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BRIEF COMMUNICATION

Temperature-dependent decay-associated fluorescence spectra in phycobilisome-thylakoid membrane complexes from *Spirulina platensis*

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Abstract

The kinetic component (39 ps) for the energy transfer from a phycobilisome (PBS) to the photosystems was temperature-dependent while the components related to the kinetic processes within PBS, photosystem 2 (PS2) or PS1 were temperature-independent. The 39 ps component possessed the amplitude maximum at 647 nm but the minimum at 715 nm (room temperature) or 685 nm (0 °C), suggesting a direct energy transfer from C-phycocyanin to PS1 at room temperature but to PS2 at 0 °C. The temperature-induced kinetic change originated from a position shift of PBS along the thylakoid membrane.

Additional key words: cyanobacterium; photosystems 1 and 2.

Cyanobacteria are photosynthetic organisms equipped with a special antenna system, phycobilisome (PBS), which greatly strengthens the efficiency for utilizing solar energy. Recently, great achievements have been made in the research of the structures of isolated phycobiliproteins (Duerring *et al*. 1991, Brejc *et al*. 1995, Stec *et al*. 1999) and photosystems (Zouni *et al*. 2001, Jordan *et al.* 2001) from cyanobacteria. The structural matches and functional association of PBSs to the photosystems are of key importance for cyanobacteria in order to utilize solar energy efficiently. PBS may be strongly attached to photosystem 2 (PS2) (Isono and Katoh 1987, Capuano *et al.* 1991, Bald *et al.* 1996). On the other hand, it was proposed that PBSs are movable on the surface of photosynthetic membrane even at physiological temperatures (Manodori and Melis 1985, Mullineaux *et al.* 1997, Sarcina *et al.* 2001). We observed previously that the temperature-induced decoupling of PBSs from the photosystems in a PBS-thylakoid membrane complex was very similar to that in the intact cells, which was ascribed to the changes of connection of PBSs with the photosystems instead of the individual structures (Li *et al.* 2001, 2003). These observations confirm the structural and functional similarities of a PBS-thylakoid membrane complex in isolated state to that in an intact cell, therefore, the results derived from the isolated complexes are generally significant. In the current work, we measured the decay-associated fluorescence spectra at room temperature (RT) and 0 °C.

The PBS-thylakoid membrane complexes were isolated from *Spirulina platensis* by the method of Katoh and Gantt (1979) with some modifications (Li *et al.* 2001). Samples were dark-adapted at either 0 °C or RT for 30 min prior to measurements. Pico-second fluorescence decays were recorded in a single-photon timing equipments. The excitation pulses were generated at frequency of 800 kHz by synchronously pumped and cavity-dumped dye laser system (*Spectra Physics*, Mountain View, USA), and the pulse width was almost 25 ps (*FWHW*). The detection system consisted of a monochromator and a conventional photomultiplier (*E3059*, *Hamamatsu*, Japan). The data were accumulated in 1 000 channels, corresponding to a time interval of 10 ns, and up to 10 000 counts in the peak channel. During the measurements, the samples were circulated through a cuvette to guarantee measurement of fresh samples. The

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Abbreviations: APC = allophycocyanin; C-PC = C-phycocyanin; DAS = decay-associated fluorescence spectra; PBS = phycobilisome; PS = photosystem; RT = room temperature (25 °C).
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laser irradiance was kept sufficiently low to ensure that PS2 centres were not closed. Fluorescence decay profiles were fitted simultaneously to the sum of exponential functions (global lifetime analysis, ASUFIT de-convolution program). The quality of the fits was assessed by global χ^2 values.

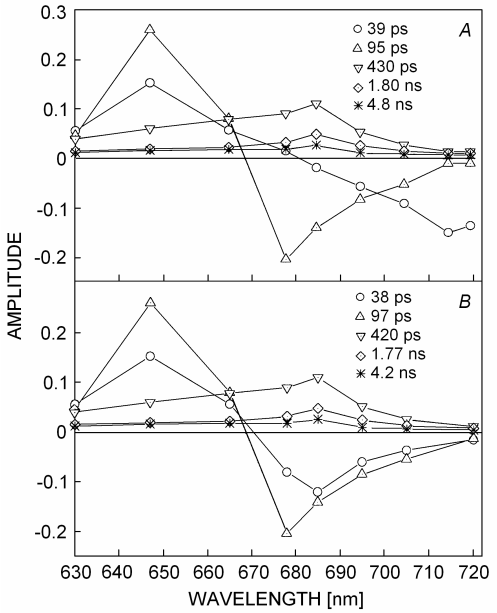

Fig. 1. Decay-associated fluorescence emission spectra (DAS) of PBS-thylakoid membrane complexes at room temperature (*A*) and at 0 °C (*B*).

The decay-associated fluorescence emission spectra (DAS) in pico-second time resolution were recorded in 10 nm intervals for the complexes excited at 610 nm at both temperatures of measurement (Fig. 1). Typical residual plots of χ^2 values from global analysis with four- and five-exponential functions are shown in Fig. 2, which confirms at least five exponentials were necessary. Among the five components, the 95 ps (or the 97 ps at 0 °C) component was dominant with the amplitude maximum at 647 nm, the fluorescence emission peak for C-PC, and the minimum at around 678 nm, the fluorescence emission peak for the terminal emitter of APC. Therefore the component can be simply assigned to the energy transfer from the C-PC in the PBS rods to the terminal emitter of the PBS core. No matter whether at RT or 0 °C, the amplitudes and lifetimes of the component are nearly the same, indicating that the intrinsic structure of a PBS is invariable during the change of temperature. In addition, the three components with time constants of 430 ps (420 ps at 0 °C), 1.8 ns (1.77 ns at 0 °C), and 4.8 ns (4.2 ns at 0 °C) have only the positive amplitudes with the maximum at around 685 nm, which can be assigned to the fluorescence decay in PS2. These components were ever observed for the isolated PS2 particles (Schatz *et al.* 1987, Mullineaux *et al.* 1991). The amplitudes and lifetimes of the three components were basically invariable no matter whether at RT or 0 °C. Therefore it can be concluded that the intrinsic structures of PS2 and PS1 are also invariable during the temperature changes and not responsible for the temperature-induced decoupling of PBSs from the photosystems.

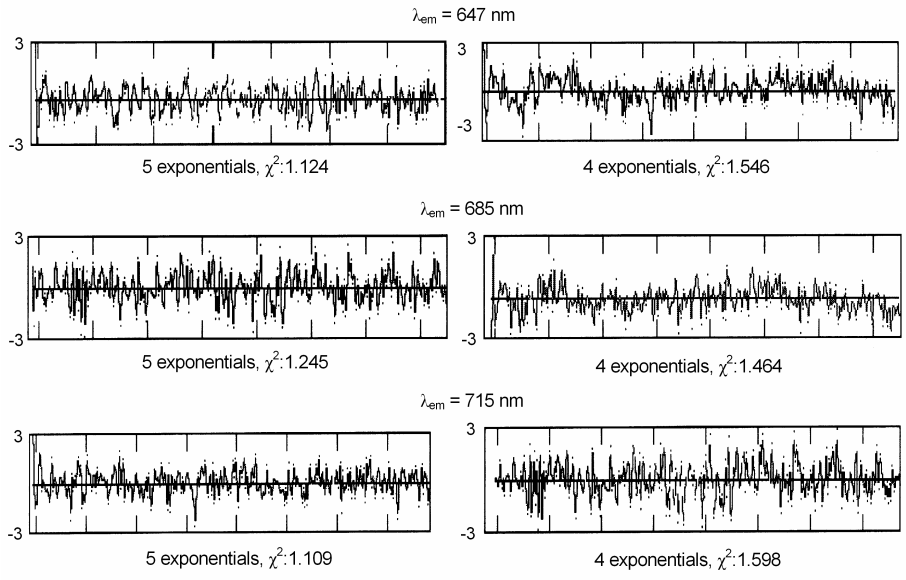

Fig. 2. The residual plots of the χ^2 values for analysis of decay-associated fluorescence emission spectra.

The only temperature-dependent component is the 39 ps one, which clearly tells what occurs during the temperature change. At room temperature (Fig. 1*A*), the component has the amplitude maximum at 647 nm, the fluorescence decay for C-PC as the energy donor, and the minimum at 715 nm, the fluorescence rising for PS1 as

the energy acceptor. This confirms a direct energy transfer from C-PC to PS1, which was previously suggested based on the steady state fluorescence spectra. At 0 °C (Fig. 1*B*), the 38 ps component still possessed the maximum (fluorescence decay) at around 647 nm while the minimum (fluorescence rising) shifted to 685 nm, exactly the wavelength for the PS2 fluorescence emission. The energy transfer from C-PC to PS2 has never been reported, however, the DAS curve suggests that the process occurs at 0 °C. Accordingly, the position shift of PBS might occur during temperature change from RT to 0 °C, which, in turn, might change the energy transfer pathway, *i.e*. from C-PC to PS1 to C-PC to PS2.

It may be argued that a fluorescence decay term in the terminal emitters and a rising term in PS2 should have been observed in the DAS spectra. However, the terminal emitters of the PBS core and the chlorophyll molecules in PS2 emit at similar wavelengths (around 680 nm), and thus the rise and decay may cancel each other and result in no observable fluorescence decay and rise. On the other hand, the fluorescence emission band of C-PC is far apart from that of PS1 (by about 80 nm) so the decay and the rise could be well observed. In addition, the initially excited C-PC is the direct donor for the energy transfer to PS1 (C-PC to PS1) while an indirect one for that to PS2 (C-PC to APC to the terminal emitter to PS2). In fact, the step by step energy transfer is probabilistic rather than deterministic, therefore, the more energy transfer steps exist, the wider the distribution of excitation energy in time and space scales (Zhao *et al.* 1995, Xie *et al.* 2002), which explains why the rising term of the fluorescence in PS1 is observable while that in PS2 not. Briefly, it can be concluded that a temperature-induced movement of PBS along the thylakoid membrane makes the PBS core move away from PS2 while the rods are closer to it at 0 °C.

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