Molecular Assembly of BChl *a* Complexes onto ITO Electrode Using Synthetic Light-harvesting Model Polypeptides Bearing Spermine Derivative

Tsuyoshi Ochiai,[1] Mitsuhiro Ota,[1] Takahide Asaoka,[1] Tomoya Kato,[1] Shinichiro Osaka,[1] Takehisa Dewa,[1] Keiji Yamashita,[1] Hideki Hashimoto,[2] and Mamoru Nango*[1]

[1]*Materials Science and Engineering, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466-8555*

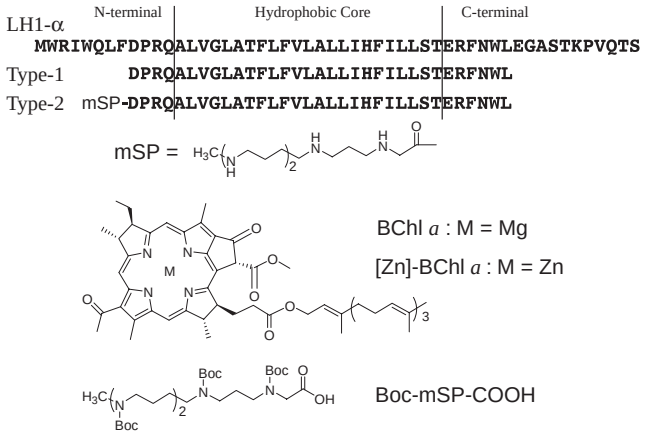
[2]*Department of Physics, Graduate School of Science, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585*

(Received August 28, 2007; CL-070926; E-mail: nango@nitech.ac.jp)

1α-Helix hydrophobic polypeptide bearing spermine, which has similar amino acid sequences to the hydrophobic core in the native photosynthetic light-harvesting (LH) 1-α polypeptide from *Rhodospirillum* (*Rsp.*) *rubrum*, was synthesized. Interestingly, an enhanced photoelectric current was observed when BChl *a* complexes together with the LH1-α model polypeptide were assembled onto an ITO electrode.

Self-assembling of polypeptides and pigments have emerged as powerful techniques in the de novo design of native protein-like structures.[1] Recently, models for the LH complex of photosynthetic bacteria have been investigated to clarify or apply of their efficient energy and electron transfer.[2,3] To perform an efficient energy and electron transfer, one should assemble the artificial LH complex with a defined orientation onto an electrodes. In our previous papers, immobilizing of artificial LH complexes on a gold electrode using *Rhodobacter* (*Rb.*) *sphaeroides* LH1-β model polypeptides bearing cysteine group and the photocurrent activity of the complexes was studied.[4] However, these model polypeptides could not use BChl *a* derivatives as an electron donor because of their self-assembling properties.[5]

We now report the molecular assembly of BChl *a* derivatives on an ITO electrode with a defined orientation, using synthetic LH model polypeptides (Type-1 and Type-2 in Scheme 1) which have similar amino acid sequences to the hydrophobic core in the native LH1-α polypeptide from *Rsp. rubrum*. The aim of this is to gain insights into the structural requirements for assembly of the LH1 complexes with a defined orientation on a solid substrate. Synthetic model polypeptides with spermine derivative at N-terminal, which can be easily bound with an ITO surface, were used. Further, we selected the LH1-α type polypeptide which either forms a stable LH1-like complex with [Zn]-BChl *a* or BChl *a* monomer complex in OG micelles.[6] The monomer complex is expected to perform enhanced photocurrent using NIR light.[4]



Scheme 1. The amino acid sequences of *Rsp. rubrum* LH1-α and its synthetic model polypeptides and the structure of BChl *a* derivatives and Boc-mSP-COOH.

BChl *a* was isolated from the carotenoidless mutant G9 of *Rsp. rubrum* and purified by HPLC as described elsewhere.[5] [Zn]-BChl *a* was prepared from BChl *a* via transmetalation as previously reported.[7] The native LH1-α and -β polypeptides, separately isolated from the LH1 complex of *Rsp. rubrum*, were prepared as previously described.[8] LH1-α model polypeptides, Type-1 and Type-2 polypeptides, were prepared by solid-phase peptide synthesis using a Wang resin, N^{α}-Fmoc amino acids, HBTU and HOBt. Type-2 polypeptide was synthesized by condensation of Boc-mSP-COOH, prepared from spermine tetrahydrochloride as previously reported on N-terminal of Type-1. The cleavage reaction was performed as described previously[9] and the purified product was confirmed by TOFMS spectrometry (Type-1: 3828.3 Da, Type-2: 4085.1 Da). The molecular assembly of pigments together with the synthetic polypeptides was carried out as previously reported.[5] Complexes were immobilized on self-assembly monolayers (SAMs) by immersing of the ITO electrode in sample solution ([polypeptides] = 3.45 μM, [pigments] = 2.41 μM, [OG] = 26.7 mM) at 4 °C for 12 h. Then, the ITO electrode was washed by MilliQ and dried by N_2 flow. Photocurrents were measured at −0.2 V (vs. Ag/AgCl) in a homemade cell that contained three electrodes as described previously.[10]

The Qy band of [Zn]-BChl *a*-monomer in OG micelles (770 nm) is red-shifted to 849 nm in the presence of Type-1 or -2 polypeptide at 4 °C (Supporting Information Table S1).[11] This is analogous to LH1-type complex that forms with either LH1-α and LH1-β or LH1-α alone. This result again shows that the [Zn]-BChl *a* complex can be organized by this synthetic hydrophobic 1α-helix polypeptide in OG micelles.[12] This result suggests that both the amino acid sequences both at N- and C-termini segments are not crucial effect on formation of the LH1-type complex (Table S1).[11,12] In contrast, similar red shift of the Qy band is not observed for BChl *a* in the presence of Type-1 or -2 polypeptide. Instead, in this case the Qy band was only red-shifted to 782 nm and aggregation peak of BChl *a* was not detected, indicating the formation of monomer complex of Type-2/BChl *a*.

FT-IR spectra of Type-2/[Zn]-BChl *a* complex immobilized on the electrode show at 1665 and 1544 cm^{-1}, Type-2/BChl *a* complex immobilized on the electrode showed absorptions 1665 and 1544 cm^{-1}. These bands can be assigned to the

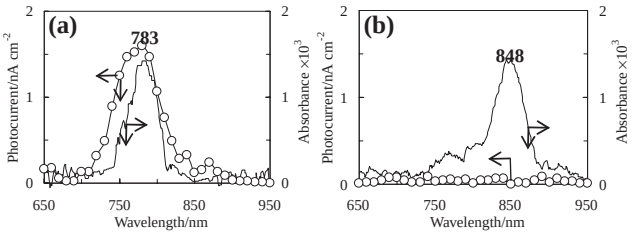


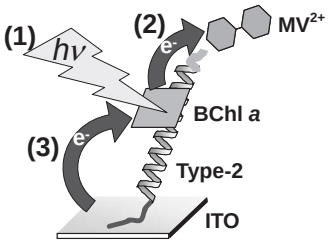
Figure 1. Action spectra (open circle and solid line) and absorption spectra (solid line) of Type-2/BChl *a* (a) and Type-2/[Zn]-BChl *a* (b) complexes assembled on an ITO electrode.

amide I and amide II band, respectively. It is also possible as previously described to use these bands to determine the tilt angle of the helices relative to the surface of the gold electrode.[13] The tilt angle of the helix in Type-2/[Zn]-BChl *a* complex is 40°. For Type-2/BChl *a* complex, the angle is 38° for the complex formed between the Type-1 polypeptide and BChl *a*. Taken together, these results show that the LH1 model polypeptide is in an α-helical conformation when assembled together with these pigments onto an ITO electrode.

Figure 1 shows the NIR absorption and photocurrent action spectra of either BChl *a* or [Zn]-BChl *a* complexes assembled with Type-2 polypeptide onto an ITO electrode at room temperature. The Qy band of the BChl *a* and [Zn]-BChl *a* complexes showed an absorption maximum at 783 and 848 nm on the ITO electrode, respectively. This absorption maximum is analogous to the Qy band for BChl *a* or [Zn]-BChl *a* complexes assembled with Type-2 polypeptide in OG micelles at 4 °C (Table S1).[11] However, no absorption band was observed for both BChl *a* and [Zn]-BChl *a* complexes with Type-1 polypeptide on the electrode (data not shown). This result indicates that mSP moiety of Type-2 polypeptide is necessary for immobilization of complexes on an ITO electrode.

Further, it is clear from Figure 1a that an enhanced photocurrent was observed for Type-2/BChl *a* complex when the electrode was illuminated with a pulse of light at the Qy band of BChl *a* (783 nm). The photocurrent responses showed maxima at wavelengths corresponding to the maxima of the main absorption bands of the complexes. In contrast, no photocurrent was observed for Type-2/[Zn]-BChl *a* complex (Figure 1b), analogous to [Zn]-BChl *a* complex with the native LH1-α/-β polypeptides immobilized on an ITO electrode modified with lipid bilayers.[10] Under the present experimental condition, a cathodic photocurrent was observed (data not shown), implying that one-way electron transfer from BChl *a* in the LH model polypeptide complex to methyl viologen occurred as shown in Scheme 2 and Supporting Information Scheme S1.[11] This result indicates that the BChl *a* complex was well immobilized by the synthetic LH model polypeptide in an α-helical configuration on the ITO electrode and the photocurrents were driven by light that was initially absorbed by the BChl *a* complex. Furthermore, the NIR absorption and FT-IR spectra of the complexes immobilized on the ITO electrode was not changed after photocurrent measurement, implying that the complexes were stable during the measurement.

In conclusion, the amphiphilic compound, mSP conjugated to LH model polypeptide was successfully synthesized. The



Scheme 2. Schematic view of light irradiation (1), electron transfer from BChl *a* to MV^{2+} (2) and electron transfer from ITO to BChl *a* (3) in Type-2/BChl *a* complex immobilized on the ITO electrode.

mSP moiety of the model polypeptide can immobilize with BChl *a* and [Zn]-BChl *a* complexes onto the ITO electrode. This method will be useful for the self-assembly of these complexes in order to study the energy-transfer and electron-transfer reactions between individual pigments in the photosynthetic LH complexes on the electrode.

M. N. and H. H. are grateful to JST, CREST for financial support. The present work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japanese Government and AOARD.

References and Notes

1 D. E. Robertson, R. S. Farid, C. C. Moser, J. L. Urbauer, S. E. Mulholland, R. Pidikiti, J. D. Lear, A. J. Wand, W. F. Degrado, P. L. Dutton, *Nature* **1994**, *368*, 425.
2 D. Noy, P. L. Dutton, *Biochemistry* **2006**, *45*, 2103.
3 L. G. Kwa, A. García-Martín, A. P. Végh, B. Strohmann, B. Robert, P. Braun, *J. Biol. Chem.* **2004**, *279*, 15067.
4 T. Ochiai, T. Asaoka, T. Kato, S. Osaka, T. Dewa, K. Yamashita, H. Hashimoto, M. Nango, *Tetrahedron Lett.* **2007**, *48*, 8468.
5 K. A. Meadows, K. Iida, K. Tsuda, P. A. Recchia, B. A. Heller, B. Antonio, M. Nango, P. A. Loach, *Biochemistry* **1995**, *34*, 1559.
6 M. Nagata, M. Nango, A. Kashiwada, S. Yamada, S. Ito, N. Sawa, M. Ogawa, K. Iida, Y. Kurono, T. Ohtsuka, *Chem. Lett.* **2003**, *32*, 216.
7 T. Dewa, T. Yamada, M. Ogawa, M. Sugimoto, T. Mizuno, K. Yoshida, Y. Nakao, M. Kondo, K. Iida, K. Yamashita, T. Tanaka, M. Nango, *Biochemistry* **2005**, *44*, 5129.
8 P. S. Parkes-Loach, J. R. Sprinkle, P. A. Loach, *Biochemistry* **1988**, *27*, 2718.
9 A. Kashiwada, H. Hiroaki, D. Kohda, M. Nango, T. Tanaka, *J. Am. Chem. Soc.* **2000**, *122*, 212.
10 M. Nagata, Y. Yoshimura, J. Inagaki, Y. Suemori, K. Iida, T. Ohtsuka, M. Nango, *Chem. Lett.* **2003**, *32*, 852.
11 Supporting Information is available electronically on the CSJ-Journal web site; http://www.csj.jp/journals/chem-lett/.
12 A. Kashiwada, H. Watanabe, T. Tanaka, M. Nango, *Chem. Lett.* **2000**, 24.
13 Y. Miura, S. Kimura, Y. Imanishi, J. Umemura, *Langmuir* **1998**, *14*, 6935.

Published on the web (Advance View) December 15, 2007; doi:10.1246/cl.2008.98