S4-013

Does Chl *b* bind to the Chl *a*–binding sites in PS1 RC complexes?

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Keywords: Photosystem I, Antenna, Chlorophyll, P700, Fluorescence

Introduction

Photosystem I in higher plants contains two types of Chl-binding proteins, LHCI and PsaA/B RC complexes. LHCI binds Chl *a* and *b*, and PsaA/B binds only about 100 Chl *a* per one reaction-center unit. Ikegami and Katoh (1975) previously found that most Chls in PSI were removed without any loss of P700 activity by ether treatment, yielding the PSI complex that have only 12 molecules of Chl *a* per P700. By the subsequent solubilization of the antenna-depleted PSI complex, the apoproteins of LHCI that binds no pigments were removed as insoluble white materials. The resultant antenna-depleted RC complexes were employed for either precise spectroscopic analysis of PSI RC components or reconstitution of antenna pigments. In the latter experiments, we found that the limited number of Chl *a* could be functionally bound on combined addition of Chl *a* and a phosphatidylglycerol (PG) (Ikegami and Katoh 1991). In order to elucidate the binding capacity of the Chl *a*-binding sites to pigments other than Chl *a*, we tried to reconstitute the antenna activity by using Chl *b*. On addition of Chl *b* with a phosphatidylglycerol, 9-14 Chl *b* per P700 bound to the Chl *a*-binding sites. We found that most of these Chl *b* bind close to P700 and transfer their excitation energy efficiently to P700.

Materials and Methods

Lyophilized spinach PSI complexes were extracted twice with diethyl ether containing water at 80% saturation. By this treatment, all the carotenoids, almost all Chl *b* and more than 90% of the Chl *a* were removed without any loss of P700 to yield the PSI complexes with 12 molecules of Chl *a* per P700 (see a review by Ikegami et al. 2000). The ether-treated materials were then dried and suspended in a 0.05 M phosphate buffer (pH 8). 50 µg Chl *b* and 400 µg phosphatidylglycerol dissolved in 20 µl ethanol were mixed to 1 ml of the buffer suspension of the antenna-depleted materials (that had originally contained about 200 µg Chl *a*/ml), incubated for 90 min at 5^{o}C and then centrifuged to remove free Chl *b* as a supernatant. The precipitate suspended in the same buffer containing 0.2% Triton X-100 was incubated for 1 hr at 5^{o}C and the Chl *b*-bound PSI RC complexes made of PsaA/B, C and other small subunits were recovered as a supernatant by centrifugation. Absorption spectra and light-induced absorption changes of P700 were measured with a Hitachi model U-3010 and Hitachi 556 dual wavelength spectrophotometer, respectively. Fluorescence emission and excitation spectra were determined as described previously (Ikegami 1983).

Results and Discussion

The absorption spectrum of the Chl *b*-bound antenna-depleted RC complexes was shown in Fig. 1B. The prominent peak at 465 nm and shoulder around 655 nm originate from the bound Chl *b*. The number of Chl *b* bound to the antenna-depleted complexes was irrespective of its amounts applied on the reconstitution and usually kept between 9 and 14 Chl *b*/P700. This indicates that only a limited number of binding sites for Chls was survived in the antenna-depleted complexes, as suggested on the reconstitution with Chl *a* (Ikegami 1983, Ikegami and Katoh 1991). The absorption spectrum of the Chl *b* bound to the complexes was isolated and shown in Fig. 1, C. Note that there is a small bump at 685 nm in this spectrum, suggesting an occurrence of some interaction of the bound Chl *b* to the Chl *a*-685. The bound Chl *b* was effective to the excitation-energy transfer to P700. The initial rate of P700 photooxidation determined with the Chl *b*-bound complexes (having 9 Chl *b*/P700 ratio) increased to 177% of that before reconstitution (data not shown). Since the excitation light used (360-480 nm) was absorbed by either Chl *a* or Chl *b*, the results indicate the quantum efficiency of the bound Chl *b* roughly equal to that of Chl *a* remaining in the antenna-depleted complexes. Fig. 2 shows the fluorescence emission spectra of Chl *b*-bound complexes determined at 10°C, of which shapes were different, each other dependently of the excitation wavelength. When excited with a 430 nm-light, the spectrum showed a peak at 675 nm with a slight shoulder around 660 nm. On the other hand,

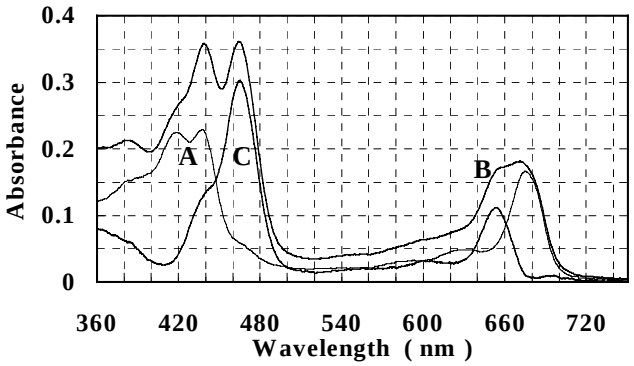

Fig.1 Absorption spectra of antenna-depleted PSI RC complexes with a Chl *a*/P700 ratio of 12 before (A) and after binding 14 Chl *b*/P700 (B). Difference spectrum (C) obtained by subtracting A from B.

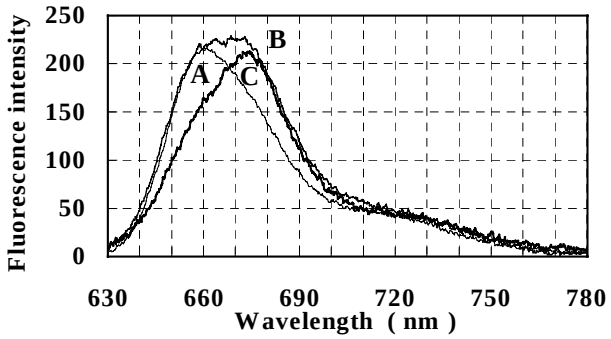

Fig.2 Fluorescence emission spectra of antenna- depleted PSI RC that bound 14 Chl *b*/P700, which was excited at 10°C by 480 (A), 450 (B) and 430 nm (C) light (half bandwidth, 12 nm and LI, 10 $\mu E \cdot m^{-2} \cdot s^{-1}$). Emission curves were normalized at their peaks.

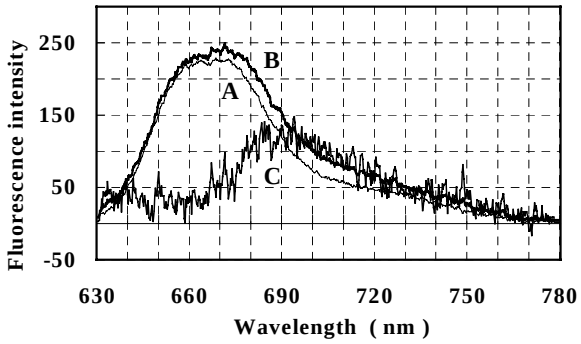

Fig.3 Fluorescence emission spectra of antenna-depleted PSI RC that bound 14 Chl *b*/P700, which was excited at 10°C by a 450 nm light with no redox reagents (A) and with 10 mM dithionite (pH10) (B). (C) Difference between A and B magnified 4 times. Other conditions were the same as in Fig. 2.

when excited with a 480 nm light, the spectrum showed a main peak at 660 nm. Fl660 is ascribed to be emitted by Chl *b*, since its excitation spectrum had the same shape as the absorption spectrum of Chl *b* (data not shown). This indicates that a small number of Chl *b* binds far from Chl *a* in terms of the energy transfer. However, a large and broad shoulder toward the longer wavelength side in the spectrum (Fig. 2, A) supports the energy transfer from Chl *b* to Chl *a*. The spectrum with the flat peak from 660 to 675 nm was obtained on excitation with a 450 nm-light. The original antenna-depleted complexes show the constant and variable fluorescence with emission peaks at 680 and 695 nm, respectively. Fl680 was identified to be emitted by Chl *a*-675. Fl695 was not observed under P700-oxidized conditions and its intensity increased stepwise with the accumulation of electrons on the PSI acceptors in the order of F_A/F_B, F_X and Q_K (Ikegami 1976, Ikegami and Ke 1983). Since the electron is not able to be transferred beyond PSI under these conditions, Fl695 was ascribed to the delayed fluorescence produced by the charge recombination between $P700^+$ and A_0^-. There was no Chl *a* with a low-energy level than P700 in the complex so that Fl695 seems to be emitted by P700 itself (Iwaki et al. 1992). On incubation of Chl *b*-bound complexes with dithionite at pH10, under which conditions F_A/F_B were reduced, the spectrum was broadened to the longer wavelength side (Fig. 3, B) and the fluorescence component induced with dithionite shows the peak around 690 nm (Fig. 3, C). These results indicate that Chl *b*-bound complexes emit Fl695, as well as Fl680 and Fl660.

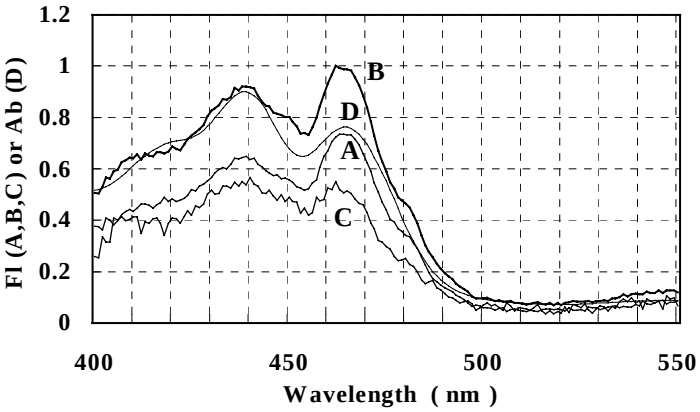

Fig.4 Excitation spectra of antenna-depleted PSI RC that bound 9 Chl *b*/P700 were measured by monitoring the fluorescence in the far red region (cutted-off with red filters; Corning 7-69 and Toshiba R-63) with no redox reagents (A) or with 10 mM dithionite (B). Monochromatic excitation light was supplied with a Jubin-Yvon monochrometer (half bandwidth, 3.2 nm, about 1 $\mu E \cdot m^{-2} \cdot s^{-1}$). (C) Difference between A and B magnified 2 times. In comparison, the absorption spectrum of the sample (D) was shown.

Next, we determined the excitation spectra of Chl *b*-bound complexes for the constant and variable fluorescence, in the far-red region in order to eliminate the contribution of Chl *b* fluorescence. In this experiment, 9 Chl *b*/P700 were bound to the antenna-depleted complexes so that the band at 465 nm was smaller than that at 440 nm in its absorption spectrum (Fig. 4, D). P700 was mostly in the oxidized state without any redox reagents. Under these conditions, we can obtain the excitation spectrum only for the constant fluorescence (Fl680) (Fig. 4, A). The spectrum shows a much larger peak at 465 nm than that at 440 nm, indicating that Chl *b* is more efficient to emit Fl680 than Chl *a* remaining in the complex. On incubation with dithionite, the variable fluorescence (Fl695) appears so that the total fluorescence intensity increases (Fig. 4, B). The excitation spectrum for the variable fluorescence was obtained by the difference between the two (Fig. 4, C). The spectrum also has the band peaking at 465 nm of which amplitude is equal to that at 440 nm. Note that there are distinct shoulders around 450 and 480 nm in the spectrum. The excitation spectrum divided by the absorption spectrum represents the wavelength-dependent quantum efficiency. There were three positive peaks at 450, 465 and 485 nm in the spectrum corresponding to the variable fluorescence (data not shown). When the quantum efficiency at 440 nm was set to be 1.00, those at 450, 465 and 485

nm were 1.20, 1.24 and 1.20, respectively. These results suggest that Chl *a* with a peak at 450 nm in the Soret region connects closely with P700 and that Chl *b* with peaks at 465 and 485 nm transfers its excitation energy to P700 as efficiently as Chl *a* closely associated to P700. The form of Chl *b* having a peak at 485 nm could not be recognized in the absorption spectrum (Fig. 1, C). Therefore, this might be a special form of Chl *b* reincorporated into a Chl *a*-binding site very close to P700.

In summary, most Chl *b* bind to the sites connected to either Chl *a*-675 or the reaction-center Chls (P700, A_0, A and/or connecting Chl) and transfer their excitation energy as efficiently as *in situ* Chl *a*. Only a few molecules of Chl *b* bind far from Chl *a* and emit strong fluorescence. These results suggest that the Chl *a*-binding sites have some ambiguity for pigment-binding, even though its binding affinity might be higher to Chl *a* than to other pigments such as Chl *b* because only Chl *a* binds to PSI RC complex under natural conditions.

Acknowledgements

This work was supported in part by a grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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