

Self-assembled monolayer of light-harvesting core complexes of photosynthetic bacteria on an amino-terminated ITO electrode

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Abstract Light-harvesting antenna core (LH1-RC) complexes isolated from *Rhodospirillum rubrum* and *Rhodopseudomonas palustris* were successfully self-assembled on an ITO electrode modified with 3-aminopropyltriethoxysilane. Near infra-red (NIR) absorption, fluorescence, and IR spectra of these LH1-RC complexes indicated that these LH1-RC complexes on the electrode were stable on the electrode. An efficient energy transfer and photocurrent responses of these LH1-RC complexes on the electrode were observed upon illumination of the LH1 complex at 880 nm.

Keywords Light-harvesting complex · Light harvesting-reaction center complex · Photosynthesis · Electrode · Energy transfer · Photocurrent

Introduction

When light energy is absorbed in vivo by purple bacterial light-harvesting (LH) complexes it is rapidly transferred to the reaction centers (RC) where the light energy is efficiently used to drive chemical reactions (Ke 2001). In most types of purple bacteria there are two types of antenna complexes: peripheral LH2 complexes and the LH1 complexes (Ke 2001). The structure of the LH2 complex of *Rhodopseudomonas acidophila* strain 10050 has been resolved to a resolution of 2.0 Å (McDermott et al. 1995). This LH2 complex consists of a ring of nine heterodimeric subunits. However, such high-resolution structure has not been determined for the LH1 complex yet. There are, however, low-resolution projection structures produced by transmission electron microscopy (TEM) (Karrasch et al. 1995) of two-dimensional (2D) crystals of the LH1 complex and a 4.8 Å X-ray crystal structure of the LH1-RC core complex (Roszak et al. 2003). TEM analysis of the LH1 complexes revealed two types of complex, monomeric complexes from *Rhodospirillum rubrum* (*R. rubrum*) (Karrasch et al. 1995) and dimeric complexes from *Rhodobacter sphaeroides* (*R. sphaeroides*) (Jungas et al. 1999). The recent crystal structure of the LH1-RC 'core' complex from *Rhodopseudomonas palustris* (*Rps. palustris*) reveals that the LH1 complex surrounds the contours of the RC so that the 'core' complex has an overall oval rather than a circular shape (Roszak et al. 2003). This structure showed the RC surrounded by the LH1 complex which consisted of 15 pairs of transmembrane helical α - and β -polypeptides and their coordinated BChls. The complete closure of the RC by the

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LH1 is prevented by a single transmembrane helix called W. Atomic force microscopy has also been used to observe antenna complexes in both natural and reconstituted membranes (Scheuring et al. 2001, 2003, 2004; Fotiadis et al. 2004; Bahatyrova et al. 2004a, b; Stamouli et al. 2003). Scheuring et al. observed the LH1 complex as a minor component together with the major LH2 complexes from *Rubrivivax geratinosus* (Scheuring et al. 2001) in reconstituted membranes and intact LH1-RC core complexes in native photosynthetic membranes (chromatophore) from *Rhodospseudomonas viridis* (Scheuring et al. 2003) and *Rhodospirillum photometricum* (Scheuring et al. 2004). Fotiadis et al. observed the LH1-RC core complex from *R. rubrum* in membranes formed from *Escherichia coli* lipids (Fotiadis et al. 2004). Bahatyrova et al. showed that the LH1 complexes of a mutant lacking the RC from *R. sphaeroides* which form circular, elliptical, and even polygonal ring shapes as well as arcs and open rings (Bahatyrova et al. 2004b) and that the LH1 complexes are positioned to function as an energy collection hub from the LH2 complexes in native membranes (Bahatyrova et al. 2004a).

Our understanding of charge separation and energy transfer in these LH2 and LH1-RC core complexes has enabled the first steps 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 (Ogawa et al. 2002) or RC (Blankenship et al. 1995) immobilized on the 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 (Ogawa et al. 2004; Das et al. 2004).

We have recently developed a procedure to create a self-assembled monolayer (SAM) of reconstituted LH1 complexes on a transparent indium tin oxide (ITO) electrode modified with 3-aminopropyltriethoxysilane (APS) using electrostatic interactions or hydrogen bonding between the electrode surface and the anionic LH1 polypeptides at pH 8.0 (Ogawa et al. 2002). The near infra-red (NIR) absorption spectrum showed that the LH1 complex was stable when immobilized onto the electrode. Our current work extends this approach to the native LH1-RC core complexes. LH1-RC 'core' complexes isolated from *R. rubrum* and *Rps. palustris* were successfully assembled on an ITO electrode modified with APS (APS-ITO). Efficient energy transfer and photocurrent responses could be observed upon illumination at 880 nm.

Materials and methods

Growth of *R. rubrum* and *Rps. palustris* bacterium

The photosynthetic bacteria, *R. rubrum* strain S1 and *Rps. palustris* strain 2.1.6 were grown anaerobically in the light in modified Hutner's media as previously described (Roszak et al. 2003; Visschers et al. 1991).

Purification of the LH1 complex of *R. rubrum*

Chromatophores of *R. rubrum* were prepared as previously described (Visschers et al. 1991). Carotenoid was extracted from chromatophores using benzene. Usually approximately 30 mM OG (1 mM Tris, pH 7.5) was added to dissolve the chromatophores until the near IR absorption band shifted from 873 to 820 nm. Then the resultant aqueous solution was applied to a Sephadex G-100 column (1.5 cm i.d. × 75 cm) to separate the RC and, LH1 complexes with carotenoids and subunit LH/BChl a complexes without carotenoid (B820 complexes). The RC was eluted immediately after the void volume and then the LH1 complexes with carotenoid. Finally the B820 complexes were collected. Absorbance (λ_{\max}) of the LH1 complex in the absence of carotenoid (15 mM OG at 25°C): 372 nm (0.86), 586 (0.23), 870 (1.14). Absorbance (λ_{\max}) of the LH complex in the presence of carotenoid (20 mM OG at 25 °C): 372 nm (0.86), 475 (0.19), 512 (0.22), 546 (0.19), 586 (0.235), 875 (0.9).

Isolation and purification of the core complex of *R. rubrum* and *Rps. palustris*

The LH1-RC core complexes isolated from *R. rubrum* and *Rps. palustris* were purified essentially as described previously (Roszak et al. 2003). These LH1-RC core complexes from *R. rubrum* and *Rps. palustris* were initially solubilized by the addition of LDAO to 0.4% or 1% v/v in 20 mM Tris-HCl pH 8.0, respectively. The LH1-RC core complex of *Rps. palustris* was then separated from the LH2 complexes by sucrose density centrifugation, and was further purified by ion exchange chromatography by DE52 cellulose column. The OD880 of the isolated LH1-RC core complexes was adjusted to 0.3.

Preparation of the core complex assembled on APS-ITO electrodes

The basic methods for this have been reported previously (Ogawa et al. 2002). Transparent indium tin

oxide electrodes were cleaned by immersion in piranha solution ($\text{H}_2\text{O}_2:\text{H}_2\text{SO}_4 = 3:7$). We obtained APS-ITO electrodes by reacting 3-aminopropyltriethoxysilane with the surface of ITO electrodes in dry benzene at 80–90°C for 4 h. The OD880 of the isolated core was adjusted to 0.3. APS-ITO electrodes were immersed in the LH complex and the core complex solutions in Tris–HCl pH 8.0 for 6 h at 4°C, and then rinsed with Tris–HCl pH 8.0. The core complexes were immobilized, as a self-assembled monolayer on an APS-ITO electrode.

Near IR, FT-IR, and fluorescence spectra

Near IR spectra were recorded with Hitachi U-2000 and U-3500. 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 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 LH1 complexes in OG. Slits were set at 1.00 mm for the LH1 complex on the electrode. The samples were measured at 25°C. FT-IR spectra were recorded with Perkin-Elmer Spectrum 2000.

Photocurrent measurements

Photocurrents were measured at –0.2 V (versus Ag/AgCl) in a home made cell that contained three electrodes; an APS-ITO electrode incorporating the core 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 0.1 M phosphate buffer (pH 7.0), containing 0.1 M NaClO_4 and 5 mM methyl viologen.

Results and discussion

Figure 1 shows the NIR absorption spectra of the isolated *R. rubrum* (a) and *Rps. palustris* (b) core complexes in 20 mM Tris–HCl buffer pH 8.0 OG micelle (dotted line) and assembled onto an APS-ITO electrode (solid line), respectively. These spectra show that these core complexes have the absorption maximum at 880 nm with two smaller peaks at 800 and 760 nm. The former peak is attributable to the overlap of bacteriochlorophyll *a* (BChl*a*) in the LH1 complex (880 nm) and the reaction center BChl*a* dimer ‘special pair’ (870 nm) and the latter two peaks to the BChl*a* called ‘accessory’ (800 nm) and bacteriopheophytin (760 nm) in the RC, respectively (Blankenship et al. 1995). The NIR absorption spectra of these core complex on the electrode indicate that these complexes were not denatured when assembled onto an APS-ITO. In the previous study it was apparent that when the RC of *R. rubrum* was assembled, by itself, on the electrode it was relatively labile (Matsumoto et al. 1999). In present study, the complete core complex proved to be quite stable, when assembled onto the electrode. The enhanced stability of the RC surrounded by the LH1 complex probably results from supportive interactions between the two complexes.

Table 1 shows the NIR absorption and fluorescence bands of the LH1 complex of *R. rubrum* and the LH1-RC core complexes of *R. rubrum* and *Rps. palustris* in OG micelles and on an APS-ITO electrode. The fluorescence bands of these core complexes on the electrode are identical to those in the OG micelles, again indicating that these complexes are stable when assembled onto the APS-ITO electrode. Interestingly, when illuminating at 880 nm the fluorescence emission of BChl*a* molecules in the LH complex of *R. rubrum* on the APS-ITO was strongly quenched, due to the presence of the RC of *R. rubrum*. This indicates that an efficient energy transfer from BChl*a* in the LH1 complex to the RC in the core complex is still occurring on the electrode (data not shown) (Ogawa et al. 2004).

Fig. 1 NIR absorption spectra of the isolated *R. rubrum* (a) and *Rps. palustris* (b) core complexes in 20 mM Tris–HCl buffer pH 8.0 OG micelle (dotted line) and assembled onto an APS-ITO electrode (solid line)

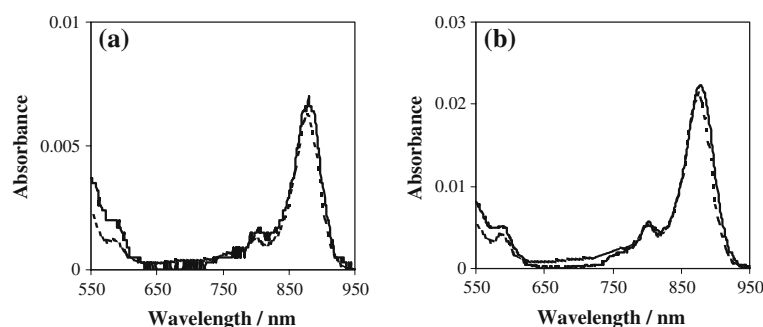


Table 1 NIR absorption and fluorescence bands of the LH1 complex of *R. rubrum* and the LH1-RC core complexes of *R. rubrum* and *Rps. palustris*

		In OG micelle		On APS-ITO	
		Absorbance (nm)	Fluorescence (nm)	Absorbance (nm)	Fluorescence (nm)
<i>R. rubrum</i>	LH1	870	890	870	890
<i>R. rubrum</i>	LH1-RC	880	900	880	900
<i>Rps. palustris</i>	LH1-RC	878	900	878	900

FT-IR spectra of the LH complex of *R. rubrum* and the LH1-RC core complexes of *R. rubrum* and *Rps. palustris* assembled on the APS-ITO show the absorptions at 1650 and 1550 cm^{-1} . These bands can be assigned to the amide I (C=O stretching vibration) and amide II (N–H deformation vibration) bands of α helical conformation, respectively (Miura et al. 1998). These results indicate that the LH polypeptides are in the same α helical configurations on the ITO electrode as in OG micelles (Parkes-Loach et al. 1988; Kashiwada et al. 2000).

In summary, all of these results indicate that the LH1 and LH1-RC core complexes were not denatured by binding to the APS-ITO surface. Similar results were obtained using Langmuir–Blodgett (LB) films to lay down LH1 complex membranes as a layer on a glass substrate (Iida et al. 2000). The LH1 complex of *R. rubrum* and the LH1-RC core complexes of *R. rubrum* and *Rps. palustris* on the APS-ITO electrode were stable enough to handle at 4°C in the dark condition least 24 h.

Figure 2 shows the time course of the photocurrent generated from the LH1-RC core complex, LH1 complex or the RC of *R. rubrum* assembled onto an APS-ITO when the electrode was illuminated with a

pulse of light at 880 nm. It is clear in Fig. 2 that an enhanced photocurrent was observed for the LH1-RC core complex. In contrast no photocurrent was observed for either LH1 complex or the RC. Under our experimental conditions a cathodic photocurrent was observed, implying that one-way electron transfer from pigments in the LH1-RC core complex to methyl viologen was occurring as shown in Fig. 3 (Nagata et al. 2003; Imahori et al. 2000).

Figure 4 shows excitation spectrum of the photocurrent density (dots) and the NIR absorption spectra (solid line) from *R. rubrum* (a) and *Rps. palustris* (b) LH1-RC core complexes assembled onto an APS-ITO, respectively. These photocurrent responses showed a maximum at the wavelength corresponding to the absorption bands of the complex. Interestingly, an enhanced photocurrent was observed especially upon illumination at 880 nm for both *R. rubrum* (a) and *Rps. palustris* (b) LH1-RC core complexes. The quantum yield of the photocurrent was 0.05% for both the *R. rubrum* and *Rps. palustris* complexes (Imahori et al. 2000). When the LH1 complex of *R. rubrum* alone, was immobilized on the electrode, the observed photocurrent was mainly generated by light absorbed at 770 nm, i.e. from monomeric BChl_a (Nagata et al. 2003). Furthermore, when the RC complex of *R. rubrum* only was immobilized on the electrode, an efficient photocurrent was not observed upon illumination at 880 nm as shown in Fig. 2. Thus, the enhanced photocurrent observed at 880 nm in the assembled LH1-RC core complex can be ascribed to energy transfer from the LH1 to the RC and then electron transfer from the electrode to the RC as shown in Fig. 3 (Ogawa et al. 2004). This data indicates that the LH1-RC core complex was well organized on the ITO and the photocurrents were driven by light that was initially absorbed by the LH components.

In conclusion, the SAM method is clearly successful in allowing assembly of functional LH1-RC core complexes on the electrode. This has been confirmed by NIR absorption spectroscopy, demonstrating that the photocurrent response, which is derived from electron transfer between the RC and the electrode, is enhanced by illumination at 880 nm.

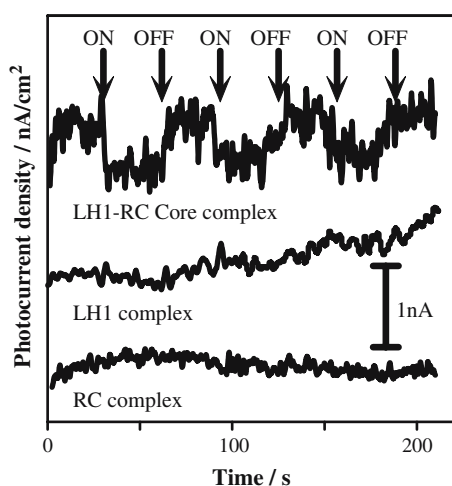


Fig. 2 Time course of the photocurrent of the LH1-RC core complex, LH1 complex or the RC complex of *R. rubrum* on an APS-ITO electrode when the electrode is illuminated with pulsed light (880 nm) firing continuously for 30 s

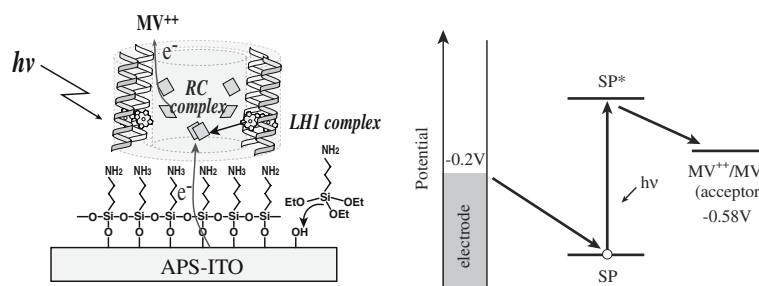
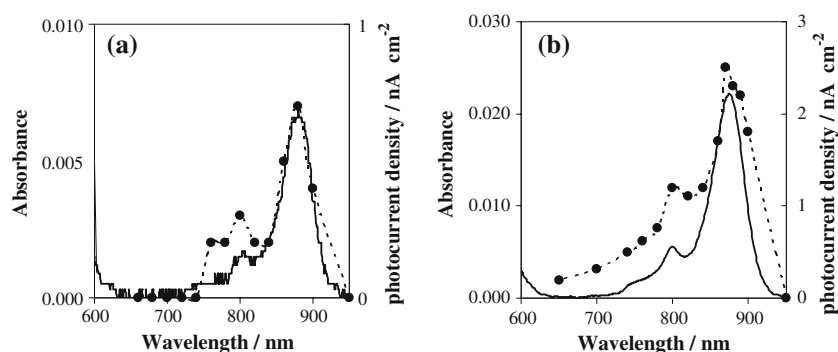


Fig. 3 Schematic drawing of LH1-RC core complexes on an APS-ITO electrode generated cathodic photocurrent which shows the electron flow from the complex to methyl viologen

(left) according to the cathodic photocurrent as shown in Fig. 2. Energy diagram for cathodic photocurrent generation by the LH1-RC core complex (right)

Fig. 4 Photocurrent density (dots) and NIR absorption spectrum (solid line) of LH1-RC core complexes form: (a) *R. rubrum* and (b) *Rps. palustris* assembled on an APS-ITO electrode



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