Sensitization of the Primary Charge Separation in Photosystem I to Green Light by an Amphiphilic Polymer Bearing Rhodamine 6G

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(Received June 5, 2006; CL-060650; E-mail: watanabe@iis.u-tokyo.ac.jp)

An amphiphilic polymer carrying an artificial fluorescent dye, Rhodamine 6G (Rh6G), acts as a good solubilizing agent for a hydrophobic membrane protein, the photosystem (PS) I complex from *Spirulina platensis*, and at the same time as an efficient sensitizing agent in the green wavelength region, resulting in an enhancement of the primary charge separation within the complex by ca. 1.5-fold.

Conversion of photon energy to chemical free energy in photosynthetic light reaction of oxygenic photosynthesis takes place within pigment–protein complexes, photosystem (PS) I and II, in thylakoid membranes. The energy transduction at an ultimate quantum yield (ca. 1) is driven by light-induced charge separation between the primary electron donor and a series of acceptors, consisting of specialized chlorophyll (Chl) *a* molecules.[1] To harvest sunlight efficiently, the light-induced charge separation is sensitized by arrays of hundreds of antenna Chl *a* molecules. Thus, the spectral window for the photochemical reactions is restricted by the spectroscopic character of Chl *a*, viz., PSs do not respond to green light at which the sunlight is most intense. In nature, cyanobacteria have developed the membrane extrinsic light-harvesting system phycobilisome to utilize green light by using pigments entirely different from Chls.

Manipulating the spectral window of PSs artificially with synthetic fluorescent dyes to harvest the sunlight more effectively than the natural one is of much importance in applying PSs to photofunctional devices.[2] Recently, we succeeded for the first time in driving the photochemistry within PS I with green light by covalent attachment of Rhodamine (Rh) B as an artificial light-harvesting antenna to lysine residues on the solution-exposed surface of PS I.[3] Energy transfer between the surface-bound RhB and the antenna Chl *a* in PS I rendered the primary electron donor (P700) responsive to green light.[3] However, the number of artificial dye molecules that can bind to the PS I complex is restricted by the number of lysine residues available on the PS I surface. Further introduction of dye molecules to PS I to increase the absorption cross section of artificial light-harvesting antenna requires development of other methods which are not limited by the number of specific amino acid residues. A promising approach would be complexation of a fluorescent dye to the hydrophobic membrane region of PS I where the natural antenna molecules are clustered, through hydrophobic interaction.

Membrane proteins in general, including PS I, are completely insoluble without surfactant molecules in water. Recently, Amphipols (APs) have been developed as novel solubilization agents.[4] An AP is a short chain polyacrylic acid with carboxylic groups modified partially with an aliphatic chain.[4] In contrast to conventional surfactants, the AP complexes tightly with the membrane integral parts of the protein via multi-point hydrophobic interactions and do not dissociate from the proteins without excess AP.[4] If we can bind a fluorescent dye to an AP-like polymer, the resulting polymer would complex tightly with PS I as an artificial light-harvesting complex. And also, the polymer would function as an artificial peripheral antenna of PS I such as membrane intrinsic light-harvesting complex I found in green algae and higher plants.

In view of these, we have attempted in this work to construct a novel artificial light-harvesting antenna, with a spectral character entirely different from that of the natural counterparts, to drive the photochemistry in PS I with green light by complexation of the polymer bearing a fluorescent dye (Figure 1).

The amphiphilic polymer bearing a fluorescent dye (AP-Rh6G; Figure 2) was synthesized from polyacrylic acid, 1-aminodecane, *N*-methyl-D-glucamine, and Rh6G at a molar ratio of 1/14/50/1 (see Supporting Information for details). *N*-methyl-D-glucamine was employed to replace anionic carboxyl groups, which potentially perturb the membrane protein, with neutral hydrophilic sugar chains through the amide linkage. Rh6G was chosen as the fluorescent dye, because it possesses an amino group, which can condense with the carboxylic group, and the absorption band is in wavelengths where the natural chlorophyllous pigments absorb poorly. To prepare the AP-Rh6G/PS I complex, native PS I prepared from a cyanobacteri-

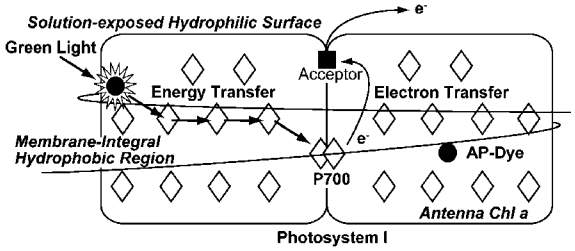

Figure 1. Schematic representation of the artificial sensitization of photochemical reaction in photosystem I, using a novel amphiphilic polymer bearing fluorescent dye (AP-Dye).

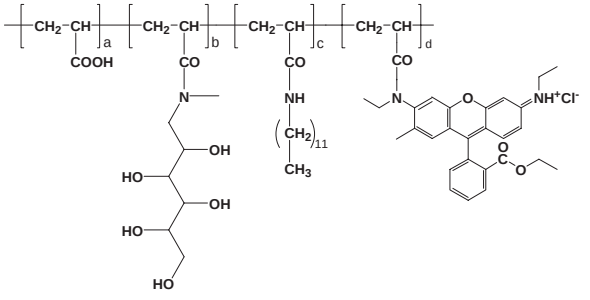

Figure 2. Structure of AP-Rh6G.

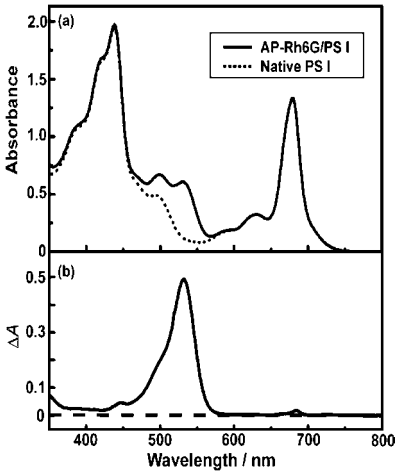

Figure 3. (a) Absorption spectra of native PS I and AP-Rh6G/PS I at the same Chl *a* concentration. (b) Difference spectrum between PS I and AP-Rh6G/PS I.

um *Spirulina platensis*[5] was concentrated to ca. 5 mM Chl *a* with a 30 kDa ultrafiltration apparatus, then an aliquot was diluted with the AP-Rh6G solution (6 μM Rh6G) to a final Chl *a* concentration of 22 μM and used without further purification. The number of Rh6G molecules contained in the solution was estimated to be ca. 26 per one unit of PS I, since native PS I contains ca. 96 molecules of Chl *a*.[5] After dilution, PS I was soluble only in the presence of AP-Rh6G; otherwise PS I tended to precipitate. This observation indicates that AP-Rh6G can, as expected, complex with PS I via multi-point hydrophobic interactions to prevent its aggregation.

Figure 3 shows the absorption spectra of native PS I and the AP-Rh6G/PS I complex. The difference spectrum between two traces shows a spectrum of Rh6G without distorting the PS I spectrum. The absorption maximum of AP-Rh6G was red-shifted by about 6 nm from that of monomeric Rh6G in the same buffer: this would reflect a change in the environment surrounding Rh6G molecules between dissolved and polymer-bound states.

The sensitization of light-induced charge separation within the PS I complex was evaluated as described previously.[3,6] Briefly, the absorbance change at 700 nm, ΔA_{700}, which is proportional to the amount of P700 radical cation generated by its one-electron oxidation, was measured against time after the onset of actinic illumination.

When excited at 630 nm, where only the native antenna Chl *a* molecules exhibit absorption, the ΔA_{700} value was the same both for native PS I and AP-Rh6G/PS I (data not shown). This demonstrates that complexation of the AP-Rh6G to PS I does not perturb the native PS I function.

By actinic excitation at 530 nm, which is near the absorption maximum of Rh6G, the native PS I showed a small ΔA_{700} value, corresponding to the photooxidation of P700 to a 13%-level of the total P700 in the solution. This is in line with a weak absorption and hence antenna function in the green wavelength range (Figure 3).

However, excitation of the AP-Rh6G/PS I at 530 nm enhanced the ΔA_{700} value by as much as ca. 1.5-fold, as is clearly seen in Figure 4. An AP-Rh6G aqueous solution showed no light-induced absorbance change (Figure 4), indicating that the enhancement of the ΔA_{700} value in AP-Rh6G/PS I should not

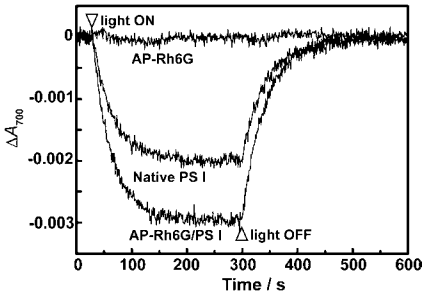

Figure 4. Light-induced absorbance change at 700 nm (ΔA_{700}) under 530 nm illumination. The actinic illumination, 6.4 μW m^{-2}, was provided from a 500-W Xe lamp through a 530-nm interference filter.

result from a photochemical process within AP-Rh6G itself; in other words, the AP-Rh6G functions as an artificial light-harvesting agent for PS I. The efficiency of the sensitization by the AP-Rh6G can be estimated as 11% from comparisons of the light-induced ΔA_{700} values (Figure 4) with the absorbance at 530 nm (A_{530}; Figure 3).[3] The estimated efficiency for the AP-Rh6G was lower than that for RhB bound to PS I in the previous report (24%). One of the reasons for the low efficiency is smaller spectral overlap between the fluorescence emission of the Rh6G and the absorption of the core antenna Chl *a* of PS I than that for RhB.

This is the first case where an amphiphilic polymer bearing an artificial fluorescent dye was shown to work as a sensitizer for the primary charge separation in the natural photosynthetic system. Improvement of the sensitization efficiency would require modification of the AP with other fluorescent dyes. Such studies are currently under way.

This work was supported in part by a Grant-in-Aid for Scientific Research (No. 17029014) 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.

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7 Supporting Information is available electronically on the CSJ-Journal web site; http://www.csj.jp/journals/chem-lett/.

Published on the web (Advance View) August 12, 2006; doi:10.1246/cl.2006.1034