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Immobilization and Photocurrent Activity of a Light-Harvesting Antenna Complex II, LHCII, Isolated from a Plant on Electrodes

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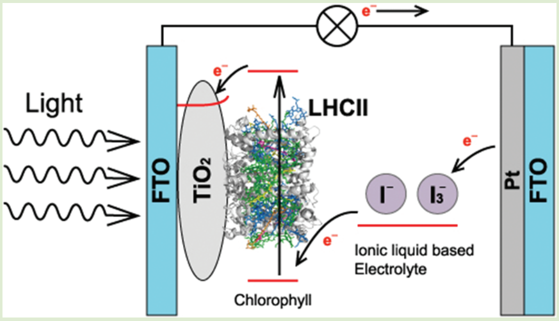
Supporting Information

ABSTRACT: A light-harvesting (LH) antenna complex II, LHCII, isolated from spinach was immobilized onto an indium tin oxide (ITO) electrode with dot patterning of 3-aminopropyltriethoxysilane (APS) by utilizing electrostatic interactions between the cationic surface of the electrode and the anionic surface of stromal side of the LHCII polypeptide. Interestingly, the illumination of LHCII assembled onto the ITO electrode produced a photocurrent response that depends on the wavelength of the excitation light. Further, LHCII was immobilized onto a TiO_2 nanostructured film to extend for the development of a dye-sensitized biosolar cell system. The photocurrent measured in the iodide/tri-iodide redox system of an ionic liquid based electrolyte on the TiO_2 system showed remarkable enhancement of the conversion efficiency, as compared to that on the ITO electrode.



Protein–pigment complexes in photosynthetic membranes convert solar light into chemical energy with high efficiency.[1] Light-harvesting (LH) antenna complexes increase the absorption of light energy, even under low illumination conditions. Energy absorbed by antenna complexes is subsequently transferred to the reaction centers (RCs), photosystem I (PSI) or II (PSII).[1−6] Interest in the assembly of these antenna complexes on substrates is fuelled by the drive to provide insight into the structure and function of the energy harvesting mechanisms, given that self-assembled chlorophyll pigments in membrane proteins play a key role in the efficient funnelling of the harvested energy.

We have recently demonstrated photo-to-current conversion systems that were created using a self-assembled monolayer (SAM) of the LH1 complex or LH1-RC complexes,[3] isolated from photosynthetic bacteria, on Au[7] or indium tin oxide (ITO)[8,9] electrodes. Furthermore, the generation of photo-induced current using the photosensitized functions of RC, PSI, and PSII assembled on electrodes has been attempted,[10−16] because these complexes perform important roles in photo-induced charge separation. However, little attention has been paid to light-harvesting complex proteins (LHCs), even though these light energy-harvesting complex proteins are known to perform the role of light harvesting and photoenergy transfer to reaction centers. Thus far, there have been few attempts at immobilizing the LHCII onto an electrode to gain insight into the photocurrent activity of the complex. Our understanding of energy transfer in these complexes has engendered the first steps toward generating artificial systems that convert light energy into usable electrical current.

The light-harvesting chlorophyll-*a*/*b*-protein complex of photosystem II (LHCII), which plays a key role in the photoenergy transfer to PSII or PSI, is obtained in pure form from spinach; it is stable in comparison with other photosynthetic proteins.[4−6] The structure of the LHCII complex has been obtained by X-ray crystallography (4.8 Å), which shows that the LHCII complex comprises a trimer containing chlorophyll-*a*/*b* and carotenoids.[4−6] LHCII consists of eight chlorophyll-*a*, six chlorophyll-*b*, two lutein, one 9′-*cis*-neoxanthin, and one violaxanthin unit as shown in Scheme 1b,c and in higher green plants is a major light harvesting complex that

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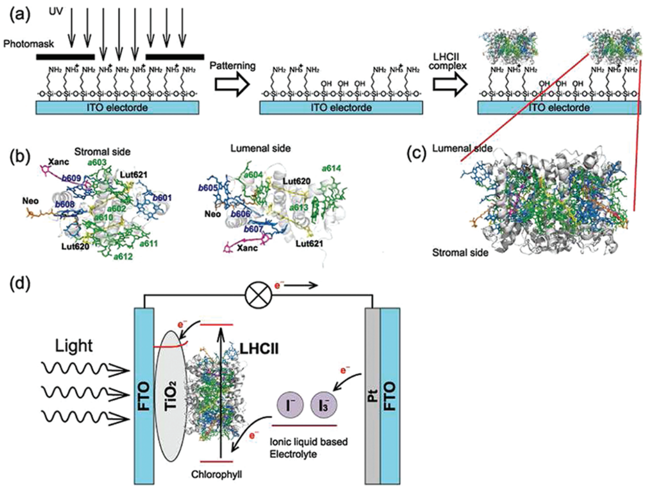
Scheme 1. (a) Schematic Model of Assembly of LHCII Complex (Protein Data Bank Code 1rwt) on an ITO Electrode Modified with the APS Pattern. (b) Pigment Location in LHCII Monomer at Lumenal and Stromal Sides: Green, Chl-*a*; Blue, Chl-*b*; Yellow, Lutein; Orange, Neoxanthin; Magenta, Xanthophyll-Cycle Carotenoids. (c) Side View of LHCII Trimer. (d) Schematic Model of the DSSC (Dye-Sensitized Solar Cells) Using LHCII Immobilized onto a TiO_2/Fluorine Doped Tin Oxide (FTO) Electrode



serves as the principal solar energy collector in the photosynthesis of green plants and presumably also functions in photoprotection under high-light conditions.[4,5]

Because LHCII contains a number of dye molecules, it is an attractive photosensitizer material with wide absorption bands. The illumination of LHCII with sunlight produces an efficient photocurrent response. All of these properties make LHCII highly appealing for potential light harvesting and electron-transfer functions in the development of photoenergy harvesting and conversion materials.[17]

Figure 1a shows the absorption spectra of LHCII isolated from spinach in 50 mM Tris-HCl buffer and after immobilization on an APS-ITO electrode. Both spectra of the LHCII complex had an absorption maximum at ca. 680 nm with two peaks at 651 nm and 674 nm, respectively. The former peak is attributable to the Qy band of chlorophyll-*b* in the LHCII complex, and the latter, to the Qy band of chlorophyll-*a*.[1] The Soret bands of the chlorophylls (chlorophyll-*a*: 435 nm, chlorophyll-*b*: 472 nm), which have molar extinction coefficients that are much larger than that of carotenoids, were also observed. The absorption spectrum of LHCII on APS-ITO was largely consistent with the spectrum in aqueous solution, indicating that the pigment complexes in LHCII were successfully assembled on the electrode without denaturation.

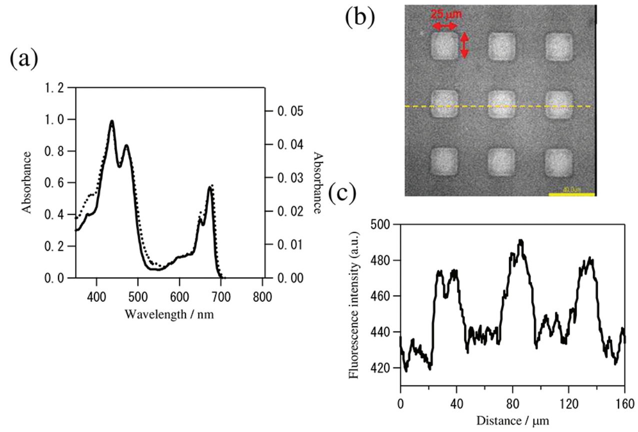


Figure 1. (a) Absorption spectra of LHCII in 50 mM Tris-HCl buffer (broken line) and after immobilization on an APS-ITO electrode (solid line). (b) Fluorescence image of LHCII assembled on ITO electrode modified with APS pattern, 160 × 160 *μ*m. (c) Fluorescence intensity profile along the dashed yellow line of panel b.

An illustration of the LHCII complex assembled on an APS-ITO electrode with patterns is presented in Scheme 1a. APS-ITO was irradiated with a UV lamp with a dot mask in the patterning of the SiOH surface. Figure 1b,c shows the fluorescence images of LHCII on the electrode with APS dot patterns and the fluorescence intensity profile, respectively. The fluorescence image of LHCII on the APS-ITO (Figure 1b) was clearly observed with patterns, indicating a 25 × 25 *μ*m dot pattern with the fluorescence emission. The fluorescence line profile also presents a width of 25 *μ*m (Figure 1c). Interestingly, no fluorescence of LHCII was observed on the SiOH domain that is not modified with APS. This selective attachment indicates that the assembly of LHCII onto the APS-ITO electrode was affected by electrostatic interactions between the cationic surface of the electrode and the anionic stromal side of the LHCII polypeptide (Scheme 1b, Figures S1 and S2 of the Supporting Information, SI) which includes anionic residues such as aspartic acid or glutamic acid in the sequence of the protein as shown in Figure S2 of the SI.[4,5,20]

Photocurrent measurements were carried out in an aqueous solution containing methyl viologen (MV^{2+}) as an electron acceptor.[7−9] Figure 2a shows the photocurrent responses of the LHCII complex on an APS-ITO electrode upon illumination of the electrode with pulsed light at 470 nm (5.2 mW/cm^2) and 670 nm (2.9 mW/cm^2), respectively. Under the employed

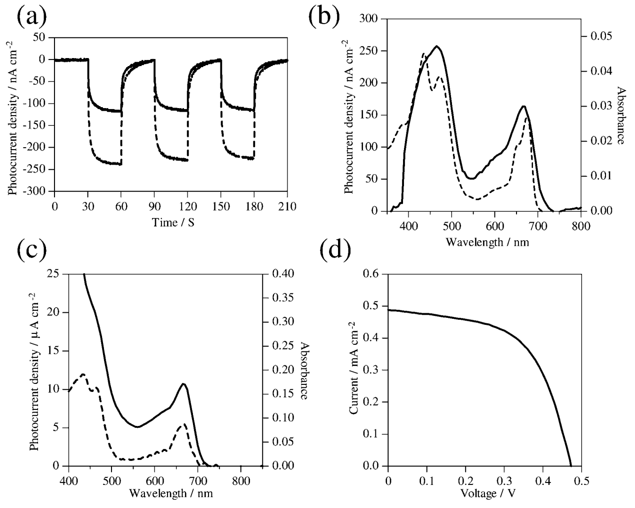


Figure 2. (a) Photocurrent response of LHCII assembled on an APS-ITO electrode in buffer solution when illuminated at 670 nm (solid line) and 465 nm (dotted line). (b) Action (solid line) and absorption spectra (dotted line) of LHCII assembled on an APS-ITO electrode. (c) Action spectra (solid line) and absorption spectra (dotted line) of LHCII assembled on a TiO_2 electrode. (d) *I*/*V* curve of DSSCs based on LHCII.

experimental conditions, cathodic photocurrents were observed, implying that photoinduced electron transfer from LHCII to MV^{2+} occurred more efficiently than the back electron transfer to the electrode.[7−9] Thus, the photocurrent resulting from initial absorption of light by the LHCII component can be ascribed to the electron transfer from the excited state of the chlorophyll pigments in the LHCII complexes to MV^{2+}, subsequent to which electron transfer from the electrode to the ground state of the pigments may occur. The reduced MV^{2+} diffuses to the counter electrode and transfers an electron, resulting in the generation of a cathodic photocurrent.[7] Figure 2b shows the action spectra expanded from the point where the largest negative current was obtained as shown in Figure 2a, in conjunction with the absorption spectrum of the LHCII complex assembled onto APS-ITO. The photocurrent response depended on the absorption wavelength in the buffer solution, which was slightly broader than the absorption spectrum of LHCII. Interestingly, efficient photocurrent responses were observed upon illumination around 470 and 670 nm corresponding to chlorophyll-*a*/*b* in the complex, and this response was particularly efficient for chlorophyll-*a*. Thus, the enhanced photocurrent observed in the assembled complex can be ascribed to electron transfer from chlorophyll-*a* in LHCII to MV^{2+}. However, there appears to be too long a distance from the surface of the electrode for electron transfer to proceed through LHCII whose height is 4.2 nm according to X-ray crystallographic structural analyses (Figure S1 of the SI).[4,5,20] In the trimeric LHCII, all 24 chlorophylls from the stromal layer are organized into two irregular circular rings (Figure S1b of the SI).[4] The remaining nine Chl-*a* and nine Chl-*b* form the outer ring and are arranged in a mosaic pattern, with three Chl-*b* alternating with three Chl-*a*. Energy transfer between two lumenal clusters is much less efficient than that within a stromal layer, due to larger separation distances in the former.[4,5] It is believed that these luminal chlorophyll clusters might serve as upstream energy collectors, absorbing energy and transmitting it to the stromal chlorophylls in a relatively independent manner.[4] The energy absorbed by the stromal chlorophylls is quickly focused on chlorophyll-*a* clusters such as Chl-*a* 612/Chl-*a* 611 and Chl-*a* 610 (Figure S1b of the SI) and is further transmitted to the neighboring LHCs or reaction centers. If the stromal side of the anionic peptides of LHCII faces the cationic surface of the electrode as a result of the electrostatic interaction, there are five chlorophyll-*a* and three chlorophyll-*b* within a distance of less than 2 nm from the electrode side, and three chlorophyll-*a* and three chlorophyll-*b* are within a distance less than 2 nm from the electrolyte side (Figure S1b of the SI).[4,5,20] Because electron transfer over a distance of 2 nm or less is possible, this transfer could occur by an electron-hopping mechanism between the pigments in LHCII, and further electron transfer may occur from these Chl-*a* derivatives, possibly from Chl-*a* 612/Chl-*a* 611 or Chl-*a* 610 to MV^{2+}.[4,20]

These data indicate that dot patterning produced a well-organized array of LHCII on the ITO and that the photocurrents were driven by light that was initially absorbed by the pigments of the LHCII components. This is the first report of the use of LHCII as a sensitizer in a photoconversion device; however, detailed experiments will be required to elucidate the exact mechanism of this reaction.

Additionally, LHCII was assembled onto a TiO_2 nanostructured film to extend the surface area for developing energy-harvesting materials.[18] Figure 2c shows the absorption and photocurrent response spectra of the LHCII complex assembled on TiO_2 in the iodide/tri-iodide redox system of an ionic liquid based electrolyte as shown in Scheme 1d. The absorption spectrum of LHCII on the TiO_2 nanostructured film was approximately consistent with the spectrum in aqueous solution, indicating that the pigment complexes in the LHCII system assembled on the TiO_2 film were stable and the native state was preserved. It is likely that assembly of LHCII on the electrode was facilitated by the many carboxyl residues such as aspartic acid or glutamic acid, contained in the protein sequence of LHCII, which binds easily to the surface of TiO_2. Figure 2b,c shows that the photocurrent response also depended on the wavelength of the illumination and the action spectrum was broadened relative to the absorption spectrum of LHCII in the buffer solution. Remarkable enhancement of the photocurrent was observed when measured in the iodide/tri-iodide redox system of an ionic liquid based electrolyte on the TiO_2 electrode in comparison to that on the ITO electrode (Table S1 of the SI). Figure 2d shows the I−V characteristics of the dye-sensitized solar cells (DSSC) for LHCII (Scheme 1d).[18,19] Table S2 of the SI summarizes the I−V characteristics [open-circuit voltages (Voc), short-circuit currents (Jsc), fill factors (FF), and conversion efficiencies (η)] of the DSSC under one-sun conditions.[18,19] LHCII could be organized on TiO_2 by electrostatic and hydrogen-bonding interactions between the anionic TiO_2 surface and the face of the protein. It can be supported by anodic current in DSSC that LHCII is adsorbing oppositely compared with an ITO system with cathodic current.

In conclusion, LHCII was successfully immobilized on an ITO electrode with APS or with APS dot patterning and on a TiO_2 electrode. Immobilization in all cases was confirmed by absorption and fluorescence spectroscopy and photocurrent responses. The photocurrent action spectrum was reasonably consistent with the absorption spectrum of LHCII, indicating chlorophyll-*a* clusters on the stromal chlorophylls play an important role on the photocurrent activity. Interestingly, the conversion efficiency of the photocurrent measured in the iodide/tri-iodide redox system of an ionic liquid based electrolyte on the TiO_2 electrode was significantly enhanced in comparison to that on the APS-ITO electrode. These results indicate that LHCII acts as an effective photosensitizer material with a wide absorption band, and it is a potential candidate for photoenergy conversion materials.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Structure and photocurrent response of LHCII and experimental section. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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