0273-2289/00/84–86/0401/$12.00

Self-Assembling Photosynthetic Reaction Centers on Electrodes for Current Generation

Chikashi Nakamura,*[,1] Miki Hasegawa,[1] Yoshiaki Yasuda,[2] and Jun Miyake[1]

[1]National Institute for Advanced Interdisciplinary Research, AIST, MITI, Tsukuba, Ibaraki 305-8562, Japan, E-mail: nakamura@nair.go.jp; and [2]Tsukuba Research Laboratory, Stanley Electric Co. Ltd., 5-9-5 Tokodai Tsukuba, Ibaraki 305-2635, Japan

Abstract

Photosynthetic reaction centers (RCs) made from photosynthetic organisms can be used in solar batteries because their molecules cause light-induced charge separation. We present a simple immobilization system of the intact RCs from *Rhodobacter sphaeroides* on an electrode that uses nickel ligand binding by the hexameric histidine tag on H subunit (HHisRC). The binding constant of HHisRC to the nickel-nitrilotriacetic acid (Ni-NTA) chip measured with a surface plasmon resonance instrument was 1.6×10^8 M^{-1}. HHisRCs were immobilized on an indium tin oxide electrode overlaid with an Ni-NTA gold substrate. The photoinduced displacement current of this electrode was measured to estimate the orientation of HHisRC on the electrode, and the detachability of HHisRC from the electrode was determined by using an imidazole solution wash. The direction of the flash-light-induced displacement current suggested that the H subunit side of the immobilized HHisRC faced the surface of the electrode. The photoinduced current disappeared after the electrode was washed in the imidazole solution. This simple immobilization and detachment of HHisRC to the electrode might be useful for making a reproducible photocurrent device.

Index Entries: Photosynthetic reaction center; purple bacteria; genetic manipulation; protein assembly; displacement current; surface plasmon resonance.

Introduction

Photosynthetic reaction centers (RCs) cause light-induced charge separation in molecules. RCs from purple bacteria have been used to construct a photo-generating electric device *(1–5)*. Orientation control and

*Author to whom all correspondence and reprint requests should be addressed.

dense packing of the RC molecules are required for optimum current generation, for which many techniques have been developed. For peptides, poly-L-lysine clusters from 10-mer to 20-mer were effective for constructing dense-packed Langmuir-Blodgett films of RC *(6)*. RC-cytochrome *c* (cyt *c*) complexes were synthesized by chemical coupling, which could be assembled either into Langmuir-Blodgett films or into liposomes and orientated in a single direction *(7)*. For orientation control of RC molecules, however, simpler materials are preferable because complex synthesis processes are difficult to control *(7)*.

To simplify the RC purification, a recombinant RC containing a six-histidine tag at the M subunit (MHisRC) was developed, and a rapid purification method using nickel-nitrilotriacetic acid (Ni-NTA) gel beads in a column chromatography was developed by Goldsmith and Boxer *(8)*. We also synthesized a new recombinant RC complex with a histidine tag by the modified gene expression using H-subunit-defected mutants PUHA1 of *Rhodobacter sphaeroides (9,10)*. The recombinant complex (HHisRC) could be purified by the method of Goldsmith and Boxer *(8)*. To investigate topological profiles of RC, horse heart cyt *c* could be used as a binding protein for RC *(11)*. The analysis for cyt *c* binding to HisRCs clarified that HHisRC was immobilized on the Ni-NTA substrate in the opposite orientation to MHisRC *(10)*. On substrates, the cyt *c* binding sites of HHisRC were exposed.

In this study, HHisRC was used to construct photoelectrochemical conversion devices. We used the displacement current measurement of the HHisRC immobilized electrode described by Yasuda et al. *(12)* to determine the orientation of HHisRC on the electrode and the detachability of HHisRC from the electrode.

Materials and Methods

Preparation of His-Tagged RC

H-subunit-defected mutant PUHA1 derived from *R. sphaeroides* was used for HHisRC production *(9,10)*. MHisRC was isolated from the SMpHis strain *(8)*. HHisRC was purified by using a previously reported method *(8,10)*. The molecular weight of His-tagged RC is about 102 kDa.

Measurement of Surface Plasmon Resonance

A surface plasmon resonance (SPR) instrument was used to analyze the molecular interaction between Ni-NTA and HHisRCs. In SPR analysis, the capturing molecule is immobilized on a sensor chip. If a sample containing a molecule that binds to the capturing molecule is passed over the chip, the binding event is detected by the SPR instrument. The rate at which molecules bind to each other (association rate) and break apart (dissociation rate) can be measured by the SPR system. The association constant can be determined from the known kinetics of this system.

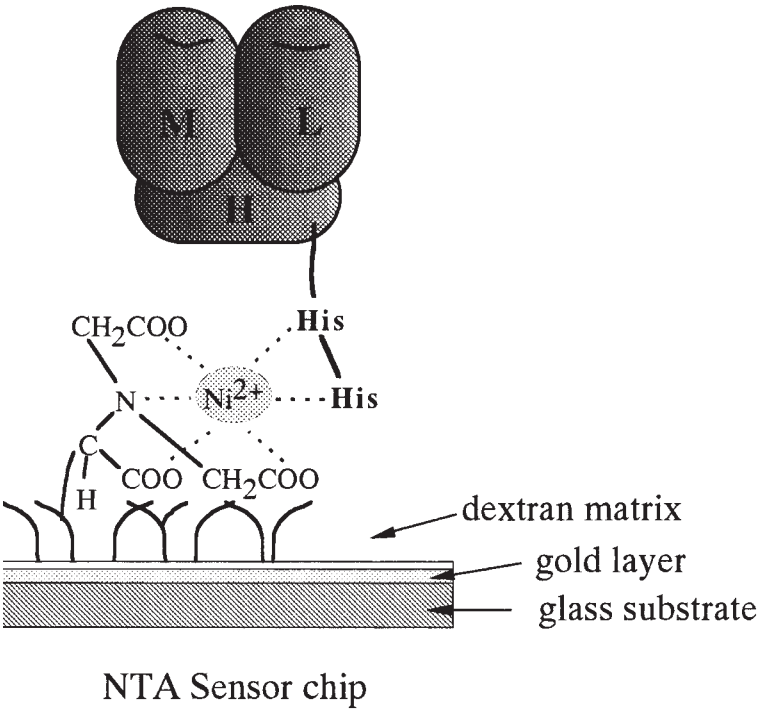

Fig. 1. Schematic of histidine-tagged RCs on an Ni-NTA chip.

An NTA sensor chip (Biacore International AB, Uppsala, Sweden) and an instrument, BIACORE X (Biacore International AB), were used for the SPR measurements. The NTA sensor chip consisted of a glass substrate, a 50-nm-thick gold layer, and a 100-nm-thick dextran matrix (Fig. 1). The density of the NTA group on the chip was about 600 fmol/mm^2. A nickel chloride solution was placed on the NTA sensor chip to ligate Ni^{2+} ions. According to the BIACORE system manual, an SPR signal of 1000 resonance units (RU) corresponds to an immobilized substance change of 1.0 ng/mm^2, and the area of the flow cell was about 1.2 mm^2. The phosphate buffer samples contained 50 m*M* NaCl and 0.2% Triton X-100, and had a pH of 8.0. Sixty microliters (12 n*M* to 1.2 μ*M*) of the HHisRC were injected into the flow cell of the SPR instrument. The binding constant (K_a) was calculated by the software supplied with the BIACORE system.

Measurement of Displacement Current

The technique for measuring the displacement current was not used to detect the conduction current, but rather to detect the displacement current that originates from a change in the electric flux through a layer between two electrodes. Figure 2 shows a schematic of the experimental setup used to measure the displacement current in the electrode we constructed. The instrument is described by Yasuda et al. *(12)* and Ueno et al. *(13)*. Electrode 1 was a glass slide coated with a semitransparent gold film

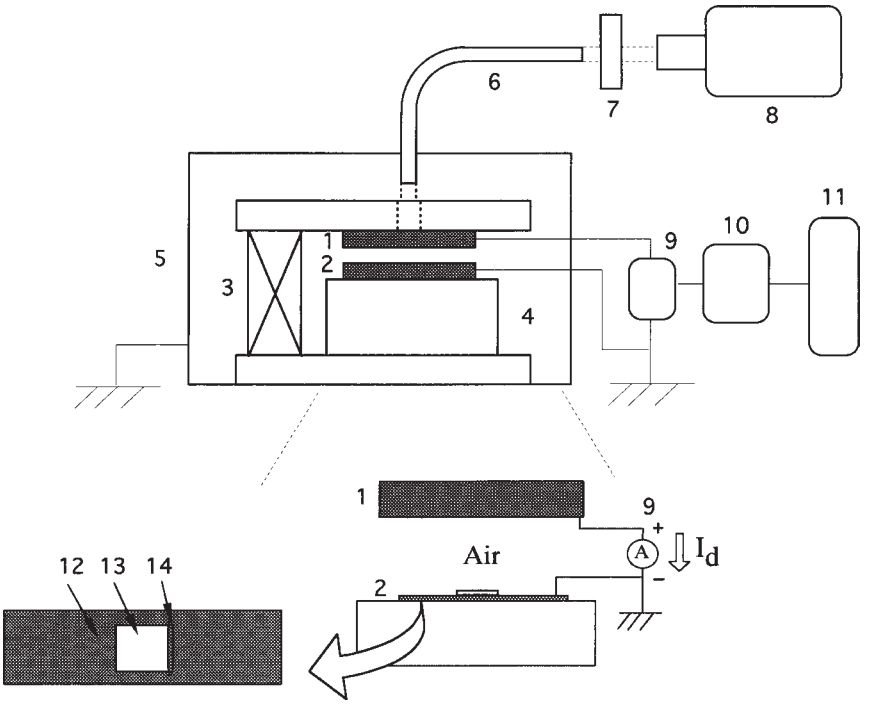

Fig. 2. Schematic of the instrument for measuring light-induced displacement current. 1, electrode 1; 2, electrode 2; 3, mechanical stage; 4, base stage; 5, shield box; 6, optical fiber; 7, lens; 8, Xe flashlamp; 9, I–V amplifier; 10, digital storage oscilloscope; 11, personal computer; 12, ITO electrode; 13, gold substrate; 14, silver paste. The gold substrate was taken off from the NTA sensor chip (Biacore International AB).

prepared by using vacuum evaporation. A substrate with a surface area of 81 mm^2 was removed from the NTA sensor chip (Biacore International AB) and attached to the indium tin oxide (ITO) electrode with silver paste; this was electrode 2. Electrode 1 was supported with a mechanical stage to obtain the desired electrical isolation from electrode 2. Electrode 1 was connected to electrode 2 with a low-noise coaxial cable through a sensitive *I-V* amplifier (Model 428; Keithley Instruments, Inc., Cleveland). The excitation light source was a xenon flashlamp (SB-20; Nikon, Co., Tokyo, Japan) with a pulse energy of 0.1 mJ and a pulse width of 60 μs. A digital storage oscilloscope (Model 2232; Tektronix, Inc., Beaverton, OR) was used to record the displacement current through the amplifier.

The buffer used in the SPR measurements was also used in this measurement. Nickel ions were introduced into the NTA group on electrode 2, washed with the buffer solution, and then dried. The blank current was then measured. Subsequently, 50 μL of HHisRC solution (1.2 μ*M*) was spotted on the NTA substrate, left for 3 min, and then removed by washing three times with a buffer solution containing 5 m*M* ascorbic acid. The displacement current of the HHisRC immobilized on the electrode was measured after drying. The HHisRCs were removed by washing with a buffer

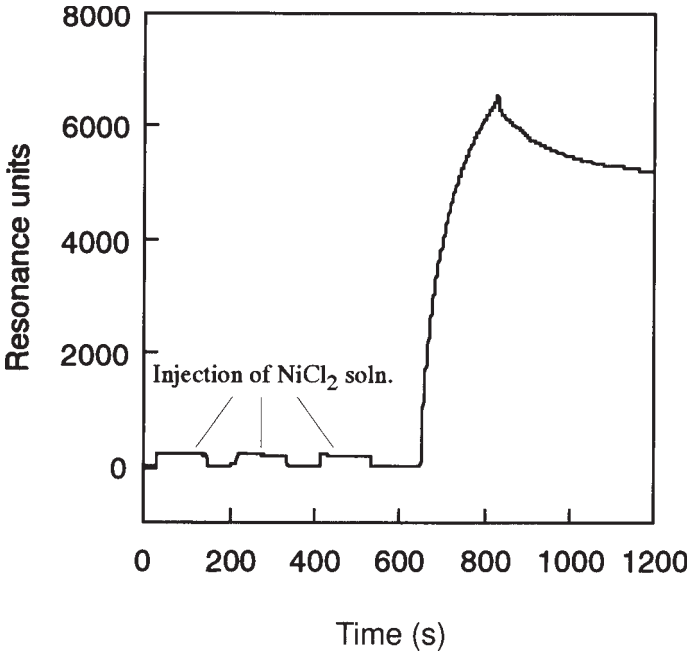

Fig. 3. Sensorgram for the maximum immobilization of HHisRC on an Ni-NTA chip. Nickel ions were introduced into a flow cell for coordinating NTA groups on the chip. After injecting Ni^{2+} three times, 1.2 μ*M* HHisRC was injected.

containing 40 m*M* imidazole. The current was measured again to estimate the detachment of the HHisRC from the electrode. The current measurements were made eight times and the measured current was averaged.

Results and Discussion

Binding Constant of HHisRC to Ni-NTA

Figure 3 shows a sensorgram of HHisRC binding to the Ni-NTA sensor chip. The maximum increase in the SPR signal was about 6000 RU at a concentration of 1.2 μ*M* (data not shown). This indicates that the surface density of the immobilized HHisRC was 6.0 ng/mm^2 (59 fmol/mm^2) and that about 10% of the Ni-NTA groups on the chip could capture HHisRCs. A similar degree of immobilization is possible on electrodes used for the displacement current measurements.

Figure 4 shows sensorgrams for various concentrations of HHisRC. The coordination binding kinetics of the histidine tag on RC to Ni-NTA was determined by curve fitting using the sensorgram data (12–120 n*M*). The K_a value of HHisRC was 1.6×10^8 M^{-1}. The binding affinity was sufficiently high to cause immobilization on the electrode surface. In the SPR measurement, nearly the same results were obtained as for MHisRC (data not shown).

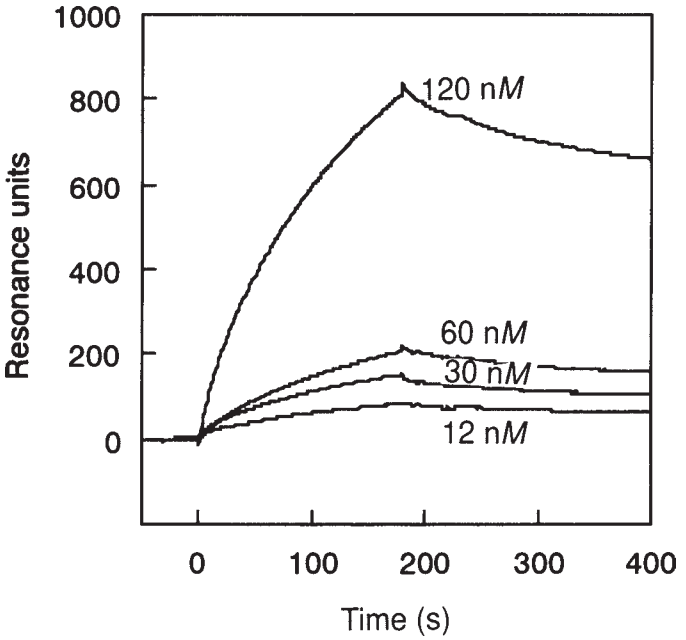

Fig. 4. Sensorgrams of HHisRC binding to Ni-NTA chips with varying HHisRC concentrations. The sensorgrams were curve fitted by using version 3.0 of the BIAevaluation software supplied with the BIACORE system.

Displacement Current of the Immobilized HHisRC

When the excitation light was irradiated onto the substrate, the displacement current resulting from charge separation of the immobilized RC flowed through the external circuit. For the immobilized HHisRC, Fig. 5 shows that from the point of xenon flash irradiation, a light-induced displacement current peak of 50 pA occurred after an 80-μs time delay. This finding is in good agreement with the data for an RC Langmuir-Blodgett film *(12)* and indicates that the current was generated by photoactivated charge separation in the HHisRC immobilized on the Ni-NTA substrate of electrode 2.

The negative current shown in Fig. 5 indicates that the HHisRC molecules had the same orientation as those in the Z-type film in which the H subunit side was close to the contact electrode *(5,12)*. This indicates that the hexameric-histidine tag on the H subunit coordinated nickel ions and that the H subunit side faced toward the substrate. We expected that a positive current would occur for MHisRC, but in fact we observed a weak negative current peak (data not shown). The RC of the *R. sphaeroides* is a polarized hydrophobic molecule because the H subunit domain is more hydrophilic than the other domains (ML subunits). This is presumably because during the drying step H subunits strongly interact with the hydrophilic dextran matrix, so that the molecular orientation of MHisRC might reverse on the electrode. These results suggest that HHisRC, rather than MHisRC, can assemble unidirectionally on the Ni-NTA substrate.

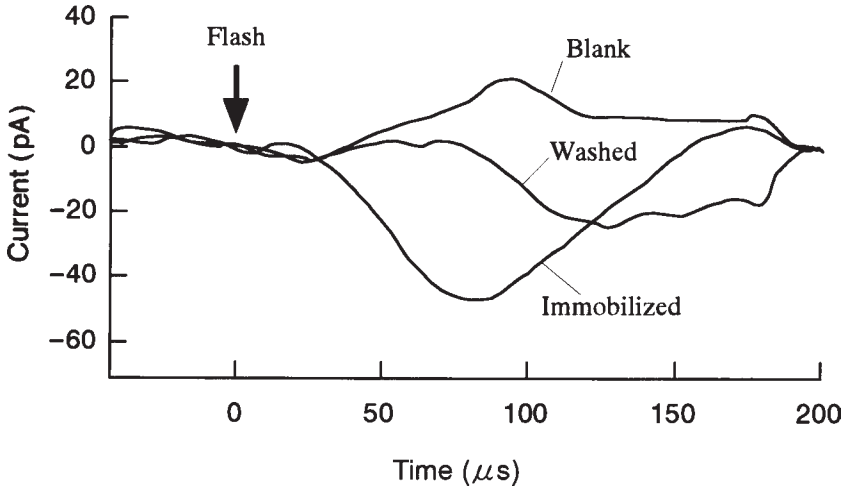

Fig. 5. Light-induced displacement current response of HHisRC immobilized on the Ni-NTA electrode. HHisRC was immobilized on the gold part of an Ni-NTA electrode (*see* Fig. 2). A chlorophyll special pair was reduced by using ascorbic acid in a buffer solution. The arrow indicates the time to trigger the xenon flash.

After the electrode was washed with the imidazole solution, the photoinduced current disappeared. Moreover, HHisRC was immobilized on the washed electrode, and the current could be reproduced (data not shown). The detachability of HHisRC from the Ni-NTA surface and the reproducibility of the Ni-NTA substrate were therefore demonstrated.

Conclusion

A photocurrent-generating device of HHisRC immobilized on an electrode by using Ni-NTA coordination was successfully constructed. The binding constant of the histidine tag to Ni-NTA was $>10^8\ M^{-1}$. The HHisRC was easily detached by washing with an imidazole solution. Therefore, this device can be used for making reproducible photocurrent-generating devices.

Acknowledgment

This work was supported by the Biomolecular Mechanism and Design Project of National Institute for Advanced Interdisciplinary Research (NAIR), Agency of Industrial Science and Technology (AIST).

References

1. Janzen, A. F. and Seibert, M. (1980), *Nature* **286,** 584–585.
2. Tiede, D. M., Mueller, P., and Dutton, P. L. (1982), *Biochim. Biophys. Acta* **681,** 191–201.
3. Hirata, Y., Nukanobu, K., Hara, M., Asada, Y., Miyake, J., and Fujihira, M. (1992), *Chem. Lett.* 2277–2280.
4. Yasuda, Y., Hirata, Y., Sugio, H., Kumei, M., Hara, M., Miyake, J., and Fujihira, M. (1992), *Thin Solid Films* **210/211,** 733–735.

5. Yasuda, Y., Sugio, H., and Toyotama, H. (1994), *Bioelectrochem. Bioenerget.* **34,** 135–139.
6. Noda, K., Akutsu, H., Miyake, J., Nakamura, C., and Hara, M. (1998), *Supramol. Sci.* **5,** 773–775.
7. Ueno, T., Hirata, Y., Hara, M., Arai, T., Sato, A., and Miyake, J. (1995), *Materials Sci. Eng. C* 99, 1–6.
8. Goldsmith, J. O. and Boxer, S. G. (1996), *Biochim. Biophys. Acta* **1276,** 171–175.
9. Sockett, R. E., Donohue, T. J., Varga, A. R., and Kaplan, S. (1989), *J. Bacteriol.* **171,** 436–446.
10. Nakamura, C., Kaneko, T., Hasegawa, M., Yang, Q., Hara, M., Shirai, M., and Miyake, J. (1998) in *Photosynthesis: Mechanisms and Effects*, Garab, G., ed., Kluwer Academic Publishers, Dordrecht, Netherlands, Vol. IV, pp. 3087–3090.
11. Ueno, T., Hara, M., Kamo, N., Fujii, T., and Miyake, J. (1998), *J. Biochem.* **124,** 485–490.
12. Yasuda, Y., Kawakami, Y., and Toyotama, H. (1997), *Thin Solid Films* **292,** 189–191.
13. Ueno, T., Miyake, J., Fujii, T., Shirai, M., Arai, T., Yasuda, Y., and Hara, M. (1998), *Supramol. Sci.* **5,** 783–786.