Molecular Assembly of Light-harvesting Antenna Complex on ITO Electrode

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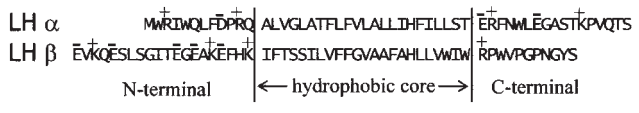
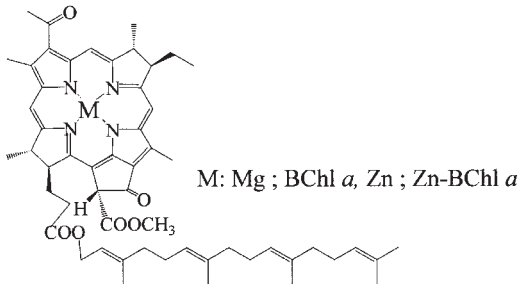
Light-harvesting (LH) antenna complex was successfully self-assembled on an ITO electrode modified with 3-aminopropyltriethoxysilane (APS-ITO). The reconstitution of the LH complex using LH polypeptides, isolated from photosynthetic bacteria, with Zn-BChl *a* formed an excitonically coupled state of Zn-BChl *a* complex on the electrode. Near infra-red (NIR) absorption and IR spectra indicated that the excitonically coupled Zn-BChl *a* and the LH polypeptides in an α-helical configuration were assembled on APS-ITO.

The light reactions occur in closely coupled pigment systems of the LH and RC complexes in photosynthetic bacteria; light energy is absorbed by excitonically coupled antenna pigments bound to the LH polypeptides, and the excitation energy is efficiently transported to RC (Reaction Center), where the energy is converted into a stable trans-membrane charge separation. However, the relation between the structure and function of the LH 1 complex in photosynthetic bacteria is not well understood because of the lack of X-ray crystallographycal analysis at an atomic resolution, although the structures of the RC and LH 2 complex have been reported.[1] Thus, the construction of the LH 1 complex monolayer, with the excitonically coupled pigments, on a transparent ITO electrode will be useful for studying the structure, the energy transfer via individual pigments, and for imaging its natural environment by the use of single molecule spectroscopy or AFM.[2,3] Recently, the techniques of self-assembled monolayers of organic molecules on the ITO or Au electrode have been developed. However, no work has yet focused on the construction of self-assembled monolayers (SAM) of LH 1 complexes on the electrodes, because the excitonically coupled pigments are not stable and are difficult to handle.

We now describe the self-assembly of reconstituted LH complexes on an ITO electrode modified with 3-aminopropyltriethoxysilane (APS-ITO). The APS-ITO surface will be useful for the SAM construction of the LH complex on the ITO using either electrostatic interactions or hydrogen bonds between the ITO and the LH complex. The native LH α and β polypeptides (Scheme 1) were separately isolated from the photosynthetic bacterium, *Rhodospirillum rubrum*. Zn-BChl *a* was obtained as described previously.[4] Zn-BChl *a* (Scheme 2) was used because its chemical stability as well as a strong association in the LH complex in comparison to BChl *a*.[6] The LH complex was formed using Zn-BChl *a* in *n*-octyl-β-D-glucopyranoside (OG) micelles as previously described.[5–7] The ITO electrode was chemically



Scheme 1. Amino acid sequences of LH polypeptides from *R. rubrum*.



Scheme 2. Structure of bacteriochlorophyll *a* (BChl *a*) derivatives.

cleaned by immersing it in Piranha solution (98% H_2SO_4/33% H_2O_2 = 7/3) for 2 min and dried with N_2 gas (bare ITO). To assemble the reconstituted LH complexes, amino groups were introduced onto the surface of ITO electrode by the silane coupling agent, APS. This reacted with –OH groups on the surface of ITO electrode, where the bare ITO were refluxed with 0.01% APS benzene solution. The surface concentration of the amino groups on the ITO was about 0.5 nmol cm^{-2} as determined by titration with 4-nitrobenzaldehyde.[8] The self-assembly of the LH complexes on the APS-ITO was performed by immersing the APS-ITO into the OG micellar solution containing the LH complex over night at 4 °C, followed by sufficient rinsing with water (and by dialysis to remove OG) and by drying it with N_2 gas.

Figure 1 shows the NIR absorption spectra of the Qy band of the Zn-BChl *a*, reconstituted with LH polypeptides in the OG micellar solution. It is clear from Figure 1 that the absorption maximum of the Qy band is red-shifted to 858 nm with LH $\alpha\beta$, to 864 nm with LH α alone, and to 830 nm with LH β alone. The Qy band of monomeric Zn-BChl *a* in organic solvent is 774 nm.[6] It is

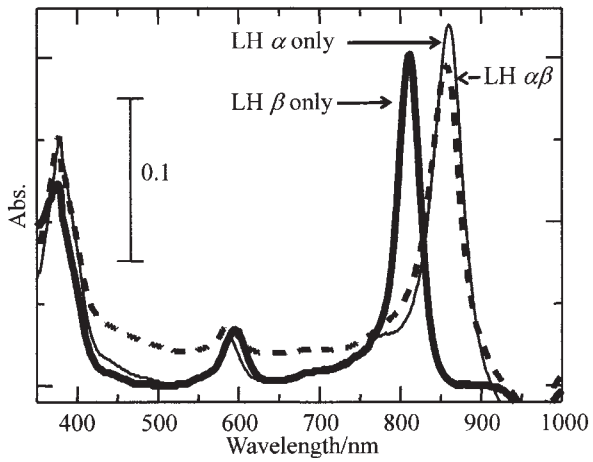


Figure 1. Visible absorption spectra of Zn-BChl *a* complex in the presence of LH α only, LH β only and LH $\alpha\beta$.

likely that the LH complex showing the absorption maximum at 820 nm is analogous to the subunit-type complex. The complex showing an absorption maximum at 858 nm is analogous to the LH 1-type complex, which is consistent with a further aggregation of the subunit-type of complex.[5–7,9] The red shift of the Qy absorption band of Zn-BChl *a* in the complexes with the LH polypeptides indicates that these Zn-BChl *a* molecules are excitonically coupled. This has been confirmed by CD spectroscopy.[5–7] These results indicate that each of the complexes described above (i.e. with LH α or LH β alone or with LH α and LH β) can be assembled on the ITO electrode.

FT-IR spectra of the LH complexes assembled on the APS-ITO show the absorptions at 1650 cm^{-1} and 1550 cm^{-1}. These bands are assigned to the amide I and amide II bands, respectively. These results indicate that the LH polypeptides are in an α-helical conformation on the ITO electrode, consistent with the configuration of the LH polypeptides in OG micelle in which α-helical content of the LH polypeptides is about 50% in OG micelle.[5,6]

Figure 2 shows the NIR absorption spectra of the Qy band of Zn-BChl *a* in reconstituted LH complexes on the APS-ITO. The absorption maximum observed at 864 nm with LH α alone, at 830 nm with LH β alone, and at 858 nm with LH $\alpha\beta$, respectively, is assigned, in the same way as described above, to the red-shift of the Qy band of the Zn-BChl *a* in the reconstituted LH complex in the OG micelle, respectively, as shown in Figure 1. These NIR absorption data indicate that the reconstituted LH complexes are assembled on the APS-ITO. Interestingly, an enhanced absorption of Zn-BChl *a* with LH β alone was observed in comparison to that of Zn-BChl *a* with LH α alone or LH $\alpha\beta$. The reason is not clear at present. In contrast, no coverage of the LH complexes was observed on the bare ITO electrode (data not shown). These results imply that modification of the surface of ITO electrode with the amino groups is useful for the stable assembly of the LH complexes on the ITO. The reconstituted LH complexes using BChl *a*, however, were not assembled on the APS-ITO, indicating that the stability and the stronger association of Zn-BChl *a* in the LH complex are helpful for the LH complex-formation on the interface. Without Zn-BChl *a* the LH complex denatures since the proteins and BChl *a* are unstable at an air-substrate interface.

In conclusion, the reconstituted LH complexes with Zn-BChl *a* are stably self-assembled on the APS-ITO. This method will be useful for the self-assembly of the LH and RC complexes in order to study the energy and electron transfer reactions between individual pigments in the supramolecular complexes on the electrode.

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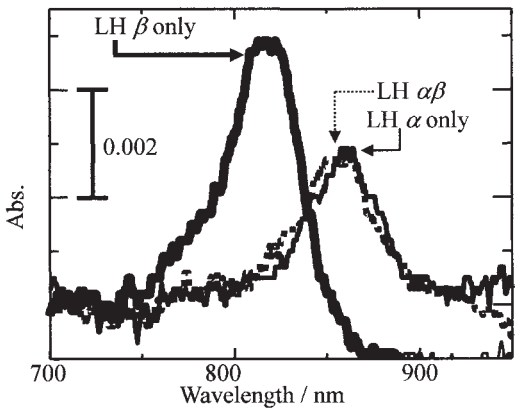

Figure 2. NIR absorption spectra of Zn-BChl *a* complex in the presence of LH α only, LH β and LH $\alpha\beta$ on the APS-ITO.

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