dark-high light shift. NaF (80 mM) was added and state 1 or state 2 was induced by pre-acclimation under blue light and dark for 50 min. After 50 min pre-acclimation, the cyanobacterial cells under state 1 or state 2 were exposed to high light (1700 µmol photons·m^{-2}·s^{-1}) for 12 min and then put in the dark 40 min for recovery. It was shown that NaF increased the sensitivity to high light when the cyanobacterial cells were under state 2, since the state 2 to state 1 transition was partly inhibited by NaF, which suggested that state 2 to state 1 transition plays a role in high light regulation.

Moreover, we investigated the interaction effect of OCP-related quenching and state transition under high light in *Synechocystis* sp. and *Microcystis aeruginosa*. These two species were treated by high light (1700 µmol photons·m^{-2}·s^{-1}) for 12 min and then exposed to darkness for 40 min. The results showed that *M. aeruginosa* had higher OCP-related quenching, lower state 1 to state 2 transition, but higher high light regulation ability compared to *Synechocystis* sp.. These results suggested that OCP-related quenching is more efficient than state 1 to state 2 transition in the studied species.

Following the results obtained in the first part of my thesis, I studied the short term response mechanisms to high zinc for one toxic and one non-toxic strains of *M. aeruginosa* and *Synechocystis* sp.. These three cyanobacteria were exposed to three concentrations of zinc for 5 h, then the photosynthetic and physiological parameters were measured. The three strains showed different sensitivities to high zinc, and we found that the inner zinc content, pigment concentrations and NPQ data explained these sensitivities. Finally, the two strains of *M. aeruginosa* were chosen for a long term study on the response mechanisms to high zinc and light treatment. The cyanobacteria were grown under two light intensities (50 µmol photons·m^{-2}·s^{-1} and 500 µmol photons·m^{-2}·s^{-1}) and two zinc conditions (7.8×10^{-7} M and 7.8×10^{-6} M). At their exponential phase, the cyanobacterial cells were exposed to high light and then dark recovery. These two strains showed different sensitivities to high light at different growth light and zinc conditions. The results of pigments, state transition and microcystin content could explain these differences.

Key words: *Synechocystis* sp., *Microcystis aerginosa*, Non-photochemical quenching, state transition, dephosphorylation, light, zinc and NaF

GENERAL INTRODUCTION

Background

Cyanobacteria are very old living organisms, and they are distributed in a variety of habitats, but are most often found in shallow, slow-moving and warm freshwater. Some species of cyanobacteria can grow fast and gather to the upper level of water, and can form a colorful thick layer, known as cyanobacterial bloom (Zanchett and Oliveira-Filho, 2013; Saad and Atia, 2014). This agglomeration of cyanobacterial cells at the surface of the waterbody will block the oxygen and CO_2 diffusion into water, causing harmful effects for the other phytoplankton and zooplankton. Moreover, some species can produce cyanotoxins (such as microcystins), which can cause a series of damages, such as the liver and kidney damage and neurotoxicity for animals and humans (Carmichael, 2001).

As the agricultural and industrial activities increased, more waste water may be discharged into freshwater ecosystems increasing nutrient concentrations. These increased nutrient concentrations combined to climate changes, may increase the possibility of cyanobacterial bloom formation. Cyanobacterial blooms are found worldwide, from South Africa, North Africa, Europe, Brazil, USA, Canada, China and Japan (Zohary, 1985; Watanabe *et al.*, 1986; Mohamed *et al.*, 2003). Most of these cyanobacterial blooms were associated with microcystin presence. In Canada, *Microcystis aeruginosa*, one species of cyanobacteria that can form blooms in summer, was often found in lakes and in the water storage sites of small farms at latitude 50 to 55 °N (Kotak *et al.*, 1993). Cyanobacterial blooms were also found in some lakes and microcystin was detected in some water areas in Canada.

Because of the adverse effect of cyanobacterial blooms, a lot of research was done to reveal the mechanisms of the formation of cyanobacterial blooms and find ways to control them (Havens, 2008; Brookes and Carey, 2011; Deng *et al.*, 2014). However, there are still some mechanisms that are not clear in cyanobacterial growth and bloom formation (Merel *et al.*, 2013). Non-photochemical quenching (NPQ) is one of the most important mechanisms of response to stress conditons, which needs to be clarified in the near future to better understand the cyanobacterial growth (Kirilovsky, 2014). When cyanobacteria grow under excess light condition, pigment-protein complex will dissipate excess energy into heat, and therefore protect the photosystem II (PSII), and this protection mechanism is kown as NPQ. NPQ is a protective mechanism in higher plants, green algae and cyanobacteria, which plays an important role in response to high light stress in these photosynthetic organisms.

In cynaobacteria, the two important components of NPQ are orange carotenoid protein (OCP)-related NPQ and state transition (Bailey and Grossman, 2008; Dong *et al.*, 2009). After more than 30 years of research, the mechanism and photoprotection role of OCP-related NPQ is almost fully understood. However, the mechanism of state transition is far to be clear and its role is still under debate (Kirilovsky, 2014). In this project, I investigate the mechanism of state transition and its role in cyanobacteria (Chapter 1, 2 and 5). Moreover, the interaction of OCP-related NPQ and state transition in high light regulation was also investigated (Chapter 3). This research provides basic information on state transition and OCP-related NPQ in cyanobacteria, and helps us to better understand the cyanobacterial growth under varied conditions.

At the same time, we wanted to investigate high light resistance mechanisms in cyanobacteria. Light is an important factor for cyanobacterial growth and excess light can cause the cells damage by producing reactive oxygen species. It is suggested that light intensity can reach up to 2000 μmol photons·m^{-2}·s^{-1} at the surface of water body in summer (Xu *et al.*, 2012). Cyanobacterial cells could then be exposed to this high light intensity when they form surface blooms or when winds bring the cells at the

surface. Therefore, the high light resistance ability may be important for the cyanobacteria to survive and may affect their dominances in water ecosystems. In order to better understand the high light resistance mechanisms, we monitored the different responses to high light stress among different cyanobacterial species and try to get a high light regulation model for cyanobacteria (chapters 3 and 5). The high light and zinc response mechanisms were also investigated for different species grown under two light and zinc conditions (chapter 5).

As we mentioned above, the increase in agricultural and industrial activities lead to more nutrients and metals into water ecosystems. Zinc is one of the most frequently found metals causing contaminations (Foy et al. 1978). Although zinc toxicity is not a common problem for humans, the toxicity of high zinc to phytoplankton has been documented (Gough *et al.*, 2008; Bbosa and Oyoo, 2013). Even if cyanobacterium is supposed to have various mechanisms to cope with high zinc stress, it is still sensitive to high zinc concentration. Therefore, zinc pollution in water bodies should affect the growth of some cyanobacteria. On the other hand, some species were found to be resistant to high zinc stress and may be used to remove zinc in some polluted water bodies (Okmen *et al.*, 2011; Goswami *et al.*, 2015). In this project, I chose three cyanobacterial strains, which were grown under two light intensities, and then exposed under different high zinc stress conditions. The different short-term high zinc acclimation mechanisms were measured in these three strains (chapter 4). Moreover, the mechanisms of long-term adaptation to high zinc were also measured in two cyanobacterial strains (chapter 5).

Cyanobacteria respond to environmental stress by adjusting their physiology, biochemistry and morphology and these responses occur at different time-scales. Short-term responses (seconds to minutes) such as NPQ quenching (by OCP-related quenching or state transition, or enzyme de/activation) are considered as regulation. Mid-term responses (few hours to days) represent an acclimation, such as the response observed under high light (increase pigment content and protein synthesis, changes in photosystem stoichiometry and antioxidant contents). Adaptation occurs only after

numerous generations by natural selection. In this thesis, I investigated the short-term responses (regulation, chapters 2, 3 and 5) and long-term responses (adaptation, chapters 4 and 5) to different light intensities in different cyanobacterial strains. Furthermore, I also investigated the mid-term (acclimation, chapter 4) and long-term (adaptation, chapter 5) response to different zinc concentrations for different cyanobacterial strains.

Cyanobacteria

Cyanobacteria are a very diverse group of gram-negative bacteria that carry out photosynthesis by splitting water and releasing oxygen, and are widely distributed in biosphere, and many species are found in freshwater environments (Catherine *et al.*, 2013; Cheung *et al.*, 2013; Merel *et al.*, 2013).

Cyanobacteria exist as unicellular or filamentous forms in the size of micrometer range, and they can also gather into colonies that can be seen when they form blooms. Cyanobacteria are very old living organism that date back to 3.5 billion years before the present according to fossil records (Schopf 2000, Falconer 2005). Moreover, many of them have the identical size and shape to current cyanobacteria (Schopf 2000). In modern times, the worldwide distribution of cyanobacteria can be found in soil, sea and freshwater, suggesting that these organisms can adapt to a lot of different habitats (Svirčev *et al.*, 2014).

Increasing attention on the physiology of cyanobacteria started in last few decades since the cyanobacterial blooms occurred frequently in many countries. Some species of cyanobacteria can grow fast and float to the surface of waterbodies forming dense blooms (Figure 0.1). These cyanobacterial blooms have adverse effects on phytoplankton, zooplankton, animals and recreational activities since it can block oxygen and carbon dioxide to diffuse in the water and these organisms can be also very toxic since they can produce toxins. Toxic cyanobacterial blooms are found almost all

over the world (Falconer 2005). Many cases of death among livestock have been reported after ingesting water containing toxic cells from a cyanobacterial bloom (Carmichael and Falconer 1993).

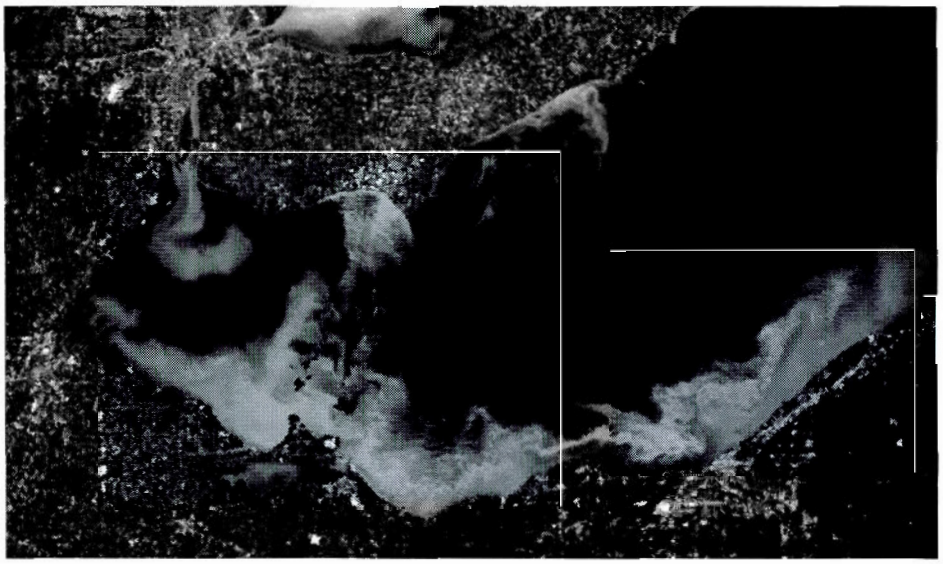

Figure 0.1. Cyanobacterial bloom in western lake Erie. The green area shows the cyanobacterial bloom. (Photo Credit: MERIS/NASA; processed by NOAA/NOS/NCCOS).

Therefore, the photosynthesis and physiology research in cyanobacteria is important to better understand the growth and the mechanism of bloom formation. If one day we can forecast the growth and bloom formation, and at last avoid cyanobacterial blooms, the problems related to toxic cyanobacterial blooms will be resolved, leading to a better water quality.

Photosynthesis

Photosynthesis is one of the most significant achievements of life on Earth, and is described as the complex process that converts light energy into stable chemical energy that is the fuel of most ecosystems (Gest, 2002).

Photosynthetic processes can be divided into two stages, which are light reactions and dark reactions. Light reactions start with the light absorption by the light harvesting complex in photosynthetic organisms. And then photosystem II (PSII) use this energy to split water and release electrons and H^+ energy. The electron transfers along a Z-scheme electron-transport chain from PSII to PSI via the cytochrome b_6f complex and is finally used for the reduction of $NADP^+$ to NADPH. This Z-scheme electron transport chain is composed of a series of transporters: PQ pool, Cyt b_6f, PC/Cyt c_{553}, P700 and at last $NADP^+$, which attach to the thylakoid membrane, as shown in Figure 0.2. During electron transport, a H+ gradient is formed across the membrane, which is used by the ATP synthase for the production of ATP. The energy kept in NADPH and ATP finally could be used for other reaction for fix carbon dioxide in dark reactions (Roberta 2012).

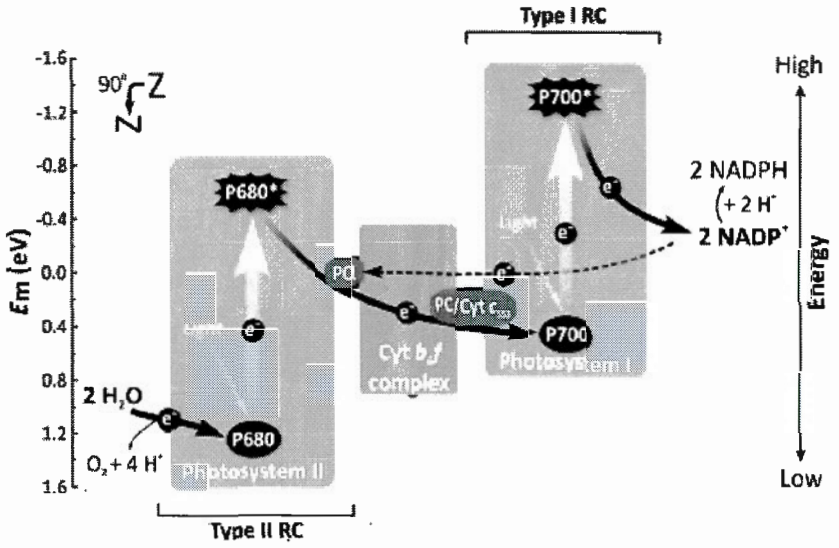

Figure 0.2. A simplified Z-scheme representing the linear (black solid arrows) and a cyclic (black dashed arrow) electron transport in cyanobacteria. Em (eV), redox potential at pH 7; Cytb$_6$f, cytochrome b$_6$f complex; Cyt c$_{553}$, cytochrome c$_{553}$; PC, plastocyanin (Govindjee and Shevela, 2014).

The photosynthetic electron transport chain (Figure 0.2) in cyanobacteria is essentially identical to that in higher plants and green algae (Vermaas, 2001). However, respiration and photosynthesis share some electron transports in cyanobacteria. This will lead to some physiological differences compared to higher plants and green algae (Campbell *et al.*, 1998). Otherwise, a major difference is the light harvesting protein between higher plants and cyanobacteria. In higher plants, the light harvesting protein is composed of chlorophyll and carotenoid molecules, which encoded by Lhc genes. Some proteins form dimmers (LHCI) or trimers (LHCII). However, in cyanobacteria, the light harvesting protein is composed of phycobilisomes, a large assembly of allophycocyanin (APC), Phycocyanin (PC) or Phycoerythrin (PE). These protein complexes are located at the periphery of thylakoid membranes (Gantt and Conti, 1966). This difference of light harvesting proteins will lead to a series of differences in Non-Photochemical Quenching (NPQ) between higher plants and cyanobacteria (see the following parts).

Non-Photochemical Quenching

Under appropriate light conditions, most of the absorbed energy will be used for photosynthesis. However, under high light, that exceed the requirement for photosynthesis, excess energy will be dissipated as heat. This process is the Non-Photochemical Quenching (NPQ).

The mechanisms of NPQ is usually compared between higher plants and cyanobacteria since some parts of the mechanisms are the same in these types of photosynthetic organisms (Karapetyan, 2007). Therefore, in order to better understand the mechanisms of NPQ in cyanobacteria, we first briefly introduce the mechanisms of NPQ in higher plants.

In higher plants, NPQ can be divided into at least three components according to the relaxation of chlorophyll fluorescence quenching (Quick and Stitt, 1989), which are qE (a fast phase; $t_{1/2}$=1-2 min), qT (a medium phase; $t_{1/2}$=5-10 min) and qI (a slow phase; $t_{1/2}$>60 min). It is generaly assumed that these three phases are related to three processes: 1) the pH-regulated energy dissipation in the antenna of PSII, 2) state transition and 3) photoinhibition (Krause and Jahns 2004). The rapidly forming qE component of NPQ occurs when a high trans-thylakoid proton gradient is build up under excessive light. A lumen pH below 6 activates the de-epoxidation that transforms violaxanthin into zeaxanthin via the intermediate pigment antheraxanthin and the reverse reaction when pH up 6 under low light or dark conditions (Yamamoto *et al.*, 1962). Zeaxanthin can attach to light harvesting complex and dissipate energy (Ahn *et al.*, 2008). Psbs, an intrinsic chlorophyll-binding protein of PSII protein, is necessary for the nonphotochemical quenching and supposed to be one of the quenching site of qE formation in higher plants (Ballottari *et al.*, 2007; Avenson *et al.*, 2008).

The qT component of NPQ, related to state transition, is activated by thylakoid protein kinase or LHCII phosphatase (Allen, 2003). When higher plants are exposed to high

light, electrons enter into the plastoquinone pool faster than they leave and therefore plastoquinones (PQ) become reduced. Reduction of PQ activates a thylakoid protein kinase that catalyzes phosphorylation of LHCII proteins, which then leave PSII and join PSI and this is state 1 to state 2 transition. If the high light intensity decreases, plastoquinone will be oxidized, the kinase is switched off, LHCII becomes dephosphorylated by an LHCII phosphatase, and LHCII returns to PSII, which is state 2 to state 1 transition.

When the first two components of NPQ cannot completely protect PSII under high light conditions in higher plants, the third component of NPQ, qI, related to photoinhibition, will be triggered by the degradation of the D1 protein of damaged PSII reaction centers (Aro *et al.*, 1993). The recovery of qI needs the new production of D1 protein, so it normally needs several hours, depending on the extent of the high light stress and species. The qI mechanism is assumed to be the same between higher plants and cyanobacteria. On the other hand, the qE and qT are quite different between higher plants and cyanboacteria, and the mechanism of qT is far to be clear in cyanobacteria.

OCP-related quenching

Similarly to NPQ in higher plants, NPQ can also be divided into three components (qE, qT and qI) in cyanobacteria. However, the Psbs, an intrinsic protein which is necessary for qE formation in higher plants, is not present in cyanobacteria (Koziol *et al.*, 2007). Moreover, light harvesting antenna is different between higher plants and cyanboacteria. Indeed, the water-soluble phycobiliproteins are organized in a large extramembranal complex known as the phycobilisome (PBS), which is the light harvesting antenna of cyanobacteria (Figure. 0.3) (Glazer, 1984; Grossman *et al.*, 1993). The energy is transferred from PBSs to the chlorophylls of PSII and PSI (Rakhimberdieva *et al.*, 2001).

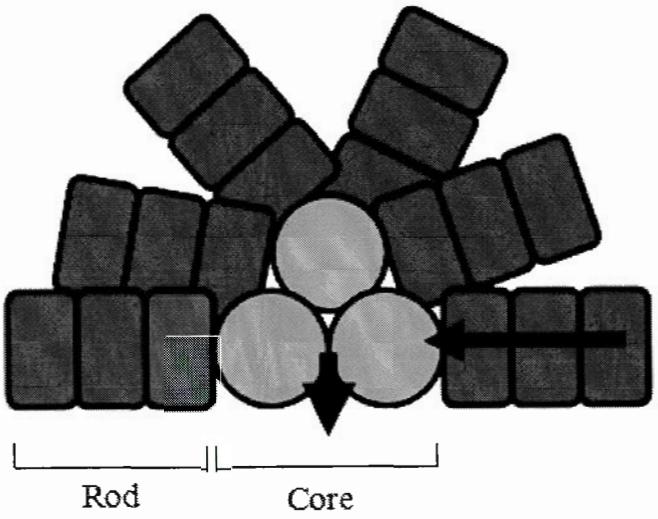

Figure 0.3. The representation of *Synechocystis* phycobilisome (Kirilovsky, 2014). The core phycobiliprotein is allophycocyanin (APC, violet and light blue color) and the rods are phycocyanin (PC, blue color).

For a long time, it was suggested that cyanobacteria do not have antenna-related NPQ (such as qE in higher plants), and that state transiton was therefore supposed to play a major role in response to high light stress (Campbell *et al.*, 1998). However, recent research demonstrated that cyanobacteria can also induce a fast component of NPQ (qE) to cope with high light stress (El Bissati *et al.*, 2000; Wilson *et al.*, 2006; Wilson *et al.*, 2007). This NPQ is related to the conformational change of the orange-carotenoid protein (OCP) (see Figure 0.4 for the mechanism). Hereafter, OCP-related NPQ will be used to refer the qE in cyanobacteria.

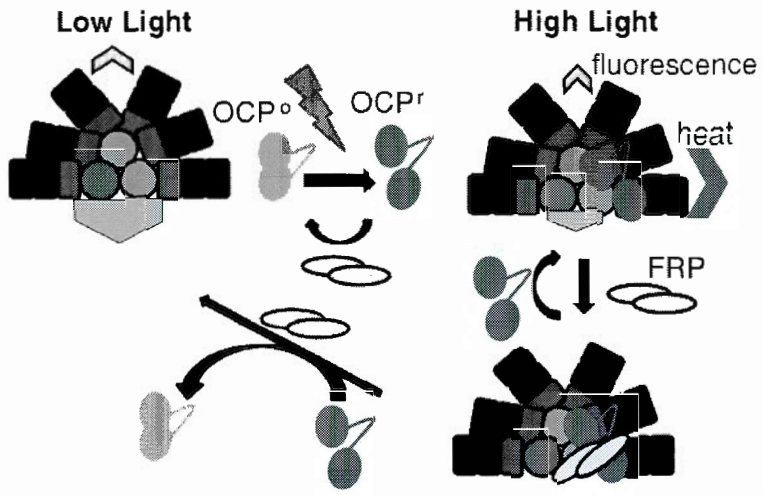

Figure 0.4. Working model of the OCP-related NPQ (Kirilovsky, 2014).

As it is shown in Figure 0.4, OCP is in an orange form (OCP^o) and is not attached to the PBS, and therefore cannot dissipate the energy through heat under dark or low light conditions. Under blue light or high white light conditions, OCP is changed to red form (OCP^r), attaches to the core of PBS (APC), and efficiently dissipates excess energy into heat and therefore preserves PSII activity. The reverse process will occur under low light or dark conditions by the help of fluorescence recovery protein (FRP).

OCP-related NPQ can be induced in few minutes (1-2 min) similarly to the xanthophyll cycle in higher plants. However, OCP-related NPQ is not affected by the pH change in lumen or the content of Psbs and zeaxanthin. Furthermore, OCP-related NPQ is much more sensitive to blue light compared to white light (Bailey *et al.*, 2005).

The amplitude of OCP-related NPQ depends on OCP concentration (Tian *et al.*, 2012). It was found that a mutant of *Synechocystis* strain containing about 8 times more OCP compared to wild type, has around 2 times the OCP-related NPQ of the wild type (Wilson *et al.*, 2008). It is worth to note that OCP is found in most species of cyanobacteria, but not all.

State transition in cyanobacteria

As the difference of OCP-related NPQ was shown between higher plants and cyanobacteria, the second component of NPQ, state transition in cyanobacteria also presents some differences compared to higher plants. As mentioned above, state transition involves the movement of LHCII between PSII and PSI in higher plants. However, in cyanobacteria there is PBS instead of LHCII as light harvesting antenna. This suggests that state transition could involve PBSs movement between two photosystems in cyanobacteria. Some research developed a model called "PBS movement" model, to explain state transition. Besides PBS movement model, another called "Spillover" was proposed to participate in state transition in cyanobacteria (Li *et al.*, 2004). These two models can change the energy transfer to PSII and PSI, therefore trigger state transition in cyanobacteria (Figure 0.5).

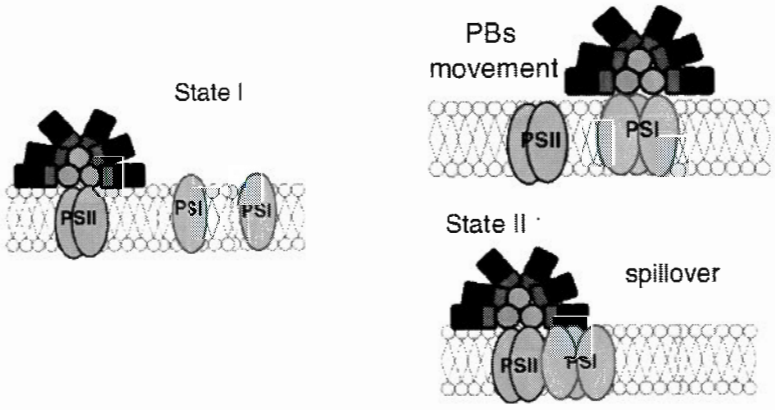

Figure 0.5. PBS movement and spillover model of state transition in cyanobacteria (Kirilovsky, 2014).

PBS movement model. This model suggests that PSII cannot move, but only PBS can move between PSII and PSI and therefore change the energy transfer from PBS to PSII and PSI (as Figure. 0.5). This model was demonstrated by many researches showing that PBS rapidly diffuse at the surface of thylakoids, while PSII movement is largely restricted (Mullineaux *et al.*, 1997; Sarcina *et al.*, 2001; Yang *et al.*, 2007; Kaňa, 2013). Moreover, it was also shown that high concentrations of phosphate, sucrose, glutaraldehyde and betain inhibit the movement of PBS, which increase the binding force of PBS to membrane also inhibit state transition (Joshua and Mullineaux, 2004; Li *et al.*, 2004). These results directly prove that PBS movement is necessary for state transition in cyanobacteria.

Spillover. This model suggests that strong coupling between PBS and PSII but variable coupling between PSII and PSI during state transition (Kirilovsky, 2014). Therefore, PSII and PSI will be closer each other causing a higher coupling under state 2, and more distant causing a lower coupling under state 1. It was demonstrated by electron microscopy that there is a re-arrangement of PSII and PSI in state 1 and state 2 adapted cells (Olive *et al.*, 1997). Secondly, it was also proposed that reversible and fast PSI monomerization/trimerization could be involved in state transition, and trimers of PSI are more abundant in state 2.than in state 1 (Aspinwall *et al.*, 2004). Moreover, it was also found that inhibition of photosystem movement prevents state transition (El Bissati *et al.*, 2000).

Nowdays, it is largely accepted that both mechanisms participate to the state transition (Li *et al.*, 2004; Kirilovsky, 2014). Indeed, state transition induced by dark-light shift is supposed to be related to both PBS movement and spillover in cyanobacteria. Moreover, state transition could be induced in 2 minutes in cyanobacteria, which is much shorter than it in higher plants (5-10 minutes). Therefore, OCP-relating NPQ and state transition can be induced in the same time scale (2 minutes), which makes it hard to distinguish between these two mechanisms in cyanobacteria.

Despite differences between higher plants and cyanobacteria, state transition shows at least one similarity. Indeed, state transition is controlled by the redox of PQ in cyanobacteria, as it is in higher plants. However, many questions need to be answered to fully understand state transition in cyanobacteria: 1) How the redox of PQ triggers the PBS movement and/or spillover during state transition in cyanobacteria?; 2) Are the kinase and phosphatase involved in PBS movement-related state transition?; 3) How PBS movement and spillover mechanism work together to cope with variety light conditions?; 4) What is the contribution of each mechanism? Moreover, for the OCP-related quenching and state transition, there are still many questions need to be answered in the future: 1) what is the separate role of OCP-related quenching and state transition under high light regulation of cyanobacteria?; 2) Can OCP-related quenching and state transition interact under high light regulation?; 3) If the answer is yes for 2), how these two mechanisms work together to cope with high light stress?

Light

In cyanobacteria, light intensity can affect sharply photosynthesis and growth. It is suggested that cyanobacteria have a higher ability to cope with low light conditions compared to other phytoplankton (Xu *et al.*, 2012). This characteristic may help cyanobacteria to dominate other phytoplankton by being present to the deep layers of the water body where the light intensity is limiting and not suitable for the growth of other phytoplankton. On the other hand, cyanobacteria have evolved a lot of mechanisms to cope with high light (Muramatsu and Hihara, 2012). Besides the increase of NPQ to resist high light condition, increase the carotenoid content is also a common response mechanism to resist high light stress in cyanobacteria.

Cyanobacteria show a variety of high light resistance ability depending on species and strains since different habitats are involved. It was found that non-toxic *Microcystis aeruginosa* is more sensitive to high light than toxic *M. aeruginosa* (Deblois and

Juneau, 2012). It was also found that a low light adapted strain of *Prochlorococcus* is more sensitive to high light compared to the high light adapted one (Bailey *et al.*, 2005). This different sensitivity to high light among different cyanobacterial species was suggested to be related to the carotenoid content and NPQ ability. However, the mechanisms of different NPQ abilities in cyanobacteria under various environmental conditions are still under debate.

Zinc

Zinc content is greatly variable in water ecosystems, and can vary from very low (0.001 µg/l) in open ocean to very high concentrations (>200 µg/l) in enriched streams floating through mineralization areas (Whitton *et al.*, 1982). For living organisms, appropriate zinc concentration is required for many biological functions, which include both structural and catalytic roles for a number of enzymes (Lipscomb and Sträter, 1996). For photosynthetic organisms, zinc is also needed for electron transport and photophosphorylation and for carbonic anhydrase activity involved in CO_2 supply to RuBisCO. It is suggested that the growth of phytoplankton is inhibited by low zinc concentration in seawater, which in turn affects the biomass production (Morel *et al.*, 1994). On the other hand, zinc may be also a toxic metal and is introduced into freshwater by the increased human activities. High zinc concentration could therefore inhibit some phytoplankton growth and affect the dominance of species in the ecosystem. As demonstrated in the Figure 0.5 for the Rhine river, the optimum zinc concentration is between 2-50 µg/L, lower or higher concentration of zinc than this optimal concentration will inhibit the growth of some species of phytoplankton.

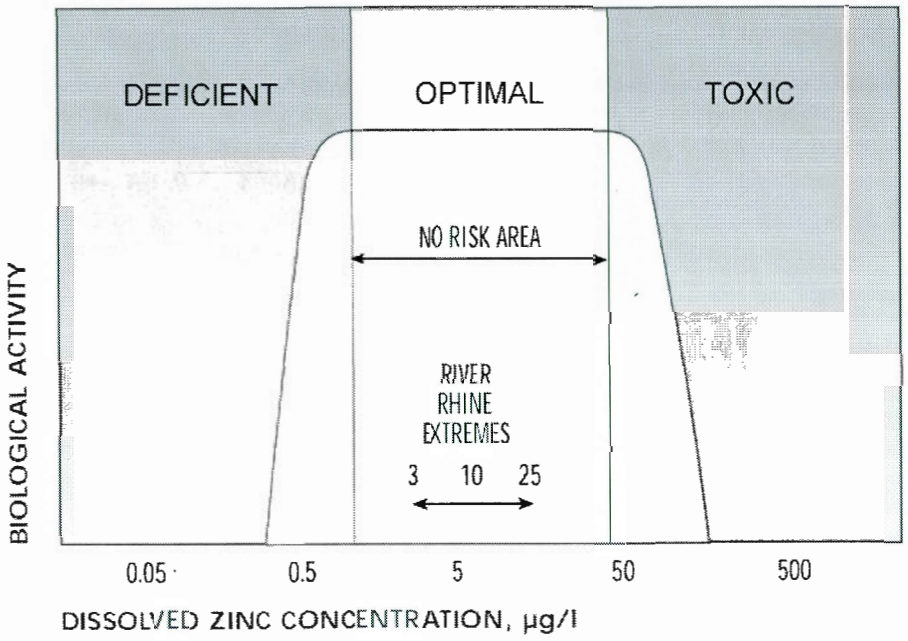

Figure 0.6. Risk assessment for zinc in the European alluvial lowland river habitat. Indicated are the "No Risk Area", bordered at the toxicity side by the PNEC (Van et al. 1996) and the mean, minimum and maximum concentrations of dissolved zinc in the Rhine river, observed over the years 1988, 1989, 1990 (Heymen and Vander Weijden 1991).

The effect of high zinc stress on photosynthesis was shown to be related to the following: 1) decrease of the maximal and operational PSII quantum yields; 2) decrease in the content of chlorophyll *a* and carotenoid. For cyanobacteria, high zinc stress also inhibited the absorbed energy transfer from PBS to the reaction centers. Although cyanobacteria possess various resistance mechanisms, they were shown to be more sensitive to high zinc stress than other phytoplankton (Takamura *et al.*, 1989; Pandey *et al.*, 2015). It was shown that a zinc concentration of 150 µg/l was toxic for most cyanobacteria (Hudek *et al.*, 2012).

However, different cyanobacterial species show different sensitivity to high zinc. It was found that *Tolypothrix tenuis* is more sensitive to high zinc than *Anabaena oryzae*, and the higher resistance of *A. oryzae* is related to the increased carotenoid content under high zinc stress (Chakilam, 2012). It was found that toxic *Microcystis aeruginosa* has similar sensitivity to high zinc compared to non-toxic *Microcystis* strains (Zeng *et al.*, 2009). High concentration of zinc (up to 1.468 mg/l) was shown to inhibit *Microcystis* spp. growth and affect the phytoplankton species dynamic in Sango Bay of Lake Victoria (Bbosa and Oyoo, 2013). Although cyanobacteria are frequently affected in polluted water ecosystems (due to their high sensitivity to zinc), response mechanisms to zinc are still not clear.

The effect of light on zinc uptake and toxicity

The effect of light on the uptake of metals by phytoplankton was previously described. It was suggested that light mediates the reduction of Fe (III) to Fe (II) playing a critical role in iron uptake in *Microcystis aeruginosa* (Fujii *et al.*, 2011). It was also found that high light can increase the zinc uptake in *M. aeruginosa* (Zeng and Wang, 2011). Consequently, it is assumed that more zinc uptake will occur under higher light, consequently zinc toxicity may be increased.

On the other hand, phytoplankton grown under different light conditions could contain different proportions of photosynthetic enzymes relative to photosynthetic pigments, which can affect the ability of energy dissipation (NPQ) (Demmig-Adams and Adams Iii, 1992). Indeed, it is normally found that higher light adaptation induced higher ratio of carotenoid relative to chlorophyll compared to lower light adaptation (Olaizola and Duerr, 1990). It is suggested that high light adaptation can help phytoplankton to resist to copper toxicity (Nielsen *et al.*, 2003; Nielsen and Nielsen, 2010). This high resistance ability to copper could be related to the higher carotenoid content, expressed by a higher NPQ in some species of algae (Nielsen *et al.*, 2003).

However, information about the effects of light on zinc uptake in different cyanobacteria is still not enough. Moreover, the effect of high light adaptation on zinc toxicity in cyanobacteria is still not clear. Furthermore, the response to high light stress of phytoplankton acclimated or adapted to different zinc concentrations was little studied.

Objectives of the present research project

As an important response mechanism for high light and other stress conditions, NPQ should be investigated in cyanobacteria. Therefore, the major goal of this project is to investigate the mechanism of NPQ, and the role of NPQ in response to high light and zinc stress in cyanobacteria. In chapters 1 and 2, the mechanism of the second component of NPQ, state transition, was investigated. For chapter 1, the research goals are the following: 1) investigate whether the phosphatase is involved in state 2 to state 1 transition induced by far red light in one species of cyanobacteria; 2) investigate whether the phosphatase is involved in state 2 to state 1 transition induced by DCMU in one species of cyanobacteria.

For Chapter 2, we continued to investigate the role of state transition in high light regulation in cyanobacteria. As it is pointed out above, state 2 to state 1 transition should be induced by dark-light shift in cyanobacteria. However, under high light, state 1 to state 2 transition will be triggered to protect the PSII activity. So, previously state 2 to state 1 transition induced by dark-light shift seems not necessary in cyanobacteria. In chapter 2, whether the state 2 to state 1 transition plays a role in high light regulation was investigated.

For chapter 3, we compared the role of state transition and OCP-related NPQ in high light regulation in two cyanobacterial species. We mainly wanted to understand when cyanobacteria, grown under different habitats, developed different NPQ mechanisms to cope with high light stress. Moreover, we wanted to propose a high light regulation model in cyanobacteria which depends on the species.

For chapters 4 and 5, we investigated the effect of light and zinc on the photosynthesis and growth in three cyanobacterial strains. Also, we investigated the interaction of light and zinc on these processes. The goals of chapter 4 were to investigate: 1) the NPQ changes of three cyanobacterial strains that were adapted under two growth light conditions. 2) the NPQ changes when three cyanobacterial strains suffered by high zinc stress. 3) the reasons of different sensitivities to high zinc in three cyanobacterial strains.

For chapter 5, we grew two cyanobacterial strains under two light intensities and zinc content regimes. Then, we investigated the response mechanisms to high light stress of the adapted cyanobacteria.

All of these five chapters are linked together and provide a better understanding of the response mechanisms to high light and high zinc stress in cyanobacteria. The obtained results can provide interesting trend of species dominance in natural freshwater ecosystems.

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CHAPTER I

EVIDENCE THAT DEPHOSPHORYLATION REGULATES STATE TRANSITION IN A CYANOBACTERIUM

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Will be submitted to Photosynthesis research

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1.1. Résumé

Chez les cyanobactéries, il est bien connu que les mécanismes de transition d'état sont contrôlés par l'état redox de la plastoquinone (PQ), de même que par le complexe du cytochrome b_6f (cyt b_6f). Par contre, les mécanismes derrière le mouvement des phycobilisomes entre le photosystème II (PSII) et le photosystème I (PSI), impliquant PQ et cyt b_6f, sont encore ambigus. Dans cette étude, il a été démontré, chez *Synechocystis* sp. FACHB898, que l'inhibiteur de phosphatase NaF peut inhiber le transfert de l'état 2 à l'état 1, lorsqu'il est induit par la lumière rouge lointaine et ce, de manière dépendante à la concentration. Cependant, chez la même cyanobactérie, NaF n'a pas inhibé le transfert à l'état 1 induit par le DCMU. Ces résultats démontrent que, chez les cyanobactéries, la déphosphorylation régule la transition d'état lorsqu'elle est induite par la lumière rouge lointaine.

Mots clé: *Synechocystis* sp., transition d'état, déphosphorylation, NaF

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1.2. Abstract

The mechanism of how plastoquinone and cytochrome b_6f complex trigger the state transition in cyanobacteria is still ambiguous. In this study, we found that phosphatase inhibitor NaF inhibited the transition to state 1 involved in phycobilisome movement, induced by far red light in *Synechocystis* sp. FACHB898, suggesting that dephosphorylation regulates state transition. However, this process does not seem to be involved when the transition to state 1 is induced by DCMU in the studied species. These results suggest that phycobilisome movement and spillover are involved in energy distribution between the photosystems when cyanobacteria are exposed to various environmental conditions.

Key words: *Synechocystis* sp., state transition, dephosphorylation, NaF

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1.3. Introduction

State transition is supposed to balance light energy between photosystem I (PSI) and photosystem II (PSII) under varying light conditions in higher plants, green algae and cyanobacteria (Allen and Forsberg, 2001; Allen, 2003; Dong *et al.*, 2009). For over thirty years, this mechanism was well studied in higher plants and green algae, but less in cyanobacteria (Mullineaux and Emlyn-Jones, 2005). In higher plants and green algae, it was found that the reduction/oxidation of plastoquinone (PQ)/cytochrome b_6f complex (cyt b_6f) directly controls the activation of light-harvesting complex II (LHCII) kinase/LHCII phosphatase, which induces the phosphorylation/dephosphorylation and redistribution of LHCII between PSI and PSII (Canaani *et al.*, 1984; Depège *et al.*, 2003). However, in cyanobacteria, the mechanism by which the redox state of PQ/cyt b_6f controls the movement of phycobilisomes (PBSs) in state transition is still controversial. Allen et al. (1985) suggested that protein phosphorylation/dephosphorylation is involved in state transition in cyanobacteria. The proposed model suggests that phosphorylation and dephosphorylation directly control the movement of PBSs, and thus state transition in cyanobacteria. However, there is no convincing evidence found to prove that phosphatase is involved in this mechanism. Canaani (1986) found that a specific inhibitor of phosphatase (NaF) inhibited the transition to state 1 induced by far red light in *Nostoc muscorum*. However, later, Shen and Wang (1998) found that NaF did not inhibit transition to state 1 induced by far red light in *Synechocystis* PCC 6803 and suggested that this state transition was not directly related to dephosphorylation in cyanobacteria. It was also advanced that protein phosphorylation/dephosphorylation are not involved in state transition in cyanobacteria since the movement of LHCII in higher plants depends on thylakoid lateral heterogeneity (Fork and Satoh, 1983; Biggins *et al.*, 1984; Miller and Lyon, 1985; Biggins and Bruce, 1989) and in cyanobacteria PSI and PSII are not partitioned into different regions (Olive *et al.*, 1997).

It was suggested that PBSs can transfer energy to PSI and PSII through different mechanisms mainly explained by the "mobile model" or the "spillover model". For the mobile model, PBSs can attach to PSI and PSII and transfer energy by moving along the surface of the thylakoid membrane (Tsinoremas *et al.*, 1989; Mullineaux, 1992; Zhao *et al.*, 2001). Therefore, under this model, state transition is related to the movement of PBSs between photosystems. For the spillover model, PBSs are firmly attached to PSII, and the energy is transferred to PSI due to the spillover (Bruce *et al.*, 1986; Bruce *et al.*, 1989). Thus, in this model, the induction of state transition does not imply the movement of PBSs. It was advanced that in cyanobacteria these two mechanisms could play a role in adaptation to different growth conditions (Li *et al.*, 2004). Indeed, pre-illumination at different wavelengths induced redistribution of excitation energy through the movement of PBSs and is named light state transition (Biggins and Bruce, 1989). On the other hand, state transition induced by redox state (dark induced state 2 and blue light induced state 1 in presence of DCMU) depends not only on the PBS mobility but also on the spillover (Li *et al.*, 2004). Similarly, it was also suggested that state 1 induced by dark to light exposure depends also on both mechanisms (Li *et al.*, 2006; Zhang *et al.*, 2009). However, there is still not enough information about these different mechanisms in cyanobacteria. Therefore, in the present paper we investigated the involvement of dephosphorylation in dark to light- and DCMU-induced transition to state 1 in *Synechocystis* sp. FACHB898.

1.4. Materials and methods

1.4.1. Cultures

Synechocystis sp. FACHB898 (hereafter FACHB898) was obtained from Freshwater Algal Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences. This species was cultured under a temperature of 22 °C in bold basal media (BBM) at pH 6.8 (Stein, 1973). The cultures were grown under a light:dark cycle of 14:10 h at 50 μmol photons·m^{-2}·s^{-1}, provided by a combination of incandescent bulbs and white fluorescent tubes (Philips F72T8/TL841/HO, USA).

1.4.2. Oxygen evolution and respiration measurements

In mid-exponential growth phase, cultures were harvested, milli-Q water (as control), 40 mM and 80 mM NaF were added and then dark acclimated for 50 min. Oxygen evolution was measured under red light illumination (Wavelengths = 650 nm) at 500 μmol photons·m^{-2}·s^{-1} with a Clark-type oxygen electrode (Chlorolab 2, Hansatech). Respiration was measured under dark condition with the same equipment.

1.4.3. PSI quantum yield measurements

PSI quantum yield was measured by using dual-wavelength pulse-amplitude-modulated fluorescence monitoring system (Dual-PAM, Heinz Walz, Effeltrich, Germany) according to Perreault et al. (2009). Used modulated, actinic and saturating pulse (800 ms) light intensities were 1 μmol photons·m^{-2}·s^{-1}, 53 μmol photons·m^{-2}·s^{-1} and 3000 μmol photons·m^{-2}·s^{-1}, respectively. The PSI quantum yield [Y(I)] was calculated according to Klughammer and Schreiber (2008) as: $Y(I) = (P_M`-P)/(P_M-P_O)$, where $P_M$ and $P_M`$ are the maximum change of P700 signal in dark

and light adapted state, respectively, P is the P700 transmittance signal caused by actinic light before the saturating flash and P_O is the minimal level of P700 transmittance change measured after the saturating pulse, when P700 is reduced by electrons from PSII.

At the same time, the redox state of P700 under far red light and saturating multiple-turnover flash (MT) was also measured in FACHB898. The far red light was turned on to oxidize P700 at the seventh second. Later, at twenty second, MT flash was turned on to reduce the P700.

1.4.4. PSII chlorophyll a fluorescence measurements

In the mid-exponential growth phase, FACHB898 was filtered (Millipore, Billerica, MA, USA) and suspended in fresh BBM medium at a concentration of 3×10^7 cells/ml (3 μg chlorophyll *a*/ml), determined with Multisizer™ 3 Coulter Counter® (Beckman Coulter Inc., Brea, CA, USA). Milli-Q water (for control), 40 mM NaF or 0.4 M betaine (inhibition of PBS movement between two photosystems) were added and then samples were dark-adapted for 50 min before measurement. The maximum fluorescence (F_{Mdark}) was measured by applying saturating pulses (800 ms, 3000 μmol photons·m^{-2}·s^{-1}) every 40 seconds during 3 minutes using a Water-PAM fluorometer (Heinz Walz GmbH, Effeltrich, Germany). Then, far red light was turned on and maximum fluorescence under light condition (F_M') was measured by applying saturating light pulses every 40 seconds. After another 3 minutes, the far red light was turned off and F_{Mdark} was again measured every 40 seconds for three minutes.

1.4.5. DCMU treatment

Samples (control, 40 mM NaF and 0.4 M betaine) were dark-adapted for 1 h and then were put in PAM cuvette for measurement. 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (100 μM) was added to each sample and then actinic light (200 μmol photons·m^{-2}·s^{-1}) was turned on to induce state 1. We collected samples for 77K measurement before and after adding DCMU.

1.4.6. Low temperature fluorescence measurements

To evaluate the energy distribution between PSI and PSII, low temperature fluorescence (77K) emission spectra under 435-nm excitation was also measured. Samples were collected directly from the PAM cuvette before measurement (dark) and after 2 minutes of DCMU and actinic light. Under same illumination conditions, the samples were put in small glass tubes, and then quickly (3 seconds) immersed in liquid nitrogen. Low temperature emission spectra were obtained by using a luminescence spectrometer (Perkin Elmer, Waltham, USA) with an excitation slit (10 nm) and emission slit (20 nm). A band-pass green filter (λ = 493nm; Edmund 46052, Barrington, NJ) and a red long-pass filter (λ > 600nm; Edmund 66065, Barrington, NJ) were used for excitation and emission light, respectively. The fluorescence signals at 693 nm (F693) and at 729 nm (F729) were chosen to represent respectively the energy associated to PSII and PSI.

1.5. Results

1.5.1. The effects of NaF on photosynthesis and respiration

The effects of NaF on photosynthesis and respiration in FACHB898 are shown in

Table 1.1. Concentrations of NaF up to 40 mM did not affect oxygen evolution and respiration. However, at concentration of NaF of 80 mM, the oxygen evolution and respiration rates were decreased respectively to 31% and 21% of the control.

Table 1.1. The effects of sodium fluoride (NaF) on oxygen evolution and respiration in *Synechocystis* sp. FACHB898. Cells were kept in the dark for measurement of respiratory oxygen uptake or illuminated with red light for measurement of photosynthetic oxygen evolution. Rates of oxygen uptake and evolution are in μmol O_2 $(10^{10}$ cell$)^{-1}$ h^{-1}. Different letters in the same column indicate significant differences (Tukey's HSD, $P < 0.05$).

Cells in:	Oxygen evolution rate	Oxygen uptake rate
Control	35.66 ± 1.43^a	3.00 ± 0.66^a
40 mM NaF	32.27 ± 2.13^a	3.06 ± 1.19^a
80 mM NaF	11.02 ± 1.51^b	0.62 ± 0.08^b

1.5.2. The effects of NaF on quantum yield of PSI

The effects of NaF on quantum yield of PSI in FACHB898 are shown in Figure 1.1. 40 mM NaF treatment increased the PSI quantum yield [Y(I)] by 21%, However, at 80 mM NaF, Y(I) was inhibited by 33% compared to the control sample.

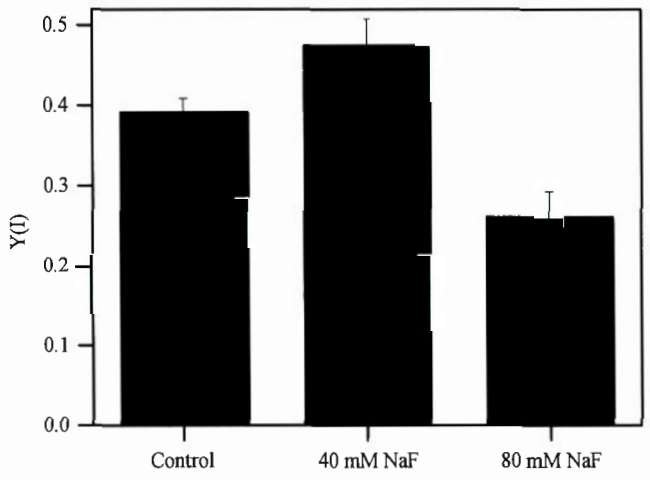

Figure 1.1. The effects of NaF on quantum yield of PSI (Y(I)) in FACBH898.

1.5.3. The effects of NaF on redox state of P700 induced by far red light and saturation pulse

In order to investigate the effects of NaF on the oxidation of P700 induced by far red light and on the electron transport rate between PSII and PSI in FACHB898, the redox state of P700 (control, 40 mM and 80 mM NaF) was recorded under far red light and MT flash (Figure 1.2). 40 mM and 80 mM NaF did not induce any significant effect on oxidation of P700 induced by far red light (Tukey's HSD, $P > 0.05$). However, 80 mM NaF treatment significantly inhibited (by 38%) electron transport between PSII and PSI compared to control sample (Tukey's HSD, $P < 0.05$). For the following

experiments, NaF concentration of 40 mM was chosen since we demonstrated that 80 mM NaF decreased the PSII and PSI activities and electron transport rate between PSII and PSI, thus that this concentration could modulate PQ redox state which is known to modify state transition.

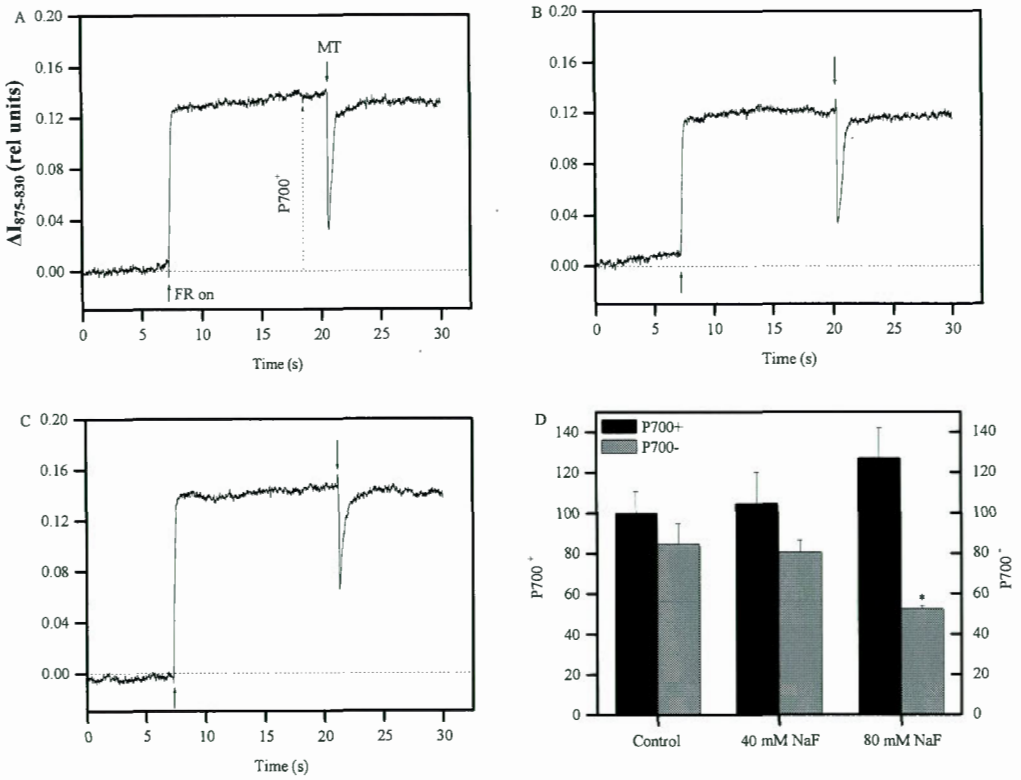

Figure 1.2. The effects of NaF (40 and 80 mM) on the change of PSI redox state induced by far red light and saturating light in FACHB898. Panel A: Control; Panel B: 40 mM NaF; Panel C: 80 mM NaF; Panel D: The oxidation and reduction of PSI under control, 40 mM and 80 mM.

1.5.4. The effects of NaF and betaine on transition to state 1 induced by far red light

The effect of NaF and betaine on transition to state 1 induced by far red light in FACHB898 is shown in Figure 1.3. Figure 1.3A shows a typical chlorophyll *a* fluorescence trace for a far red light induced transition to state 1 in the studied species: Far red light induced an increase of fluorescence from a minimum fluorescence level (F_0) to steady-state fluorescence level (F_S), and this was accompanied by an increase of the saturating light induced maximum fluorescence level (F_{Mdark}) to obtain F_M'. Removal of the far red light leaded to the relaxation of F_S and F_M' values toward the initial dark-adapted values, F_0 and F_{Mdark} respectively, indicating that transition to state 2 was induced by darkness. These results suggest that PBSs moved and/or that spillover occurs under these conditions, resulting in state transition. A concentration of 40 mM NaF reduced the increase of the maximal fluorescence signal (F_M') by half compared to the control (Figure 1.3A), suggesting an inhibition of the state transition due to the inhibition of phosphatase by NaF (Figures 1.3B and 1.3D). Similarly, figures 1.3C and 1.3D show that 0.4 M betaine partly inhibited transition to state 1 induced by far red light since the increase of the maximal fluorescence level (F_M') was also reduced.

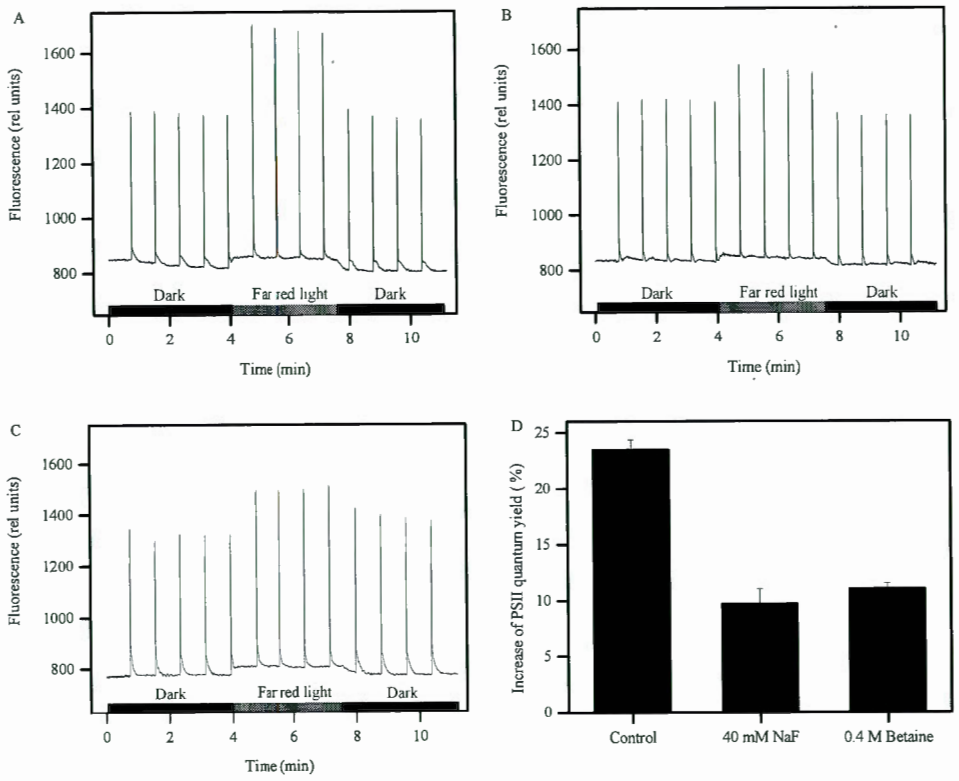

Figure 1.3. The results of transition to state 1 induced by far red light in FACHB898 under control, NaF and Betaine. Panel A: Control; Panel B: 40 mM NaF; Panel C: 0.4 M betaine; Panel D: the increase of PSII quantum yield by far red light in compared to dark for control, 40 mM NaF and 0.4 M betaine treated samples (increase of PSII quantum yield = $(Y_{red\ light}-Y_{dark})/Y_{dark}\times 100$. $Y_{red\ light}$ and Y_{dark} are the PSII quantum yield under far red light and dark condition respectively).

1.5.5. The effects of NaF on transition to state 1 induced by DCMU

In order to determine whether NaF can inhibit the transition to state 1 induced by DCMU in FACHB898, we treated the cyanobacteria by 40 mM NaF before adding DCMU (Figure 1.4). As seen, in presence of DCMU, actinic light illumination quickly induced transition to state 1 since the room temperature fluorescence increases

to the maximal as in the control (Figure 1.4A) and the F693/F729 increased by 28% and 44% compared to control and NaF treatment (Figure 1.4B). In these conditions, PQ are completely oxidized since DCMU blocks electron transport from PSII to PQ, therefore, transition to state 1 induced by DCMU can be considered as the maximum state 1 extent. However, our study showed that this complete transition to state 1 was not inhibited by 40 mM NaF (Figure 1.4).

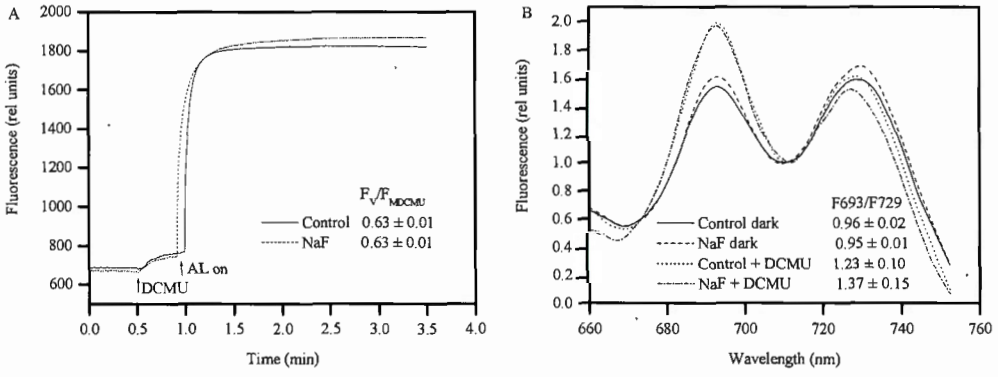

Figure 1.4. The effects of NaF (40 mM) on transition to state 1 induced by DCMU according to room temperature fluorescence measurements (panel A) and 77K fluorescence measurements (panel B) in FACBH898. The fluorescence data of 77K measurements were normalized at 711 nm.

1.5.6. The effects of betaine on transition to state 1 induced by DCMU

The room temperature fluorescence emission and 77K emission fluorescence spectra were used to determine the effect of betaine on transition to state 1 induced by DCMU (Figure 1.5). We can notice that state 1 induced by DCMU was not inhibited by 0.4 M betaine since the room temperature fluorescence emission increased to the maximum, as for the control (Figure 1.5A), and the F693/F729 ratio increased also similarly to the control (Figure 1.5B).

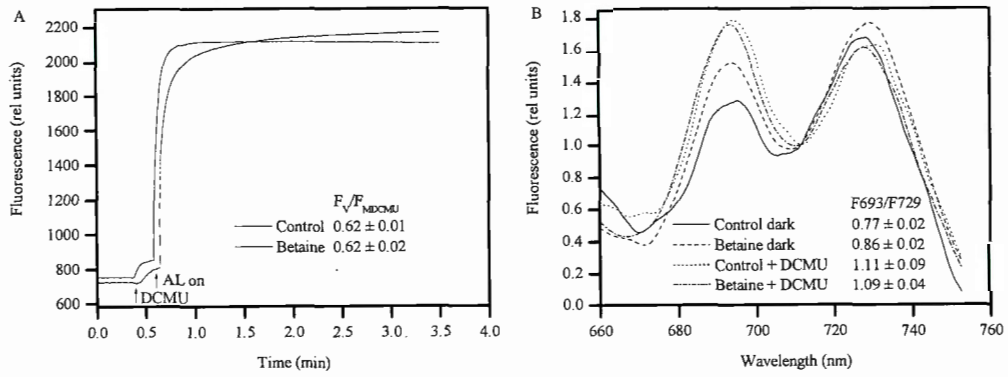

Figure 1.5. The effects of betaine (0.4 M) on transition to state 1 induced by DCMU according to room temperature fluorescence measurements (panel A) and 77K fluorescence measurements (panel B) in FACBH898. The fluorescence data of 77K measurements were normalized at 711.5 nm.

1.6. Discussion

We have provided evidences that phosphatase may be involved in state transition in *Synechocystis* sp. FACHB898 when transition to state 1 is induced by far red light. This result is in accordance with what was previously shown in higher plants and green algae (Bennett, 1979; Telfer *et al.*, 1983; Canaani *et al.*, 1984; Veeranjaneyulu *et al.*, 1991; McCormac *et al.*, 1994; Delphin *et al.*, 1995; Gans and Wollman, 1995). For the last 30 years, whether phosphorylation and dephosophorylation are involved in state transition in cyanobacteria lead to controversial results despite the research aiming to better understand state transition in cyanobacteria (Mullineaux, 1999; Puthiyaveetil *et al.*, 2012). Canaani (1986) suggested that NaF inhibited the transition to state 1 induced by far red light in *Nostoc muscorum* according to room temperature fluorescence experiments. Harrison et al. (1991) also found that NaF inhibited transition to state 1 in thylakoid membrane isolated from *Synechococcus* sp.. However, Delphin et al. (1995) compared the effect of NaF on state transition in green alga (*Chlamydomonas reinhardtii*) and red alga (*Rhodella violacea*), and found that NaF can inhibit the transition to state 1 induced by far red light in *Chlamydomonas reinhardtii* but not in *Rhodella violacea.* Moreover, it was also found that NaF cannot inhibit transition to state 1 induced by far red light in *Synechocystis* PCC 6803 (Shen and Wang, 1998). Therefore, these contradictory results occasioned ambiguity in the understanding of phosphatase role in the transition to state 1 mechanism in cyanobacteria. In the present study, we showed that at a NaF concentration (40 mM), not inhibiting oxygen evolution and uptake, quantum yield of PSI and electron transport rate between PSII and PSI, this phosphatase inhibitor partly blocked transition to state 1, suggesting that dephosphorylation may play a role in the far red light transition to state 1 in FACHB898. We can explain the contradictory results between our study and Shen and Wang's (1998) paper by the much higher

cyanobacterial cell density used in this previous study (the Chl *a* concentrations in Shen and Wang's paper and our study are respectively 40 µg/ml and 3 µg/ml). Indeed, the ratio NaF and cell concentration may have been too low to observe inhibition of the transition to state 1 induced by far red light, since we found that the extent of this state transition inhibition is dependent on the concentration of NaF (data not shown).

As mentioned previously, state transition in cyanobacteria is mainly related to two different mechanisms (mobile PBS and spillover models). It is suggested that dark-light and light-dark state transitions involve these two mechanisms. Indeed, dark to blue light induced transition to state 1 is partly (65%) linked to the movement of PBSs at room temperature (Zhang *et al.*, 2007), suggesting that spillover participates also in this state transition. However, it was also proposed that dark-light induced state transition does not involve spillover (Joshua and Mullineaux 2004; Tsimilli-Michael et al., 2009). It was previously shown that betaine can immobilize PBSs on the thylakoid membranes, therefore inhibiting state transition involving PBS movement (Li *et al.*, 2004; Zhang *et al.*, 2007). In the present study, we found that betaine can also partly inhibit transition to state 1 induced by far red light. This result tends also to confirm that transition to state 1 induced by far red light involves PBS movement. Moreover, dark to far red light induced transition to state 1 seems also to be regulated by the dephosphorylation (since it was inhibited by NaF). Therefore, we hypothesize that transition to state 1 induced by dark to light exposure involves PBS movement in cyanobacteria similarly to transition to state 1 found in higher plants which is controlled by dephosphorylation of light harvesting complexes, except that in cyanobacteria PBSs are involved instead of LHCII.

On the other hand, it was surprising that DCMU-induced transition to state 1 was not inhibited by NaF in the studied species, suggesting that there is another mechanism than dephosphorylation involved in transition to state 1 in FACHB898. This mechanism could be related to spillover. Indeed, the transition to state 1 induced by DCMU does not include a complementary change in the distribution of energy

between PSI and PSII in the studied species (Fig. 1.4B and Fig. 1.5B). These results also seem to suggest that PBS movement is not involved for the transition to state 1 induced by DCMU in the studied species. It was suggested that DCMU-induced state 1 was related to movement of PBSs and to spillover in *Spirulina platensis* (Li *et al.*, 2004). However, in our study, NaF did not inhibit the transition to state 1 induced by DCMU indicating that the movement of PBSs induced by phosphatase was not necessary for the DCMU-induced state 1 in FACBH898.

To our knowledge, for the first time, we provided evidence, based on fluorescence data, that dephosphorylation is probably involved in the transition to state 1 induced by far red light in intact cells of *Synechocystis* sp.. We have also shown that PBS movement seems to be not necessary for the transition to state 1 induced by DCMU in the studied species. It is however necessary to further investigate the phosphorylation/dephosphorylation site of PBS and to better understand how different mechanisms involved in state transition (PBS movement and spillover) work together to allow efficient adaptation of cyanobacteria when exposed to different environmental conditions.

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CHAPTER II

STATE 2 TO STATE 1 TRANSITION INDUCED BY DARK-HIGH LIGHT SHIFT PLAYS A ROLE IN HIGH LIGHT REGULATION IN *SYNECHOCYSTIS* SP.

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2.1. Résumé

Il est connu que la transition de l'État II vers l'État I est induite par le passage de l'obscurité à la forte lumière chez les cyanobactéries. Cependant, le rôle de cette transition n'a pas encore été élucidé. La présente étude a pour but d'examiner le rôle de la transition de l'État II vers l'État I dans l'acclimatation à la forte lumière chez *Synechocystis* sp. FACHB898. En induisant la transition des cyanobactéries vers l'État II par l'obscurité, le NaF, connu pour jouer un rôle dans l'inhibition de la déphosphorylation, provoqua une baisse significative du rendement quantique du photosystème PSII durant une exposition à la lumière intense. Par rapport aux témoins, une récupération du rendement quantique du PSII a été par la suite observée à l'obscurité. Cependant, aucun effet du NaF sur le rendement du PSII ainsi qu'aucune récupération du rendement à l'obscurité n'ont été constatés lorsque les échantillons ont été prétraités à la lumière bleue. Ces résultats indiquent donc que la transition de l'État II vers l'États I qui est induite par le passage de l'obscurité à la forte lumière jouerait un rôle important dans l'acclimatation à la lumière intense chez cette souche de cyanobactérie. Une conclusion similaire peut etre obtenue pour les autres intensités lumineuses, suggérant que les transitions d'états peuvent jouer un rôle important pour l'acclimatation à la lumière dans l'environnement.

Mots clé: Transition d'états, *Synechocystis* sp., lumière intense

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2.2. Abstract

It is known that state 2 to state 1 transition is induced by dark-high light shift in cyanobacteria, but its role is still not clear. Therefore, we investigated the role of state 2 to state 1 transition during high light regulation in *Synechocystis* sp. FACHB898. When state 2 was induced by pre-dark acclimation, NaF (inhibitor of dephosphorylation) significantly decreased the operational PSII quantum yield under high light and the PSII quantum yield recovery in dark following this high light treatment. However, when samples were pre-acclimated to blue light, NaF did not affect the PSII quantum yields under high light treatment and dark recovery. These results suggest that state 2 to state 1 transition induced by dark-high light shift influence the resistance to high light in the studied species. Similar conclusion can also be drawn for other light intensities suggesting that this state transition may play an important role in photoprotection in natural environment.

Key words: State transition, *Synechocystis* sp., High light

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2.3. Introduction

State transition is known to balance light energy between photosynthesis II (PSII) and photosynthesis I (PSI) under varying light conditions in cyanobacteria (Post *et al.*, 1986; Biggins and Bruce, 1989; Schreiber *et al.*, 1995). This photo-regulation mechanism is controlled by redox state of plastoquinone (PQ) and can be divided into two types in cyanobacteria: light and redox state transition (Mullineaux and Allen, 1986; Allen, 2003). Light state transition is induced by the change of light quality and only involves the phycobilisome (PBS) movement between PSII and PSI (Li *et al.*, 2004). On the other hand, the redox state transition refers to the PBS movement and the conformational change of PSI (monomer/trimer state) induced by the change from light to dark condition (and dark to light condition) or DCMU (Li *et al.*, 2006). For both types of state transition, reduced PQ induces state 2 and PBS movement toward PSI. On the contrary, oxidized PQ induces state 1 and PBS will be more attached to PSII.

It is known that in cyanobacteria respiration and photosynthesis share some electron transporters, such as PQ, cytochrome b_6f and plastocyanin (Campbell *et al.*, 1998; Kirilovsky, 2014; Mullineaux, 2014). Therefore, dark-acclimated cyanobacterial cells will be under state 2 due to a reduced PQ pool by respiratory processes (Aoki and Katoh, 1982; Mullineaux and Allen, 1986). Upon illumination, electrons accumulated in the PQ pool in darkness will be transferred to PSI, therefore re-oxidizing the PQ pool inducing state 2 to state 1 transition (Mullineaux and Allen, 1990), thus the PBS movement and the PSI conformational change. However, to our knowledge, there is no information about the role of this state 2 to state 1 transition in the regulation of cyanobacteria to high light condition.

In the present work, we used a phosphatase inhibitor, NaF, to block the state 2 to state 1 transition involving the PBS movement induced by dark-high light shift in

Synechocystis sp.. We investigated whether this state 2 to state 1 transition plays a role for high light regulation in cyanobacteria, thus providing evidences to better understand the role of state transition under these conditions.

2.4. Materials and methods

2.4.1. Culture

Synechocystis sp. FACHB898 was obtained from Freshwater Algal Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences. This species was cultured under a temperature of 22 °C in bold basal media (BBM) at pH 6.8 (Stein, 1973). The culture was grown under a light:dark cycle of 14:10 h at 50 $\mu E \cdot m^{-2} \cdot s^{-1}$, provided by a combination of incandescent bulbs and white fluorescent tubes (Philips F72T8/TL841/HO, USA).

2.4.2. Pre induction of state 1 and state 2

In mid-exponential growth phase, *Synechocystis* sp. was filtered (Millipore, Billerica, MA, USA) and re-suspended in fresh BBM medium at a concentration of 3×10^7 cells/ml, determined by a Multisizer™ 3 Coulter Counter® (Beckman Coulter Inc., Brea, CA, USA). Milli-Q water (as control) and NaF (80 mM) were added and then samples were acclimated 50 minutes to dark or blue light (20 $\mu E \cdot m^{-2} \cdot s^{-1}$) to induce state 2 and state 1. In this chapter, we used 80 mM NaF to completely inhibite the state 2 to state 1 transition induced by dark-high light shift in the studied species.

2.4.3. Chlorophyll a fluorescence measurements

After pre-dark or pre-blue light acclimation, the chlorophyll *a* fluorescence was measured by FMS-1 fluorometer (Heinz Walz GmbH, Effeltrich, Germany). The maximum fluorescence (F_M) was measured by applying saturating pulses (800 ms, 3000 $\mu E \cdot m^{-2} \cdot s^{-1}$). Then, actinic white light (1700 $\mu E \cdot m^{-2} \cdot s^{-1}$) as high light was turned

on and maximum fluorescence under light condition (F_M') was measured by applying saturating light pulses every 1 minute for 12 minutes. After this high light illumination, the actinic light was turned off and F_M was again measured at 2, 5, 10, 20, 30 and 40 minutes during dark recovery.

2.4.4. Low temperature fluorescence measurements

To evaluate energy distribution between PSII and PSI, low temperature fluorescence (77K) emission spectra under 436-nm excitation was measured. Samples were collected directly from the measurement cuvette before measurement (dark) and after actinic high light treatment. Under same illumination conditions, the samples were put in small glass tubes, and then quickly (3 seconds) immersed in liquid nitrogen. Low temperature emission spectra were obtained by using a luminescence spectrometer (Perkin Elmer, Waltham, USA) with an excitation slit (10 nm) and emission slit (10 nm). A short-pass green filter ($\lambda < 600$ nm; Edmund 46052, Barrington, NJ) and a red long-pass filter ($\lambda > 600$ nm; Edmund 66065, Barrington, NJ) were used for excitation and emission light, respectively. The fluorescence signals at 693 nm (F693) and at 729 nm (F729) were chosen to represent respectively the energy associated to PSII and PSI.

2.5. Results

2.5.1. The effect of NaF on state transition

Dark acclimated cyanobacteria have lower PSII quantum yield compared to blue light acclimated sample, suggesting that state 2 was induced by dark and state 1 was induced by blue light in the studied species (Figure 2.1A). Sodium fluoride (NaF) decreased the PSII quantum yield under dark and blue light by both 10% compared to control samples, but it is not influencing the state transition in these conditions. Indeed, the 77K fluorescence spectra confirmed that different states were induced by acclimation to dark and blue light since the F693/F729 ratios in the dark and NaF + dark samples were lower than in the blue light and NaF + blue light samples (Figure 2.1B).

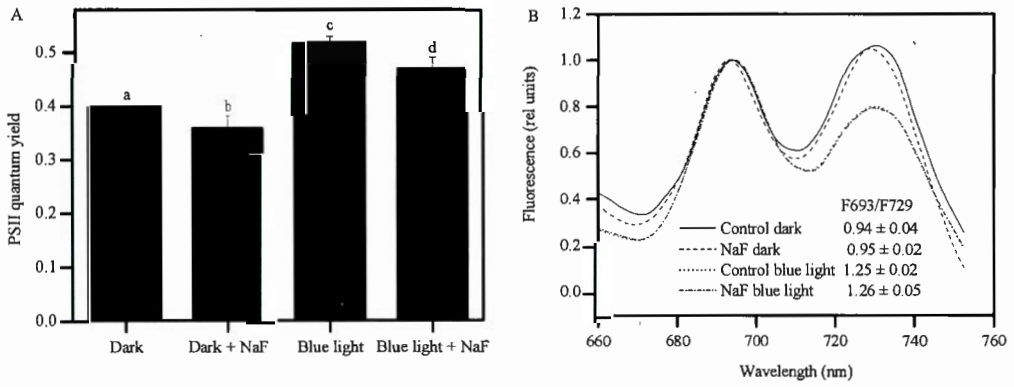

Figure 2.1. The effect of NaF on PSII quantum yield under dark and blue light (A) and state transition (B) of *Synechocystis* sp. pre-acclimated under dark and blue light. The 77K fluorescence spectra were normalized to the fluorescence emitted at 693 nm.

The effect of NaF on the state 2 to state 1 transition induced by dark-high light shift was also investigated (Figure 2.2). After moving *Synechocystis* sp. from dark to high light, NaF treated sample has lower F693/F729 value compared to control, indicating that state 2 to state 1 transition induced by dark-high light shift was inhibited by 46% compared to the control.

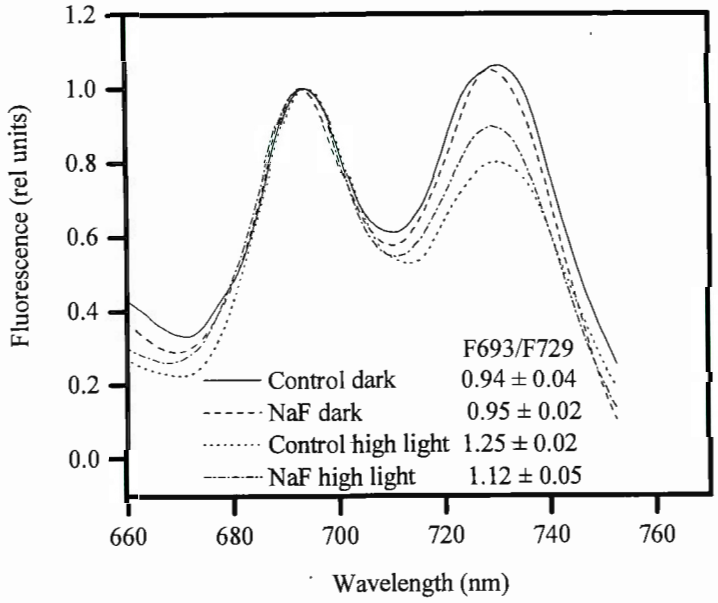

Figure 2.2. The effect of NaF on state 2 to state 1 transition induced by dark-high light (illumination under high light for 30 senconds) shift for *Synechocystis* sp. pre-acclimated under dark condition according to 77K measurements. The 77K fluorescence spectra were normalized to the fluorescence emitted at 693 nm.

2.5.2. The fluorescence changes under high light and dark conditions

Figure 2.3A represents a typical chlorophyll *a* fluorescence change under high light treatment and dark recovery for *Synechocystis* sp. pre-acclimated to dark. Under high light condition, the decrease in the fluorescence yield and in F_M' suggests induction of NPQ. After 40 minutes of dark recovery, F_0 can completely return to its initial level and F_M to 65% of its initial level. These results suggest that dark acclimated cyanobacteria can rapidly induce NPQ to acclimate high light treatment and this NPQ was mainly related to qE and qT. Figure 2.3B shows that NPQ can also be induced by high light when the studied species was initially under state 1 (blue light illumination). For the pre-dark acclimated samples in presence of NaF (Fig. 2.3C), NPQ was induced by high light, but after 40 minutes dark recovery, F_0 could not completely recover and the F_M was able to return only at 50% of its initial level. These results suggest that more photoinhibition was induced in the dark + NaF acclimated samples. Figure 2.3D shows that NaF did not affect the ability for high light regulation in the studied species when state 1 was pre-induced under low blue light.

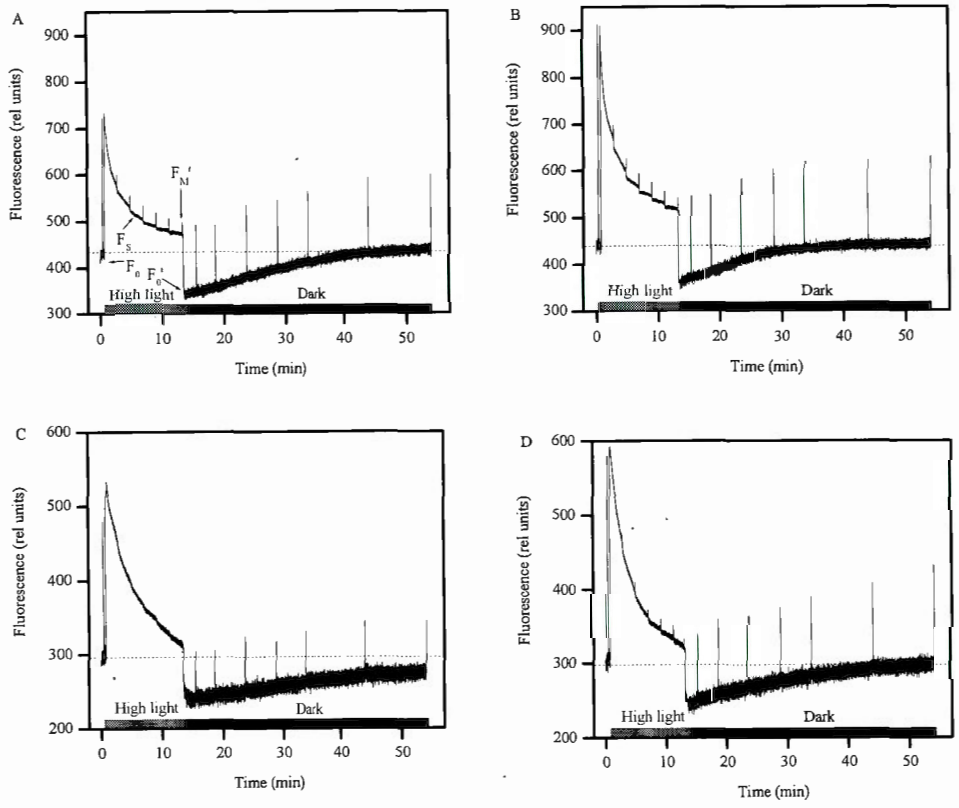

Figure 2.3. The effect of NaF on fluorescence yield changes under high light and dark recovery conditions for *Synechocystis* sp. pre-acclimated to dark or blue light. Panel A: pre-acclimated to dark; Panel B: pre-acclimated to blue light; Panel C: pre-acclimated to dark in presence of NaF; Panel D: pre-acclimated to blue light in presence of NaF.

2.5.3. The PSII quantum yields under high light and dark conditions

For the pre-dark acclimated samples, the PSII quantum yields were significantly decreased in presence of NaF during the high light treatment and dark recovery compared to the control (Figure 2.4A). However, for the blue light acclimated *Synechocystis* sp., NaF had no effect on the PSII quantum yields under high light treatment and dark recovery (Figure 2.4B), suggesting that the state 2 to state 1 transition induced by dark-light shift is highly involved in the high light regulation process of the studied species.

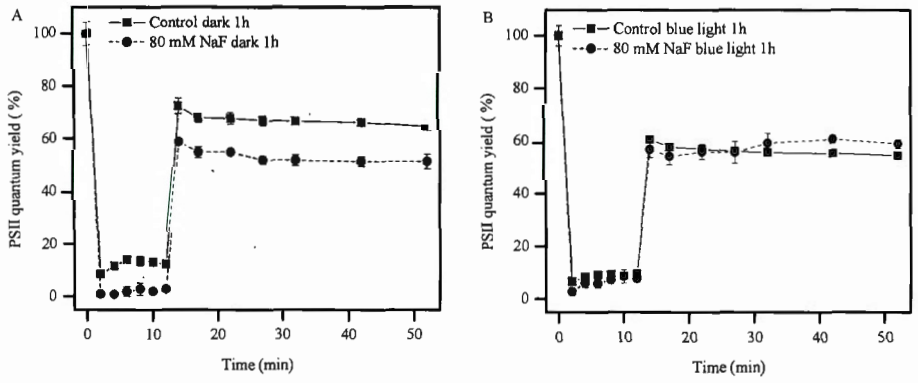

Figure 2.4. The effect of NaF on PSII quantum yield changes under high light (1700 $\mu E \cdot m^{-2} \cdot s^{-1}$) and dark conditions for *Synechocystis* sp. pre-acclimated to dark (A) or blue light (B). The initial PSII quantum yield is 0.40 $\pm$ 0.00, 0.36 ± 0.02, 0.52 ± 0.01 and 0.47 ± 0.02 for control dark, NaF dark, control blue light and NaF blue light samples respectively.

2.5.4. The role of state transition to regulate different light intensities

In order to investigate the role of state 2 to state 1 transition induced by dark-light shift under different light intensity treatments, other light intensities (200 $\mu E \cdot m^{-2} \cdot s^{-1}$ and 700 $\mu E \cdot m^{-2} \cdot s^{-1}$) were also chosen to expose the different pre-acclimated samples. The difference of the PSII quantum yield between dark and dark + NaF pre-acclimated samples was not affected by the three investigated light intensities (Figure 2.5). This suggests that state 2 to state 1 transition plays a similar regulation role when the cyanobacteria was exposed to moderate and high light conditions.

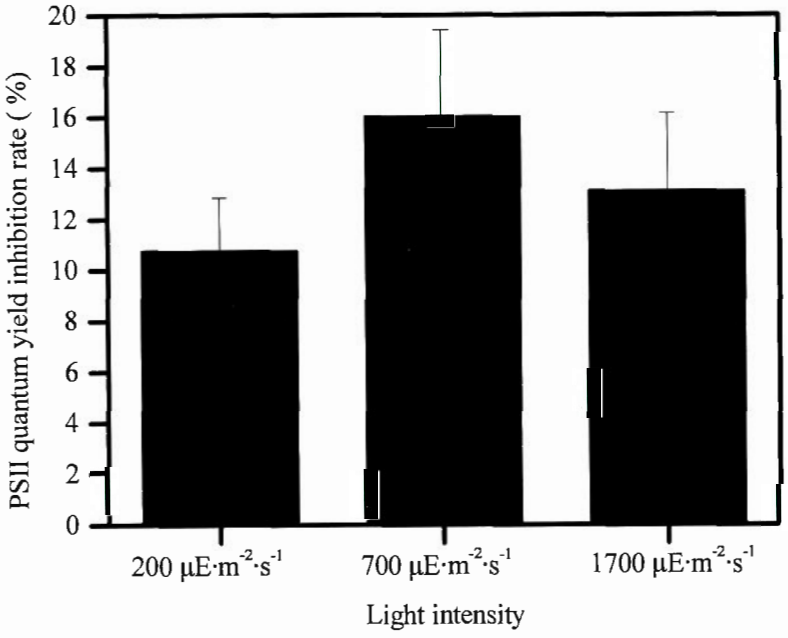

Figure 2.5. The inhibition rate of PSII quantum yield by NaF in *Synechocystis* sp. during 40 minutes of dark recovery after 12 minutes exposure to different high light treatments.

2.6. Discussion

Until now, high light regulation studies in cyanobacteria focused mainly on the role of state 1 to state 2 transition (PBSs movement from PSII to PSI). It was suggested that state 1 to state 2 transition plays a role in high light regulation since more energy will be transferred to PSI and therefore can protect PSII under state 2 (Dong *et al.*, 2009). However, it is also known that when cyanobacterial cells move from dark or low light to high light condition, state 2 to state 1 transition will occur (Campbell *et al.*, 1998), but to our knowledge, no information exists on the role of state 1 transition under these conditions. We clearly showed here that state 2 to state 1 transition induced by dark-high light shift could play an important role for high light regulation in *Synechocystis* sp..

It was previously demonstrated that F_0 quenching is related only to the quenching of PBS fluorescence, which is associated to the orange carotenoid protein (OCP) non-photochemical quenching (Wilson *et al.*, 2006; Karapetyan, 2007; Wilson *et al.*, 2007). OCP-related quenching is another mechanism (beside state transition) that plays an important role in high light resistance in cyanobacteria (Wilson *et al.*, 2006), while F_M' quenching is related to PBS and chlorophylls (Kirilovsky, 2007; Rakhimberdieva *et al.*, 2007; Rakhimberdieva *et al.*, 2007). In the studied species, all the fluorescence levels (F_0, F_s and F_M') decreased after the 12 minutes high light illumination for the four pre-acclimated types of cells (Figure 2.3). These results suggest that different pre-acclimation conditions did not affect the type of fluorescence quenching induced. However, for the dark + NaF sample, the F_M' was much lower than the one in the control sample under high light treatment and it recovered less during the dark recovery period. Moreover, F_0 was not able to recover to its initial level for the dark + NaF sample after 40 minutes dark recovery (Figure 2.3C). These results indicate that photoinhibition was more induced after 12 minutes high light treatment in dark + NaF sample compared to the dark pre-acclimated samples. In *Synechocystis* sp., the state

1 to state 2 transition was not affected by NaF (data not shown), therefore, if we compare the effect of NaF on high light regulation between pre-dark and pre-blue light acclimated samples, state 1 to state 2 transition does not seem to be involved in the decrease of high light regulation ability in the dark + NaF sample. Moreover, we showed that the PBS-related quenching is different between the dark + NaF sample and the other three acclimation conditions (Fig. 2.3A, 2.3B and 2.3D). We can therefore advance that state 2 to state 1 transition induced by dark-high light shift affects the OCP-related quenching in *Synechocystis* sp..

It was suggested recently that usually only one of the photo-protection mechanisms (state transition or OCP-related quenching) is triggered to cope with high light in cyanobacteria (Zhao *et al.*, 2015). Therefore, when OCP-related quenching is induced, state 1 to state 2 transition should be inhibited, and vice versa. Based on the results presented in the present work, we may conclude that pre-blue light acclimated samples mainly trigger OCP-related quenching to cope with high light since NaF does not affect high light regulation. On the other hand, we demonstrated that the pre-dark acclimated sample mainly triggers state 1 to state 2 transition to cope with high light. It is known that the main difference between the pre-dark and pre-blue light acclimated samples is the extent of PBSs attaching to PSII, where more PBSs attached to PSII in pre-blue light acclimated sample compared to pre-dark acclimated sample (Campbell *et al.*, 1998). Therefore, we may advance that when more PBSs attach to PSII, the OCP-related quenching will be more induced and that a threshold concentration of PBSs attaching to PSII could be necessary for triggering the OCP-related quenching under high light. A similar situation was found in iron limited cyanobacterial cells, where under state 1 (most PBSs attached to PSII) OCP-related quenching was easily induced under high light (Bailey *et al.*, 2005).

We demonstrated here that the state 2 to state 1 transition induced by dark-high light shift affects the high light regulation ability of *Synechocystis* sp., and that is probably linked to the OCP-related quenching. These results suggest that state transition and OCP-related quenching could interact and work together to provide optimal regulation to high light in cyanobacteria, which can help us to understand the mechanism of high light acclimation in cyanobacteria.

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CHAPTER III

RELATIVE INVOLVEMENT OF THE ORANGE CAROTENOID PROTEIN (OCP)-RELATED NON-PHOTOCHEMICAL QUENCHING AND THE STATE TRANSITION DURING HIGH LIGHT REGULATION OF TWO CYANOBACTERIAL SPECIES

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3.1. Résumé

Les mécanismes de photo-protection impliquant l'*orange carotenoid protein* (OCP) et les transition d'états constituent des mécanismes de protection importants qui permettent aux cyanobactéries de réagir à des conditions de stress lumineux élevés. Cependant, la manière dont ces deux mécanismes fonctionnent ensemble pour une réponse optimale n'est toujours pas claire. Afin de mieux comprendre la complémentarité entre le *quenching* associé aux OCPs et les transition d'états, nous avons évalué ces mécanismes chez deux espèces de cyanobactéries (*Synechocystis* sp. FACHB898 et *Microcystis aeruginosa* FACHB905), lorsqu'exposées à un stress de lumière élevé. Nous avons démontré que les mécanismes de photo-protection associés aux OCPs sont plus élevés chez *M. aeruginosa*, alors qu'elle présente une plus faible capacité de transition entre l'état 1 à 2, comparativement à *Synechocystis* sp. De plus, *M. aeruginosa* a démontré une plus grande capacité de régulation au stress de lumière élevé. Ces résultats suggèrent que pour une régulation à un stress de lumière élevé, la photo-protection associée aux OCPs constitue un mécanisme plus efficace que l'état de transition chez les espèces étudiées.

Mots clé: Processus non-photochimique de protection impliquant l'*orange carotenoid protein*, États de transition, Stress lumineux, *Synechocystis* sp. et *Microcystis aeruginosa*

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3.2. Abstract

Orange Carotenoid Protein (OCP)-related non-photochemical quenching and state transition are important mechanisms permitting light stress regulation of cyanobacteria. However, how these two mechanisms work together for optimal regulation of high light is still unclear. Therefore, we evaluated these mechanisms in *Synechocystis* sp. FACHB898 and *Microcystis aeruginosa* FACHB905. We found that *M. aeruginosa* had higher OCP-related quenching, but lower ability for state 1 to state 2 transition compared to *Synechocystis* sp.. Moreover, *M. aeruginosa* showed a higher ability to resist to high light stress then *Synechocystis* sp., suggesting that OCP-related quenching is more efficient than state transition to cope with high light stress.

Keywords: Orange carotenoid protein (OCP)-related non-photochemical quenching, state transition, high light stress, *Synechocystis* sp. and *Microcystis aeruginosa*

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3.3. Introduction

Although light is essential for cyanobacterial growth, too much light can be harmful by inducing production of damaging reactive oxygen species. In natural environment light intensity may fluctuate rapidly causing possible stress to photosynthetic organisms. Therefore, cyanobacteria have developed different protective mechanisms to cope with excess of light (Biggins and Bruce 1989; Allen 1992; Karapetyan et al. 1999; Müller et al. 2001). One of them is the non-photochemical quenching (NPQ) process, which permits to dissipate excess excitation energy as heat through pigment-protein complexes. It is known that NPQ plays a major role to regulate and protect photosynthetic apparatus in environments in which light energy absorption exceeds the capacity for light utilization (Karapetyan 2007; Joshua and Mullineaux 2004). In cyanobacteria, NPQ can be divided into at least three components, which are Orange Carotenoid Protein (OCP)-related NPQ, state transition and D1 protein damage-related photoinhibition (Karapetyan 2007; Wilson et al. 2006). OCP-related NPQ and state transition were proposed to play a role for the regulation to high light stress in cyanobacteria (Wilson et al. 2007; Kirilovsky 2007), but their relative contribution is still unclear.

The OCP is a photoactive protein being orange in darkness (inactive form) and being converted to active red form under blue-green light or high white light (Wilson et al. 2008). This red OCP form binds to the core of phycobilisome (PBS) and decreases the excess energy transferred to the reaction centers by increasing thermal dissipation (Wilson et al. 2008; Kirilovsky 2015; Tian et al. 2012), and thus protecting cyanobacteria against high light stress. Indeed, it was found that ΔOCP *Synechocystis* mutant (lacking OCP) was more sensitive to high light stress than the wild type (Wilson et al. 2006). Although most cyanobacterial species possess OCP, some species are lacking this protein and were shown to be more sensitive to high irradiance and lose their photosystem II (PSII) activity faster than species containing the OCP

(Boulay et al. 2008). However, there is not enough information about the different abilities to induce OCP-related NPQ under high light stress among various cyanobacterial species, since most of the studied species were "model" cyanobacteria such as *Synechocystis* sp. and *Synechococcus* sp..

State transition is controlled by the redox state of plastoquinone (PQ) and plays a role in keeping the balance of absorption energy between PSII and photosystem I (PSI). Early studies demonstrated that state transition does not play a high light regulation role in cyanobacteria, but on the contrary suggested that this mechanism is needed to balance light absorption between PSII and PSI only under low light intensity (Emlyn-Jones et al. 1999; Allen 2003; Mullineaux and Emlyn-Jones 2005). However, some more recent works suggested that state transition plays a role for cyanobacterial regulation to high light condition (Fujimori et al. 2005; Dong et al. 2009; Wang et al. 2010; Xu et al. 2012b), but further research is needed to better understand the possible involvement of state transition under high light stress condition in cyanobacteria. Moreover, to our knowledge, it is not known how OCP-related NPQ and state transition work together to permit optimal high light regulation in cyanobacteria.

We therefore investigated the high light regulation ability of two cyanobacterial species in relation to the OCP-related NPQ and state-transition. We demonstrated that the two studied species have different abilities to induce OCP-related NPQ and state transition and therefore show different abilities to cope with high light.

3.4. Materials and methods

3.4.1. Cultures

Synechocystis sp. FACHB898 and *Microcystis aeruginosa* FACHB905 were obtained from Freshwater Algal Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences. These two species were grown under a temperature of 22 °C in bold basal media (BBM) at pH 6.8 (Stein 1973), with a light:dark cycle of 14:10 h at 50 μmol photons·m^{-2}·s^{-1}, provided by a combination of incandescent bulbs and white fluorescent tubes (Philips F72T8/TL841/HO, USA). The cell number and volume were measured by a Multisizer™ 3 Coulter Counter® (Beckman Coulter Inc., Brea, CA, USA) every two days.

3.4.2. Chlorophyll a fluorescence measurement

At exponential phase, cells were collected on a 0.8 μm filter (Xingya Purifying Materials Factory, Shanghai, China), and then resuspended in fresh BBM to get a chlorophyll *a* concentration of approximately 3 μg/ml. Chlorophyll *a* fluorescence measurements were recorded by a FMS-1 fluorometer (Hansatech Instruments Ltd., Norfolk, UK). All samples were dark acclimated for 15 min prior to measurement. Saturating light was provided with white light (3000 μmol photons·m^{-2}·s^{-1}) for 1 s duration. Actinic irradiance was provided with white light (1700 μmol photons·m^{-2}·s^{-1}), and was used for high light treatment during 12 min. During this period, maximal fluorescence yield under light was measured by triggering saturating light every 2 min. Then, actinic light was turned off, and maximal fluorescence yield under dark was measured at 2, 5, 10, 15 20, 30 and 40 min by using saturating light. At the end of the measurement, DCMU was added to the samples to get the maximal

fluorescence yield (F_M) in presence of DCMU and under light illumination (200 µmol photons·m^{-2}·s^{-1}). The non-photochemical quenching (NPQ) was calculated with the following equations: NPQ = (F_M- F_M')/ F_M' (Bilger and Björkman, 1990).

3.4.3. Room and low temperature fluorescence measurements

In order to investigate the fluorescence quenching associated with chlorophyll *a* and PBSs (Wilson et al. 2006; Kirilovsky 2007), room temperature fluorescence was measured with a Cary Eclipse spectrofluorometer (Varian, USA). Before and after high light treatments, samples were immediately transferred in the spectrofluorometer and fluorescence emission spectra from 600-800 nm were recorded. The excitation light was 436 nm and 580 nm to excite chlorophyll *a* and PBSs respectively (Kirilovsky 2007).

In order to evaluate energy distribution between PSI and PSII before and after high light treatment, low temperature fluorescence (77K) emission spectra under 436-nm excitation were also measured according to Li et *al.* (2004), by transferring samples in small glass tubes which were quickly (less than 3 seconds) immersed in liquid nitrogen. Low temperature emission spectra were obtained by using a luminescence spectrometer (Perkin Elmer, Waltham, USA) with an excitation slit (10 nm) and emission slit (10 nm). A short-pass filter ($\lambda < 600$nm; Edmund 64665, Barrington, NJ) and a red long-pass filter ($\lambda > 600$nm; Edmund 66065, Barrington, NJ) were used for excitation and emission light, respectively. The fluorescence signals at 693 nm (F693) and at 729 nm (F729) were chosen to represent respectively the energy associated to PSII and PSI.

3.5. Results

3.5.1. Room temperature fluorescence transient

The fluorescence transients during high light illumination and darkness are shown in Figure 3.1A (*Synechocystis* sp.) and 3.1B (*M. aeruginosa*). For *Synechocystis* sp., a fast quenching of the maximal fluorescence (F_M') was observed under high light treatment. The steady state (F_S) and minimal fluorescence yields were also decreased. During the dark recovery, the maximal and minimal fluorescence yields (F_M' and F_0') increased respectively to 60% and 100% of the original levels (F_{MDARK} and F_0). For *M. aeruginosa* (Figure 3.1B), the F_M', F_S and F_0 decreased under high light treatment, but more importantly than in *Synechocystis* sp.. However, the recovery of F_M' is much faster and more complete in *M. aeruginosa* compared to *Synechocystis* sp.. These results suggest that NPQ is induced in the two studied species, to permit their regulation to high light condition. For both cyanobacterial species, the change of the PSII quantum yield under high light and dark recovery conditions and the NPQ under high light treatment are shown in Figure 3.1C and Figure 3.1D. *M. aeruginosa* has higher PSII quantum yield under high light and dark conditions compared to *Synechocystis* sp. (Figure 3.1C), suggesting that *M. aeruginosa* has a higher ability to resist high light stress than *Synechocystis* sp. Concomitantly, *M. aeruginosa* has much higher NPQ than *Synechocystis* sp. after 12 min high light treatment (Figure 3.1D).

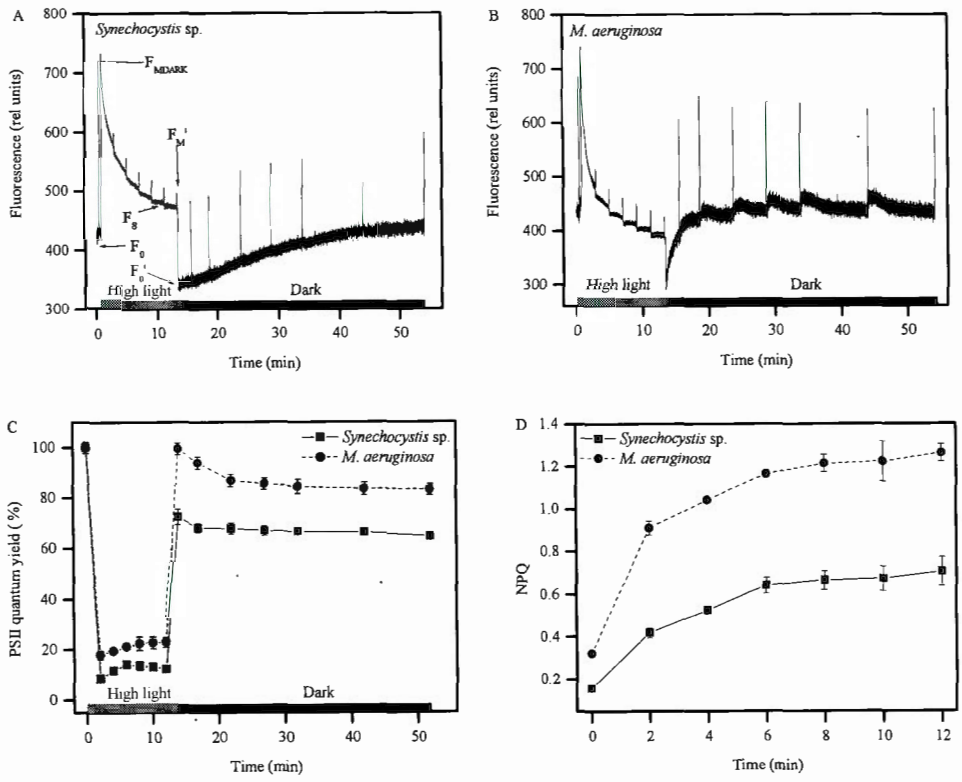

Figure 3.1. High light-induced fluorescence quenching in two cyanobacterial species. A: *Synechocystis*. sp FACHB898. B: *Microcystis aeruginosa* FACHB905. C: The PSII quantum yield change under high light and dark conditions. D: Increased of NPQ during high light treatment in two cyanobacterial species. F_{MDARK}: the maximal fluorescence yield under dark; F_M': the maximal fluorescence yield under illumination; F_S: steady state fluorescence yield; F_0: the minimal fluorescence yield and F_0': the minimal fluorescence yield after illumination.

3.5.2. Room and low temperature fluorescence measurements

In order to investigate the role of the major components of NPQ (OCP-related NPQ and state transition) in the observed different abilities to cope with high light, room

temperature fluorescence emission spectra were recorded before and after high light treatment for the two studied species (Figure 3.2A-D). OCP-related quenching under high light was evaluated by measuring fluorescence emission under an excitation wavelength at 580 nm which mainly excites PBS (Kirilovsky 2007; Tian et al. 2011). After 12 min high light treatment, the fluorescence peak at 660 nm decreased for both species (Figure 3.2A and 3.2B). This result suggests that OCP-related quenching is induced by high light treatment in both species. However, we can notice that *M. aeruginosa* showed a higher (2 times) fluorescence quenching compared to *Synechocystis* sp., suggesting that OCP-related quenching is more induced in *M. aeruginosa* than in *Synechocystis* sp.. On the other hand, under excitation wavelength exciting mainly chlorophyll (436 nm) (Kirilovsky 2007), no significant change of chlorophyll quenching was found after high light treatment for both species.

The ability for state transition under high light treatment of the two studied species is shown in Figure 3.3. The F693/F729 ratio decreased by 10% and 26% after high light treatment in *M. aeruginosa* and *Synechocystis* sp. respectively, suggesting that high light induced a stronger state 1 to state 2 transition in *Synechocystis* sp. than in *M. aeruginosa.*

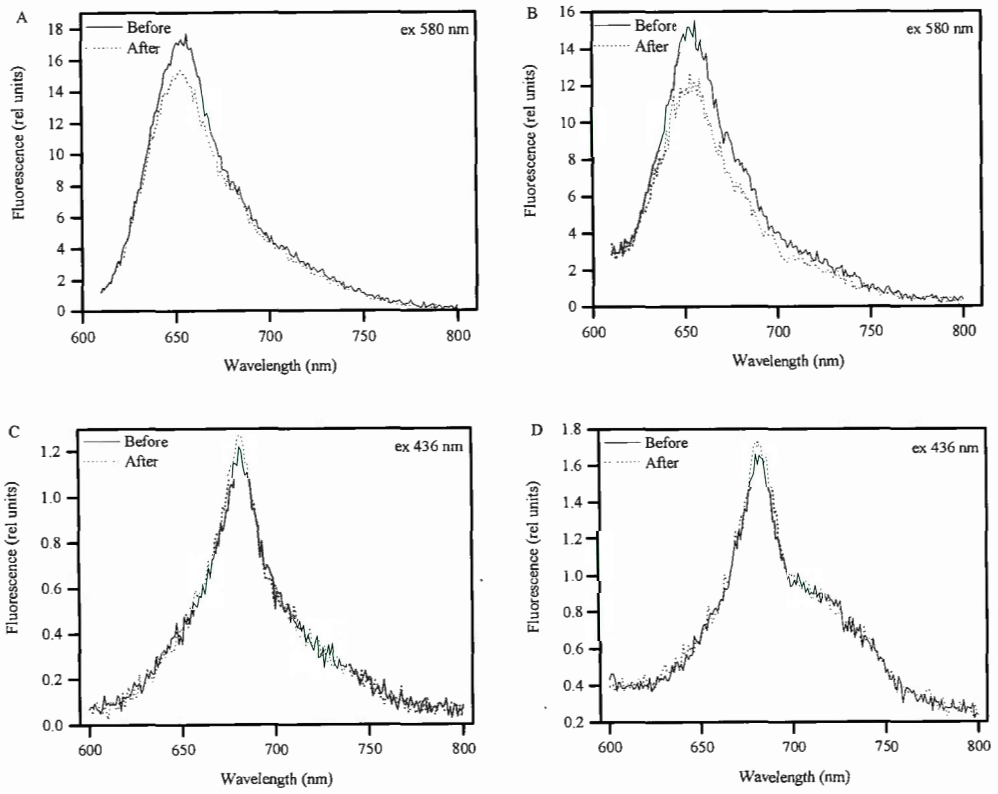

Figure 3.2. Room temperature fluorescence spectra of two cyanobacterial species before and after high light treatment. Excitation is performed at 580 nm (A: *Synechocystis* sp. FACHB898 and B: *M. aeruginosa* FACHB905) and at 430 nm (C: *Synechocystis* sp. FACHB898 and D: *M. aeruginosa* FACHB905).

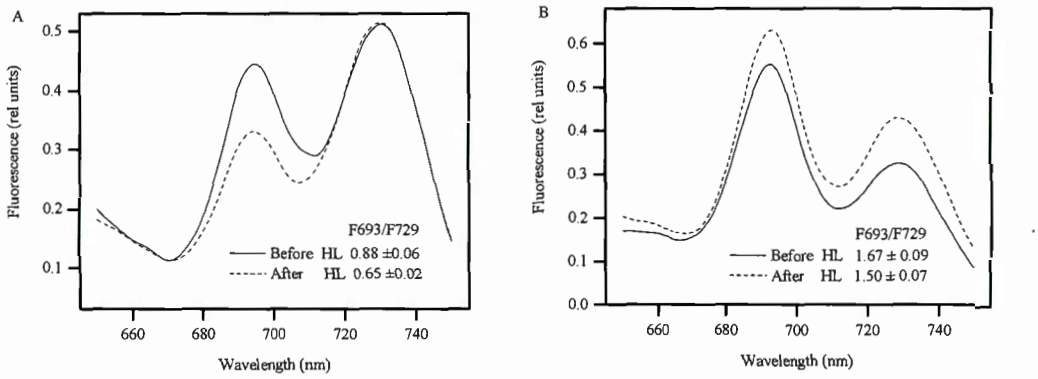

Figure 3.3. Low temperature fluorescence spectra of two cyanobacterial species before and after high light treatment.A: *Synechocystis* sp. FACHB898. B: *M. aeruginosa* FACHB905.

3.6. Discussion

Similarly to what was found for laboratory cultures of cyanobacteria exposed to high white light intensity (Bailey et al. 2005; Xu et al. 2012a; Wilson et al. 2006), we showed here that NPQ was also induced for *M. aeruginosa* and *Synechocystis* sp.. We demonstrated that *M. aeruginosa* induced higher NPQ, and showed a higher ability to resist high light stress compared to *Synechocystis* sp.. It is known that *M. aeruginosa* can form harmful blooms and float at the surface of waterbodies and therefore have more chance to be exposed to higher light intensities compared to *Synechocystis* sp. which is not known to form such kind of blooms at the surface (Paerl and Otten 2013). Consequently, *M. aeruginosa* may have developed protective mechanisms, such as NPQ, to deal with the potentially excessive light energy found periodically at the water surface in natural habitats. Although these two species were grown in laboratory conditions for a long time (more than 4 years), *M. aeruginosa* still tends to gather at the surface, while *Synechocystis* sp. is more uniformly distributed in

the growth medium under our growth conditions. Photosynthesis-irradiance light curves indicated that *M. aeruginosa* has higher saturating light for photosynthesis and relative electron transport rate under high light compared to *Synechocystis* sp. (data not shown). Therefore, it suggests that these two cyanobacterial species evolved to be able to induce different extents of NPQ to grow in their different habitats. We can advance that the observed higher NPQ value for *M. aeruginosa* mainly results from a higher OCP-related NPQ (Figure 3.2B), since the state 1 to state 2 transition was not strongly induced (Figure 3.3B). The larger difference in F_0 yield (quenching from F_0 to F_0') induced by high light treatment in *M. aeruginosa* compared to *Synechocystis* sp. also suggests a higher OCP-related quenching for this species since F_0 quenching was shown to be related only to phycobilisome (allophycocyanin) fluorescence (Bailey et al. 2005; Wilson et al. 2006; Wilson et al. 2007). On the other hand, *Synechocystis* sp. induced a stronger state 1 to state 2 transition and a lower OCP-related quenching after high light treatment compared to *M. aeruginosa* (Figure 3.3). Therefore, NPQ induced in *Synechocystis* sp. is mainly linked to the state 1 to state 2 transition and not to the OCP-related quenching. However, it appears that this higher state 1 to state 2 transition cannot protect completely the PSII activity under high light treatment for this cyanobacterial species. Therefore, OCP-related quenching seems to have a higher efficiency to protect PSII activity compared to state 1 to state 2 transition under high light illumination.

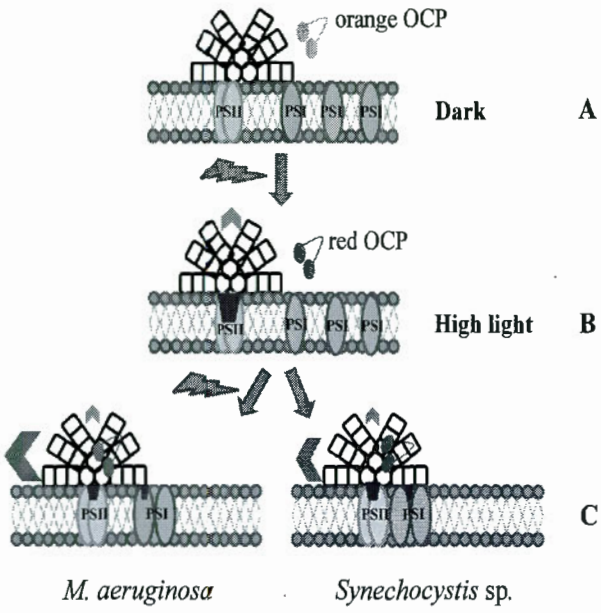

Figure 3.4. Model of OCP-related quenching and state transition mechanisms in response to high light in cyanobacteria. In dark condition (A), state 2 is induced and phycobilisomes are attached to PSII and PSI. Then when cyanobacteria are exposed to high light (B), state 2 to state 1 is induced and the orange OCP is converted to red OCP (B) and attached to PBS (C) inducing OCP-related quenching. When this quenching is sufficient to cope with high light, state transition is less induced (such as in *M. aeruginosa*). Otherwise, state transition will be more induced (such as in *Synechocysits* sp.). In this figure, we use the contact between components to show the extent of OCP-related quenching and state transition. When red OCP are more attached to the core of phycobilisomes, it indicates that more OCP-related quenching occurs. When phycobilisomes are more connected to PSI, it indicates that state 1 to state 2 transition is more induced. The heat and fluorescence emission are represented respectively be the red and orange arrows. The energy transfer from phycobilisomes to PSII for photosynthesis is represented by purple trapezoid. The size of the different symbols (arrow or trapezoid) indicates the relative extent of theses energy dissipation processes.

OCP-related quenching and state 1 to state 2 transition are associated with changes in the PSII fluorescence quantum yield (Kirilovsky 2007; Scott et al. 2006). Therefore, as we demonstrated in the present study, both mechanisms can decrease PSII fluorescence yield and therefore their distinction in cyanobacteria may be difficult. For a long time, NPQ induced under high light was interpreted as a mixture of these two mechanisms, resulting in difficulties to evaluate their specific contribution by simple methodological approach such as Pulse Amplitude Modulated fluorometry (Kirilovsky 2007). In the present study we showed that, by using a combination of room temperature and 77K fluorescence measurements, we were able to distinguish the different components of NPQ permitting us to compare their efficiencies as two regulation strategies used by cyanobacteria to cope with high light intensity.

Based on the data presented in this paper together with previous published results, we propose, for cyanobacteria exposed to high light stress, an regulation model involving OCP-related quenching and state transition (Figure 3.4). Firstly, in the dark (A) cyanobacteria are known to be in state 2 (phycobilisomes attached to PSII and PSI) due to respiratory processes active in these conditions and reducing the PQ pool (Fork and Satoh 1983; Mullineaux and Allen 1986; Mullineaux and Allen 1990). Then, as previously demonstrated, state 2 to state 1 transition (phycobilisomes attached only to PSII) occurs when light conditions changed from darkness to high light intensities (B), since electrons accumulated in the PQ pool in darkness will be transferred to PSI, therefore re-oxidizing the PQ pool (Kirilovsky 2015; Campbell et al. 1998). If high light conditions continue (C), causing an excess of energy absorbed by PSII, the OCP-related quenching will be triggered to cope with this stressful condition (present study). Concomitantly, if OCP-related NPQ is efficient to dissipate excess energy, state 1 to state 2 transition is not or only slightly induced (phycobilisomes strongly attached to PSII but slightly attached to PSI trimers), as we have shown for *M. aeruginosa* (C). However, if OCP-related quenching is not able to dissipate excess energy efficiently, PQ will be reduced and therefore state 1 to state 2 transition will be triggered (phycobilisomes strongly attached to PSII and PSI) to improve the PSII

photoprotection, as we found for *Synechocystis* sp. (C). We can therefore conclude that, based on the different sensitivities to high light between the two studied species, the OCP-related quenching is more efficient to dissipate excess energy than the state 1 to state 2 transition. This model indicates that OCP-related quenching and state transition interact closely in a specific manner depending on the cyanobacterial species, determining their high light resistance abilities, and could explain, in part, the distribution of cyanobacterial species in freshwater ecosystems.

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CHAPTER IV

DIFFERENT PHYSIOLOGICAL AND PHOTOSYNTHETIC RESPONSES OF THREE CYANOBACTERIAL STRAINS TO LIGHT AND ZINC

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4.1. Résumé

La pollution au zinc dans les écosystèmes aquatiques est un problème commun dans plusieurs pays et son effet sur le phytoplancton peut être influencé par d'autres facteurs environnementaux. Dans l'environnement, l'intensité lumineuse varie continuellement en fonction des saisons ou du couvert nuageux. De plus, l'effet du zinc au niveau mécanistique sous différentes conditions lumineuses n'est pas connu chez les cyanobactéries. Dans cette étude, nous investiguons l'effet du zinc sur trois souches de cyanobactéries (*Microcystis aeruginosa* CPCC299, *Microcystis aeruginosa* CPCC632 et *Synechocystis* sp. FACHB898) et ce, sous deux régimes d'intensité lumineuse. Sous condition de forte lumière (FL), une augmentation du ratio Car/Chl *a* a été observée et une induction du *quenching* non photochimique a été observé chez les trois souches de cyanobactéries étudiées. Par contre, des trois souches, c'est CPCC299 qui montre le plus grand taux de croissance. Ces résultats suggèrent que CPCC299 possède une plus grande habileté à s'adapter à la forte lumière que les deux autres espèces. Sous un traitement de forte concentration de zinc, les valeurs de l'efficacité photochimique maximale (Φ_M) et opérationnelle (Φ'_M) du PSII, de l'efficacité photochimique du PSI [Y(I)] et du *quenching* non photochimique (NPQ) ont été diminuées. Pour cette étude, l'ordre suivant de sensibilité entre les souches peut être établis : CPCC299 > CPCC632 > FACHB898. Cette différence de sensibilité peut être en partie expliquée par la forte concentration interne en zinc retrouvé chez CPCC299 comparativement à la concentration mesurée chez les deux autres souches. La forte intensité lumineuse aurait donc augmenté la concentration interne de zinc et ainsi augmenté la toxicité du zinc chez les deux types de *M. aeruginosa*, mais avec une plus grande ampleur chez CPCC299. De plus, chez CPC299, la valeur du ratio de Car/Chl *a* a été diminuée par la forte concentration de zinc sous une forte intensité lumineuse alors qu'elle ne l'a pas été sous une faible intensité lumineuse. Ces résultats suggèrent que les trois espèces ont développé une réponse mécanistique différente à un fort stress de zinc lors d'exposition à différentes intensités lumineuses. Dans cette étude, nous avons démontré que l'interaction entre l'intensité lumineuse et la concentration de zinc doit être considérée lors de l'étude du dynamisme de fleurs de cyanobactéries dans les écosystèmes d'eau douce.

Mots clé: *Mircocystis aeruginosa*, *Synechocystis* sp., forte concentration de zinc, stress lumineux et photosynthèse.

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4.2. Abstract

Zinc pollution in freshwater aquatic ecosystems is a common problem in many countries and its effect on phytoplankton may be influenced by other environmental factors. In the natural environment, light intensity varies continuously depending on cloud cover and seasons. However, for cyanobacteria, the response mechanism to high zinc stress under different light conditions is not clear. In this research, we investigated the effect of high zinc on three cyanobacteria (*Microcystis aeruginosa* CPCC299, *Microcystis aeruginosa* CPCC632 and *Synechocystis* sp. FACHB898) grown under two light regimes. Grown under high light condition (HL), the three cyanobacteria increased their Car/Chl *a* ratio and Non-photchemical quenching (NPQ) to adapt to HL. However, CPCC299 has the highest growth rate among three studied strains, suggesting that CPCC299 has higher ability to adapt HL compared to other two strains. At high zinc treatment, the values of maximal (Φ_M) and operational (Φ'_M) PSII quantum yields, PSI quantum yield [Y(I)] and NPQ were decreased. The following order of sensitivity to high zinc can be established for the studied strains: CPCC299 > CPCC632 > FACHB898. This different sensitivity can be partly explained by the higher inner zinc content found in CPCC299 compared to the other two strains. HL increased the inner zinc content and therefore increased zinc toxicity in both *Microcystis aeruginosa* strains, but at a higher extent for CPCC299. Moreover, the ratio of Car/Chl *a* ratio was decreased by high zinc concentration under HL, but not under low light (LL) in CPCC299. These results suggest that the three strains have developed different response mechanisms to high zinc stress when grown under two light regimes. In this study, we demonstrated that the interaction between light intensity and zinc concentration needs to be considered when studying cyanobacterial bloom dynamics in freshwater ecosystems.

Keywords: *Mircocystis aeruginosa*, *Synechocystis* sp., high zinc, light and photosynthesis

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4.3. Introduction

Cyanobacteria are widely distributed in freshwater and seawater ecosystems, and some species can form blooms that may decrease the water quality, and therefore have negative effects on phytoplankton, zooplankton and animals (Codd, 1995; Carmichael, 2001; Metcalf and Codd, 2009; Paerl and Otten, 2013). Cyanobacterial blooms are formed by fast growing species and can be found at the surface of waterbodies that may be influenced by various environmental factors, such as light, temperature, nutrients and pollutants (Watanabe and Oishi, 1985; Yamamoto and Nakahara, 2005; Xu *et al.*, 2012; Peters *et al.*, 2013).

It has been reported that in polluted aquatic ecosystems zinc concentrations may range from 61 µg/l (Taihu lake, China (Su *et al.*, 2012)) to 1468 µg/l (Sango Bay of Lake Victoria, Africa) (Bbosa and Oyoo, 2013). Consequently, high zinc concentration found in lakes and rivers may affect physiological processes of phytoplankton, their species distribution and the cyanobacterial bloom formation (De Magalhães *et al.*, 2004; Chaloub *et al.*, 2005; Bbosa and Oyoo, 2013). Various studies have been conducted to better understand the physiological response of cyanobacteria to zinc stress. It has been reported that high zinc concentrations can cause decreases in the pigment (chlorophyll *a*, carotenoids and phycobilisomes) content, decrease the PSII activity, inhibit the energy transfer from phycobilisomes to PSII centers, and consequently inhibit photosynthesis and growth of various cyanobacterial species (Zeng *et al.*, 2009; Okmen *et al.*, 2011; Xu *et al.*, 2013). It has also been suggested that different cyanobacterial species showed different sensitivities to high zinc concentrations (Okmen *et al.*, 2011). For example, *Nostoc punctiforme* can tolerate to a zinc concentration of 150 µg/l, which are toxic for most cyanobacteria (Hudek *et al.*, 2012). Even within the same genera, differences have been observed. For instance, *Spirulina indica*, *S. maxima* and *S. platensis* are differently sensitive to high zinc concentrations (Balaji *et al.*, 2014).

The toxicity of zinc is not only species dependent, it is also affected by co-occurrence with environmental factors, such as light (Chaloub *et al.*, 2005; Zeng and Wang, 2011). Since any change in light intensity may affect photosynthesis and growth of cyanobacteria, it has been suggested that light can alter the distribution of cyanobacteria in freshwater ecosystems (Ibelings *et al.*, 1994). Furthermore, light has been shown to affect the metal uptake and toxicity in phytoplankton (Zeng and Wang, 2011; Xu *et al.*, 2013). However, most of the studies conducted assessing zinc toxicity to cyanobacteria focused on the effect of zinc alone (without combination with other environmental factors) or mainly focused on one physiological process (such as zinc uptake efficiency or PSII-PSI electron transport rate). Therefore, our understanding of the different abilities of various cyanobacterial species to acclimate to high zinc under various environmental factors, such as light, still needs to be improved.

In this research, we investigated the physiological and acclimation responses to short-term high zinc stress of three cyanobacterial strains grown under two light intensities. We found that zinc uptake rate is different among the three studied strains, which leads to different sensitivities and responses to high zinc stress.

4.4. Materials and methods

4.4.1. Cultures

Microcystis aeruginosa CPCC299 (hereafter CPCC299) and *Microcystis aeruginosa* CPCC632 (hereafter CPCC632) were obtained from the Canadian Phycological Culture Centre. *Synechocystis* sp. FACHB898 was obtained from Freshwater Algal Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences. These three strains were cultured under a temperature of 22 °C in 250 ml flask which contain 120 ml modified bold basal media (BBM) at pH 6.8 (Stein 1972). In order to avoid zinc contamination, all flasks were soaked with 10% HCl overnight and rinsed thoroughly (at least three times) with Milli-Q water before used. In the modified BBM, the micronutrient content (except zinc) was decreased to 10% of the original BBM, which is beneficial for controlling the metal species in the solution (Twiss *et al.*, 2001). The zinc concentration in modified BBM was 0,78µM (pZn 8.9). The cultures were grown under a light:dark cycle of 14:10 h at 50 µmol photons·m^{-2}·s^{-1} (LL) and 300 µmol photons·m^{-2}·s^{-1} (HL), provided by a combination of incandescent bulbs and white fluorescent tubes (Philips F72T8/TL841/HO, USA). Sub-samples were taken every day for measurements of cell density with a Multisizer™ 3 Coulter Counter® (Beckman Coulter Inc., Brea, CA, USA). The specific growth rate is calculated as: $\mu=(\ln(N_6)-\ln(N_2))/4$. N_6 is the cell concentration at the 6^{th} day, while N_2 is the cell concentration at the 2^{nd} day.

4.4.2. High zinc treatment

At exponential phase, cultures were harvested by a gentle filtration on 0.8 µm filters (Xingya Purifying Materials Factory, Shanghai, China) and re-suspended in fresh BBM

contained three different zinc concentrations. For all experiments, cell concentrations were kept the same (3.5×10^5 cells/ml). All the cyanobacterial cells were exposed to three zinc concentrations (0.78 μM (pZn 8.9 as control), 7.8 μM (pZn 5.6) and 39 μM (pZn 4.8)) for 4.5 h under two growth light conditions, and then were moved to dark condition for 30 min before further measurments. Free zinc concentrations were calculated by Visual MINTEQ Version 3.0 (Gustafsson JP, http://www.lwr.kth.se/english/OurSoftware/vminteq/). The real zinc concentrations in the medium were confirmed by the atomic absorption spectrometry (see 4.4.6).

4.4.3. Pigment measurements

The pigment profiles of the three cyanobacterial strains were measured spectrophotometrically. Cell density was initially determined with 1 ml sub-sample as the above. Samples were harvested by filtration, re-suspended in methanol and boiled for 5 min, then the extract was stored overnight at -80 ℃. After filtration through a GFF filter (Whatman, Piscataway, NJ, USA), the absorbance spectra of the extract was measured with a Cary 300 UV-VIS spectrophotometer (Varian Australia Pty Ltd, Mulgrave, VIC, Australia). The contents of chlorophyll *a* (Chl *a*) and carotenoids (Car) were calculated according to Ritchie (2008), using the absorbance at 632 nm, 652 nm, 665 nm and 696 nm.

4.4.4. PSII activity and non-photochemical quenching determination

After 30 min dark acclimation, sub-samples were taken to measure PSII activity with a WATER-PAM Chlorophyll Fluorometer (Walz GmbH, Effeltrich, Germany). The minimal fluorescence yield (F_0) was determined in the absence of actinic illumination. Then, the actinic light was turned on (same intensity than the growth light intensity) for 1 minute, and the fluorescence yield F' and F_M' were respectively determined

briefly before or during the saturating light pulse (800 ms, 3000 μmol photons·m^{-2}·s^{-1}) respectively. Then the actinic light was turned off and DCMU was added to the samples to get the maximal fluorescence yield in presence of DCMU (F_M) and under light illumination (200 μmol photons·m^{-2}·s^{-1}). The maximal PSII quantum yield with DCMU (Φ_M) and light (Φ'_M), and non-photochemical quenching (NPQ) were calculated with the following equations: $\Phi_M = (F_M - F_0) / F_M$ (Kitajima and Butler, 1975); $\Phi'_M = (F_M' - F')/ F_M'$ (Genty *et al.*, 1989) and $NPQ = (F_M - F_M')/ F_M'$ (Bilger and Björkman, 1990).

4.4.5. PSI activity measurements

PSI transmittance was measured by using dual-wavelength pulse-amplitude-modulated fluorescence monitoring system (Dual-PAM, Heinz Walz, Effeltrich, Germany) according to Perreault et al. (2009). Used modulated, actinic and saturating pulse (800 ms) light intensities were 1 μmol photons·m^{-2}·s^{-1}, 53 or 300 μmol photons·m^{-2}·s^{-1} and 3000 μmol photons·m^{-2}·s^{-1}, respectively. The PSI quantum yield was calculated according to Klughammer and Schreiber (2008): $Y(I) = (P_M` - P)/(P_M - P_O)$.

4.4.6. Intracellular zinc concentration measurements

After 5 h treatment to different zinc concentrations, samples were filtered and washed by 10 ml of 0.1 mM EDTA for 3 min to remove any loosely adsorbed metal (Zeng and Wang, 2011). Then the samples were collected by the same filter and kept in virgin polypropylene digestion tubes (SCP science corporate, Quebec, Canada) at 4 °C until measurement. 10 ml nitric acid (analytical grade) was added to each sample and digested at 100°C for 25 min. All analyses were done by using a GBC 906AA atomic absorption spectrometer with furnace (GBC Scientific Equipment LLC, USA). In order to avoid any zinc contamination, all the tubes were soaked in 10% nitric acid for one day and rinsed by Milli-Q water for at least three times.

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4.5. Results

4.5.1. The growth rate of three cyanobacterial strains under two light intensities

The specific growth rate of the three studied strains grown under two light intensities is shown in Figure 4.1. The specific growth rate was increased by 15% under high light compared to low light for CPCC299. However, it was decreased by 20% and 33% under high light compared to low light respectively for CPCC632 and FACHB898.

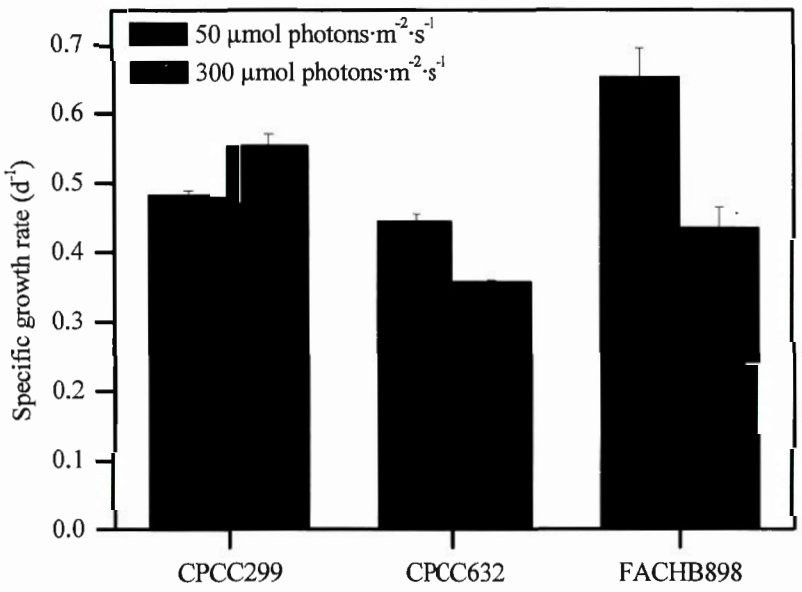

Figure 4.1. The specific growth rates of three studied strains grown under two light intensities. Data are means ± SD ($n = 6$)

4.5.2. The effect of zinc on pigment content

Under low growth light condition, the content of Chl *a* and Car decreased to 68% and 66% of the control at pZn 4.8 zinc for CPCC299 (Table 4.1). Consequently, there is no significant change in the Car/Chl *a* ratio for CPCC299 exposed to the three studied zinc concentrations. For CPCC632, pZn 4.8 decreased the Chl *a* content to 71% compared to the control (Tukey's HSD, $P < 0.05$). However, for this strain, high zinc treatment did not affect the Car content and Car/Chl *a* ratio (Tukey's HSD, $P > 0.05$). For FACHB898, high zinc treatment did not influence the Chl *a* and Car content and therefore the Car/Chl *a* ratio (Tukey's HSD, $P > 0.05$). Under high growth light condition, pZn 4.8 decreased the Car content and Car/Chl *a* ratio of CPCC299 to 66% and 61% compared to the control (Tukey's HSD, $P < 0.05$). For CPCC632 and FACHB898, the Chl *a* and carotenoid contents tended to decrease under high zinc concentration, although not significantly (Tukey's HSD, $P > 0.05$).

Table 4.1. The effects of zinc on pigment content in three studied strains grown under two light intensities. Chl *a* and Car contents are expressed as $\mu g/10^8$ cells.

		LL			HL		
		pZn 8.9	pZn 5.6	pZn 4.8	pZn 8.9	pZn 5.6	pZn 4.8
CPCC299	Chl *a*	6.08±0.81^{a}	3.55±0.20^{b}	4.11±0.18^{b}	3.59±0.76^{a}	3.88±0.83^{a}	3.88±0.17^{a}
	Car	2.78±0.33^{a}	1.73±0.26^{b}	1.83±0.18^{b}	3.00±0.33^{a}	2.35±0.41^{ab}	1.99±0.10^{b}
	Car/Chla	0.46±0.04^{a}	0.49±0.05^{a}	0.44±0.04^{a}	0.85±0.08^{a}	0.61±0.04^{b}	0.52±0.01^{b}
CPCC632	Chl *a*	7.86±1.05^{a}	7.02±0.86^{ab}	5.56±0.22^{b}	5.13±0.52^{a}	5.02±0.18^{a}	4.08±0.90^{a}
	Car	3.08±0.29^{a}	2.84±0.41^{a}	2.60±0.17^{a}	3.21±0.40^{a}	3.30±0.11^{a}	2.27±0.85^{a}
	Car/Chla	0.39±0.03^{a}	0.41±0.06^{a}	0.47±0.02^{a}	0.63±0.02^{a}	0.66±0.00^{a}	0.55±0.11^{a}
FACHB898	Chl *a*	2.33±0.12^{a}	2.35±0.22^{a}	2.54±0.31^{a}	1.51±0.33^{a}	1.34±0.09^{a}	1.32±0.04^{a}
	Car	0.85±0.34^{a}	0.72±0.10^{a}	0.92±0.06^{a}	0.89±0.28^{a}	0.92±0.05^{a}	0.77±0.02^{a}
	Car/Chla	0.37±0.12^{a}	0.31±0.07^{a}	0.36±0.05^{a}	0.58±0.05^{ab}	0.68±0.05^{a}	0.56±0.01^{b}

$^{a, b, c}$ Those with different superscript letters for each strains in the same line at same growth light are significantly different (Tukey's HSD, $P < 0.05$). Data are means ± SD ($n = 6$).

4.5.3. The effect of zinc on PSII activity

Under low growth light condition (Figure 4.2A), pZn 5.6 treatment slightly decreased the maximal PSII quantum yield (Φ_M) of CPCC299 by 16% compared to control, and no decrease was observed for the other two cyanobacteria. On the other hand, the highest Zn concentration (pZn 4.8) decreased the Φ_M by 100%, 53% and 17% compared to control for CPCC299, CPCC632 and FACHB898 respectively. Under high growth light condition (Figure 4.2B), pZn 5.6 treatment decreased the Φ_M by 100% and 11% compared to control for CPCC299 and CPCC632 respectively. When the Zn concentration is increased to pZn 4.8 the Φ_M decreased by 100% and 45% compared to control respectively for CPCC299 and CPCC632. No effect of high zinc concentration on Φ_M was noted for FACHB898 (Tukey's HSD, $P > 0.05$).

Under low growth light condition (Figure 4.2C), pZn 5.6 treatment decreased the operational PSII quantum yield (Φ'_M) by 20% and 11% compared to the control for respectively CPCC299 and CPCC632. For the highest tested zinc concentration (pZn 4.8), Φ'_M decreased by 100%, 73% and 40% for CPCC299, CPCC632 and FACHB898 respectively. Under high growth light condition (Figure 4.2D), pZn 5.6 treatment inhibited completely the operational PSII quantum yield of CPCC299 and decreased it by 40% for CPCC632. When the zinc concentration was increased to pZn 4.8, Φ'_M decreased by 100%, 83% and 16% for CPCC299, CPCC632 and FACHB898 respectively.

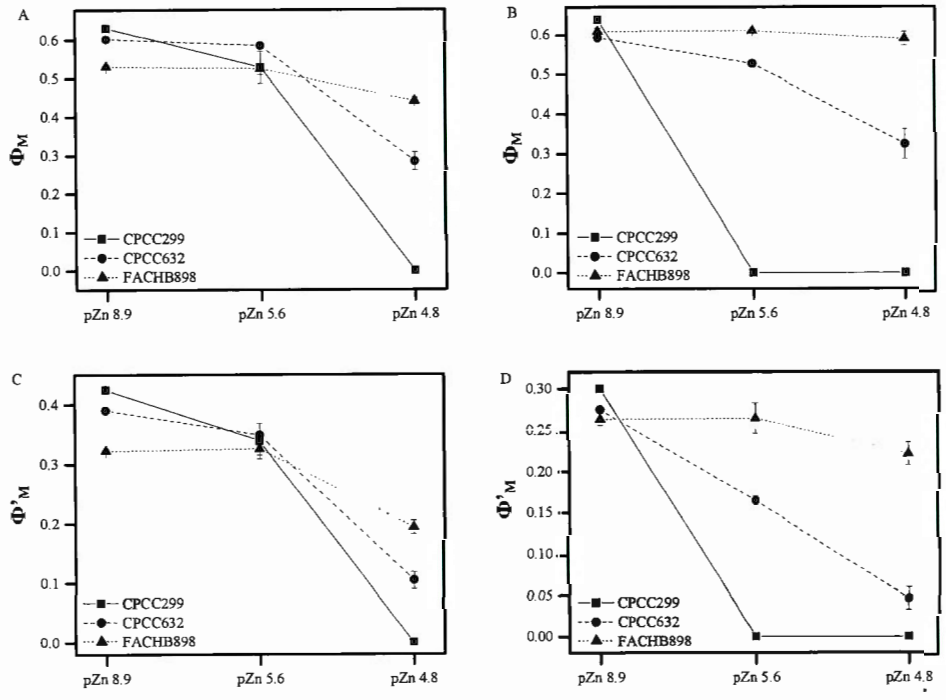

Figure 4.2. The effects of zinc on maximal PSII quantum yield (Φ_M) and operational PSII quantum yield (Φ'_M) of three studied strains grown under two light intensities. Panel A and C: grown under 50 μmol photons·m^{-2}·s^{-1}; Panel B and D: grown under 300 μmol photons·m^{-2}·s^{-1}. Data are means ± SD (n = 6).

As seen in Figure 4.3A, the highest Zn concentration increased the minimal fluorescence yield (F_0) of CPCC299 and CPCC632 grown under low light by 400% and 54% respectively, but did not influence the F_0 of FACHB898. Under high growth light (Figure 4.3B), both zinc treatments increased the F_0 of CPCC299 (by 209% and 323% for pZn 5.6 and pZn 4.8 respectively). However, high zinc treatment did not significantly affect F_0 of CPCC632 and FACHB898 of high light grown cells.

The effect of zinc on maximal fluorescence yield (F_M) of the three studied cyanobacteria is shown in Figure 4.3C and 4.3D. Under low growth light condition, pZn 4.8 treatment increased F_M to 187% of the control for CPCC299. However, this high zinc treatment decreased F_M to 86% for both CPCC632 and FACHB898 (Figure 4.3C). Under high growth light condition, pZn 4.8 treatment increased F_M to 152% of the control in CPCC299, however, decreased the F_M to 85% of the control in CPCC632 and did not influence this fluorescence yield for FACHB898 (Figure 4.3D).

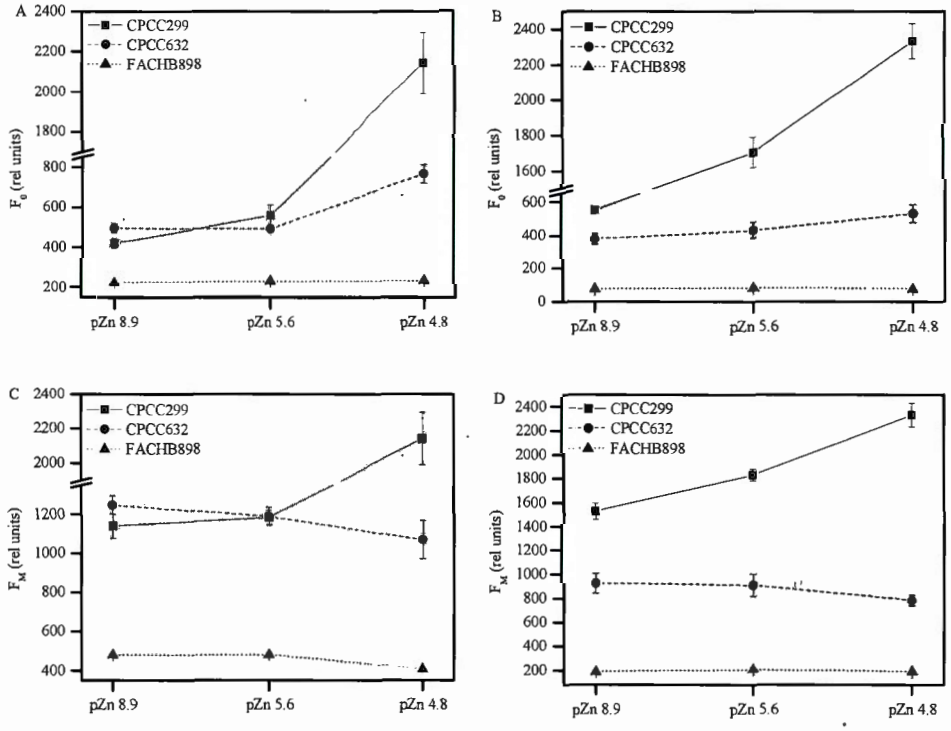

Figure 4.3. The effects of zinc on minimal (F_0) and maximal (F_M) fluorescence yield of three studied strains grown under two light intensities. Panel A and C: grown under 50 μmol photons·m^{-2}·s^{-1}; Panel B and D: grown under 300 μmol photons·m^{-2}·s^{-1}. Data are means ± SD (n = 6).

4.5.4. The effect of zinc on PSI activity

Under low growth light condition, pZn 5.6 and pZn 4.8 treatments decreased the PSI activity [Y(I)] of CPCC299 by 40% and 45% respectively (Figure 4.4A). On the other hand, there was no influence of Zn on PSI activity of CPCC632 and FACHB898. Under high growth light condition, pZn 5.6 and pZn 4.8 inhibited the Y(I) by 31% and 100% for CPCC299. Zinc concentration of pZn 5.6 did not affect PSI activity of CPCC632 and FACHB898. The highest Zn concentration increased the Y(I) by 27% for FACHB898, but not for *M. aeruginosa* 632.

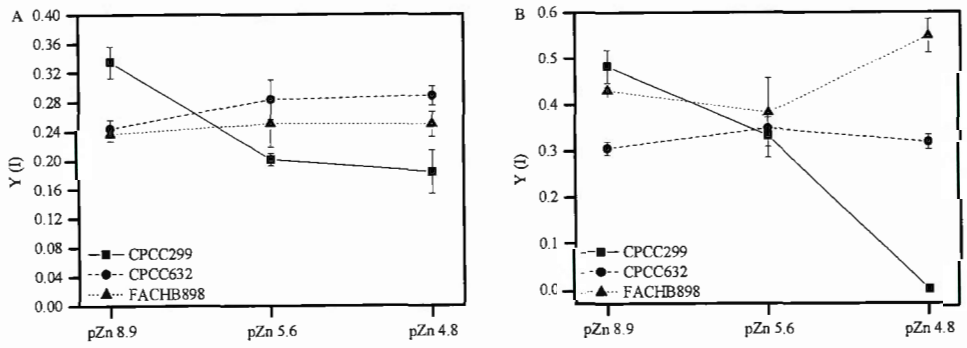

Figure 4.4. The effects of zinc on PSI activity of three studied strains grown under two light intensites. Panel A: grown under 50 μmol photons·m^{-2}·s^{-1}; Panel B: grown under 300 μmol photons·m^{-2}·s^{-1}. Data are means ± SD (n = 6).

4.5.5. The effect of zinc on Non-Photochemical Quenching (NPQ)

As seen in Figure 4.5A, under low growth light condition, pZn 5.6 induced a decrease of the Non-Photochemical Quenching (NPQ) only for CPCC299 (40% decrease). Higher Zn concentration (pZn 4.8) induced a decrease of NPQ for all studied strains, but at a different extent (CPCC299 = 100%; CPCC632 = 66%; FACHB898 = 22%). Under high growth light condition (Figure 4.5B), pZn 5.6 decreased NPQ of both CPCC299 (by 100%) and CPCC632 (by 15%). Higher zinc concentration (pZn 4.8) induced strong decrease of the NPQ (100% and 69% for CPCC299 and CPCC632 respectively). Interestingly, NPQ of FACHB898 was not affected by high zinc treatment under this light condition (Figure 4.5B).

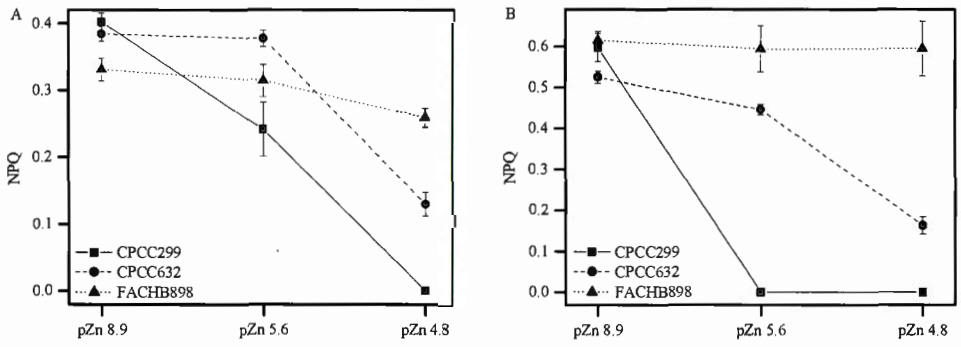

Figure 4.5. The effects of zinc on Non-photochemical Quenching (NPQ) of three studied strains grown under two light intensities. Panel A: grown under 50 µmol photons·m^{-2}·s^{-1}; Panel B: grown under 300 µmol photons·m^{-2}·s^{-1}. Data are means ± SD (n = 6).

4.5.6. Intracellular zinc content

The intracellular zinc content of the three studied cyanobacteria increased as light intensity and zinc concentration increased (Figure 4.6). Under low light intensity, after 5 h treatment to pZn 5.6 the intracellular zinc contenta was 3.2, 2.5 and 4.3 times of the ones of the control cells for CPCC299, CPCC632 and FACHB898 respectively. When the zinc concentration increase to pZn 4.8, the intracellular zinc concentrations were 7.2, 4.7 and 10.4 times higher than in the control cells for CPCC299, CPCC632 and FACHB898 respectively (Figure 4.6A). Higher light intensity further increased the Zn uptake and intracellular zinc contents were increased by 4.2, 3.1 and 4.4 times (for pZn 5.6) and 19.2, 12.2 and 12.3 (for pZn 4.8) for CPCC299, CPCC632 and FACHB898 respectively (Figure 4.6B).

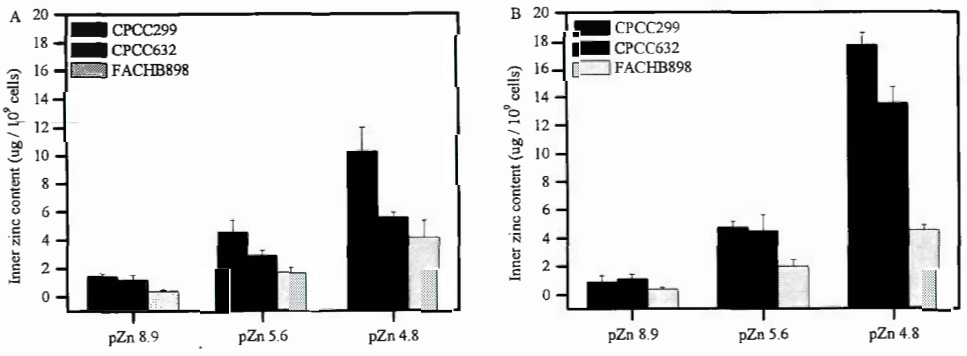

Figure 4.6. After treated by three zinc concentrations for 5 hours, the inner zinc content of three studied strains grown under two light intensities. Panel A: grown under 50 μmol photons·m^{-2}·s^{-1}; Panel B: grown under 300 μmol photons·m^{-2}·s^{-1}. Data are means ± SD (n = 3-6).

4.6. Discussion

Effect of light. Almost all environmental factors like light intensity and temperature variations may affect cyanobacterial photosynthesis and physiology. Indeed, as we have shown here, growth rate and electron transport rate were increased or decreased (depending on strains and light intensity) by high growth light intensity (Brookes *et al.*, 2003; Huisman *et al.*, 2004; Deblois and Juneau, 2012).

In the case of CPCC299, our results showed that high growth light increased its specific growth rate by 15% compared to when this strain grown under low growth light condition, while, interestingly, the operational PSII quantum yield (Φ'_M) was decreased by 30%. On the other hand, for CPCC632 and FACHB898, the specific growth rate and photosynthetic activity were lower under higher growth light intensity. These results suggest that, for CPCC299, growth under high light may be also influenced by other biochemical processes (Bañares-España *et al.*, 2013), and that this strain has higher ability to adapt to high light compared to the other two studied cyanobacteria. This conclusion that toxic cyanobacteria cells have a better ability to cope with high light was also found in previous work (Renaud *et al.*, 2011; Deblois and Juneau, 2012; Xu *et al.*, 2013). Concomitantly to the decrease of Φ'_M under HL for the studied species, the value of PSI quantum yield [Y(I)] increased (in CPCC632 and FACHB898) or was less inhibited (in CPCC299) suggesting that cyanobacteria can regulate the activity of their two photosystems to optimize their electron flow.

We also showed that NPQ was higher under HL compared to LL for the three studied strains (Figure 4.5). This modification of NPQ may be linked to the change of Orange Carotenoid Protein (OCP)-related quenching and state transition (Kirilovsky, 2007). OCP-related quenching and state transition are known to be two distinct components of NPQ of cyanobacteria participating in high light photoprotection in these organisms (Kirilovsky and Kerfeld, 2012; Kirilovsky, 2014). Furthermore, the

Car/Chl *a* increased under HL in the studied strains, suggesting that cyanobacterial cells may dissipate more energy through heat to protect PSII reaction center (Deblois and Juneau, 2012). Since Car/Chl *a* is higher in CPCC299 compared to the other studied cyanobacteria, it suggests that this strain has a higher carotenoid-related energy quenching (such as OCP-related NPQ). We recently demonstrated that CPCC299 decreased its ability to perform state transition under high light compared to low light (Xu *et al.*, 2013), therefore, the observed higher NPQ under HL in the present study, mainly results from the higher Car/Chl *a* ratio which leads to a higher carotenoid energy quenching (such as OCP-related NPQ).

Effect of zinc. In the present work, we have shown that pZn 4.8 (39 μM) inhibited photosynthesis in all three strains (Figure 4.2). This is in agreement with previous studies showing that zinc content higher than 23 μM inhibited photosynthesis and growth in most of cyanobacterial species (De Magalhães *et al.*, 2004; Chaloub *et al.*, 2005; Okmen *et al.*, 2011). Among the tested cyanobacteria here, CPCC299 was the most sensitive to high zinc stress, while FACHB898 the least (Figure 4.2). This result is different compared to a previous study showing that toxic and non-toxic *Microcystis* sp. had similar tolerance to high zinc (Zeng *et al.*, 2009). This difference could be linked to the different strains and experimental conditions used in our compared to Zeng's experiments. We also demonstrated that F_0 increased under high zinc stress for both *M. aeruginosa* strains, but F_M was increased by high zinc stress in CPCC299, while decreased in CPCC632 (Figure 4.3). It was suggested that an increase in F_0 coupled with no obvious change in F_M implies that energy transfer among phycobilisomes and/or from phycobilisomes to reaction centers could be blocked under high zinc stress (Chaloub *et al.*, 2005). Therefore, our results indicate that these types of energy transfers could be inhibited by high zinc concentrations in CPCC632. However, for CPCC299, besides the F_0 increase, F_M was also increased by high zinc stress, which suggests that both an inhibition of these types of energy transfers and a damage to the electron transport chain were observed under high zinc stress (Figure 4.3). We noticed that PSI activity was inhibited by high zinc concentration only for

CPCC299, suggesting that PSII is more sensitive to high zinc stress compared to PSI in the studied species. Our results are in accordance with a previous study demonstrating that PSII activity was more affected than PSI in *Synechocystis aquatilis f. aquatilis* under high zinc concentration (De Magalhães *et al.*, 2004).

It was surprising to find out that NPQ was decreased by pZn 4.8 treatment in the three studied strains, except for FACHB898 when growing under HL. Indeed, it is generally assumed that NPQ should increase to protect PSII under stress conditions (such as high light and xenobiotic presence) (Campbell *et al.*, 1998; Juneau *et al.*, 2007; Ralph *et al.*, 2007). As we mentioned above, in cyanobacteria, NPQ is mainly linked to the OCP-related quenching and state transition (Karapetyan, 2007). Under high zinc treatment, there was no increase of the Car/Chl *a* for three studied strains, suggesting that carotenoid energy quenching (such as OCP-related NPQ) is not more induced under high zinc treatments compared to the control condition. We found that PSII was more sensitive to high zinc stress than PSI, which could suggest that PQ will be more oxidized under high zinc condition compared to control. Since state transition is controlled by the redox of PQ in cyanobacteria (Mullineaux and Emlyn-Jones 2004), more oxidized PQ pool should induce state 2 to state 1 transition under zinc stress condition. Therefore, the observed NPQ decrease may result from the lower carotenoid content and the lower ability for state 1 to state 2 transition under high zinc stress for three studied strains.

Light influence on the zinc toxicity. We showed here that HL increased the zinc toxic effect for *M. aeruginosa* but not for *Synechocystis* sp. (Figure 4.2). These results can be explained by the higher zinc absorption under HL for these two *M. aeruginosa* strains compared to *Synechocystis* sp. (Figure 4.6). Higher Zn accumulation was also found for other *Microcystis* sp. when grown under higher light intensity (Zeng and Wang, 2011). Although HL does not affect the zinc absorption in FACHB898 (Figure 4.6), it seems to obviously decrease its toxicity (Figure 4.2). Therefore, FACHB898 grown under HL may have developed mechanisms to better acclimate to high zinc stress compared to when this species grows under LL condition. These mechanisms

could be related to the higher Car/Chl *a* ratio found in HL grown cells compared to LL. It was previously suggested that higher carotenoid content can help *Fucus serratus* to resist high copper stress (Nielsen *et al.*, 2003). We can also hypothesize that higher Car/Chl *a* increased the resistance to high zinc also for CPCC299 and CPCC632, but this increased resistance was counteracted by the much higher zinc absorption found under HL compared to LL. In the case of CPCC299, on top of the effect of HL on zinc absorption, which leads to the increase in sensitivity to zinc, the decrease Car/Chl *a* could also increase its zinc sensitivity (Table 4.1). Indeed, lower carotenoid content will decrease the ability of energy dissipation through heat and therefore, energy can accumulate at the PSII donor side. This can lead to reactive oxygen species accumulation and PSII damage (Campbell *et al.*, 1998), and consequently a higher sensitivity to zinc, especially for HL grown cells. These results suggest that a combination of high light and zinc stress could be critical for the growth of toxic *M. aeruginosa* strains. In conclusion, we clearly showed that high light has different effects on zinc toxicity for the studied cyanobacterial strains, which suggests that the combination of light intensity and zinc concentrations are conditions to be considered when studying cyanobacterial bloom dynamic in freshwater ecosystems, since some cyanobacterial strains have higher zinc uptake rates than others. Furthermore, energy dissipation processes needed for photoprotection are influenced by light intensity and zinc concentration and are variable among the studied strains.

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CHAPTER V

DIFFERENT RESPONSES TO HIGH LIGHT STRESS OF TOXIC AND NON-TOXIC *MICROCYSTIS AERUGINOSA* ADAPTED TO TWO LIGHT INTENSITIES AND ZINC CONCENTRATIONS

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5.1. Resume

Dans cette étude, l'interaction entre les effets de la haute lumière (HL) et ceux du zinc ont été étudiés sur deux souches de *Microcystis aeruginosa* (la souche toxique CPCC299 et la souche non-toxique CPCC632). Ces deux souches ont été cultivées sous deux concentrations en zinc (une concentration moyenne en zinc (MZn): 7.8×10^{-7} M, et une concentration élevée en zinc (HZn): 7.8×10^{-6} M) et sous deux intensités lumineuses (lumière faible (LL): 50 µmol photons·m^{-2}·s^{-1} et haute lumière (HL): 500 µmol photons·m^{-2}·s^{-1}) puis exposées à un stress lumineux élevé (2000 µmol photons·m^{-2}·s^{-1}) pendant 20 minutes. Nos résultats suggèrent que le taux de croissance spécifique de CPCC632 était plus sensible au HZn que celui de CPCC299 lorsqu'elles étaient acclimatées à HL. Lorsque *M. aeruginosa* a été soumise à un stress lumineux pendant 20 minutes, les taux maximal et opérationnel du PSII (Φ_M et Φ'_M) des deux souches ont diminué, et CPCC632 cultivée à LL et HZn avait la plus forte baisse parmi les quatre conditions de croissance. En condition de croissance sous LL, CPCC632 était plus sensible au stress lumineux que CPCC299. Toutefois, cette différence entre les deux souches disparaît lorsqu'elles sont cultivées en condition de HL. Ces résultats suggèrent que CPCC632 possède une faible capacité à s'acclimater au stress lumineux lorsqu'acclimatée à des conditions de LL et HZn. Nous avons également démontré que les mécanismes d'action du stress lumineux chez *M. aeruginosa* étaient liés aux changements dans les pigments, aux processus de dissipation de l'énergie et au contenu en microcystine (pour la souche toxique). Nos données indiquent que les différences dans l'effet de la haute lumière et du zinc sur les souches de *Microcystis aeruginosa* peuvent influencer la dominance de certaines souches dans les systèmes aquatiques.

Mots clé: *Microcystis aeruginosa*; haute lumière; zinc; photosynthèse

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5.2. Abstract

In this study, interaction effects of high light (HL) and zinc on two *Microcystis aeruginosa* strains (toxic CPCC299 and non-toxic CPCC632) were investigated. Our results suggested that the specific growth rate of CPCC632 was more sensitive to high zinc (HZn; 7.8×10^{-6} M) concentration than it is in CPCC299 when acclimated to HL (500 μmol photons·m^{-2}·s^{-1}). When *M. aeruginosa* were treated 20 min to high light (2000 μmol photons·m^{-2}·s^{-1}), the maximal and the operational PSII quantum yields (Φ_M and Φ'_M) of the two strains decreased, but CPCC632 (50 μmol photons·m^{-2}·s^{-1} (low light (LL)) + HZn) has the biggest decrease among the four growth conditions. Under LL growth conditions, CPCC632 was more sensitive to high light stress than CPCC299. However, these differences disappeared when they were grown under HL condition. These results suggested that CPCC632 had the lowest ability to acclimate to high light stress under LL + HZn conditions. We also showed that the response mechanisms to high light stress of *M. aeruginosa* were linked to the change of pigments, energy dissipation processes and microcystin content (for the toxic strain). Our data indicated that different effects of high light and zinc on *M. aeruginosa* strains may influence the dominance of different strains in aquatic system.

Keywords: *Microcystis aeruginosa*; high light; zinc; photosynthesis

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5.3. Introduction

Microcystis is known to be one of the major genuses involved in cyanobacterial blooms around the world (Wu *et al.*, 2007). *Microcystis* spp. are problematic since they have the potential to produce microcystins that reduce water quality and have adverse effects on aquatic ecosystems, livestocks, human water supplies and recreational activities (Sigee *et al.* 1999; Nakamura *et al.* 2003; Xu *et al.* 2012). The occurrence of *Microcystis* blooms is known to be linked to many environmental factors, such as temperature, light and nutrients. These factors may not only play a role in growth of *Microcystis* spp., but also in the proportion of toxic and non-toxic strains in water ecosystems (Xie *et al.*, 2003; Yamamoto and Nakahara 2005; Imai *et al.*, 2009; Deblois and Juneau 2010, 2012).

Although metal pollution is a concern for many decades, this type of water pollution is still important due to increased industrial and agricultural activities of many countries (Das *et al.*, 2008; Zhou *et al.* 2008). Zinc is one of the metals essential for growth of phytoplankton due to its involvement as cofactor of many enzymes (e.g. in carbonic anhydrase, superoxide dismutase and RNA polymerase). However, when present at high concentration, this metal becomes toxic to phytoplankton by decreasing growth rate, oxygen production, photosynthetic electron transport, and chlorophyll *a* content (Davies and Sleep 1979; Assche and Clijsters 1986; Chaloub, Magalhaes, and Santos 2005; Zeng *et al.*, 2009; Sayyed and Bhosle 2010). Despite the usual high degree of stress tolerance of cyanobacteria, it has been shown that they are highly sensitive to metal pollution, including zinc (Brand *et al.* 1986; Juneau *et al.* 2001; Miao *et al.*, 2005). However, although zinc is one of the most common metal pollutants of terrestrial and aquatic environment (Foy *et al.*, 1978; Collins, 1981), only few studies were done to investigate the effect of high zinc concentrations on growth and photosynthesis of *M. aeruginosa* (Zeng *et al.*, 2009; Zeng and Wang 2011), and no studies has been conducted on zinc toxic effect on *M. aeruginosa* grown under

different light intensities. Furthermore, during the life cycle of *M. aeruginosa*, cells may be exposed to high light intensity up to 2000 µmol photons·m^{-2}·s^{-1} during sunny summer days. It was proposed that this species has a high ability to acclimate to this high light stress (Zhang *et al.* 2008). Recently, Deblois and Juneau (2012) suggested that toxic *M. aeruginosa* strains have higher ability to cope with high light stress than the non-toxic ones. However, no information exists on the response to high light stress of *M. aeruginosa* acclimated to different light and zinc conditions, conditions occurring in natural ecosystems.

The objective of this work was to evaluate the involved mechanisms and the ability of two *M. aeruginosa* strains to cope with high light stress when these two strains were acclimated to different zinc and light conditions.

5.4. Materials and methods

5.4.1. Cultures

Microcystis aeruginosa CPCC632 (non-toxic strain) and *Microcystis aeruginosa* CPCC299 (toxic strain) were obtained from Canadian Phycological Culture Centre. These two *M. aeruginosa* strains were cultured in 250-ml Erlenmeyer flasks containing 150 ml fresh bold basal media at pH 7.8 (BBM; Stein 1973) having two zinc concentrations (7.8×10^{-7} M is the medium zinc concentration (MZn) and 7.8×10^{-6} M the high zinc concentration (HZn)). These cultures were grown under two light intensities (50 µmol photons·m^{-2}·s^{-1} (LL) and 500 µmol photons·m^{-2}·s^{-1} (HL)) on 14-h light:10-h dark cycle, at 22 ± 1 °C. The light was provided by Philips cool white fluorescence tubes and light intensity was measured by a US-SQS/WB spherical Micro Quantum sensor (Walz GmbH, Effeltrich, Germany). The two *M. aeruginosa* strains were acclimated under four growth conditions more than four generations and one month before measurements of all the parameters. Sub-samples were taken every day for the measurements of cell density and biovolume with a Multisizer™ 3 Coulter Counter® (Beckman Coulter Inc., Brea, CA, USA).

5.4.2. Photoinhibitory treatment

For photoinhibition experiments, cultures in their exponential phase were put into a thermoregulated cylindrical glass vessel exposed to an irradiance of 2000 µmol photons·m^{-2}·s^{-1} for 20 min at 22 °C. Cell densities at the beginning of the treatment were similar for all replicates. The temperature in the cylindrical glass vessel was controlled by a polystat refrigerated bath (Cole-Parmer Instrument Co., Vernon Hills, IL, USA).

5.4.3. Chlorophyll fluorescence measurements

Cultures in exponential phase were dark acclimated for 15 min and used for the measurements of rapid light response curves with a WATER-PAM Chlorophyll Fluorometer (Walz GmbH, Effeltrich, Germany). The maximal PSII quantum yield (Φ_M) was determined in the absence of actinic illumination. Then, the actinic light intensity was automatically increased from 71 to 1098 μmol photons·m^{-2}·s^{-1} in eight steps. The minimum fluorescence yield (F_O) was recorded before actinic light was opened. The cells were kept at each actinic light intensity for 1 minute before making measurements, and the fluorescence yields F_S and F_M' were respectively determined briefly before or during a saturating light pulse (800 ms, 3000 μmol photons·m^{-2}·s^{-1}) at the end of each illumination period. The F_M of *M. aeruginosa* were obtained by adding DCMU at the end of measurement since non-photochemical quenching (NPQ) may occur in the dark for cyanobacteria because of the interconnection between respiratory and photosynthetic electron transport chain (Campbell *et al.* 1998). Φ_M, the operational PSII quantum yield (Φ'_M) and NPQ were calculated by formulae: $\Phi_M = (F_M - F_0)/F_M$ (Kitajima and Butler 1975); $\Phi'_M = (F_M' - F_S)/ F_M'$ (Genty *et al.*, 1989); $NPQ = (F_M - F_M')/ F_M'$ (Bilger and Björkman 1990).

The 77K fluorescence emission spectra under 435-nm excitation were measured by Hitachi F-4500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). Samples were put in small glass tubes before and after 20 min high light exposure (photoinhibitory treatement), then quickly immersed in liquid nitrogen before measurements. The EX Slit (10 nm) and EM Slit (20 nm) were used for the measurements. A band-pass green filter (λ = 493nm; Edmund 46052, Barrington, NJ) and a red long-pass filter (λ > 600nm; Edmund 66065, Barrington, NJ) were used for excitation and emission light, respectively. The fluorescence intensity of 685 nm and 720 nm were used to represent the PSII and PSI content of two studied strains.

5.4.4. Pigment measurements

The pigment profiles of two *M. aeruginosa* strains grown at four growth conditions were measured spectrophotometrically. The 95% ethanol was used to extract pigments and the absorbance was measured with a Cary 300 UV-VIS spectrophotometer (Varian Australia Pty Ltd, Mulgrave, VIC, Australia). The contents of chlorophyll *a* (Chl *a*) and carotenoids (Car) were calculated using the equations of Lichtenthaler and Buschmann (2001).

5.4.5. Microcystin measurements

Samples were collected by 0.8 μm pore size membrane filter under exponential phase and put in -80 ℃ until measurement. Methanol was used to extract microcystin (Deblois and Juneau 2010) and QuantiPlate Kit for Microcystis-EP 022 (EnviroLogix Inc, Portland, ME, USA) was used to measure microcystin content in CPCC299. The microcystin content measured in this paper represents the total microcystins content in algae.

5.4.6. Statistical analyses

All experiments were performed using at least three replicates. All data are presented as the mean ± standard deviation (SD). Statistical analyses were carried out using STATISTICA® 6.0 (StatSoft Inc., Tulsa, OK, USA) and Origin® 6.1 (Originlab Corporation, Northampton, MA, USA). The one-way analysis of variance was used to determine the effect of treatments and Tukey's honestly significant difference (HSD) test was conducted to test the statistical significance of the differences between means of various treatments.

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5.5. Results

5.5.1. Effect of light and zinc acclimation on the growth and biovolume of two *M. aeruginosa* strains

The specific growth rate and cell biovolume of the two studied *M. aeruginosa* strains are shown in Table 5.1. As seen, growth rate of CPCC632 was significantly higher when cultured at HL conditions (Tukey's HSD, $P < 0.05$) compared to when this strain grows under LL conditions. Under LL conditions, HZn acclimation did not affect significantly growth of the studied strains. When CPCC632 strain was acclimated to HL, high Zn concentration decreased significantly its growth rate compared to HL + MZn acclimated cells (Tukey's HSD, $P < 0.05$). We can notice that the growth rate of *M. aeruginosa* CPCC299 was not influenced by any of the growth conditions tested here (Tukey's HSD, $P > 0.05$).

Table 5.1. Specific growth rate and cell biovolume of two *M. aeruginosa* strains grown under different conditions. Data are means ± SD (n =3). Different superscript letters for each strain in the same row are significantly different (Tukey's HSD, $P < 0.05$).

		LL		HL	
	Strains	MZn	HZn	MZn	HZn
Growth rate	CPCC632	0.38 ± 0.01^{a}	0.34 ± 0.02^{a}	0.60 ± 0.03^{b}	0.50 ± 0.04^{c}
($\mu \cdot day^{-1}$)	CPCC299	0.35 ± 0.01^{a}	0.37 ± 0.02^{a}	0.35 ± 0.04^{a}	0.36 ± 0.01^{a}
Cell volume	CPCC632	15.60 ± 0.72^{a}	15.42 ± 1.40^{a}	24.45 ± 0.43^{b}	39.94 ± 2.31^{c}
($\mu m^3 \cdot cell^{-1}$)	CPCC299	16.69 ± 0.78^{a}	19.03 ± 0.86^{a}	21.94 ± 3.58^{a}	23.20 ± 0.61^{a}

The cell biovolume of both strains grown under HL were bigger (although not significantly for CPCC299) than cultures grown at LL for both MZn and HZn conditions (Tukey's HSD, $P < 0.05$) (Table 5.1). Moreover, the cell biovolume of CPCC632 grown under HL + HZn was the biggest among the four growth conditions (Tukey's HSD, $P < 0.05$).

5.5.2. Effect of light and zinc acclimation on pigments

The Chl *a* and Car content of two studied strains grown at four growth conditions are shown in Table 5.2. When grown at LL and HL conditions, zinc content did not affect the Chl *a* and Car content of two studied strains (Tukey's HSD, $P > 0.05$). For CPCC632, HL acclimated decreased the Chl *a* content of algae compared to LL acclimated algae (Tukey's HSD, $P < 0.05$). HL acclimated decreased Car content of algae compared to LL + MZn acclimated algae. For CPCC299, HL acclimated algae had lower Chl *a* content than LL acclimated algae under both zinc conditions (except LL + HZn). However, there was no significantly difference of Car content of CPCC299 grown under four conditions (Tukey's HSD, $P > 0.05$). As seen the Car/Chl *a* ratios of *M. aeruginosa* acclimated to HL were significantly higher than the cultures grown under LL except for CPCC632 in MZn condition (Tukey's HSD, $P < 0.05$). When grown at 50 μmol photons·m^{-2}·s^{-1}, HZn acclimated cells decreased their Car/Chl *a* ratio for CPCC632, but there was no significant difference for CPCC299 between the LZn and HZn conditions (Tukey's HSD, $P > 0.05$). Under HL condition, HZn acclimated CPCC299 and CPCC632 cells had higher Car/Chl *a* ratio than cultures grown under LZn condition (Tukey's HSD, $P < 0.05$).

Table 5.2. Pigment content of two *M. aeruginosa* strains grown under different conditions. Data are means ± SD (n =3). Different superscript letters for each strain in the same row are significantly different (Tukey's HSD, $P < 0.05$).

		LL		HL	
		MZn	HZn	MZn	HZn
CPCC632	Chl *a* 10^{-15} g/μm^3	5.91±0.59^a	6.29±0.86^a	3.84±0.77^b	2.79±0.11^b
	Car 10^{-15} g/μm^3	2.54±0.32^a	2.13±0.30^{ab}	1.66±0.32^b	1.51±0.09^b
	Car:Chla	0.43±0.03^a	0.34±0.02^b	0.43±0.03^a	0.58±0.04^c
CPCC299	Chl *a* 10^{-15} g/μm^3	6.00±1.25^a	4.87±0.13^{ac}	3.32±0.43^{bc}	2.07±0.10^b
	Car 10^{-15} g/μm^3	2.92±0.73^a	2.36±0.07^a	3.00±0.39^a	2.18±0.07^a
	Car:Chla	0.48±0.02^a	0.49±0.02^a	0.90±0.04^b	1.05±0.02^c

5.5.3. Effect of high light stress on photosynthetic activity of *M. aeruginosa* acclimated two light and zinc conditions

After 20 min high light treatment, the maximal PSII quantum yield, Φ_M, of CPCC632 grown under the four acclimation conditions tested in this study, was significantly reduced by 22-45% compared to the non-HL-exposed cells (Tukey's HSD, $P < 0.05$) (Figure 5.1a). We can also notice that cells grown under 50 μmol photons·m^{-2}·s^{-1} were more affected by 20 min high light treatment than the ones grown under 500 μmol photons m^{-2} s^{-1}. For CPCC299, Φ_M was decreased by 26-32% after 20 min high light treatment, but no significant difference was observed among the four different acclimation conditions (Tukey's HSD, $P > 0.05$; Figure 5.1a). These results indicate that *M. aeruginosa* strains may have different regulatory mechanisms to cope with high light stress when acclimated to different light and zinc conditions.

The operational PSII quantum yield (Φ'_M) followed a similar trend to Φ_M when cells were exposed to 20 min high light treatment (Figure 5.1b). Indeed, after high light treatment, Φ'_M was reduced by 32-63% for CPCC632 (Tukey's HSD, $P < 0.05$). We can also notice that this strain grown under LL + HZn was the most sensitive to HL stress. For CPCC299, Φ'_M was reduced by 29-38% after high light treatment, but no significantly difference was found among the four acclimation conditions (Tukey's HSD, $P > 0.05$; Figure 5.1b).

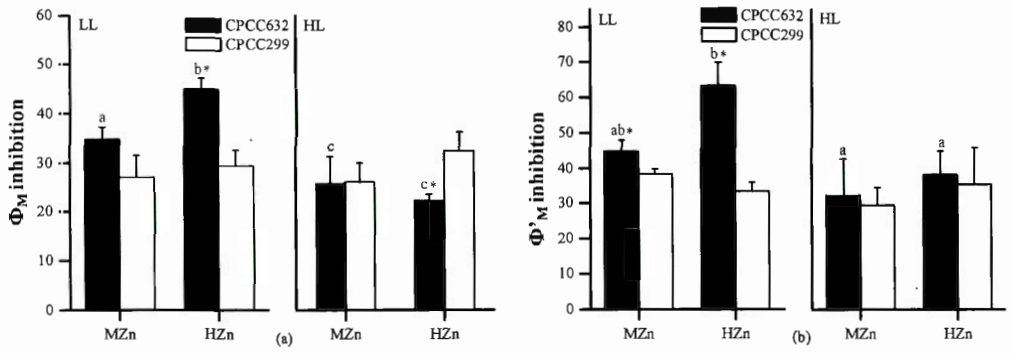

Figure 5.1. Maximal PSII quantum yield (Φ_M; a) and operational PSII quantum yield (Φ'_M; b) of the two studied *M. aeruginsa* strains grown under different conditions and after 20 min high light treatment. The bars with different letters on error bars are significantly different (Tukey's HSD, $P < 0.05$). The bars with asterisk (*) on error bars represent significantly different in two strains under the same growth condition. Data are means ± SD (n =3).

5.5.4. Effect of high light stress on non-photochemical energy dissipation of M. aeruginosa acclimated to different light and zinc conditions

The non-photochemical quenching (NPQ) of the two studied *M. aeruginosa* strains are shown in Figure 5.2a. After 20 min high light treatment, the NPQ of CPCC632

acclimated to LL was lower than for cultures grown under HL. On the other hand, the NPQ of CPCC299 cultured at LL was higher than for cultures grown at HL. We found also that the NPQ of CPCC299 was significantly higher than CPCC632 when these strains were grown at LL + MZn and LL + HZn respectively (Tukey's HSD, $P < 0.05$).

5.5.5. Effect of light and zinc acclimation on state transition

As seen in Figure 2b, after 20 min high light treatment, the decreased ratio of PSII/PSI of CPCC299 acclimated to LL + MZn was generally higher than for cultures grown under LL + HZn and HL. For low light grown CPCC632 and CPCC299, high zinc acclimation led to lower change of PSII/PSI ratio after 20 min high light treatment than cultures acclimated to normal zinc concentration (where no difference was observed). This trend was also found when these two strains were acclimated to HL condition. The effect of the 20 min high light stress on the PSII/PSI ratio was significantly higher for LL + MZn acclimated CPCC299 than CPCC632 (Tukey's HSD, $P < 0.05$).

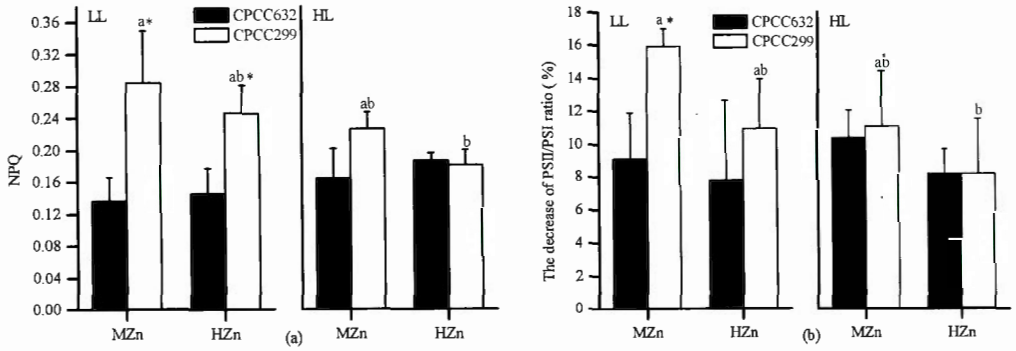

Figure 5.2. Effect of 20 minutes treatment of high light (2000 μmol photons·m^{-2}·s^{-1}) on Non-photochemical quenching (NPQ; a) and ratio of PSII/PSI (b) for the two studied *M. aeruginosa* strains grown under different conditions. The bars with different letters on error bars are significantly different (Tukey's HSD, $P < 0.05$). The bars with asterisk (*) on error bars represent significantly different in two strains under the same growth condition. Data are means ± SD (n =3).

5.5.6. Effects of light and zinc on microcystin content of CPCC299

The microcystin content of CPCC299 cultured under the four growth conditions are show in Figure 5.3. We can notice that growth under 500 μmol photons·m^{-2}·s^{-1} significantly decreased the microcystin content compared to growth under 50 μmol photons·m^{-2}·s^{-1} (Tukey's HSD, $P < 0.05$). However, for both growth light conditions, zinc concentration did not affect microcystin content in CPCC299.

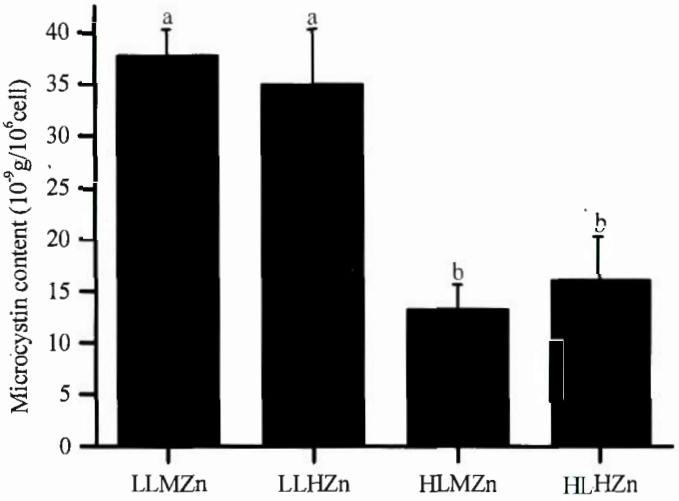

Figure 5.3. Microcystin content of *M. aeruginosa* CPCC299 grown under different conditions. The bars with different letters on error bars are significantly different (Tukey's HSD, $P < 0.05$). Data are means ± SD (n =3).

5.6. Discussion

Zinc pollution becomes more serious in aquatic environment worldwide, and zinc concentration as high as 10^{-6} M was found in some aquatic water systems (Cheng, 2003; Sayyed and Bhosle 2010). Algae and cyanobacteria are therefore exposed to high zinc concentrations when they grow at various light intensities caused by seasonal variations. We have shown in this study that *Microcystis aeruginosa* acclimated to two light intensities and zinc concentrations depicted changes in their specific growth rates (Table 5.1). When grown under HL condition, the growth rate of the non-toxic strain CPCC632 was higher than cultures grown under LL condition at both low and high zinc concentrations. Moreover, under HL condition, the specific growth rate of this strain was decreased by high zinc concentration. Our results are in accordance with previous studies indicating that long term high zinc concentration exposure can decrease growth rate of diatoms and cyanobacteria (Colwell, Hornor, and Cherry 1989;

Loez, Topalian, and Salibian 1995). Our results indicate that growth light intensity may play a role in the long term zinc toxicity on *M. aeruginosa* CPCC632, but not on the toxic strain CPCC299, suggesting an interaction between these two factors for the non-toxic strain. Concomitantly to these changes in growth rates, cell biovolume was also influenced by growth conditions, where HL increased the biovolume of both strains and HZn further increased the cell biovolume of CPCC632 grown under HL. This suggest that increasing biovolume may be a strategy to cope with stress conditions in the non-toxic strain, but not necessarily in the toxic strain, since biovolume was not influenced by zinc concentration. This strategy was previously observed in *Chlorella* sp. to deal with high copper and zinc stress (Wilde *et al.*, 2006).

Contrary to what we observed for *M. aeruginosa* CPCC632, we have shown that the growth rate of the toxic strain, CPCC299, was not modified under all four growth conditions, which may indicate a role of microcystin in the observed response. It was shown recently that microcystin content was linked to growth light intensity in *M. aeruginosa* strain (Wiedner *et al.*, 2003; Deblois and Juneau 2010). Under LL conditions, as expected, CPCC299 had high toxin content compared to when this *M. aeruginosa* strain grows under higher light intensity (Figure 5.3). Since zinc concentration did not influence microcystin content at two light intensities and that under high light growth rate did not increase like in CPCC632, we may advance that CPCC299 uses the higher energy provided by higher growth light to adjust its physiology to cope with the higher zinc concentration. We may hypothesize that one possible role of microcystin is to make complexes with metals present in the cells (Zinc in our study), which was also suggested previously (Humble *et al.*, 1997; Saito *et al.* 2008). Therefore, this may explain why under high light intensity the content of microcystin of CPCC299 was sufficient to protect against zinc toxic effect as it was observed in CPCC632.

Based on the difference observed in growth rates and biovolumes of these two strains of *M. aeruginosa* acclimated to different light and zinc conditions, it was of interest to

investigate their response to high light stress, since in summer the occurrence of *Microcystis* blooms is frequent and these organisms may float at the surface of the water bodies where high light intensity can be observed (Zhang *et al.* 2008; Xu *et al.* 2012). In this context, it is important to understand the regulation of photosynthetic apparatus of *M. aeruginosa* strains upon the exposure of high light when acclimated to different zinc concentrations and light intensities. The Φ_M, representing the maximal PSII quantum yield of phytoplankton, is known to be a good proxy of the health (photosynthesis and growth) of the photosynthetic organisms and any modification indicates that the organism experiences stress conditions (Björkman and Demmig 1987). After 20 min high light treatment, the Φ_M significantly decreased for the studied *M. aeruginosa* strains acclimated to the four growth conditions evaluated (Figure 5.1a). The inhibition of Φ_M after 20 min high light treatment was significantly less for CPCC632 grown under HL compared to LL grown cyanobacteria (Figure 5.1a), indicating that acclimation to HL may protect photosynthetic apparatus to subsequent high light stress as it was also demonstrated for higher plants (Gray *et al.*, 1996). This protective effect induced by HL acclimation was not seen for the toxic strain, although maximal quantum yield was not more affected by high light stress in CPCC299. Deblois and Juneau (2012) suggested that toxic *M. aeruginosa* strains have higher ability to acclimate to high light compared to non-toxic strains when exposed more than 60 min to high light. In the present study, this trend was respected for *M. aeruginosa* grown under low light conditions, but was not totally true for cells grown under higher light intensity, suggesting a complex interaction between light, zinc and potentially the toxin content (as suggested above). For LL grown CPCC632, the combined effect of zinc toxicity and HL stress may explain the stronger inhibition of photosynthesis observed under these conditions compared to normal zinc concentration and high light stress. Again, as we have discussed for growth of *M. aeruginosa* CPCC299, microcystin seems to play a role to protect the LL grown toxic *M. aeruginosa* when they are exposed to high light stress under high zinc concentration, and this might be due to the chelator capacity of microcystin (Saito et al. 2008). The change of Φ'_M in *M. aeruginosa* was similar as the Φ_M, although more affected by the

stress condition as demonstrated previously (Macinnis-Ng and Ralph 2003; Juneau *et al.*, 2007; Chalifour et al. 2009).

In order to gain more insights on the mechanisms triggered to cope with high light stress, energy dissipation processes of *M. aeruginosa* were investigated. One of the most important energy dissipation processes in phytoplankton is the non-photochemical energy dissipation process (NPQ), and it was shown to be highly induced under stress conditions (Guo et al. 2006). Surprisingly, for the non-toxic strain (CPCC632), the NPQ induced by high light stress was not modified by the acclimation condition. On the other hand, for CPCC299, the NPQ induced after high light stress was higher for cells acclimated to LL than the ones acclimated to HL. For both strains, we showed that zinc concentration used in our study was probably not high enough to induce any change of NPQ values as it was previously demonstrated for various phytoplankton species treated to low (lower than 10 $\mu g\ L^{-1}$) concentrations of copper (Juneau *et al.*, 2002). Since in cyanobacteria, there is no xanthophyll cycle, which is the main part of NPQ in plants and green algae, state transition was advanced to be the main part of NPQ in these organisms (Rakhimberdieva *et al.* 2004). In our study, toxic strain of *M. aeruginosa* grown under LL had higher ability to induce state transition after 20 min high light treatment compared to cultures acclimated to HL (Figure 5.2b). For the non-toxic strain, no change in the state transition after high light stress was observed between the different acclimation conditions. These results indicate again a strong difference in the response to high light stress between the two studied strains.

Protection of the photosynthetic apparatus could also occur from photoprotective pigments (carotenoids) associated to the photosynthetic apparatus (Wilson et al. 2006; Kirilovsky and Kerfeld 2012). It was shown recently that *M. aeruginosa* having high Car/Chl *a* ratio had a higher ability to acclimate to high light than when this ratio was low (Deblois and Juneau 2012). In the present study, Car/Chl *a* may also help to understand the response of the studied strains to high light stress. Indeed, CPCC632

grown under HL had higher Car/Chl *a* ratio than cultures grown under LL and this may be the main reason why photosynthetic activity was less inhibited by high light stress (since the induction of NPQ and state transition by high light stress were not changed by acclimation conditions). For CPCC299, the increase of Car/Chl *a* ratio accompanied the decrease of NPQ and state transition under HL compared to LL acclimated algae. Therefore, the ability to acclimate to high light stress was not changed by high light acclimation since the involvement of the photoprotective pigments may be combined to the role of the NPQ and state transition.

This study demonstrated that high light can affect zinc toxicity on *M. aeruginosa*, and the response mechanisms to cope with high light stress of *M. aeruginosa* acclimated at four growth conditions were linked to the change of pigments, energy dissipation processes. Microcystin content seems also to play a role in the response to HL stress. These results help to understand the different abilities of *M. aeruginosa* strains to cope with high light stress under different growth conditions and may therefore bring new knowledge on the underlying processes explaining the dominance of specific strains in aquatic ecosystems.

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GENERAL DISCUSSION

This thesis has demonstrated that dephosphorylation indeed is involved in state transition induced by far red light in cyanobacteria. Also, state transition and OCP-related quenching can work together to cope with stress conditions and their abundance vary between cyanobacterial species. There is not a specific rule for these two mechanisms responses to high light for all cyanobacteria. Every cyanobacterium can induce NPQ to cope with stress conditions. However, the extent of different components of NPQ is not the same, and depends not only on species, but also on the growth light conditions. Moreover, toxic metals can affect the capability of NPQ in cyanobacteria and this effect is different between species.

Main results

In chapter I, dephosphorylation regulated the state 2 to state 1 transition induced in far red light in *Synechocystis* sp. FACHB898. However, when state 2 to state 1 transition is induced by DCMU, dephosphorylation is not necessary. This information firstly links the redox of PQ pool and PBS movement in cyanobacteria and also suggests that PBS movement could not be involved in state 2 to state 1 transition induced by DCMU. Moreover, these results provide the important information to elucidate the whole mechanism of state transition in cyanobacteria. On top of the result that NaF can inhibit the PBS movement in state 2 to state 1 transition in *Synechocystis* sp. FACHB898, I used NaF to partly inhibit the state 2 to state 1 transition induced by dark-high light shift, then investigated the role of this state transition under high light stress condition. The results of Chapter II showed that PBS reorganization between the two photosystems is important for regulating high light stress in *Synechocystis* sp. FACHB898. This result suggests that state transition could affect the other high light

quenching mechanisms (such as OCP-related quenching) in cyanobacteria.

In order to continue to investigate the interaction of state transition and OCP-related quenching under high light, two cyanobacterial species (*Microcystis aeruginosa* and *Synechocystis* sp.) having significantly different growth habitats were chosen for Chapter III. *M. aeruginosa* showed higher ability of OCP-related quenching but much less state transition compared to *Synechocystis* sp.. It was also found that OCP-related quenching is more efficient to protect PSII compared to state transition under high light stress conditions for the two studied species. According to these results and previous publications, I summarized the high light regulation model in cyanobacteria. This model indicates that cyanobacterium has evolved regulation mechanism according to its habitat and could induce one of the two mechanisms (OCP-related quenching and state transition) to cope with high light. Therefore, this model can help us to better understand the high ligh regulation mechanisms in cyanobacteria grown under a variety of conditions.

In Chapter IV, we found that NPQ was decreased by short term high zinc treated in *M. aeruginosa*, but not in *Synechocystis sp.*. High zinc decreased of the NPQ could result from the lower Car/Chl *a* ratio and also the lower state transition capability in *M. aeruginosa*. At the same time, we found that high light increased the zinc uptake in *M. aeruginosa*, but not in *Synechocystis sp.*. These results suggest that *Synechocystis sp.* could tolerant higher zinc concentration compared to *M. aeruginosa* under high light conditions. It is really important to point out that high zinc concentration decreases the NPQ and Car/Chl *a*, which could be lethal for some cyanobacterial species under high light conditions. It was already found that phytoplankton dominance could be influenced by zinc concentration in some freshwater ecosystems, but my research provide important information proving that high zinc and high light could favor the non-toxic cyanobacterial species.

At last, Chapter V showed that long-term high zinc and high growth light adaptation could affect the high light regulation ability of *M. aeruginosa*. The NPQ and state

transition was decreased when the toxic *M. aeruginosa* CPCC299 was grown under high growth light and high zinc, but not for the non-toxic *M. aeruginosa* CPCC632. This result is somewhat different for the NPQ changes between the short-term and long-term high zinc treatment in CPCC632 since the short-term high zinc treatment also lead the decrease of NPQ. It was also found that after *M. aeruginosa* adapted to the high zinc concentration, this species can increase the Car/Chl *a* to cope with this high zinc stress condition. This thesis also showed that some different response mechanisms in short and long-term zinc resistance in *M. aeruginosa*. This conclusion reminds us that the understanding of the effect of metals on the physiology and photosynthesis of aquatic photosynthetic organisms need more caution.

Prospect

Although more experiments can be done to provide a clearer view of the mechanism of state transition in cyanobacteria, the results showing that desphosphorylation is involved in state 2 to state 1 transition induced by far red light, open a new window for the researchers in this domain. According to these results, we can anticipate that state transition is not composed of only one mechanism in cyanobacteria induced by far red light as opposed to higher plants. It has been suggested that PBS movement and spillover are involved together in state 2 to state 1 transition in cyanobacteria (Li *et al.*, 2004). However, our results suggested that this state transition can be triggered without PBS movement, which improve our understanding about the interaction of PBS movement and spillover, working together in state transition in cyanobacteria. Moreover, this result gives an opportunity to clarify the whole mechanism of state transition in cyanobacteria.

It was interesting to find that various NPQ mechanisms are present in different cyanobacterial species, since cyanobacteria were supposed to have no harvesting-antenna quenching (such as xanthophyll cycle in higher plants) (Campbell

et al., 1998). Also, the results that high light regulation model will provide the basic information is important when we anticipate the physiological and photosynthetic process of cyanobacteria in natural ecosystem.

It is suggested that zinc emission to environmental increased around 5 times in 1989 compared to 1898 year (Nriagu, 1996). It is therefore obvious that cyanobacteria may suffer from this high zinc concentration in some polluted waters under various light conditions. Although it is hard to mimic the natural ecosystem in laboratory experiments done with isolated species, the results of the present thesis still can give the trend for the natural ecosystem. According to the result that high light and high zinc could be lethal for *M. aeruginosa* according to decrease the Car/Chl *a* ratio and NPQ for both *M. aeruginosa* CPCC632 and CPCC299 in short-term high zinc stress experiment, and decrease the NPQ for CPCC299 under long-term high zinc stress experiment, we can anticipate that high zinc pollution could decrease the biomass of the *M. aeruginosa* in polluted water ecosystem. This trend has been also found in some freshwater ecosystems (Bbosa and Oyoo, 2013).

As we pointed out, metals can be toxic to phytoplankton (Bbosa and Oyoo, 2013), but they can also have adverse effects for human through the food chain. This is particularly problematic because metals are non-biodegradable in nature, and they tend to accumulate in various life forms (Šmejkalová *et al.*, 2003). Therefore, it is important to look for an economical, effective and environmental-friendly way for moving metals from various types of water. Conventional ways including chemical precipitation, ion exchange, electrochemical removal, are ineffective, costly or produce secondary sludge that are also toxic. Hence, more and more research focus on the more effective biological ways for metals removal (Won *et al.*, 2014). It was found that *Nostoc muscorum*, a cyanobacterial species can represented a maximum removal of Zn(II) (71 %) at the end of the 60-h culture period (Roy *et al.*, 2015). Genetic engineering could be used to create or select more resistant mutants or more effective ones to remove metals, and therefore to decrease the metal content below permissible limits.

On the other hand, as we found that high zinc combined with high light could be lethal for *Microcystis aerugionsa*, but less *synechocystis* sp.. These results inspire a way to again *M. aeruginosa* bloom by combining metals with high light. The further research could find a concentration of metal that can kill *M. aeruginosa* under high light, but less effects on other phytoplankton.

In brief, understanding the response mechanisms to high light and high zinc concentration (together with other environmental factors) can help to better understand the formation of cyanobacterial blooms and the influence of metals in aquatic environments.

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