Randomization of the EF particles in thylakoid membranes of Synechocystis 6714 upon transition from state I to state II

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In the cyanobacterium *Synechocystis* 6714 we show that changes in light energy distribution are controlled by the redox state of the PQ pool and we report on the deep ultrastructural modifications caused by these changes. We conclude that State I-State II transitions correspond to an increased energy transfer between PS II and PS I through a randomization of the EF particles of the thylakoid membranes. This reorganization could involve a change in the interaction between PS II centers and the phycobilisomes.

Cyanobacteria *State I-State II transition* *Thylakoid chlorophyll fluorescence* *Freeze-fracture*

1. INTRODUCTION

Light harvesting in green algae and higher plants is mainly accomplished by intrinsic membrane protein complexes (LHC I and II), binding Chl *a* and Chl *b*, whereas it is accomplished by water-soluble phycobiliproteins organised in large extrinsic complexes at the thylakoid outer surface, the phycobilisomes, in red algae and cyanobacteria [1,2].

These differences have major implications on the overall organization of the thylakoid membranes: they are separated from each other in phycobilisome-containing organisms but are frequently stacked in LHC-containing organisms giving rise to two membrane domains, for instance the grana and stroma lamellae regions in higher plants [3].

In spite of these differences, a regulation of light energy distribution between the two photosystems, referred to as State I-State II transitions, exists in both types of photosynthetic organisms [4,5]. This is a very intriguing observation since the mechanism responsible for the regulation in higher plants and green algae involves a change in kinase activity, dependent on the redox state of the PQ pool, which causes the reversible migration of LHC II from the stacked membrane regions, enriched in PS II, to the unstacked membrane regions, enriched in PS I (review [6]). Whether the regulation observed in cyanobacteria and red algae involves a totally different mechanism remains controversial [7–9].

Using intact cells of the cyanobacteria *Synechocystis*, we used pretreatments giving rise either to PQ pool reduction (State I) or to PQ pool oxidation (State II) and compared the ultrastructural membrane organization in both states.

Abbreviations: PQ, plastoquinone; PS, photosystem; LHC, light-harvesting complex; Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_v, variable fluorescence; EF, exoplasmic fracture face

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2. MATERIALS AND METHODS

The wild-type strain of the cyanobacterium *Synechocystis* sp. strain PCC 6714 was obtained from Dr R.Y. Stanier [10]. The cells were grown on minimum medium under standard photosynthetic growth conditions [11]. The cells were collected in exponential phase at 1.2×10^8 cells per ml (about 12 μg Chl per ml).

Fluorescence experiments at room temperature and at 77 K were performed as in [12].

Anaerobic conditions were produced either by addition of 3 mM sodium dithionite under an argon atmosphere or by addition of glucose oxidase and glucose as in [13].

3. RESULTS AND DISCUSSION

As reported by others on phycobilisome-containing organisms [4,14], State I-State II transitions can be monitored by a preillumination at 440 and 620 nm, respectively. 440 nm light is mainly absorbed by Chl *a* and sensitizes preferentially PS I whereas 620 nm light is mainly absorbed by phycocyanin and sensitizes preferentially PS II. Both states reverse rapidly to an intermediate state in darkness or dim light. Therefore, we looked for conditions where State I and State II would be stable enough to elicit a comparative ultrastructural study of the thylakoid membranes in the two states. This was achieved by pretreatments which proved efficient with the green algae *Chlamydomonas reinhardtii* [13]: either production of an anaerobic state in the dark (PQ pool reduced) or pre-illumination in the presence of DCMU (PQ pool oxidized).

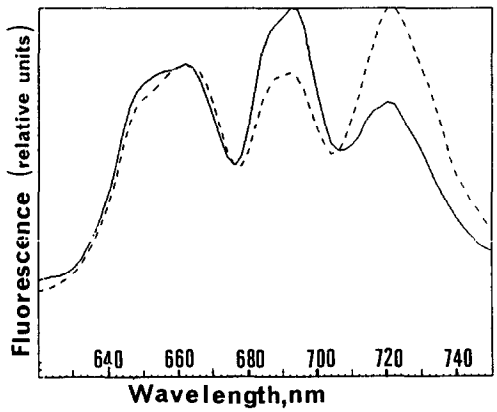

Fig.1. Fluorescence spectra at 77 K. Same conditions as in table 1. (——) Cells in State I, induced by preillumination in the presence of DCMU. (– – –) Cells in State II induced by 2 min in the anaerobic state in the dark. The three maxima correspond to phycocyanin (660 nm), PS II (695 nm) and PS I (720 nm).

As shown in fig.1, these pretreatments induced the classical changes in PS I and PS II

Table 1

Fluorescence characteristics of *Synechocystis* 6714 in States I and II

	Treatments	Room temperature		77 K F_{695}/F_{720}
		F_v	$t_{1/2}$ (ms)	
State II	preillumination at 620 nm	1.00	25.00	0.91
	anaerobic state	1.05	26.50	0.81
State I	preillumination at 440 nm	2.45	22.50	1.23
	DCMU + light	2.41	22.00	1.32

Room temperature: excitation wavelength, 440 nm ($\Delta\lambda$, 50 nm). 10^{-5} M DCMU was added to the cells (1 μg Chl/ml) in the dark, 15 s before recording fluorescence induction, except in the last condition where DCMU was already present. F_v, variable fluorescence; $t_{1/2}$, half-time of fluorescence rise. 77 K: excitation wavelength, 560 nm ($\Delta\lambda$, 15 nm). Emission $\Delta\lambda$, 3 nm. Cells (60 μg Chl/ml) were frozen immediately after treatment

fluorescence emission at 77 K [14]. The changes were of even larger amplitude than with a conventional State I-State II transition (table 1). Thus changes in light energy distribution between the two photosystems occurred in *Synechocystis* under the same conditions as in green algae in vivo, i.e. upon changes of the redox state of the PQ pool.

Studies of the fluorescence induction curves in the presence of DCMU at room temperature further confirmed that State I-State II transitions, obtained either by a change in the wavelength of the preilluminating light or by a DCMU + light to anaerobic state transition, were similar (table 1).

The fluorescence changes observed in *Synechocystis* were however different from those observed in the presence of 5 mM $MgCl_2$ with higher plant thylakoids [15,16]. In the latter case, one observes a large decrease in the half-time ($t_{1/2}$) of the fluorescence rise, without significant change in variable fluorescence, due to the movement of some LHC from PS II to PS I. In contrast, State I-State II transitions in *Synechocystis* produced little changes in $t_{1/2}$ but a large decrease in F_v. These effects were similar to those observed upon high salt to low salt transitions in higher plant thylakoids [17] and indicated that reduction of the PQ pool in *Synechocystis* produced an increased energy transfer from PS II to PS I rather than a net change in PS II antenna size.

In phycobilisome-containing organisms, freeze-fractured thylakoid membranes frequently exhibit rows of EF particles [3]. In *Synechocystis* most of the EF faces showed these characteristic rows in State I but these were only occasionally seen in State II (fig.2). We performed a quantitative estimation assuming that rows group more than 3 particles. This computation gave 40% of EF particles in rows in State I but less than 20% in State II. It is now well established that the EF particles in the thylakoid membranes from any type of algae and from higher plants contain the PS II reaction centers [18,19]. Therefore, the randomization of the EF particles in State II leads to an increase in the average distance between PS II centers (EF particles) but should conversely decrease the average distance between PS I and PS II centers. Such a change in organization supports an increased energy transfer from PS II to PS I in State II as compared to State I and is consistent with the fluorescence characterization shown in table 1.

A possible change in the interaction between PS II and the phycobilisomes should also be con-

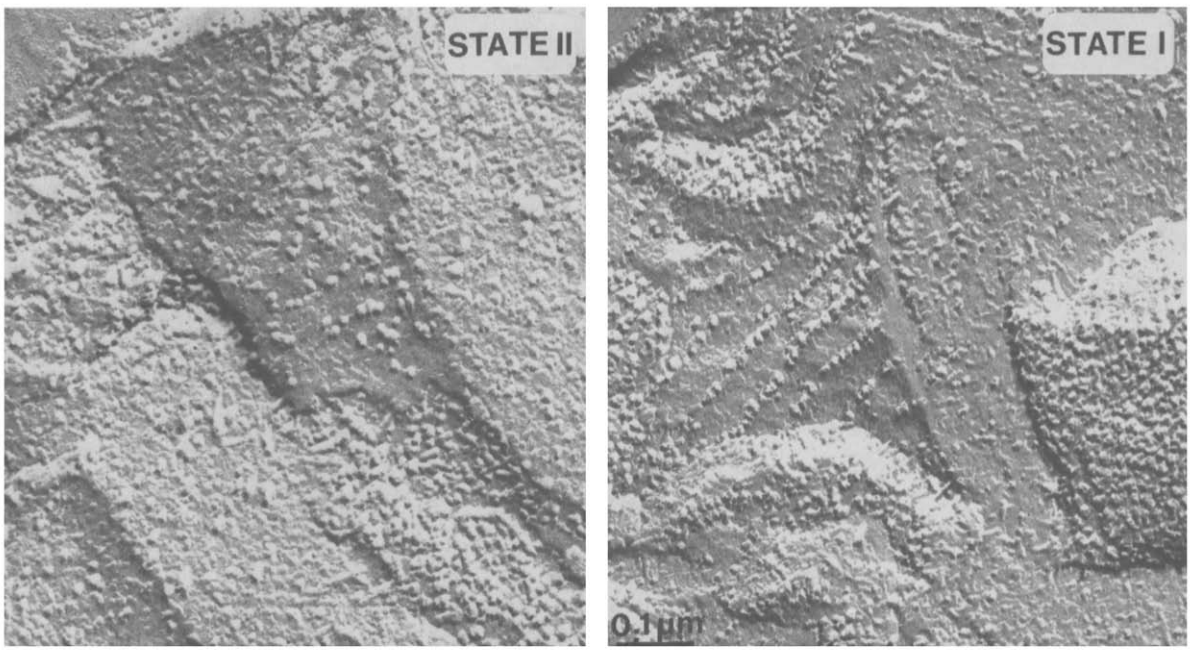

Fig.2. EF fracture faces of *Synechocystis* thylakoids, in State I and in State II.

sidered. Phycobilisomes are organized on the thylakoid outer surface in rows with the same interspacing arrangements as that of the EF particles [3]. The absence of EF rows in the III-C mutant of *C. caldarium* lacking the phycobilisomes [20] as well as their lower frequency in cells having less phycobilisomes on the thylakoid outer surface [3] are consistent with the view that EF particles are maintained in rows by their association with the overlying phycobilisomes. Beside the lower ability of EF particles to form rows in State II, we observed an increase in EF particle densities and sizes in State II as compared to State I (fig.3). In particular, an increased population of EF particles, 14.0 nm in diameter, was observed in State II. A similar size increase was observed in the III-C mutant of *C. caldarium* [20]. On the other hand, an increase in EF particle density was reported in cells having less phycobilisomes [21] as well as in the III-C mutant of *C. caldarium* [20]. Thus, the changes in organization, size and density of the EF particles from State I to State II could reflect the decreased ability of these particles to associate with the phycobilisomes. Comparison of excitation spectra of PS II fluorescence in both states should allow us to distinguish between this hypothesis and a mere randomization of PS II-phycobilisome complexes in State II.

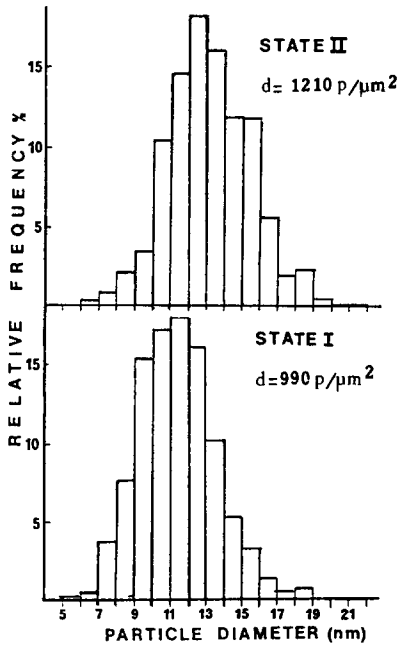

Fig.3. Histogram of the EF particle diameters and corresponding densities, in State I and State II.

4. CONCLUSION

The molecular mechanism for State I-State II transitions in cyanobacteria is still unknown. Our results are consistent with a dependence of these transitions on the redox state of the PQ pool. As is the case in higher plants and green algae, these redox changes might regulate the activity of a kinase. Such a change in phosphorylation of a phycobilisome linker and of a subunit of the PS II reaction center has indeed been observed by Allen et al. [9] on a *Synechococcus* strain. The increased negative charges produced by this phosphorylation could induce electrostatic repulsion between the phycobilisomes and PS II reaction centers as stated by Allen et al. [9] as well as between adjacent PS II reaction centers. Both phenomena can account for the randomization of the EF particles that we have observed in State II in *Synechocystis*.

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