Light-induced Charge Separation in Photosystem I can be Sensitized by an Artificial Fluorescent Dye Covalently Linked to the Photosystem I Complex Surfaces

Akimasa Nakamura,*# Shinji Mizoguchi, Emi Yoshida, Yuki Kato, and Tadashi Watanabe*
Institute of Industrial Science, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8505

(Received July 25, 2005; CL-050959)

To expand the spectral window for the light-induced charge separation in photosystem (PS) I of oxygenic photosynthetic organisms artificially, Rhodamine B (RhB) was covalently linked to the solution-exposed surfaces of PS I. By introduction of RhB to PS I, the activity of light-induced charge separation for excitation wavelength at which the light absorption is dominated by RhB increased by about 1.8-fold, showing that RhB covalently attached to PS I can serve as the artificial light-harvesting antenna.

In the light reaction of oxygenic photosynthetic organisms, conversion of photon energy to chemical free energy at an ultimate quantum yield (ca. 1.0) is achieved by light-induced charge separation occurring at the reaction centers of photosystem (PS) I and II in thylakoid membranes. The light-induced charge separation between the primary electron donors and the acceptors, consisting of specialized chlorophyll (Chl) *a*, is sensitized by large arrays of core antenna Chl *a* molecules in PS I and II.[1] Though Chl *a* shows strong light absorption at blue and red spectral regions, light absorption at the green region is significantly weak. To utilize efficiently the sunlight under which they live, oxygenic photosynthetic organisms expand the spectral window for the light-induced charge separation by developing various external light-harvesting systems with such pigments as Chl *b*, carotenoids and bilins[2] whose spectroscopic characters are different from those of Chl *a*.

Whether the spectral window for the light-induced charge separation in PS can be expanded by using synthetic dyes entirely different from natural pigments, remains unaddressed so far. One of the promising approaches for this question may be attachment of artificial fluorescent dyes to the solution-exposed surfaces of a PS complex (Figure 1). Three-dimensional structures of PS I[3] and PS II[4] revealed that many of the core antenna Chl *a* molecules exist within relatively shallower positions from the solution-exposed surfaces, ca. 10 Å depth. We can expect that the relatively short distance enables excitation energy transfer from artificial fluorescent dyes on the surfaces to the core antenna Chl *a*, which then transfers the excitation energy to the primary electron donor. In nature, the external light-harvesting system of cyanobacteria, phycobilisome, is attached also on the solution-exposed surfaces of PS and transfers its excitation energy to the core antenna Chl *a*.[5] This indicates that the arrangement of the core antenna Chl *a* has been optimized for the energy transfer from the surface-bounded fluorophores. These considerations have led us to bind artificial fluorescent dyes on the PS surface to expand the spectral window for the light-induced charge separation, limited by the natural photosynthetic pigments.

We chose a combination of PS I as the photosystem and a fluorescent dye, Rhodamine B (RhB), to facilitate evaluation of the spectral sensitization. RhB was introduced covalently with

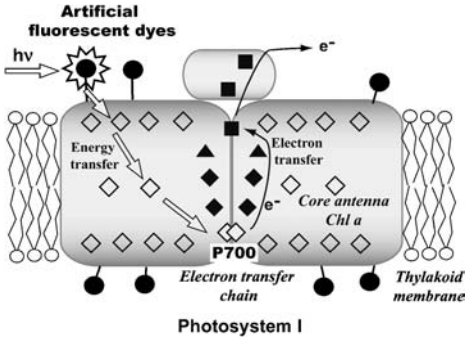

Figure 1. Schematic representation of the spectral sensitization of light-induced charge separation in photosystem by covalent modification of the solvent-exposed surfaces with artificial fluorescent dyes.

a thiourea linkage to the PS I complex prepared from a cyanobacterium *Spirulina platensis*.[6] RhB isothiocyanate (RITC; Sigma), which reacts primarily with the NH_2 groups of lysine residues and N-terminal amines,[7] was incubated with solubilized PS I, containing ca. 100 molecules of Chl *a*,[6] at an RITC/Chl *a* molar ratio of ca. 1 in 50 mM $NaHCO_3$ (pH = 9.8) and 0.025% dodecyl β-D-maltoside for 30 min at room temperature. The PS I-RhB was purified by gel permeation chromatography and sucrose density gradient centrifugation.

Figure 2a shows the absorption spectra of native PS I and PS I-RhB. In PS I-RhB, an absorption peak of RhB appeared at around 550 nm without distorting the PS I absorption spectrum itself as shown in the difference spectrum (Figure 2b), which agrees well with the absorption spectrum of RhB solution. When PS I-RhB was treated with acetonitrile/water = 9/1 which can extract pigments in PS I quantitatively, less than 10% of RhB in PS I-RhB was found in the solvent. The activity for the light-induced charge separation evaluated from photooxidation of the primary electron donor, P700,[6] was the same for both native PS I and PS I-RhB, and agrees well with previous results on cyanobacterial PS I.[3,6] These findings ensure that RhB was indeed attached covalently to PS I without perturbing the activity for the light-induced charge separation. The number of RhB molecules introduced to a PS I complex was found to be ca. 10 under an assumption that the molar extinction coefficient of RhB was not altered by binding to PS I. These RhB molecules would distribute exclusively on the solution-exposed surfaces of PS I, because membrane-integral regions of PS I do not contain lysine residues.[3]

Whether RhB molecules attached to PS I sensitize the light-induced charge separation can be examined by monitoring the absorbance change at 700 nm (ΔA_{700}) upon P700 oxidation under continuous illumination at 550 nm, the absorption maximum of RhB. When PS I was illuminated in the presence of an artifi-

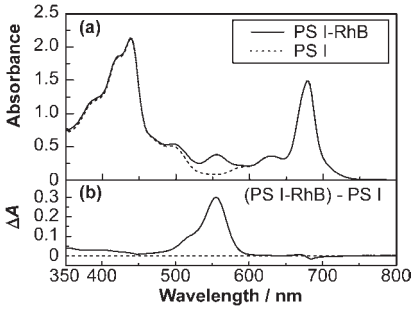

Figure 2. (a) Absorption spectra of native PS I and PS I-RhB at the same Chl *a* concentration. (b) Difference spectrum between PS I-RhB and native PS I.

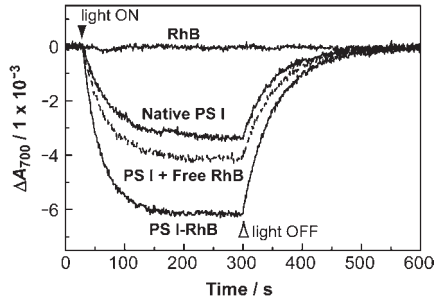

Figure 3. Light-induced absorbance change at 700 nm[6] under 550 nm illumination. The actinic illumination, 15 μW m^{-2}, was provided from a 500 W Xe lamp through a 550-nm interference filter.

cial electron acceptor, methyl viologen (MV), the oxidized P700 was accumulated as a result of the light-induced charge separation and subsequent PS I-mediated reduction of MV by competing with re-reduction of P700$^+$ by reductants added in solution.[8] Because energy transfer and the light-induced charge separation in PS I complete within ps and μs time range after photon absorption, the ΔA_{700} value under continuous illumination is determined from an equilibrium between the photon absorption by antenna molecules giving rise to P700$^+$ formation and the re-reduction of P700$^+$, taking place throughout the process.[9] If RhB attached to PS I can serve as the light-harvesting antenna and sensitize the light-induced charge separation, the ΔA_{700} value in PS I-RhB would increased from that in native PS I under 550-nm excitation, as a consequence of the increased photon absorption rate for 550-nm excitation caused by RhB.

Figure 3 shows light-induced ΔA_{700} under 550-nm actinic illumination. When the actinic illumination was adjusted to an intensity where ca. 20% of P700 was photooxidized in native PS I, the ΔA_{700} value in PS I-RhB increased by 1.81-fold from that in native PS I for a common P700 concentration (Figure 3). Because the RhB solution itself did not show any light-induced absorbance change (Figure 3), the increased ΔA_{700} in PS I-RhB is not an artifact arising from interference of RhB fluorescence emission. Thus, the increased ΔA_{700} in PS I-RhB demonstrates that RhB covalently attached to the PS I surface can function as the artificial light-harvesting antenna and sensitize the light-induced charge separation in PS I. The ΔA_{700} value was increased also by simply adding a RhB solution to native PS I (PS I + Free RhB) at the same RhB concentration as in PS I-RhB. However, the increase in ΔA_{700} was smaller than that for PS I-RhB (Figure 3), showing that the covalent linking of RhB to the PS I surfaces significantly enhances the P700 sensitization, though the sensitization itself can occur via RhB physisorbed to the PS I surface.

The efficiency of the sensitization by RhB can be estimated from comparisons of the light-induced ΔA_{700} values (Figure 3) with the absorbance at 550 nm (A_{550}; Figure 2). The contribution from bound RhB to the P700 sensitization in PSI-RhB can be obtained from the difference in the ΔA_{700} values between PS I-RhB and native PS I. The contribution to the P700 photosensitization (Figure 3) was 81% of the core antenna Chl *a*, though contribution from bound RhB to photon absorption for 550-nm illumination was 3.34-fold larger than that of the core antenna Chl *a*, as judged from the A_{550} values (Figures 2a and 2b). Because the quantum yield for the photochemical reactions within PS I is almost unity,[1] the apparent efficiency of energy transfer from bound RhB to the core antenna Chl *a* is estimated to be 24% by using a ratio of the contribution from RhB to the P700 sensitization against the photon absorption for 550-nm illumination. Though the efficiency of the energy transfer between bound RhB and the core antenna Chl *a* is relatively low, the fluorescence emission from RhB was quenched significantly in PS I-RhB, as compared to the free RhB solution at the same concentration (not shown). This indicates that one of the reasons for the lower efficiency is thermal deactivation of excited RhB molecules through attachment on the PS I surfaces.

Here, we have shown, for the first time, that light-induced charge separation taking place within a photosynthetic membrane-integral protein, PS I, can be sensitized by modifying the solution-exposed surfaces with an artificial fluorescent dye. Further improvement of the sensitization efficiency by choosing other dyes is under way.

This work was supported in part by a Grant-in-Aid for Scientific Research (No. 15033214) on Priority Area (417) and a COE project for "Human-Friendly Materials Based on Chemistry" from the Ministry of Education, Culture, Sports, Science and Technology.

References

Present address: Technology R & D Department, Central Japan Railway Company, 1545-33 Oyama, Komaki, Aichi 485-0801

1 N. Nelson and A. Ben-Shem, *Nat. Rev. Mol. Cell Biol.*, **5**, 1 (2004).

2 D. J. Simpson and J. Knoetzel, "Oxygenic Photosynthesis: The Light Reactions," ed. by D. R. Ort and C. F. Yocum, Kluwer, Dordrecht (1996), Chap. 27, p 493.

3 P. Jordan, P. Fromme, H. T. Witt, O. Klukas, W. Saenger, and N. Krauß, *Nature*, **411**, 909 (2001).

4 K. N. Ferreira, T. M. Iverson, K. Maghlaoui, J. Barber, and S. Iwata, *Science*, **303**, 1831 (2004).

5 N. Adir, *Photosynth. Res.*, **85**, 15 (2005).

6 A. Nakamura, M. Akai, E. Yoshida, T. Taki, and T. Watanabe, *Eur. J. Biochem.*, **270**, 2446 (2003).

7 M. Brinkley, *Bioconjugate Chem.*, **3**, 2 (1992).

8 B. Ke, *Biochim. Biophys. Acta*, **301**, 1 (1973).

9 M. L. Girardi and A. Melis, *Plant Physiol.*, **74**, 993 (1984).

Published on the web (Advance View) September 28, 2005; DOI 10.1246/cl.2005.1472