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# The osmolality of the cell suspension regulates phycobilisome-to-photosystem I excitation transfers in cyanobacteria

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## Abstract

The chlorophyll *a* (Chla) fluorescence of cyanobacteria, which at physiological temperature originates from photosystem (PS) II holochromes, is suppressed in hyperosmotic suspension, and enhanced in hypo-osmotic suspension (G.C. Papageorgiou, A. Alygizaki-Zorba, *Biochim. Biophys. Acta* 1335 (1997) 1–4). We investigated the mechanism of this phenomenon by comparing *Synechococcus* sp. PCC 7942 cells that had been treated with *N*-ethylmaleimide (NEM) in order to inhibit electronic excitation transfers from phycobilisomes (PBS) to Chlas of PSI (A.N. Glazer, Y.M. Gindt, C.F. Chan, K. Sauer, *Photosynth. Res.* 40 (1994) 167–173) with untreated control cells. The NEM-treated cells were indistinguishable from the control cells with regard to PSII-dependent oxygen evolution, reduction of post-PSII oxidants, and osmotically induced volume changes, but differed in the following properties: (i) they could not photoreduce post-PSI electron acceptors; (ii) they diverted more PBS excitation to PSII; (iii) the rise of Chla fluorescence upon light acclimation of darkened (state 2) cells was smaller; and (iv) the Chla fluorescence of light-acclimated (state 1) cells was insensitive to the cell suspension osmolality. These properties suggest that osmolality regulates the core-mediated excitation coupling between PBS and PSI, possibly by influencing mutual orientation and/or distance between core holochromes (ApcE, ApcD) and PSI holochromes. Thus, in hyper-osmotic suspension, PBS deliver more excitation to PSI (hence less to PSII); in hypo-osmotic cell suspension they deliver less excitation to PSI (hence more to PSII). © 2001 Elsevier Science B.V. All rights reserved.

## 1. Introduction

Cyanobacteria employ two membrane-intrinsic pigment complexes of chlorophyll *a* (Chla)/Car holochromes (photosystem (PS) I, PSII) and two associated photoreaction centers (reaction center (RC) I,

RCII) in order to perform plant-like oxygenic photosynthesis. There are ~45 Chlas per PSII [1], ~100 Chlas per PSI [2], and 1–5 PSI per PSII (depending on culturing conditions; reviewed in [3]). Intrinsic Chlas feed singlet electronic excitation to Chla dimers in RCI and RCII, where primary charge separation reactions take place. The main light harvester of cyanobacteria and red algae, however, is the phycobilisome (PBS), a large extrathylakoid organelle consisting primarily of phycobiliproteins ([4]; reviews [5–8]). The PBS of freshwater *Synechococcus* (spp. PCC 6301 and PCC 7942) is a semicylindrical structure having a two-cylinder core that consists of allophycocyanin (APC) trimers, and six peripheral rods that consist of phycocyanin (PC) hexamers. Linker

Abbreviations: APC, allophycocyanin; Chla, chlorophyll *a*; DCMU, 3-(3',4'-dichlorophenyl)-1,1'-dimethylurea; HEPES, *N*-2-(2-hydroxyethyl)-*N'*-ethanesulfonic acid; NEM, *N*-ethylmaleimide; PBS, phycobilisome; PBQ, phenyl-*p*-benzoquinone; PC, phycocyanin; PS, photosystem; RC, reaction center

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polypeptides join phycobiliprotein multimers in the rods and in the core, and two linker holochromes (ApcE, or L<sub>CM</sub>) anchor the core cylinders to the stroma side of PSII.

The major part of the PBS excitation is transferred to PSII along the route PC → APC → (ApcF, ApcD) → ApcE → PSII with high efficiency [9–11]. PBS also donate a minor part of their excitation to PSI Chlas via two, possibly co-existing, routes. One visualizes the excitation transfer to be mediated by core pigments, along the route PC → APC → (ApcF, ApcD) → |ApcE| → PSI [10,11]; the other visualizes direct PC → PSI transfers [12–14]. At physiological temperature, virtually all Chla fluorescence originates from Chlas of PSII. Some PSI fluorescence has been detected, however, in decay associated fluorescence spectra [15]. PSI fluorescence is readily detectable at cryogenic temperatures [16].

The fractions of PBS excitation that are apportioned between PSII and PSI, hence the Chla fluorescence intensity at room temperature, depend on the light history of cyanobacterial cells (reviews [17–21]). PBS-sensitized Chla fluorescence is more intense in light-acclimated cells, when the electron transport intermediates between RCII and RCI are oxidized (state 1) than in dark-acclimated cells, when the intersystem intermediates are reduced (state 2). However, this so-called state 2 to state 1 fluorescence transition occurs only when the cell suspension is hypo-osmotic. In hyper-osmotic suspension, Chla fluorescence does not rise upon light acclimation of the cells. Moreover, hyper-osmotic shocks depress the Chla fluorescence of light-acclimated cells to the level of dark-acclimated cells [22,23]. After treating the cyanobacteria with *N*-ethylmaleimide (NEM), the Chla fluorescence becomes osmotically insensitive [24].

Glazer et al. [14] reported that the treatment of *Synechococcus* sp. PCC 6301 cells with NEM blocks electronic excitation transfers between PBS and PSI. NEM derivatizes –SH groups, and in this case it modified two PBS proteins in particular, the rod-associated CpcD linker polypeptide of the distal PC disk, and the ferredoxin-NADP<sup>+</sup> oxidoreductase, which is located in the proximal PC disk [25]. The treatment with NEM had no effect on photoinduced electron transport from water to RCI (through RCII), but it caused more PBS excitation to be di-

verted to PSII, resulting in stronger Chla fluorescence.

In the present work we investigated the mechanism of osmotic effects on Chla fluorescence in cyanobacteria by comparing NEM-treated *Synechococcus* cells with untreated control cells. We will show that Chla fluorescence is osmotically sensitive only when it is excited by energy transfer from PBS, and not when it is excited directly. Also, we shall show that, although NEM-treated cells are capable of osmotic volume adjustments (indistinguishably from control cells), their Chla fluorescence is osmotically inert. To account for these phenomena, we propose an osmotic regulation of exciton exchanges between donor holochromes (ApcD, ApcF, ApcE) of PBS cores, and Chla holochromes of PSI of thylakoid membranes.

## 2. Materials and methods

### 2.1. Cell cultures and preparations

Cyanobacteria (*Synechococcus* sp. PCC 7942, *Synechocystis* sp. PCC 6803, *Nostoc* sp. PCC 7413) were cultured in BG11 [26], that contained additionally 20 mM HEPES NaOH, pH 7.5 (basal medium). The cultures were provided with white fluorescent light (100 μE m<sup>-2</sup> s<sup>-1</sup>), and 5% v/v CO<sub>2</sub> in air. Cells were harvested after 4 days (exponential phase), and they were suspended in basal medium at 20 μg Chla ml<sup>-1</sup>. Except where indicated differently, all assays were conducted with cells suspended in basal medium, in the presence of 20 μM DCMU in order to keep the RCII closed.

Cells were reacted with 0.1 mM NEM for 40 min, as in [14]. Unreacted NEM was removed with two washes with basal medium.

### 2.2. Measurements of Chla fluorescence

We measured Chla fluorescence of cell suspensions with two different fluorimeters: a modulated excitation–emission fluorometer (PAM; Heinz Walz, Effeltrich, Germany), with fixed excitation and emission bands, but capable of good time resolution, and a continuous excitation–emission fluorometer (Model MPF-3L, Perkin-Elmer, Norwalk, USA), affording

excitation and emission band selection, but of poor time resolution.

The PAM fluorometer provided periodic excitation pulses (650 nm;  $\Delta\lambda = 25$  nm; 1.6 kHz; 1  $\mu$ s flashes; 70 nmoles  $m^{-2} s^{-1}$ ; hereafter called *measuring light*), and detected only synchronous Chla fluorescence signals at  $\lambda > 690$  nm. The measuring light was absorbed both by Chla and by PBS chromophores, so it excited Chla both directly and indirectly. Actinic light was provided either as Xe flash (50 ms; 8 mE  $m^{-2} s^{-1}$ ), or as continuous illumination from an incandescent source (1.3 mE  $m^{-2} s^{-1}$ ; KL1500 Electronic; Schott Glasswerke, Germany). On the way to the sample, the latter passed through a heat reflecting mirror (Oriol No. 5740; steady transmittance from 440 to 780 nm), and a 620 nm ( $\Delta\lambda = 12$  nm) interference filter. The fluorescence signals of the aperiodic actinic excitations were not detected by the PAM fluorometer.

The MPF-3L fluorometer provided continuous excitation at 435 nm ( $\Delta\lambda = 4$  nm), or at 620 nm ( $\Delta\lambda = 4$  nm), and detected continuous Chla fluorescence at 685 nm ( $\Delta\lambda = 5$  nm). Actinic light was provided to the sample normally to the excitation–emission plane by an incandescent source (QH-150, PBL International, Newburyport, MA, USA). Its output passed either through broadband blue filter (Corning CS 5-58; 28  $\mu$ E  $m^{-2} s^{-1}$ ), or through orange interference filter (620 nm,  $\Delta\lambda = 12$  nm, 30  $\mu$ E  $m^{-2} s^{-1}$ ).

### 2.3. Suspension media of defined osmolality

Suspension media of defined osmolality consisted of sorbitol solutions in basal medium. Cyanobacteria are impermeant to sorbitol [22,23]. Osmolalities were measured cryoscopically as in [27]. Cell suspensions will be designated as *hypo-osmotic*, or *hyperosmotic* by reference to the turgor threshold of the cells (maximal external osmolality at which cells are capable of maintaining turgor; 0.22 Osm  $kg^{-1}$  for *Synechococcus* sp. PCC 7942 [27]). The basal medium (0.080 Osm  $kg^{-1}$ ) was, therefore, hypo-osmotic. Osmolalities of cell suspensions and suspension media were considered equal.

### 2.4. Determination of packed cell volumes

Twice washed cells with basal medium were resus-

pending in defined osmolality media at 3–4 mg Chla  $ml^{-1}$ . After 10 min, aliquots were transferred to capillaries, and centrifuged in a Hawksley microhematocrit centrifuge (Gallenkamp, London, UK) at  $12\,000 \times g$  for 15 min [27].

### 2.5. Photosynthetic oxygen evolution

Photosynthetic oxygen evolution was measured with an oxygen concentration electrode (Hansatech Instruments, Norfolk, UK). The reaction mixture was made in basal medium and contained cells (5  $\mu$ g Chla  $ml^{-1}$ ), and phenyl-*p*-benzoquinone (PBQ; 0.3 mM) or methyl viologen (MV; 0.1 mM) as electron acceptors. PBQ accepts electrons from RCII [28]. MV accepts electrons from the post-RCI iron–sulfur center  $F_x$  [29]. The samples were incubated in darkness (3 min) prior to the assay. Actinic light (4.0 mE  $m^{-2} s^{-1}$ ; Hansatech LS2 Illuminator), passed through an orange glass filter (Corning CS 3-69; transmission  $\geq 510$  nm). Neutral density filters (Corion, Holliston, MA, USA) were used to grade the actinic light intensity.

### 2.6. Other methods

The Chla concentration was determined in *N,N*-dimethylformamide extracts of cell pellets [30]. Light intensities were measured with a Li-Cor Quantum Radiometer (Li-Cor, Lincoln, NE, USA).

## 3. Results

Fig. 1 displays kinetic traces of Chla fluorescence during light acclimation of three cyanobacteria, *Synechococcus* sp. PCC 7942 (Fig. 1A), *Synechocystis* sp. PCC 6803 (Fig. 1B), and *Nostoc* sp. PCC 7413 (Fig. 1C). We compared NEM-treated cells, and untreated control cells, suspended either in hypo-osmotic medium (0.08 Osm  $kg^{-1}$ ; traces denoted as I), or in hyperosmotic medium (0.34 Osm  $kg^{-1}$ ; traces denoted as II). In the hypo-osmotic suspension of control *Synechococcus* cells (Fig. 1A), the periodic *measuring light* (650 nm) excited detectable Chla fluorescence initially to level  $F_2$ . Subsequent illumination with continuous actinic light (620 nm) raised fluorescence to level  $F_1$ . The  $F_1$  level contained no

fluorescence contribution from actinic light excitation. The actinic illumination, however, acclimated the cells (state 2 to state 1 transition) and as a result it increased the fraction of PBS excitation (generated

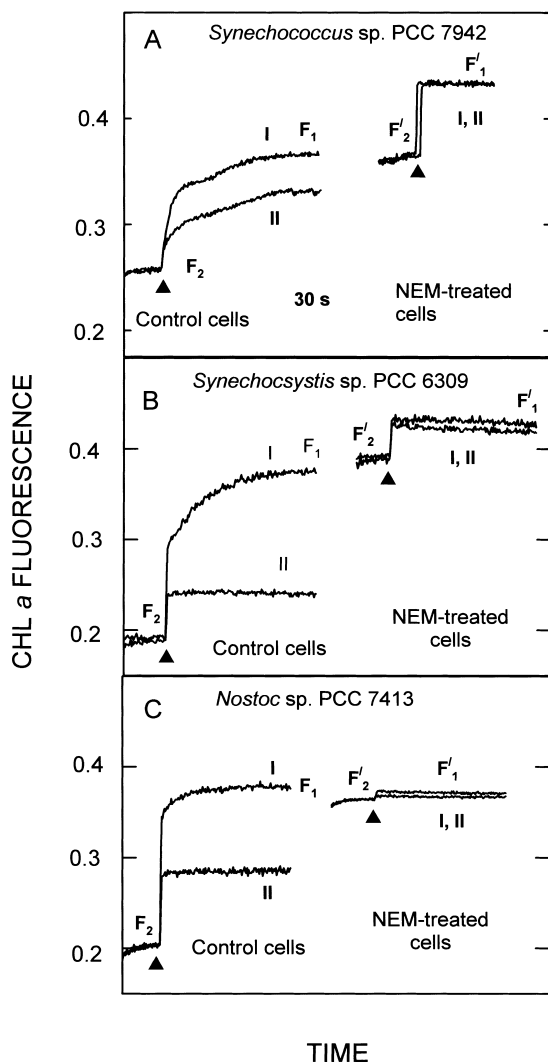


Fig. 1. Light-induced Chla fluorescence kinetics in three cyanobacteria, *Synechococcus* sp. PCC 7942 (A), *Synechocystis* sp. PCC 6803 (B), and *Nostoc* sp. PCC 7413 (C). Control cells and NEM-treated cells were suspended either in basal medium medium (BG11 plus 0.02 HEPES NaOH, pH 7.5; 0.08 Osm kg<sup>-1</sup>; traces denoted as I), or in basal medium containing also 0.24 M sorbitol (0.34 Osm kg<sup>-1</sup>; traces II). DCMU, 20 μM, was present in all samples. The sample suspensions were first acclimated to darkness (4 min), then illuminated with measuring light (650 nm, 30 s) to excite Chla fluorescence ( $F_2$ ; state 2), and lastly with measuring light and actinic light (620 nm, 120 s) in order to elicit the light-acclimative rise of Chla fluorescence to level  $F_1$  (state 1). ▲ indicates onset of actinic light illumination.

Table 1

Photoinduced electron transport activities of control and NEM-treated *Synechococcus* sp. PCC 7942 cells

Electron transport	Rate of evolution or uptake of oxygen (μmol O <sub>2</sub> (mg Chla) <sup>-1</sup> h <sup>-1</sup> )	
	Control cells	NEM-treated cells
H <sub>2</sub> O → PBQ	421	400
H <sub>2</sub> O → MV	121	ND <sup>a</sup>
H <sub>2</sub> O → CO <sub>2</sub>	152	ND

Control (untreated) cells, and cells treated with 0.1 M NEM for 40 min were washed twice with basal medium and resuspended in the same at 5 μg Chla ml<sup>-1</sup>. Oxygen evolution was measured at 27°C, at saturating illumination. Electron acceptors were PBQ (0.3 mM), or MV (0.1 M), or CO<sub>2</sub>.

<sup>a</sup>Not detectable.

by measuring light) that was apportioned to Chlas of PSII. In the hyperosmotic cell suspension, the  $F_2$  level was the same as in the hypo-osmotic cell suspension, but the  $F_1$  level after light acclimation was lower. The difference  $\Delta F F_2^{-1} = (F_1 - F_2) F_2^{-1}$  is known to relate linearly to osmotically induced cell volume changes [31].

In the case of NEM-treated *Synechococcus*, the Chla fluorescence excited by measuring light ( $F'_2$ ) was higher than in control cells (Fig. 1A). Actinic illumination raised Chla fluorescence to a higher level ( $F'_1$ ), but in contrast to the control cells, the  $F'_1$  fluorescence of the NEM-treated cells was the same both in the hypo-osmotic and in the hyperosmotic suspension.

Qualitatively similar results were obtained with another unicellular cyanobacterium, *Synechocystis* sp. PCC 6803 (Fig. 1B), and a filamentous cyanobacterium *Nostoc* sp. PCC 7413 (Fig. 1C). In all three genera, the Chla fluorescence of light-acclimated cells was lower at higher osmolality, but after treatment with NEM it became independent of osmolality. The results suggest that these phenomena are common in many cyanobacteria, and perhaps in all cyanobacteria (see also [22–24]).

In spite of the omnipresence of the thiol group, remarkable activities survive in *Synechococcus* after derivatization with NEM. Thus, phycobiliproteins and Chla proteins were not modified to an extent that it would influence their fluorescence spectra, and the reduction of P700<sup>+</sup> radicals by intersystem intermediates was not prevented [14]. In Table 1,

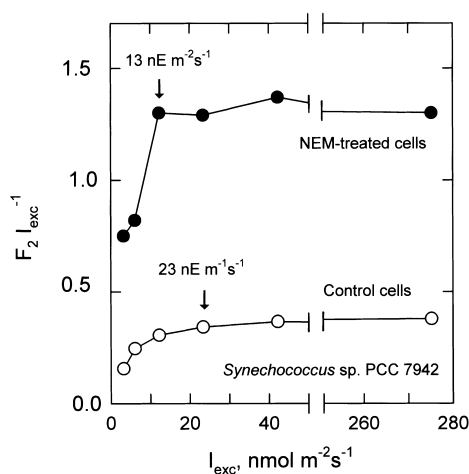


Fig. 2. The relative quantum yield ( $F_2 I_{exc}^{-1}$ ) of Chla fluorescence of dark-acclimated *Synechococcus* sp. PCC 7942 cells as a function of measuring light intensity ( $I_{exc}$ ).  $\circ$ , control cells;  $\bullet$ , NEM-treated cells. Samples contained  $18 \mu\text{g Chla ml}^{-1}$  and  $20 \mu\text{M DCMU}$ .

we compare control and NEM-treated cells for photoinduced electron transport across PSII ( $\text{H}_2\text{O} \rightarrow \text{PSII} \rightarrow \text{PBQ}$ ), and across both PSII and PSI ( $\text{H}_2\text{O} \rightarrow \text{PSII} \rightarrow \text{PSI} \rightarrow (\text{MV}, \text{CO}_2)$ ). Activities were measured either as photoinduced oxygen evolution (with PBQ, or  $\text{CO}_2$  as electron acceptors), or as oxygen uptake (with MV as electron acceptor), at saturating illumination. Results show that NEM-treated cells could photoreduce PBQ as actively as control cells, but they were unable to photoreduce MV, or  $\text{CO}_2$ . The inability to photoreduce MV indicates inhibition of electron transport at, or before, the post-PSI electron transport intermediate  $F_x$  [29].

It is plausible to assume that a block in the  $\text{PBS} \rightarrow \text{PSI}$  excitation transfer cascade will divert surplus PBS excitation to PSII. This event will manifest most clearly in the reduction rate of  $\text{Q}_A$  by RCII under conditions of limiting exciton supply to PSII. We investigated this possibility in *Synechococcus* sp. PCC 7942 by plotting  $F_2 I_{exc}^{-1}$  (relative quantum yield of  $\text{Q}_A$  photoreduction) against the measuring light intensity ( $I_{exc}$ ). According to Fig. 2,  $F_2 I_{exc}^{-1}$  rose to a higher level with  $I_{exc}$  in both kinds of cells, but the rise was faster and more extensive in NEM-treated cells. The faster rise of  $F_2 I_{exc}^{-1}$  is consistent with increased exciton supply to the RCII of NEM-treated cells, while the higher end fluorescence level (also evident in Fig. 1) indicates a higher quantum yield

of Chla fluorescence that may be due to chemical modifications of thylakoid membrane holochromes.

If the PBS of NEM-treated cells deliver more excitation to PSII than the PBS of control cells, then at limiting excitation supply photoinduced electron transport across RCII is expected to be faster in the former cells, while at non-limiting excitation supply it is expected to be the same in both kinds of cells. Both expectations were indeed borne out by experiments, in which DCMU-free cell suspensions were used. In the experiment shown in Fig. 3, the rate of oxygen evolution at limiting actinic light and with PBQ as electron acceptor rose faster in the NEM-treated cells than in control cells. In Fig. 4, Chla fluorescence of dark-acclimated cells was first excited to level  $F_2$  with weak measuring light, and then the cells were illuminated with a saturating Xe flash ( $8 \text{ mE m}^{-2} \text{ s}^{-1}$ ; 50 ms), that photoreduced  $\text{Q}_A$ ,  $\text{Q}_B$ , and PQ quantitatively and obliterated photochemical quenching completely. The flash caused approximately equally fast fluorescence rises ( $t_{1/2} \approx 2\text{--}3$  ms) in control and NEM-treated cells, indicating approximately equal rates of  $\text{Q}_A^-$  accumulation. Characteristically, however, the fluorescence rise amplitude was 25% larger in NEM-treated cells, evidencing a higher quantum yield of Chla fluorescence.

As shown in Fig. 1, the PBS-sensitized Chla fluorescence of light-acclimated cyanobacteria was suppressed at hyperosmotic conditions. In Fig. 5 we

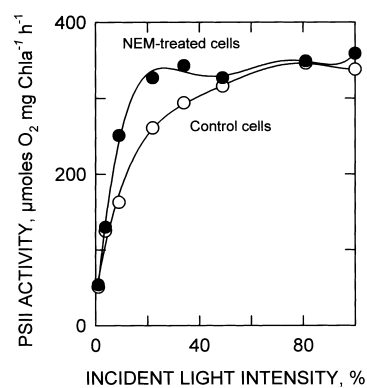


Fig. 3. Light curves of the PSII activity of control ( $\circ$ ), and NEM-treated ( $\bullet$ ) *Synechococcus* sp. PCC 7942 cells. The PSII activity was quantitated in terms of the rate of light-induced oxygen evolution, with PBQ as electron acceptor, at  $27^\circ\text{C}$ . Samples contained  $5 \mu\text{g Chla ml}^{-1}$  and  $0.3 \text{ mM PBQ}$ . Full power (100%) incident actinic light was  $4.0 \text{ mmol m}^{-2} \text{ s}^{-1}$ . The actinic light was attenuated with neutral density filters.

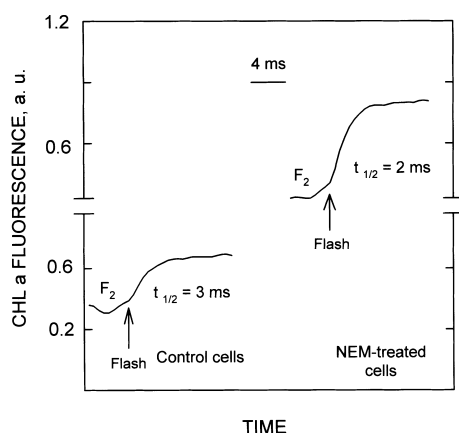


Fig. 4. Flash-induced kinetics of Chla fluorescence in suspensions of control and NEM-treated *Synechococcus* sp. PCC 7942 cells. DCMU-free samples were first acclimated to darkness (4 min), then they were excited with measuring light to yield the  $F_2$ ,  $F'_2$  fluorescence levels, and finally they were illuminated with a saturating light flash (50 ms;  $8 \text{ mE m}^{-2} \text{ s}^{-1}$ ) to elicit the indicated rises of the fluorescence signal. Displayed traces are averages of 10 recordings.

asked if this is also true for directly excited Chla fluorescence. The fluorometer we used in this experiment (Perkin-Elmer MPF3A) provided continuous fluorescence excitation and continuous fluorescence detection, and allowed selection of excitation and emission bands. DCMU,  $20 \mu\text{M}$ , was present in the samples to prevent the photochemical reduction of intersystem intermediates by RCII, while their photochemical oxidation by RCI was allowed.

Illumination of *Synechococcus* cells with 620 nm light (absorbed by PBS) excited Chla fluorescence (detected at 685 nm) which rose to a higher level ( $F_1$ ) as a result of light acclimation in hypo-osmotic suspension but not in hyperosmotic cell suspension (Fig. 5A). In contrast, the Chla fluorescence of cells excited and acclimated to  $F_1$  with broad-band blue light (380–480 nm, mainly absorbed by Chla) remained at the same level, regardless of the cell suspension osmolality (Fig. 5B). These results indicate that cell suspension osmolality regulates the PBS-sensitized Chla fluorescence, but not the directly excited Chla fluorescence.

The fluorescence magnitude  $\Delta F F_2^{-1}$  (see Fig. 1 for notation) is a measure of osmotically induced cell volume changes in cyanobacteria [22,23]. Fig. 6 shows that in control cells,  $\Delta F F_2^{-1}$  is a function of suspension osmolality, while the corresponding mag-

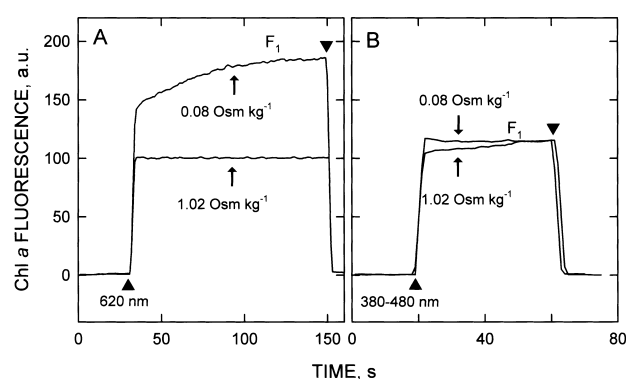


Fig. 5. Dependence of the light-induced rise of Chla fluorescence of *Synechococcus* sp. PCC 7942 on the osmolality of the cell suspension and the spectral quality of exciting light. (A) Indirectly excited Chla fluorescence (via PBS,  $\lambda = 620 \text{ nm}$ ,  $\Delta\lambda = 12 \text{ nm}$ ,  $30 \mu\text{E m}^{-2} \text{ s}^{-1}$ ). (B) Directly excited Chla fluorescence (380–480 nm,  $\lambda_{\text{max}} = 410 \text{ nm}$ ,  $28 \mu\text{E m}^{-2} \text{ s}^{-1}$ ). Chla fluorescence was monitored at 685 nm,  $\Delta\lambda = 5 \text{ nm}$ . Cells were suspended in basal medium ( $0.08 \text{ Osm kg}^{-1}$ ; hypo-osmotic suspension), or in basal medium plus 0.8 M sorbitol ( $1.02 \text{ Osm kg}^{-1}$ ; hyperosmotic suspension). Measurements were performed with a continuous excitation/detection fluorometer (Perkin-Elmer Model MPF-3A).

nitude  $\Delta F' (F'_2)^{-1}$  of NEM-treated cells is independent of osmolality. This may have two explanations: either the NEM-treated cells are incapable of osmotic volume adjustment, or in these cells the PBS-sensitized Chla fluorescence is incapable of reporting osmotic volume changes.

To resolve the dilemma, we measured packed vol-

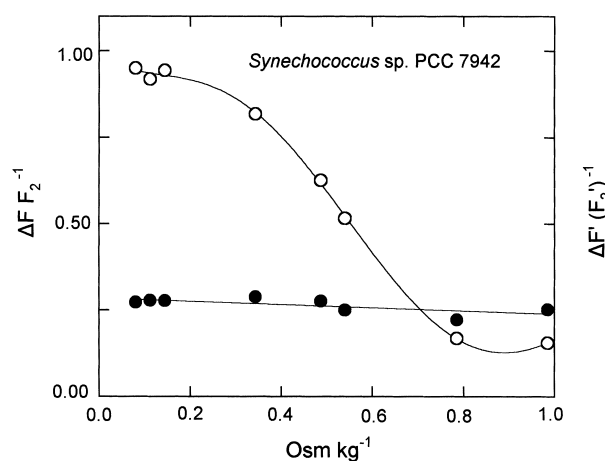


Fig. 6. Light-induced rises of Chla fluorescence ( $\Delta F F_2^{-1}$ ) in *Synechococcus* sp. PCC 7942 cell suspensions as a function of suspension osmolality.  $\circ$ , control cells ( $\Delta F F_2^{-1}$ );  $\bullet$ , NEM-treated cells ( $\Delta F' F_2'^{-1}$ ). Actinic light, 620 nm ( $\Delta\lambda = 12 \text{ nm}$ ).

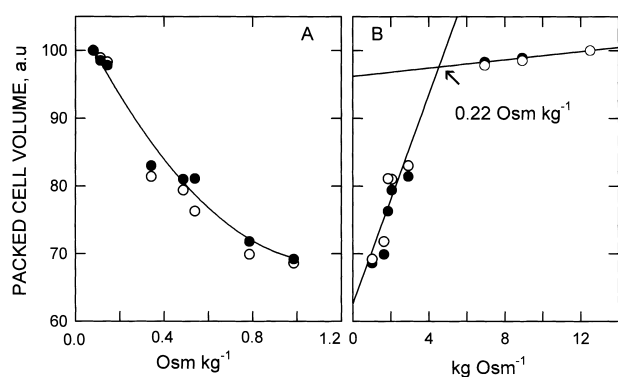


Fig. 7. Effect of cell suspension osmolality on packed cell volumes of *Synechococcus* sp. PCC 7942 cells. ○, control cells; ●, NEM-treated cells. (A) Packed cell volumes (relative) plotted against suspension osmolality. (B) Packed cell volumes (relative) are plotted against reciprocal suspension osmolality.

umes of control and NEM-treated cells as a function of suspension osmolality. The packed cell volume is the sum of osmotic cell spaces, and of non-osmotic contributions (cell walls, periplasm, membranes, solid inclusions, surface-bound water, and intercellular spaces [23]). Osmotically induced differentials in packed cell volumes represent, however, changes in the internal osmotic space. According to Fig. 7A, packed volumes of control cells and of NEM-treated cells exhibited exactly the same dependence on suspension osmolality. When packed cell volumes were plotted against reciprocal osmolality (Fig. 7B), all data points fell on two linear segments, whose intersection yielded the same turgor threshold ( $0.22 \text{ Osm kg}^{-1}$ ) for both kinds of cells. A similar turgor threshold value has been reported for *Synechococcus* sp. PCC 7942 in [22]. From the results shown in Figs. 6 and 7 we infer that NEM-treated cells are capable of osmotically induced volume changes, just as control cells. However, in the NEM-treated cells volume changes are not reported by corresponding changes in the PBS-excited Chla fluorescence.

#### 4. Discussion

In this research, we sought to advance our understanding about the mechanism with which the suspension osmolality regulates the quantum yield of Chla fluorescence of cyanobacterial cells. We shall base the discussion of our results on two experimen-

tally supported assumptions. First, we shall assume that treatment with NEM interrupts the excitation transfer from PBS to PSI, but not to PSII. According to Glazer et al. [14], light absorbed by PBS (590 nm) does not photo-oxidize P700 in NEM-treated *Synechococcus*, while light absorbed by Chla (680 nm) does. Second, we shall assume that when the PBS to PSI excitation transfer is blocked, surplus excitation is diverted to PSII. Indeed, in the weak excitation limit both the Chla fluorescence (Fig. 2) and the photochemical rate of RCII (Fig. 3) rise faster with increasing excitation intensity in the NEM-treated cells than in control cells.

Another consequence of the treatment with NEM was an increase of the quantum yield of Chla fluorescence. This was observed both at conditions of limiting excitation ( $F_2$  levels in Figs. 1 and 2), as well as at conditions of non-limiting excitation (Fig. 4). Glazer et al. [14] reported a  $\sim 32\%$  increase in the quantum yield of Chla fluorescence with 590 nm excitation, after treating cyanobacteria with NEM. This corroborated their hypotheses (a) of a block in PBS-to-PSI excitation transfers, and (b) of diversion of PSI-destined PBS excitation to PSII. However, they also reported a 12% increase in the directly excited Chla fluorescence (at 430 nm) in the NEM-treated cells. Since PBS are not involved, the increase in the directly excited Chla fluorescence could mean that, as a result of the NEM treatment, either some PSI excitation was diverted to the fluorescing Chlas of PSII, or the quantum yield of PSII fluorescence increased. We consider the first alternative unlikely, because of the distance between PSII and PSI holochromes and because the first excited level of PSI Chlas lies below that of PSII Chlas; and we consider the second alternative more likely, because of the omnipresence of thiol groups in amino acid side chains. Our results (Figs. 1, 2 and 4) are consistent with the second alternative.

It should be remarked, however, that although the treated cells wasted more Chla excitation as fluorescence, at saturating illumination they were as active in photoreducing post-PSII electron acceptors as the control cells (Table 1). Therefore, the derivatization of thiols with NEM did not materially influence PBS  $\rightarrow$  PSII  $\rightarrow$  RCII excitation transfers, nor the functionalities of the oxygen evolving complex, and of the photoinduced electron transport from water to RCI.

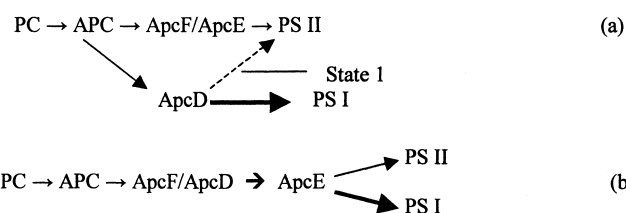
Cells respond to external osmotic pressure shifts by importing or exporting water passively, in order to equalize mechanical forces that are applied on plasma membranes. In hypo-osmotic suspensions of cyanobacteria and other walled cells, the external osmotic pressure is balanced both by the osmotic pressure of the cytoplasm, and the resistance of the cell wall to elastic area expansion. In a hyperosmotic suspension, the cell wall resistance vanishes, and water transport across the plasma membrane suffices to equalize the internal and external osmotic pressures (i.e., turgor also vanishes). In hyperosmotic suspension, therefore, cell volumes change only in response to the difference in osmotic pressure [23,24]. In these ways, changes in the cell suspension osmolality are communicated to the cytoplasm.

Macromolecules are surrounded by proximal aqueous phases of characteristically different thermodynamic properties from the distal (or bulk) aqueous phases (reviewed in [32]). In particular, ratios of solute molecules to water molecules in the two phases may differ. A shift in the chemical potential of distal phase solutes, as for example after osmotic perturbation, may exert different effects on the chemical potentials of water in the two aqueous phases, and this may have an impact on macromolecular conformation. Such conformational effects have been described for simple triadic systems (water, low molecular weight osmoticum, and macromolecule [32]), and may well form the physical basis of the osmotic regulation of PBS-sensitized Chla fluorescence in cyanobacteria.

According to Förster [33], the frequency of resonance electronic excitation transfers between dipoles depends on the overlap of donor emission and acceptor absorption, and on two geometric factors, donor–acceptor distance and mutual orientation. In the PBS/Chla system, the position of the emission bands of terminal core holochromes (ApcD, ApcE, ApcF) ensures good overlap with the absorption band of Chla [34,35], while the overlap between rod PC emission and Chla absorption is poor [36]. Thus, core-mediated excitation transfer PBS to Chla [10,11] is more likely, although rod-mediated excitation transfer may also occur to some extent [12–14]. Core-mediated excitation transfer to PSI requires the

PBS (or a subset of them) to bind to the PSII and the PSI complexes alternately. Mullineaux et al. [37] employed pigment photobleaching to demonstrate rapid diffusion of PBS on the surface of the thylakoid membrane, while the intramembrane PSII complexes did not diffuse.

Experiments with mutant cyanobacteria having defective PBS cores have indicated the following core-mediated pathways for PBS to PSII and to PSI excitation transfers in *Synechococcus* sp. PCC 7002 (a [10]), and *Synechocystis* sp. PCC 6803 (b [11]).



The essential difference is that in pathway (a) holochrome ApcD mediates excitation transfer from APC to PSI directly (but part of its excitation is transferred to PSII in light-acclimated cells), while in pathway (b) holochrome ApcE is the terminal mediator of excitation transfers to both photosystems. ApcD, in the latter case, was proposed to lie in a path of secondary importance.

As shown in this work, osmotically induced cell volume changes are reported by PBS-excited Chla fluorescence in control cyanobacterial cells, but not in NEM-treated cells (Figs. 6 and 7). These results are consistent with an osmotic regulation of excitation transfer at a site between PBS and PSI, which regulates indirectly also the supply of PBS excitation to PSII, and the quantum yield of Chla fluorescence at room temperature. Likely sites for osmotic regulation are indicated with bold arrows in pathways (a) and (b) above. We consider the mutual geometry of holochromes ApcF and ApcE less likely to be influenced by shifts in the chemical potential of proximal water, since the two proteins are interlocked in the B trimers of core cylinders. More likely sites are those between ApcE and PSI (or ApcD and PSI), since the interacting chromophores are located on different proteins, so their spatial relations may be subject to change after osmotic perturbation.



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