PHOTOSYNTHETICA 40 (3): 383-387, 2002

Effect of sodium thiosulfate on the depletion of photosynthetic apparatus in cyanobacterium *Synechocystis* sp. PCC 6803 cells grown in the presence of glucose

Zeneng WANG, Yinong XU, Zhenle YANG, Haitong HOU, Guizhen JIANG, and Tingyun KUANG

Key Laboratory of Photosynthesis and Environmental Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, P. R. China

Abstract

Fluorescence spectroscopy at 77 K showed that the application of glucose lead to the depletion of phycobilisomes (PBS) and photosystems (PS) 2 and 1, and that PS2 was more sensitive to glucose than PS1. The application of sodium thiosulfate, an effective scavenger of reactive oxygen intermediates, counteracted the effects of glucose. Sodium thiosulfate effectively protected photosynthetic apparatus, PS2, PS1, and PBS against glucose-induced depletion. Sodium thiosulfate showed strong capability to inhibit the disappearance of chlorophyll induced by glucose. At a relatively low concentration of glucose, the application of sodium thiosulfate can even be helpful for the assembly of photosynthetic apparatus. Hence the reactive oxygen species might be involved in the depletion of the photosynthetic apparatus in the cyanobacterium *Synechocystis* sp. PCC 6803 cells grown in the presence of glucose.

Additional key words: allophycocyanin; chlorophyll; fluorescence; oxygen evolution; photosystems 1 and 2; phycobilisome; phycocyanin.

Introduction

Photosystem 2 (PS2) uses photons to oxidise water and reduce plastoquinone, and PS1 uses it to reduce $NADP^+$ (Arnon and Barber 1990). Phycobilisomes (PBSs), composed of phycobiliproteins, are bound to the outer side of the photosynthetic membrane and funnel excitation energy to the PS2 reaction centre (Sidler 1994). There are two kinds of phycobiliproteins in *Synechocystis*, phycocyanin (PC) and allophycocyanin (APC), both of them characterised by the co-valently bound, open-chain tetrapyrrole chromophores, phycocyanobilins (Sidler 1994). Photosynthetic apparatus can be distinguished from each other by 77 K fluorescence emission spectroscopy. Strong irradiance, low temperature, and salt stress lead to the depletion of photosynthetic apparatus (Deshnium *et al.* 1997, Gombos *et al.* 1997, Hayashi *et al.* 1997). Whether photosynthetic apparatus can be depleted by exogenous sugar will be answered in this study.

Sodium thiosulfate, an agent rapidly neutralising reactive oxygen intermediates (Stewart *et al.* 1999), can be used to demonstrate whether reactive oxygen species are involved in a given physiological process. Tichy and Vermaas (1999) reported that freshly autoclaved BG-11 medium contains up to 5 μM H_2O_2 and the sodium thiosulfate applied is an efficient H_2O_2 scavenging agent. The occurrence of other reactive oxygen species is associated with H_2O_2. Superoxide anion ($O_2^{\cdot-}$) can spontaneously dismutate to H_2O_2 and oxygen, and H_2O_2 can be converted in a spontaneous reaction catalysed by Fe^{2+} (Fenton reaction) or by reaction with superoxide anion (Harber Weiss reaction) to the highly reactive hydroxyl radical ($^{\cdot}OH$) (http://www.rndsystems.com/asp/g_sitebuilder.asp?bodyId=222#over). Therefore, sodium thiosulfate can scavenge other reactive oxygen species indirectly.

In this study, sodium thiosulfate was also added to liquid BG-11 medium to test whether reactive oxygen species were involved in changes in photosynthetic apparatus induced by glucose.

Received 11 April 2002, *accepted* 27 June 2002.
Fax: +86-10-82594105; e-mail: yinongxu@ns.ibcas.ac.cn
Acknowledgements: We thank Mr. Qun Zhang and Ms. Lijie Feng for their help with fluorescence spectroscopy. We also acknowledge China Postdoctoral Scientific Foundation, the State Key Basic Research and Development Plan (G1998010100), One-Hundred-Scientist Program of the Chinese Academy of Sciences, and the Innovative Foundation of Photosynthesis Basic Research, Institute of Botany, Chinese Academy of Sciences, for their support of our research work.

Materials and methods

Cells, culture conditions: Cells of *Synechocystis* sp. PCC 6803, a gift from the laboratory of Professor Qingyu Wu, Tsinghua University, purified previously, were cultivated at 30 °C under constant irradiation from fluorescent lamps (about 70 μmol m^{-2} s^{-1} in BG-11 medium (pH 7.5) (Allakhverdiev *et al.* 1999), which contained various concentrations of glucose. 0.3 % (m/v) sodium thiosulfate was added to some BG-11 media containing different concentrations of glucose. The density of cells was measured with a *UNIKON 943* spectrophotometer at 730 nm (OD_{730}).

Chlorophyll (Chl) content was determined as described in Dai *et al.* (2000). Results were normalised to the absorbance of *Synechocystis* sp. PCC 6803 cells at 730 nm.

Measurement of photosynthetic activities: Oxygen evolution of *Synechocystis* cells was measured with a Clark-type oxygen electrode under an irradiance of 2.9 W m^{-2} at 30 °C. No exogenous donor or acceptor of electrons was added during monitoring photosynthetic oxygen evolution of intact cells. Intact cells were collected by centrifugation and washed twice with the BG-11 medium and were suspended in the same medium for measurements (Hagio *et al.* 2000).

Fluorescence spectroscopy: Fluorescence emission spectra of intact cells were measured at 77 K using a *Hitachi F-4500* fluorescence spectrophotometer. The cells were suspended in 60 % glycerol in 25 mM Hepes-NaOH, pH 7.0, 2 min before freezing. Two excitation wavelengths, 436 and 580 nm, were used (Wu and Vermaas 1995, Wu *et al.* 1999). The different spectra were normalised to the same density of cell suspension.

Results

Changes in Chl content (Fig. 1): In BG-11 media with 0, 1, or 5 mM glucose applied, Chl contents increased within the initial 2 d, and then they decreased similarly to those in media with 20 or 100 mM glucose. At the same growth age, Chl content decreased with glucose concentration. At ages of 4, 6, and 8 d, Chl could hardly be observed in cells grown in the presence of 20 or 100 mM glucose.

However, in the presence of 0.3 % sodium thiosulfate, Chl content increased with glucose concentration of no more than 20 mM at ages of 6 and 8 d (Fig. 1*B*). Cells grown in the medium with 100 mM glucose contained more Chl than those grown without glucose applied at the age of 6 d. At age of 8 d, the former cells contained less Chl than the latter ones. Under the same growth condition, 6-d-old cells contained more Chl than the 8-d-old ones. In the absence of glucose, cells grown in the presence of sodium thiosulfate contained less Chl than those grown in the absence of sodium thiosulfate at the same age (Fig. 1). However, in the presence of glucose, the results were just the opposite.

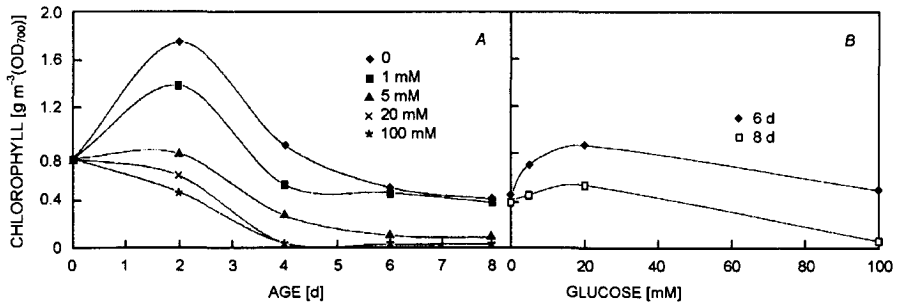

Fig. 1. Changes in chlorophyll content of *Synechocystis* sp. PCC 6803 cells grown in BG-11 media with different concentrations of glucose applied during growth. *A*, no sodium thiosulfate added; *B*, 0.3 % sodium thiosulfate added.

Changes in fluorescence intensity: The fluorescence emission peaks at 685, 695, and 725 nm excited at 436 nm had characteristics of PS2 (CP43), PS2 (CP47), and PS1, respectively (Wu *et al.* 1999). At an age of 2 d, the fluorescence amplitude at 725 nm of *Synechocystis* sp. PCC 6803 cells grown in BG-11 medium without glucose was very similar to that in BG-11 medium with 1 mM glucose (see Fig. 2*A*). At ages of 4, 6, and 8 d, the amplitudes at 725 nm all were larger for the latter than those for the former (see Fig. 2*B,C,D*). However, with further increase in glucose concentration (5, 20, and 100 mM) in the BG-11 medium, the fluorescence amplitudes at 725 nm were smaller than those in BG-11 medium without glucose added at ages of 2, 4, 6, and 8 d (see Fig. 2*A-D*). The fluorescence amplitudes at 685 and 695 nm decreased with glucose concentration at ages of 2, 4, 6, and 8 d (see Fig. 2*A-D*). PS2 and PS1 gradually disappeared with growth time in the presence of glucose. The fluorescence emission peaks at 648, 665, and 685 nm excited at 580 nm are attributed to PC, APC, and the

terminal emitter of PBS, APC-B, respectively (Yu *et al.* 1999, Schneider *et al.* 2001). In Fig. 3*A-D*, the fluorescence amplitudes at the same growth age decreased with glucose concentration added to the BG-11 medium.

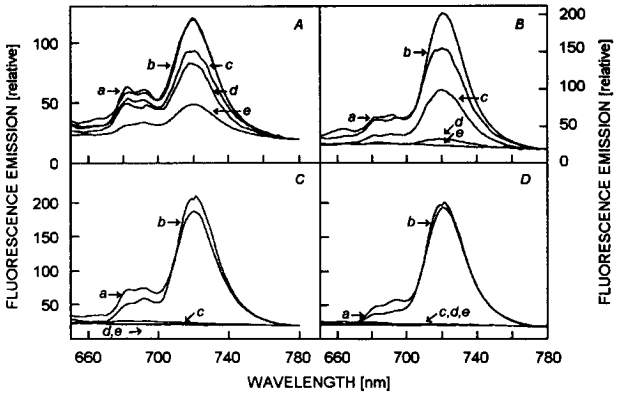

Fig. 2. 77 K fluorescence emission spectra excited at 436 nm of *Synechocystis* sp. PCC 6803 cells grown in the presence of 0 (*a*), 1 (*b*), 5 (*c*), 20 (*d*), and 100 (*e*) mM glucose for 2 (*A*), 4 (*B*), 6 (*C*), and 8 (*D*) d. Spectra were normalised to the same cell density (OD_{730}).

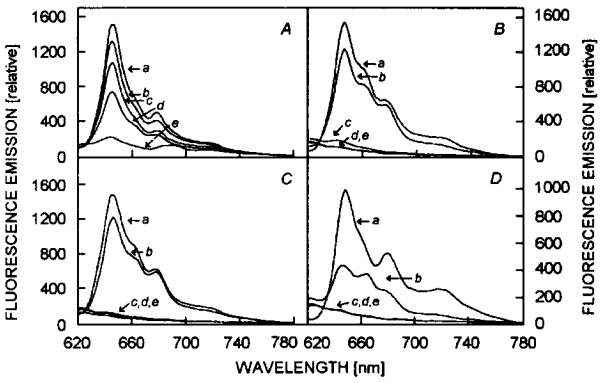

Fig. 3. 77 K fluorescence emission spectra excited at 580 nm of *Synechocystis* sp. PCC 6803 cells grown in the presence of 0 (*a*), 1 (*b*), 5 (*c*), 20 (*d*), and 100 (*e*) mM glucose for 2 (*A*), 4 (*B*), 6 (*C*), and 8 (*D*) d. Spectra were normalised to the same cell density (OD_{730}).

If sodium thiosulfate was applied to media for *Synechocystis* sp. PCC 6803, the fluorescence amplitudes of PS2 and PS1 were larger in cells grown in the presence of glucose than in those grown in the absence of glucose, and the amplitudes of cells grown in the presence of 20 mM glucose were greater than in those grown in 5 and 100 mM glucose at an age of 6 d (see Fig. 4*A*). The fluorescence amplitudes of PS2 and PS1 in cells declined at an age of 8 d. Amplitudes in decreased order for cells grown in the presence of glucose were 5, 0, and 20 mM (Fig. 4*C*). In this phase, no fluorescence emissions associated with PS2 and PS1 were detected in cells grown in the presence of 100 mM glucose and sodium thiosulfate, and the amplitudes of PS2 and PS1 in the medium with sodium thiosulfate were greater than those without this agent applied in the absence of glucose (see Fig. 4*C*). The uneven emission spectrum for cells grown in the presence of both 100 mM glucose and sodium thiosulfate may be associated with the presence of a red substance in cells. The red substance might be a product of thiosulfate reacting with free radicals. The fluorescence spectra of Fig. 4*C* suggest that the disappearance of PS2 be earlier than that of PS1.

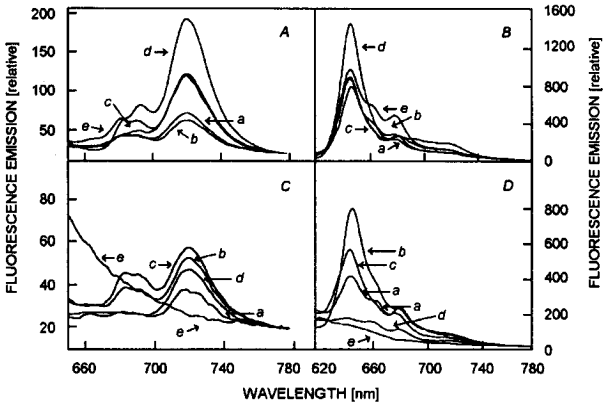

Fig. 4. 77 K fluorescence emission spectra of *Synechocystis* sp. PCC 6803 cells at growth ages of 6 (*A, B*) and 8 (*C, D*) d, respectively. Excitation wavelengths were set at 436 nm (*A, C*) and 580 nm (*B, D*), respectively. Curves *b, c, d,* and *e* correspond to cells grown in BG-11 media containing different concentrations of glucose [mM], 0, 5, 20, and 100 in the presence of 0.3 % (m/v) sodium thiosulfate, respectively. Curve *a* represents cells grown in BG-11 medium without glucose or sodium thiosulfate applied. Spectra were normalised to the same cell density (OD_{730}).

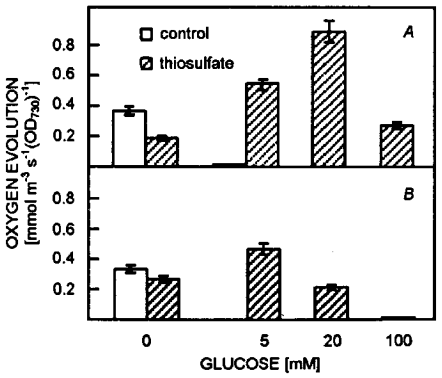

Fig. 5. Oxygen evolution activities of cells grown in BG-11 media with different concentrations of glucose and with or without 0.3 % sodium thiosulfate applied at growth ages of 6 (*A*) and 8 (*B*) d. Means ± SE for two independent replicates.

In addition, in the presence of sodium thiosulfate, fluorescence spectroscopy at 77 K indicated that at an age of 6 d, there existed fluorescence emissions from PC, APC, and the terminal emitter of PBS in the spectra of *Synechocystis* cells grown in BG-11 medium with glucose applied at all concentrations. The emission peaks of PBS terminal emitter in cells grown in the presence of

both sodium thiosulfate and different concentrations of glucose were higher than those for cells grown in the absence of sodium thiosulfate (Fig. 4*B*). At an age of 8 d, no significant fluorescence emissions from PC, APC, and PBS terminal emitter were detected in *Synechocystis* cells grown in the presence of 100 mM glucose (Fig. 4*D*).

In the presence of sodium thiosulfate, the fluorescence amplitudes of PC, APC, PBS terminal emitter, PS2, and PS1 in *Synechocystis* cells were not always decreased with the concentration of glucose applied to BG-11 medium. Sometimes the amplitudes got even larger with increase in glucose concentration, which was contrary to the case in the absence of sodium thiosulfate.

Changes in oxygen evolution activity: Oxygen evolution was not detected in 6- and 8-d-old cells grown in the presence of 20 or 100 mM glucose. Cells grown in the presence of 5 mM glucose showed only a slight oxygen evolution activity at an age of 6 d compared with those grown in the absence of glucose (Fig. 5). However, when 0.3 % sodium thiosulfate was applied to the BG-11 medium, oxygen evolution activity was detected in cells grown in the presence of all concentrations of glucose both at ages of 6 and 8 d (Fig. 5). Comparing the results, we found that sodium thiosulfate can effectively recover the effect of glucose on the oxygen evolution activity of cells and that in the presence of 0.3 % sodium thiosulfate, glucose is even helpful for oxygen evolution activity.

Discussion

Cyanobacteria perform in the same membrane aerobic respiration and oxygenic photosynthesis, both sharing some common components, such as plastoquinone pool or cytochrome (Cyt) b_6f complex (Alfonso *et al.* 2000). In the presence of irradiation and glucose, both photosynthesis and respiration proceed simultaneously in cells, which may lead to the production of an excessive amount of NADPH. If the utilisation of NADPH is sub-optimal and the content of $NADP^+$ is low, O_2 rather than $NADP^+$ may accept an electron from PS1 and therefore superoxide anion and other reactive oxygen radicals appear (Tichy and Vermaas 1999). D1 protein can be chemically cleaved by reactive oxygen and a newly synthesised protein (Yamamoto 2001) can replace the damaged D1 protein. If the rate of synthesis of D1 protein is smaller than the damage rate, PS2 decomposes slowly (Singh 2000). PS2 disappeared with time and glucose concentration in BG-11 medium in *Synechocystis* cells, and the application of sodium thiosulfate to the BG-11 medium with glucose effectively prevented the disappearance of PS2, which suggests that reactive oxygen species is involved in the degradation of PS2. Comparing the fluorescence amplitudes from PS2 and the oxygen evolution activities with the concentration of glucose applied to BG-11 medium in the presence of sodium thiosulfate (Fig. 4*A,C*), we suppose that the effects of glucose could be divided into two steps. Firstly, glucose could be used to provide substrates for the assembly of PS2, which led to the increase in PS2 fluorescence amplitude and oxygen evolution activity with glucose concentration. Then reactive oxygen species induced by glucose could not be scavenged by the limited content of sodium thiosulfate with growth time, which led to the decrease in PS2 fluorescence amplitude and oxygen evolution activity with glucose concentration.

In addition, low amounts of Chl occur in *Synechocystis* sp. PCC 6803 cells grown in the BG-11 medium with glucose. Chl availability is important for the translation, stability, and assembly of Chl-binding proteins and PBSs (He and Vermaas 1998, Yu *et al.* 1999). Therefore the reduced content of Chl in *Synechocystis* sp. PCC 6803 cells grown in the BG-11 medium with glucose applied can inevitably lead to the disappearance of photosystems and PBS, which provides another explanation why the application of glucose leads to the disappearance of PS2, PS1, and PBS. The presence of sodium thiosulfate showed strong capability to inhibit *Synechocystis* Chl damage with glucose in the BG-11 medium, therefore, PS2, PS1, and PBS disappeared fairly slowly.

The application of 0.3 % sodium thiosulfate greatly changed the ion intensity of BG-11 medium. In the absence of glucose, cells grown in the BG-11 medium with sodium thiosulfate had a lower content of Chl and lower oxygen evolution activity than those grown without sodium thiosulfate. This may be ascribed to the salt effect of sodium thiosulfate. But in the presence of glucose, the effect of sodium thiosulfate lies in the scavenging of reactive oxygen species.

This study revealed that sodium thiosulfate can protect photosynthetic apparatus against glucose-induced depletion. The simultaneous application of sodium thiosulfate and glucose to BG-11 medium is very helpful for growth of *Synechocystis* sp. PCC 6803 cells that can utilise photon energy to synthesise ATP and NADPH without accumulation of reactive oxygen species. On the other hand, cells can utilise glucose as carbon source for cell material.

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