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Regulation mechanism of excitation energy transfer in phycobilisome-thylakoid membrane complexes

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Abstract

Regulation mechanism of excitation energy transfer between phycobilisomes (PBS) and the photosynthetic reaction centres was studied by the state transition techniques in PBS-thylakoid membrane complexes. DCMU, betaine, and N-ethylmaleimide were applied to search for the details of energy transfer properties based on the steady fluorescence measurement and individual deconvolution spectra at state 2 or state 1. The closure of photosystem (PS) 2 did not influence on fluorescence yields of PS1, *i.e.*, energy could not spill to PS1 from PS2. When the energy transfer pathway from PBS to PS1 was disturbed, the relative fluorescence yield of PS2 was almost the same as that of PS2 in complexes without treatment. If PBSs were fixed by betaine, the state transition process was restrained. Hence PBS may detach from PS2 and become associated to PS1 at state 2. Our results contradict the proposed "spill-over" or "PBS detachment" models and support the mobile "PBS model".

Additional key words: absorption spectra; allophycocyanin; betaine; DCMU; fluorescence spectra; models of energy transfer; N-ethylmaleimide; photosystems 1 and 2; state transition.

Introduction

In cyanobacteria, red algae, and higher plants, the amount of excitation energy absorbed by antenna pigments and directed toward photosystem (PS) 2 and PS1 is regulated by a mechanism that maximises the efficiency of utilisation of photons (Bonaventura *et al.* 1969, Bennett *et al.* 1980). In green plants, light-induced state transition involves the redistribution of light-harvesting complex (LHC) 2 between PS1 and PS2. This occurs as a result of protein phosphorylation catalysed by a membrane bound kinase (Allen *et al.* 1981). However, this model, without modification, could not be applied to the cyanobacteria and red algae because the Chl *a* antenna of PS2 is relatively small (Glazer *et al.* 1984). The major LHC for PS2 in cyanobacteria and red algae is the phycobilisome (PBS), which is perpendicularly attached to the surface of thylakoid membrane. Therefore, the mechanism of state transition of the organisms containing PBSs differs markedly from that of higher plants (Fork *et al.* 1983, Biggins and Bruce 1989). Three main models have been proposed for the state transition in PBS-containing organism (Ley and Butler 1980, Biggins *et al.* 1984, Allen and Holmes 1986, Mullineaux and Allen 1988). In these models, state 1 is characterised by a strong energetic coupling between the PBS and PS2, while the major differences are in completion of the state 2. In the "spill-over" model, the excitation energy is transferred directly from PS2 to PS1. In order to increase this rate, PS2 and PS1 need a closer association of the two photosystems. In the "mobile phycobilisome" model, PBS could detach from PS2 and move to PS1 at state 2. The "PBS detachment" model is an improved "spill-over" model. Some other models even proposed that PBS might not play an active role in state transition; this is based on the study of a PBS-less cyanobacterium mutant (Bruce *et al.* 1989). The organisation of photosystems was changed from an ordered pattern in state 1 to a relatively random pattern in state 2 (Olive *et al.* 1986, Stowell *et al.* 1997). Obviously, state transition relates to many recent controversial problems. For instance, how do PBSs associate with the two photosystems? Could excitation energy be transferred directly from PBS to PS1? Are PBSs mobile or not on the thylakoid membrane? Is the connection between PBS and photosystems alternated during the state transition?

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Abbreviations: APC, allophycocyanin; Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; NEM, N-ethylmaleimide; PBS, phycobilisome; PS, photosystem.
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According to literature, energy transfer from PBS to PS1 may be disturbed by NEM while that from PBS to PS2 is not (Glazer *et al.* 1994). Betaine fixes PBS firmly on thylakoid membrane (Zheng *et al.* 1995). DCMU may cause closure of PS2 and therefore it has widely been used for investigations of state transition (Mullineaux and Allen 1986). In this work, the PBS-thylakoid membrane complexes were separated, treated with DCMU, betaine, and NEM, and subjected to state transition.

Materials and methods

Preparation of PBS thylakoid membrane complex: *Spirulina platensis*, a cyanobacterium, was grown in 10 000 cm^3 batch cultures at room temperature, bubbled with air, and irradiated with 40 W fluorescent lamps. The cells were harvested after 2 weeks. 5 g fresh cells of *S. platensis* was suspended in an SPC buffer (0.5 M sucrose, 0.5 M KH_2PO_4, 0.3 M sodium citrate, pH 7.0) and stirred with a magnetic stirrer at room temperature for 15 min, then centrifuged at 3 000×*g* for 15 min. The sediment was collected and ultrasonically broken in the SPC buffer. Afterwards, the preparation was centrifuged at 183 rps in a *Ti-70 Beckman* rotor for 1 h. The supernatant was subjected to further centrifugation at 300 rps in the same rotor for 1 h. The sediment was the PBS-thylakoid membrane complex. Separated PBS-thylakoid membrane complexes were suspended in the SPC buffer (containing 0.1 M NEM) for 40 min. At the end of incubation period, the complex was washed twice with 500 mM potassium phosphate buffer and immediately used for spectroscopic analysis. The complexes were diluted to a Chl *a* absorbance of about 0.4. DCMU was added to a final concentration of 20 μM. Betaine was added to a final concentration of 50 mM. After a 30-min incubation, betaine-treated complex was washed with 500 mM potassium phosphate buffer and immediately analysed spectroscopically.

Spectroscopy and deconvolution: Absorption spectra were measured with a *UV-2001 Ultra-Vis* spectrophotometer (*Hitachi*, Japan). 77 K fluorescence emission spectra were determined on a *F4500* spectrofluorimeter (*Hitachi*, Japan). By using non-linear optimisation approach in a computer, fluorescence spectra were separated into components that were imitated with Gaussians, except for the longest wavelength one (Gaussian/ Lorentzian mixture). Two restriction conditions were used for deconvolution: relative error less than 3 %, and the number of deconvolution components as small as possible.

State transition: Samples were incubated at room temperature for 5 min either in dark or under blue radiation (435 nm, 430 μmol m^{-2} s^{-1}) (Zhang *et al.* 1997). These conditions usually lead to adaptation to state 1 and state 2, respectively. The state transition was accomplished in 0.3 M potassium phosphate buffers in order to minimise the influence of sodium citrate, sucrose, and high concentration of potassium phosphate.

Results and discussion

Absorption spectra of PBS-thylakoid membrane complexes, untreated or treated with DCMU, betaine, or NEM (Fig. 1) showed five partially resolved sub-bands that may be attributed to Chl *a* (418, 436, and 678 nm), carotenoids (490 nm), and phycobilisomes (624 nm). All four absorption spectra almost overlapped within the experimental error range. Thus NEM, betaine, or DCMU could alter neither the organisation of Chl *a* complexes in photosystems nor that of phycobiliproteins.

Deconvolution of fluorescence spectra of PBS-thylakoid membrane complex in state 2 at excitation wavelength of 580 nm: Fluorescence intensity was normalised before deconvolution. The peak wavelength for each component remained constant, while the area changed. The area for each band and its ratio to the total area can be considered as the relative fluorescence yield. Formerly we found that the PBS-thylakoid membrane complex may be applied to research on state transition (Zhang *et al.* 1997). Because the main difference among the three models of state transition is how the transition to state 2 is accomplished, properties of the steady fluorescence spectra at state 2 are most concerned. Fig. 2 shows the deconvoluted fluorescence spectra of the untreated PBS thylakoid membrane complex (*A*) and those treated with DCMU (*B*), betaine(*C*), and NEM (*D*) at state 2. The 647 nm component should originate from phycocyanins and the 660 nm one from allophycocyanins. The 683, 697, and 757 nm fluorescence emission bands may originate from PS2 core complexes while the component at 720 nm must originate from PS1 core complexes (Salehian and Bruce 1992). The relative fluorescence yield of individual components at state 1 is also listed (Table 1) for comparison with the results at state 2. As for DCMU-treated complexes, the relative fluorescence yield of PBS increases while that of PS2 decreases and that of PS1 remains almost constant. It implies that the excitation energy is only redistributed between PBS and PS2.

As for betaine-treated complexes, the relative fluorescence yields of PBS, PS1, and PS2 are almost the same as those of complexes without treatment. As for NEM-treated complexes, the relative fluorescence yield of PS1 decreases greatly compared with that without treatment, which means that only a part of excitation energy can be transferred to PS1 from PBS. After treatment with NEM, the relative yield of PBS increases because energy transfer process from PBS to PS1 is disturbed. Afterwards, it leads to transfer of more excitation energy to PS2. Therefore, state 1 may be characterised by a strong energy coupling between the PBS and PS2.

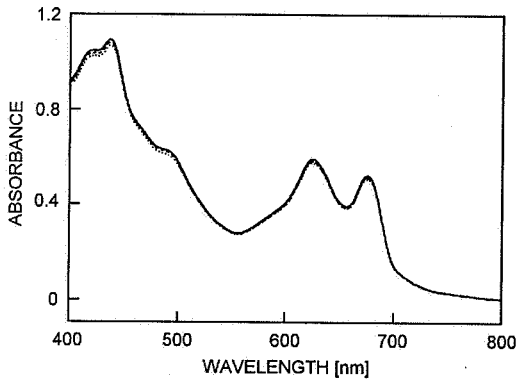

Fig. 1. Absorption spectrum of untreated phycobilisome-thylakoid membrane complexes (•••), and complexes treated with DCMU (- - -), betaine (• - • - •), and NEM (——).

Influence of DCMU on PBS-thylakoid membrane complexes at state 2: In the presence of DCMU, PS2 reaction centres become closed and correspond to the state 2. In Table 1 and Fig. 2*B* the relative fluorescence yield of PBS increases while that of PS1 remains almost constant. It implies that the excitation energy can only be transferred between PBS and PS2. On the other hand, the closure of PS2 reaction centres can greatly increase the lifetime of excitons in PS2 (Mullineaux *et al.* 1990, Salehian and Bruce 1992) which should correspondingly induce an increase of the probability for spill-over. However, the closure of PS2 does not influence the fluorescence yield of PS1. Therefore, the "spill-over" model is not a probable explanation.

Influence of betaine on PBS-thylakoid membrane complexes at state 2: The fluorescence emission spectra were obviously different from that of the PBS-thylakoid complexes without special treatment (Fig. 2*A*). The decrease of relative fluorescence yield of PS1 and increases of those of PBS and PS2 (Table 1) were

Table 1. The relative fluorescence yields of untreated phycobilisome-thylakoid membrane complexes, and complexes treated with DCMU, betaine, and NEM at state 2 (S2) and state 1 (S1). Excitation wavelength 580 nm.

	Untreated		DCMU		Betaine		NEM	
	S1	S2	S1	S2	S1	S2	S1	S2
PBS	0.307	0.252	0.318	0.261	0.305	0.338	0.362	0.441
PS2 core complexes	0.363	0.289	0.353	0.277	0.364	0.326	0.373	0.285
PS1 core complexes	0.330	0.459	0.329	0.452	0.331	0.336	0.265	0.274

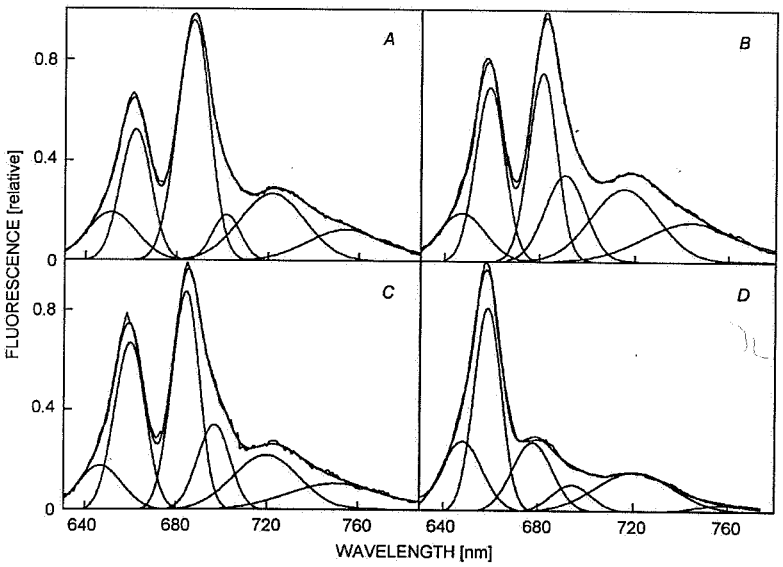

Fig. 2. Fluorescence spectra of untreated phycobilisome-thylakoid membrane complexes (*A*) and complexes treated with DCMU (*B*), betaine (*C*), and NEM (*D*) at state 2. Excitation wavelength 580 nm.

observed. The "spill-over" model requires closer association of PBS with PS2, while both the "PBS detachment" and "mobile PBS" models imply detachment of PBS at state 2. Once PBS is fixed on thylakoid membrane by betaine, the detachment process of PBS is restrained. On the other hand, if the state transition were completed according to the "spill-over model", betaine should not affect state 2. In fact, under betaine treatment the relative fluorescence yield of PS1 in state 2 was very similar to that of complexes without special treatment at state 1 (Table 1). Hence the state transition process is probably restrained. The slight growth of fluorescence yields of PBS and a little decrease of fluorescence yields of PS2 may be explained simply by energy redistribution between PBS and PS2. Therefore, there must exist a process of detachment of PBSs from PS2 during the state transition. It was proved that energy could not be spilled from PS2 to PS1 (Mullineaux 1992), which also implied that the "detachment PBS" model was not a possible explanation because it is a modified "spill-over" model. On the other hand, the "mobile phycobilisomes" model explained the transition to state 2 by the dissociation of PBS from PS2 and subsequent association to PS1. The fluorescence yield of PS1 in state 2 would not change when PBS was fixed on thylakoid membrane by betaine. Therefore, the "mobile phycobilisomes" model is in accord with our experimental results.

Influence of NEM on PBS-thylakoid membrane complexes at state 2: It was expected that NEM could disturb the pathway of energy transfer from PBS to PS1. Really, the relative fluorescence yields of PS1 in NEM-treated PBS-thylakoid membranes complex decreased largely, while that of PS2 was almost invariable (Fig. 2*D*, Table 1). If state transition were completed by the "spill-over" model, the fluorescence yield of PS1 should not decrease considering the expected function of NEM at state 2. Besides, the fluorescence yield of PS2 should have been larger and accompanied with a significant increase of fluorescence emission of PBS, which should also have led to a larger fluorescence yield of PS1. We found that the yields of PS2 were almost the same as that of a complex without special treatment at state 2, and the yields of PS1 decreased. Therefore, both the "spill-over" model and its modified version may not be a reasonable explanation. The results show that a part of PBSs may decouple from PS2 and become associated in some way with PS1, but a part of PBS may still associate with PS2 in state 2. After association with PS1, the relative yield of PBS increases because energy transfer process from PBS to PS1 is disturbed. The relative yields of PS2 (originating from coupled PBS in PS2) in NEM-treated complexes should be almost the same as that of a complex without special treatment at state 2. Consequently, the current experimental results could be reasonably explained by the "mobile PBS" model instead of the "spill-over" or "PBS detachment" models.

Table 2. The relative fluorescence yields of untreated phycobilisome-thylakoid membrane complex at state 2 (S2) and state 1 (S1) and that of betaine-treated complexes at state 2. Excitation wavelength 436 nm.

	Untreated		Betaine
	S1	S2	S2
APC core	0.130	0.125	0.145
PS2 core complexes	0.240	0.165	0.180
PS1 core complexes	0.630	0.710	0.675

Deconvolution of fluorescence spectra of the PBS-thylakoid membrane complex in state 2 at excitation wavelength of 436 nm: The fluorescence emission spectra (excited at 436 nm) of untreated PBS thylakoid membrane complex (Fig. 3*A*) and of the complex treated with DCMU (Fig. 3*B*), betaine (Fig. 3*C*), and NEM (Fig. 3*D*) can be deconvoluted into four components. At 436 nm, only the Chl *a* molecules in thylakoid membranes were excited. The 660-nm component belongs to PBS core, which originated from back transfer from PS2 to PBS. The peaks of 685 and 695 nm belong to PS2 and that of 723 nm to PS1. At state 2, the fluorescence emission spectra of betaine-treated PBS-thylakoid complex were compared with those of the complex without treatment (Fig. 3, Table 2). The back energy transfer from PS2 to PBS increased at state 2 because PBSs were fixed on the membrane in betaine-treated PBS-thylakoid complex so that they could not detach from PS2. The results also imply that PBS may decouple from PS2 and move to PS1 at state 2 during the state transition, which also supports the "mobile PBS" model. As for NEM- or DCMU-treated complexes, the fluorescence spectra excited at 436 nm were almost the same as those of the untreated complexes at state 2 within experimental error range, because the back transfer was unaffected (Fig. 3). Our recent research proved that linkage between PBS and PS2 was unstable and temperature could induce partial detachment of PBS from PS2 (Li *et al.* 2001). In addition, PBS was mobile on the thylakoid membrane (Mullineaux *et al.* 1997). All these results imply that the movement of PBS may regulate energy redistribution between PBSs and photosystems during the state transition, that is, radiant energy-induced decoupling of PBSs from PS2 and moving to PS1 is a reasonable mechanism to complete the state transition.

Conclusion: At state 2, the closure of PS2 did not influence fluorescence yields of PS1, *i.e.*, energy could not spill to PS1 from PS2. Following the disturbing of

energy transfer from PBS to PS1 by adding NEM, the relative fluorescence yield of PS1 decreased, that of PBS increased, and that of PS2 was almost invariable. Hence the "spill-over" and "PBS detachment" models are not a plausible explanation of the state transition. Once PBS is fixed on the thylakoid membrane by betaine, the state transition process does not occur. We conclude that PBS may detach from PS2 and become associated with PS1 at state 2. At state 2, the increase of relative fluorescence intensity of PS1 may originate from contribution of PBS rather than the spill-over from PS2. It supports the proposed "mobile PBS" model.

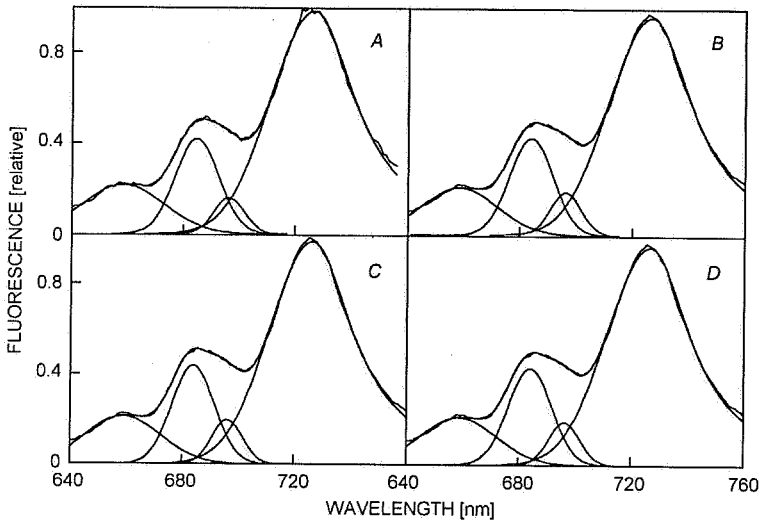

Fig. 3. Fluorescence spectra of untreated phycobilisome-thylakoid membrane complexes (*A*) and complexes treated with DCMU (*B*), betaine (*C*), and NEM (*D*) at state 2. Excitation wavelength 436 nm.

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