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Construction and Photocurrent of Light-harvesting Core Complexes of Photosynthetic Bacteria in Lipid Bilayers

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Light-harvesting antenna core (LH1-RC) complex isolated from *Rhodospirillum rubrum* (*R.rubrum*) and the assembled LH-RC complex were successfully self-assembled in lipid bilayers on an ITO or a GC electrode at room temperature. The formation of these LH1 complexes in the lipid membrane was confirmed by near infrared (NIR) absorption spectra on an ITO electrode. Interestingly, an efficient energy transfer and photocurrent responses of these complexes on a GC electrode were observed upon illumination of the light-harvesting (LH1) complex at 880nm. In contrast, the photocurrent response of the reconstituted LH1-type complex in which the RC was not contained was hardly observed on the electrode upon illumination of the complex at 880nm.

Key words: Light-harvesting complex, Photosynthesis, Electrode, Photocurrent, Lipid bilayer

1. INTRODUCTION

Artificial assembly of photosynthetic membrane can be useful for development of nanodevices as well as for studying photo-excitation of electron and the subsequent electron transfer in biological process. The electron transfer has been systematically explored by using self-assembly of proteins like Cytochrome c, Heme and photosynthetic reaction center (RC) on electrodes [1,2]. To attain an efficient photo-excitation, one should introduce a light-harvesting system into the artificial membrane. It is interesting to note that the light-harvesting (LH) polypeptides organize pigment complexes so that an efficient energy transfer between pigments may occur [3]. For example, when light energy is absorbed in vivo by purple bacterial LH complexes, it is rapidly transferred to the RC where the light energy is efficiently used to drive chemical reactions [3]. In most types of purple bacteria there are two types of antenna complexes: peripheral LH2 complexes and the LH1 complexes [3]. The structure of the LH2 complex of *R. acidophila* strain 10050 has been resolved to a resolution of 2.0 Å [4]. The LH 2 complex consists of a ring of nine heterodimeric subunits. However, such high resolution structure has yet been determined for the LH1 complex. There are low-resolution projection structures produced by transmission electron microscopy (TEM) [5] of two-dimensional (2D) crystals of the LH1 complex and a 4.8 Å X-ray crystal structure of the LH1-RC core complex [6]. TEM analysis of the LH1-RC complex revealed two types of the complex, monomeric complex from *R. rubrum* [5] and dimeric complexes from *R. sphaeroides* [7]. AFM has also been used to observe the antenna core complex in both natural and reconstituted membranes [8-14].

Our understanding of energy transfer and charge separation in these LH2 and LH1-RC complexes has enabled the first step to be taken towards generating artificial systems that convert light energy into usable electrical current. Previous attempts to produce an artificial, energy-converting electrode system used either the LH1 complexes [15] or the RC [16] immobilized on electrodes. Until now, there have only been a few attempts to immobilize intact 'core' complexes, consisting of both the LH1 and the RC components together, onto an electrode [17,18].

We have recently developed a procedure to create a self-assembled monolayer (SAM) of reconstituted LH1 complex on a transparent indium tin oxide (ITO) electrode modified with amino propyl silane (APS) [15,19]. The NIR absorption spectrum showed that the LH1 complex was stable when immobilized onto the electrode. Our current work extends this approach to the LH1-RC core complex. LH1-RC core complex isolated from *R. rubrum* and the assembled LH1-RC complex were successfully assembled on an ITO or a glassy carbon (GC) electrode modified with lipid bilayers. Efficient energy transfer and photocurrent responses on the electrode could be observed upon illumination of these complexes at 880 nm.

2. EXPERIMENTAL

Growth of R. rubrum bacterium.

The photosynthetic bacteria *R. rubrum* was grown anaerobically in the light in modified Hutner's media as described previously [19].

Isolation and purification of the RC or the LH1-RC core complex of R. rubrum.

RC or LH1-RC core complex isolated from *R. rubrum* was purified essentially as described previously [6]. These complexes from *R. rubrum* were initially solubilized by addition of N,N-Dimetyldodecylamine N-oxide (LDAO) to 0.4% v/v in 20 mM Tris HCl pH 8.0. The OD_{880} of the isolated complex was adjusted to 0.3.

Reconstitution of the LH1 complex.

BChl*a* or ZnBChl*a* was extracted and then purified by the method described in Wakao et al [20]. LH-α and

LH-β polypeptides were extracted from *R. rubrum* by $CHCl_3$ / MeOH and purified by Sephadex LH60 gel chromatography and then by HPLC [20]. The LH complex containing ZnBChl*a* was formed by using n-octyl-β-$_D$-glucopyranoside (OG) micelle as described in Parkes-Loach et al [20]. The LH1 complex was reconstituted by the method described previously [20]. Briefly, the polypeptides of LH-α (1.7μM) and -β (1.7μM) were inserted with ZnBChl*a* (2.2μM) into 0.78% OG micelle at 25 °C to form a subunit-type complex revealing absorption maximum at 810 nm in NIR spectrum. The subunit-type complex is reconstituted to the LH1-type complex, revealing absorption maximum at 860 nm on cooling at 4 °C [15].

Incorporation of the LH1 or the RC or the LH1-RC complex into lipid memblene.

The OG micelle containing the LH subunit-type complex or the RC complex was mixed with the liposome of dimyristoyl phosphatidyl glycerol (DMPG) (0.2mM) in aqueous phosphate buffer. The resulting solution was filtered by a dialysis membrane through which substances with molar weight smaller than 12,000 g/mol was removed and the remaining solution was the liposome containing the complex [17].

Preparation of the LH1 or the RC or the LH1-RC complex assembled on an ITO or a GC electrode.

The basic methods for this have been reported previously [6]. The electrode modified with the lipid bilayers was prepared by a cast method with this liposome solution: 0.1 mL of the liposome solution containing the complex was dropped on electrodes with a surface area of 0.785 cm^2. The solvent was then removed from the solution under reduced pressure at room temperature to form a thin layer of the lipid bilayers incorporating the complex.

NIR and Fluorescence spectra.

NIR spectra were recorded with Hitachi U-2000 and U-3500. CD spectra were recorded with JASCO J-820. Fluorescence spectra were measured with a Nippon Roper fluorometer by using a halogen tungsten light bulb (TS-428 DC), a single monochromator (SP-150M) for selection of the excitation wavelength, a double monochromator (SP-306) and a CCD detector (Spec 10-100 BR / LN) to detect the emitted fluorescence. The slits were set at 0.50 mm for the complex in OG. Slits were set at 1.00 mm for the complex on the electrode. The samples were measured at 25 °C.

Photocurrent measurements.

Photocurrents were measured at -0.2V (vs Ag/AgCl) in a home made cell that contained three electrodes; on an ITO electrode and a GC electrode incorporating the complex as a working electrode, an Ag/AgCl (saturated KCl) as a reference electrode, and a platinum flake as a counter electrode. The working electrode was illuminated with a halogen lamp unit, AT-100HG, through a monochromator, SPG-120S (Shimadzu). The solution consisted of 0.1M phosphate buffer (pH 7.0), containing 0.1M $NaClO_4$ and 5mM methyl viologen.

3. RESULTS AND DISCUSSIONS

Figure 1 shows the NIR absorption spectra of the Qy band of ZnBChl*a* in OG micelle containing the subunit-type complex at 4 °C and in DMPG liposome containing the LH1-type complex at 25 °C [15]. ZnBChl*a* was used because its chemical stability as well as a strong association in the LH complex in comparison to BChl*a*. The Qy band was red-shifted from 810 nm in the OG micelle solution to 860 nm in DMPG 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 transferred from OG micelle to DMPG liposome. An exciton coupling CD signal of ZnBChl*a* was observed around 860 nm corresponding to the absorption band of LH1-type complex in the liposome. The NIR and CD spectra indicated that the LH1-type complex was much more stable in the liposome than the subunit-type complex in OG micelle. For an example, the LH1-type complex in the liposome was stable for a few months although the subunit-type complex in the OG micelle was stable only for a few weeks. ZnBChl*a* was probably associated and placed in a hydrophobic environment surrounded by the LH polypeptides because the Qy band of ZnBChl*a* monomer shows the absorption maximum at 772 nm in the liposome. Similar results were also observed for the LH1-type complex using BChl*a*.

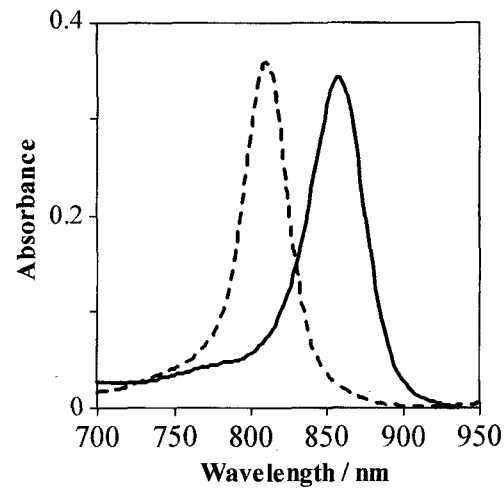

Fig.1 NIR absorption spectra at the Qy band of ZnBChl*a* in the solutions of the OG micelle with the subunit-type complex (dotted line) and the DMPG liposome containing LH1-type complex (solid line).

Further, similar Qy bands of BChl*a* or ZnBChl*a* in DMPG liposome between the native LH1-RC core complex and the assembled LH1-RC complex were observed (see Figs 2 and 3). The band at 860 or 880 nm corresponds to the LH1-type complex using ZnBChl*a* or the special pair of the RC, respectively. And the band at 800 or 770 nm corresponds to accessary BChl*a* or bacteriopheophytin of the RC, respectively. The fluorescence of ZnBChl*a* in the reconstituted LH1-type complex was strongly quenched in DMPG liposome with addition of the RC (data not shown). This result indicated that an efficient energy transfer from ZnBChl*a* in the reconstituted LH1 complex to the RC in the liposome occurred analogous to that in the native LH1-RC core complex [15].

Figure 2 shows the NIR absorption and the photocurrent action spectra of the RC and the native LH1-RC core complex in DMPG lipid bilayers on electrodes. These NIR absorption maxima of the Qy band of BChl*a* in these complexes in DMPG lipid bilayers on an ITO were assigned in the same way in the liposome solution, indicating that these complexes were stable and assembled on the electrode.

Further, photocurrent responses of these complexes showed maxima at the wavelength corresponding to the Qy absorption bands of these complexes. Interestingly, an enhanced photocurrent at 880nm was observed for the LH1-RC core complex in comparison to the native RC complex. When the RC complex only was immobilized on the electrode, an efficient photocurrent was not observed upon illumination at 880 nm (Fig. 2a). Thus, it is likely that this enhanced photocurrent observed in the native LH1-RC core complex can be ascribed to energy transfer from the LH1 to the RC and then an electron transfer from the RC to the electrode may occur.

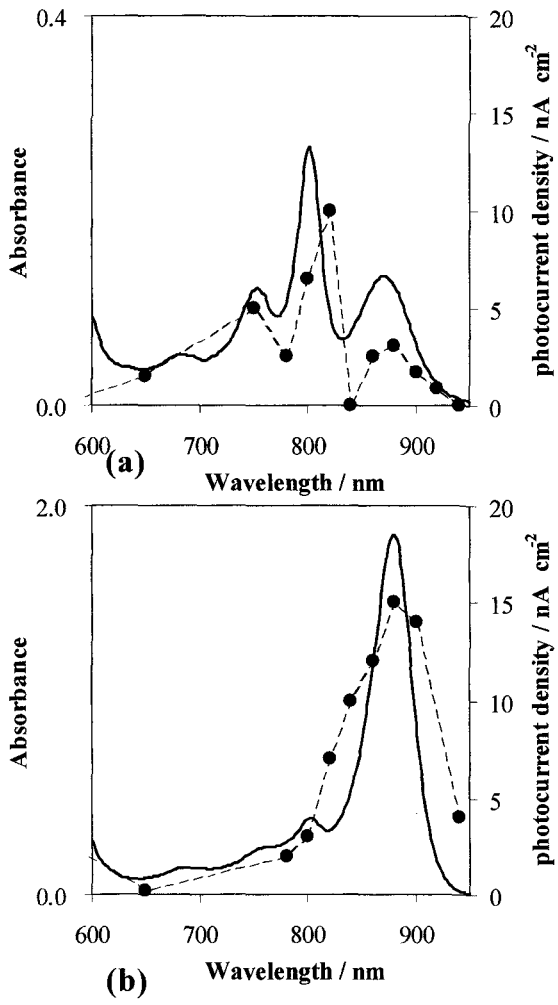

Fig.2 NIR absorption spectrum (solid line) in DMPG lipid bilayers and photocurrent density (dots) on a GC of the native RC (0.46μM) (a) and the native LH1-RC core complex (0.59μM) (b) in DMPG (0.2mM) bilayers.

Figure 3 shows the NIR absorption and the photocurrent action spectra of the reconstituted LH1 and the assembled LH1-RC complex in DMPG lipid bilayers on electrodes. The NIR absorption spectra of the assembled LH1-RC complex revealed a large peak at 860 nm as well as for the reconstituted LH1 complex, indicating that the LH polypeptides and ZnBChl*a* formed LH1-type complex in the lipid bilayers on the electrode, analogous to the native LH1-RC complex (Fig. 2b) [19].These absorption maxima of the Qy band of ZnBChl*a* or BChl*a* in the LH 1 and the assembled LH1-RC complex in DMPG lipid bilayers on an ITO were assigned in the same way in the liposome solution. These NIR absorption data indicated that these complexes were also stable and assembled on an APS-ITO [19]. The photocurrent maximum observed at 880nm may occur from the BChl*a* special pair of RC in the assemble LH1-RC complex (Fig.3b), being inconsistent with the reconstituted LH1 complex (Fig.3a) where the photocurrent density observed at 860 nm occurs from ZnBChl*a* complex in the reconstituted LH1 complex.

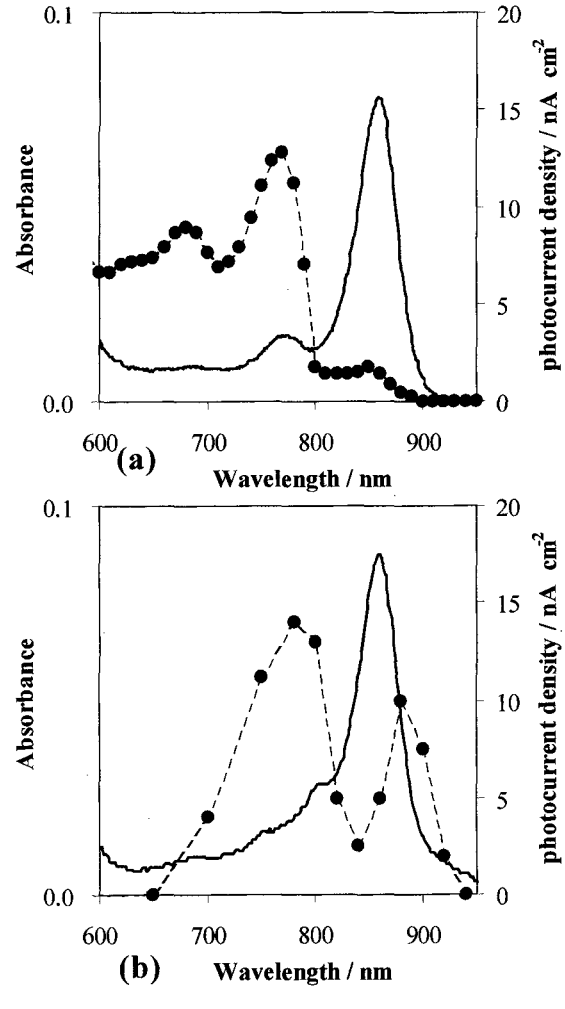

Fig. 3 NIR absorption spectrum (solid line) in DMPG lipid bilayers and photocurrent density (dots) on a GC of reconstituted LH1 complex (0.10μM) (a) and assembled LH1-RC core complex (0.09μM) (b) in DMPG (0.2mM) bilayers.

Interestingly, an enhanced photocurrent was also observed for the assembled LH1-RC complex in comparison to the reconstituted LH 1 complex upon illumination at 880 nm. This enhanced photocurrent in the assembled LH1-RC complex can be ascribed to energy transfer from the LH1-type complex to the RC, being consistent with the native LH1-RC core complex as described above (Fig. 2b). This result related to a large quenching of the fluorescence of ZnBChl*a* in the LH1 type complex with addition of the RC in the liposome solution as described above. These data indicate that the photocurrents were driven by light that was initially absorbed by the LH components, and the incorporation of the RC in the LH1 type complex may be essential to construct an efficient transfer system from the photo-excited electron to the acceptor levels. However, the photocurrent of the assembled LH1-RC complex at 860nm was smaller than that at 770nm which corresponded to the absorption of ZnBChl*a* monomer (Fig. 3b). This photocurrent was mainly generated by light absorbed at 770 nm i.e. from monomeric ZnBChl*a*, analoguous to that in the reconstituted LH1 (Fig. 3a).

In conclusion, LH1-RC core complexes were stably formed in the lipid bilayer membrane on electrodes, showing an enhanced photo-induced electric current on the electrode.

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