Construction and Photocurrent of Light-harvesting Polypeptides/Zinc Bacteriochlorophyll *a* Complex in Lipid Bilayers

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(Received May 29, 2003; CL-030478)

Light-harvesting (LH) 1-type complexes consisting of LH polypeptides and zinc bacteriochlorophyll *a* (Zn–BChl*a*) were stably assembled in DMPC lipid bilayers on an electrode at room temperature. Photoinduced electronic currents of which action spectra revealed two peaks corresponding to the Qy absorption bands of Zn–BChl*a* complex due to the presence of LH1-type complex were observed.

Artificial assembly of photosynthetic membrane can be helpful for studying photoexcitation of electron and the subsequent electron transfer in biological process. Electrochemistry is a suitable technique for the photoexcitation and electron transfer, when one constructs the membrane on an electrode. The electron transfer has been systematically explored by using self-assembly of proteins like cytochrome *c*, heme, and photosynthetic reaction center on electrodes.[1] To attain an efficient photoexcitation, one should introduce a light-harvesting system into the artificial membrane. It has, however, been suggested to be a difficult task that light-harvesting (LH) complex 1 be assembled on an electrode because the complex easily separates into peptides and bacteriochlorophylls.[2]

We now report the construction of LH1-type complex in photosynthetic bacterial membrane on an electrode modified with liposomal membranes. The photoinduced electronic current was measured by using an electrode system to construct an artificial photosynthetic antenna core model system.

We prepared an electrode covered by the liposomal membrane containing LH1-type complexes which consisted of zinc bacteriochlorophyll *a* (Zn–BChl*a*) and polypeptides of LH-α and -β. Zn–BChl*a* was extracted and then purified by the method described in Wakao et al.[3] LH-α and LH-β polypeptides were extracted from *Rhodospirillum rubrum* by $CHCl_3$/MeOH and purified by Sephadex LH60 gel chromatography and then by HPLC.[4] The LH complex containing Zn–BChl*a* was formed by using *n*-octyl-β-D-glucopyranoside (OG) micelle as described in Parkes–Loach et al.[4] The LH1-type complex was reconstituted by the method described previously.[4] Briefly, the polypeptides of LH-α and -β were inserted with Zn–BChl*a* into OG micelle at 25 °C to form a subunit-type complex revealing absorption maximum at 809 nm in UV–vis spectrum. The subunit-type complex is reconstituted to the LH1-type complex revealing absorption maximum at 858 nm on cooling at 4 °C.[6] In this paper, we further moved the LH1-type complex into lipid bilayer membrane; that is, the OG micelle of the subunit-type complex or the OG micelle of the LH1-type complex was mixed with the liposome of DMPC (dimyristoylphosphatidylcholine) in aqueous phosphate buffer. The resulting solution was filtered by a dialysis membrane through which substances with molar weight smaller than 12000 g/mol was removed and the remaining solution was the liposome of DMPC containing the LH1-type complex. The electrode modified with the lipid bilayer was prepared by a cast method with this liposome solution: 0.1 mL of the liposome solution was dropped on a transparent indium tin oxide (ITO) electrode with a surface area of 1.0 cm^2. The solvent was then removed from the solution under reduced pressure at room temperature to form a thin layer of the DMPC incorporating LH1-type complex.

Figure 1 shows the absorption spectra at the Qy band of the solutions of the OG micelle with the subunit-type complex and the DMPC liposome containing LH1-type complex at 25 °C. The Qy band was red-shifted from 809 nm for the OG micelle solution to 858 nm for the DMPC liposome solution. This shift indicates that the subunit-type complex in the OG micelle was converted to the LH1-type complex when the subunit-type complex was moved from the OG micelle to the liposome. A split CD signal was observed around 860 nm wavelength corresponding to the absorption bond of LH1-type in the liposome. The UV–vis and CD spectra indicated that LH1-type complex was much more stable in the liposome than the subunit-type complex. For an example, the LH1-type complexes in the liposome were stable for a few months; however, the subunit-type complex in the OG micelle was stable only for a few weeks. Zn–BChl*a* was probably associated and placed in a hydrophobic environment surrounded by LH1-type complex because monomers of Zn–BChl*a* show absorption maximum at 772 nm in the liposome. Similar results were also observed for BChl*a* in which a Mg atom occupies the center of the porphyrin unit.

Figure 2 shows the absorption spectrum and the photocurrent action spectrum on an ITO electrode modified with the DMPC lipid bilayers at −0.1 V (vs Ag/AgCl) in aqueous phosphate buffer containing 5 mM methyl viologen, pH 7.0 and 25 °C. The absorption spectrum in the lipid bilayer revealed only one peak at 860 nm, indicating that the LH polypeptides and Zn–BChl*a* formed LH1-type complex in the lipid bilayers on the electrode. The absorption spectrum did not change before and after photocurrent measurements where the cathodic photocurrent was observed. On the action spectrum, a large peak was observed at 770 nm, corresponding to the absorption of Zn–BChl*a* monomer, in spite of the fact that the absorption spectrum corresponded to that of the LH1-type complex. The photocurrent at 860 nm was much smaller than that at 770 nm. The photonenergy absorbed at 860 nm wavelength was probably accumulated inside the LH1-type complex, but little photoexcited electrons can transfer to acceptor levels in methyl viologen. This result may be related to a large quenching of fluorescence

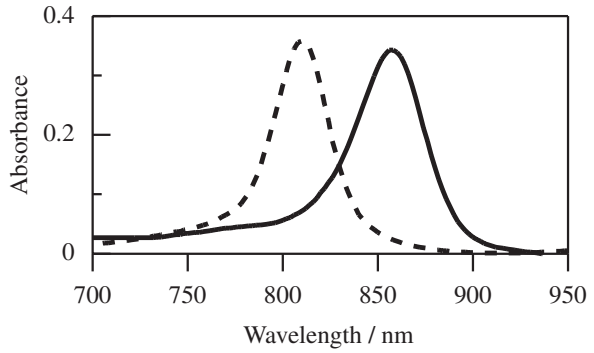

Figure 1. Absorption spectra of solutions containing 0.78% OG micelle (dotted line) with the subunit-type complex and DMPC liposome (solid line) with LH1-type complex at 25 °C.

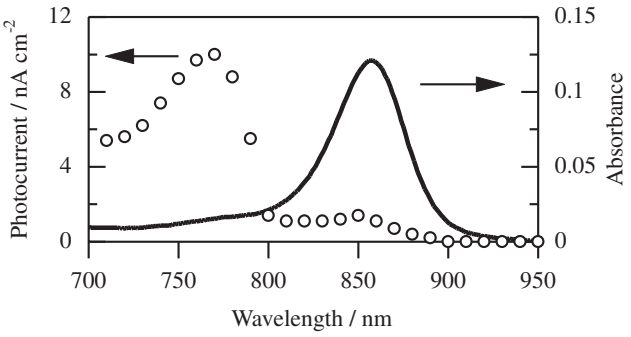

Figure 2. Absorption spectrum (solid line) and action spectrum of photocurrent (◯) of ITO electrode modified with the DMPC lipid bilayer containing LH1-type complex at 25 °C.

of zinc mesoporphyrin, which was observed in presence of the LH polypeptides in OG micelle and in lipid bilayers.[6] It may be essential to construct an efficient transfer system from the photoexcited electron to the acceptor levels to incorporate the reaction center of photosynthetic bacteria in the LH1 complex.

In conclusion, the LH1-type complex containing of LH polypeptides and Zn–BChl*a* was stably formed in the lipid bilayer membrane on an ITO electrode, showing photoinduced current on the electrode. The formation of LH1-type complex in the lipid membrane was confirmed by UV–vis absorption spectrum.

The present work was partially supported by a Grant-in-Aid for Scientific Research Areas (417) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and NEDO International Joint Grant.

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Published on the web (Advance View) August 18, 2003; DOI 10.1246/cl.2003.852