Photocurrent generation properties of Histag-photosystem II immobilized on nanostructured gold electrode

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ABSTRACT

It is well known that photosystem II (PSII) can produce electrons, oxygen, and protons simultaneously via a water-splitting photoreaction. These photochemical properties are expected to exhibit photoconductive function for PSII. In the present study, we have first observed a stable photocurrent due to the photoexcitation of PSII and the subsequent water-splitting reaction, by successfully immobilizing PSII on the self-assembled monolayer

(SAM) of a nickel-nitrilotriacetic acid complex (Ni-NTA) prepared on a gold surface via a polyhistidine tag (Histag) as a linker molecule. We have further succeeded in the fabrication of PSII-gold nanoparticle multistuctures on the surface of gold electrode, and significant enhancement of photocurrents was achieved due to increased number of immobilized PSII.

KEYWORDS: Photosystem II, gold nanoparticle, water-splitting reaction, photocurrent

1. Introduction

Biocomponents constituting the living body are very attractive nanomaterials because they have already achieved ultrahigh performance as the result of many cycles of natural selection and mutation. In photosynthesis, for example, it is well known that the quantum yield of charge separation at the reaction center is almost unity [1–2]. In particular, considerable attention has been paid to photosystem II (denoted as PSII, Fig. 1(a)) because it can perform the useful function of splitting water to produce electrons, oxygen and protons in addition to possessing ultrahigh conversion efficiency [3–8]. The splitting of water in PSII has been considered to be a promising method to perform photoenergy fixation and photoelectric conversion and to simultaneously utilize the clean solar energy.

In order to elucidate and replicate this clean energy generation system, the

phototechnological utilization of such natural biocomponents as well as construction of artificial photosynthetic systems has been extensively studied in the past several decades [9–11]. Recently, the orientation of a photosynthetic protein in a molecular thin film was controlled by using a self-assembled monolayer (SAM) as an interlayer of the film, and the effects on the photocurrent responses from the photosynthetic protein were investigated [12–15]. In particular, the genetic introduction of a polyhistidine tag (Histag) on the surface of a biocomponent has been investigated as a promising method to immobilize the biocomponent on the Ni-NTA-terminated SAM on electrodes with positional selectivity in a molecular order [16–19]. Based on this background, we have investigated the immobilization of Histag-PSII on the Ni-NTA-terminated SAM.

Gold nanoparticle has been one of the most fascinating nanomaterials in nanotechnology because it has several attractive characteristics in terms of high surface area, chemical stability, biocompatibility, optical property based on localized surface plasmon oscillation, etc. [20–23]. Thus, it is expected that they can be applied for various purposes such as catalysis, in high-surface-area electrodes, in biocompatible materials, and in analytical probe materials.

In spite of PSII and gold nanoparticles being attractive and useful nanomaterials, their hybridization has not been reported so far. In this study, for the first time, we have achieved the hybridization of PSII and gold nanoparticles via the direct linkage of the

site-selective Histag on PSII and the Ni-NTA-terminated SAM on a gold electrode. We successfully obtained a stable photocurrent from the photoexcitation of PSII and the subsequent electron transfers originating from the water-splitting reaction. Further, significantly large photocurrent responses with a satisfactory signal-to-noise ratio were successfully observed in the PSII-immobilized nanostructured gold electrodes as compared with the case of a PSII-immobilized planar gold electrode.

2. Experimental

We prepared a stable PSII complex by applying the Histag methodology to the thermophilic cyanobacterium *Thermosynechococcus vulcanus*. The carboxy terminus of the CP43 subunit of PSII was tagged with six histidine residues (Fig. 1(b)). The PSII complex was isolated from thylakoids by Ni²⁺ affinity column chromatography as described by Sugiura and Inoue (1999) [24]. The oxygen-evolving reaction of a PSII Mn cluster is carried out as a result of the efficient cascades of electron transfer reactions in the PSII electron relay system (P680 → Chl → Q_A → Q_B and Mn cluster → Ty → P680⁺*) [3, 4]. Therefore, the bioactivity of PSII was confirmed by monitoring the photoresponses of oxygen concentration (Fig. 2), in the presence of Histag-PSII (5 µg of Chl/ml), 0.4 M sucrose, 15 mM calcium chloride (CaCl₂), 15 mM magnesium chloride (MgCl₂), and 15 mM sodium chloride (NaCl) in MES-NaOH (pH6.4) buffer solution with ferricyanide and phenyl-p-benzoquinone as the electron acceptors [24].

An aqueous solution of gold nanoparticles (denoted as AuP in the Fig. 3) was prepared by the reduction of HAuCl_4 with sodium citrate [21, 25], and the absorption spectrum of the red solution had a clear peak at around 520 nm that originated from the localized plasmon of gold nanoparticles. From the TEM image, the average diameter of the gold nanoparticles was estimated to be approximately 18 nm. The preparation procedures of the Histag-PSII-immobilized planar (**PSII/A**, Fig. 3(a)) and nanostructured gold electrodes (**PSII/B** and **PSII/C**, Fig. 3(b)) are illustrated in Fig. 3. The planar gold electrode (denoted as **A**) was obtained from Geomatec (Japan); it was prepared by vacuum deposition of gold onto transparent mica plate (Furuuchi chemical Co. , Japan). The preparation procedures of nanostructured gold electrodes by the sedimentation of gold nanoparticles using NaClO_4 have been described previously [20–21, 26]. Briefly, the planar gold electrode was placed at the bottom of a flat-bottomed glass vessel (3.2 cm inner diameter), and 20 or 40 mL of colloidal gold solution was added. Subsequently, 2 mL (for 20 mL of the colloidal solution) or 4 mL (for 40 mL) of saturated aqueous NaClO_4 solution was added to induce the sedimentation (salting out) of gold nanoparticles on the surface of the planar gold electrode. After leaving it overnight, the solution was removed and the electrode was rinsed with water; this was followed by drying under nitrogen gas, to obtain nanostructured electrode **B** (for 20 mL of the colloidal solution) or **C** (40 mL).

PSII was immobilized on the gold surface via direct linkage between the Histag on PSII

and the Ni-NTA-terminated SAM on the gold surface [16–19]. The Ni-NTA-terminated SAM was in turn prepared on the gold surface by the immersion of each electrode (**A**, **B** and **C**) into three solutions as follows; (i) 2 mg/mL 3,3'-dithio-bis(propionic acid N'-hydroxysuccinimide ester (DTSP, SIGMA) dimethylsulfoxide (DMSO) solution for 1 h, (ii) 0.15 M N-(5-Amino-1-carboxypentyl)- iminodiacetic acid (AB-NTA, DOJONDO) and 0.5 M potassium carbonate (K_2CO_3) aqueous solution for 3 h, and (iii) 40 mM nickel (II) sulfate ($NiSO_4$) for 1 h. As the next step, Ni-NTA-terminated SAM electrodes were immersed into the MES-NaOH buffer solution (pH 6.4) of 580 $\mu gChl/mL$ Histag-PSII for 4 days at 0 °C, to obtain PSII-immobilized planar (**PSII/A**) and nanostructured (**PSII/B**, and **PSII/C**) gold electrodes.

Cyclic voltammetric (CV) measurements on the planar (**A**) and nanostructured (**B**, **C**) gold electrodes were carried out using platinum counter and Ag/AgCl (sat. KCl aq.) reference electrodes in 0.1M H_2SO_4 aqueous solution to evaluate the electrochemically active surface area (sweep rate: 0.05 V/s) [26]. Photocurrent measurements were also carried out using the three-electrode electrochemical cell in the presence of 0.4 M sucrose, 15 mM $CaCl_2$, 15 mM $MgCl_2$, and 0.1 M NaCl in the MES-NaOH (pH 6.4) buffer aqueous solution [24, 26–27]. The light from a xenon lamp (LAX-Cute, Asahi Spectra Co.) passed through an optical filter (Asahi Spectra Co.) and irradiated the modified (working) electrode (light intensity: 3.3 mW at 680 nm, irradiation area: 0.16 cm²). The current responses with the light irradiation were

measured and recorded as the photocurrent by using a potentiostat (FUSO HECS318C).

All measurements were carried out at room temperature.

Scanning electron micrograph (SEM) measurements of PSII modified nanostructured gold electrodes were carried out by using a Hitachi S-5200. Absorption spectra were recorded on a Jasco V-660 spectrophotometer.

3. Results and Discussion

The inset of Fig. 2 shows the photoresponses of the evolved oxygen concentration and the oxygen evolved from the isolated Histag-PSII complex at $2100 \mu\text{mol mgChl}^{-1} \text{h}^{-1}$ at 25°C with the light irradiation ($10000 \mu\text{photon m}^{-2} \text{s}^{-1}$ at around 400–700 nm). This result clearly shows that PSII can sufficiently cause a water-splitting reaction and the oxygen evolving under these conditions; the bioactivity was maintained even after the purification and genetic introduction of Histag on PSII. Figure 2 shows the time course of PSII activity in the oxygen-evolving reaction at a few different temperatures (-80°C , 4°C , and 25°C). This result indicates that the activity of Histag-PSII was sufficiently maintained during the period of 4 days for immobilization on the gold electrodes and photocurrent measurements.

Photocurrent measurements were carried out using the three-electrode electrochemical cell in the presence of 0.4 M sucrose, 15 mM CaCl_2 , 15 mM MgCl_2 , and 0.1 M NaCl in the MES-NaOH (pH 6.4) buffer aqueous solution (Fig. 3). This solution for the photocurrent measurements possessed almost the same composition as the solution in the oxygen-evolving

experiment involving PSII in Fig. 2; in particular, and there were no sacrificial electron donors. By subjecting the PSII-immobilized planar gold electrode (**PSII/A**) to light irradiation of 680 nm (3.3 mW), a stable anodic photocurrent was observed and the value was maintained constant for at least several tens of minutes. The action spectrum of the photocurrent value in **PSII/A** shows clear photocurrent peaks at around 670~680 nm (Fig. 5(c), denoted by ●) that overlap with the absorption spectrum of PSII. On the other hand, the photocurrent value of the Ni-NTA-terminated SAM-modified gold electrode (**D**, reference electrode without PSII) was negligible. The comparison of the photocurrent value in the two electrodes (**PSII/A** and **D**) and the action spectrum in **PSII/A** clearly indicate that the photocurrent in this system was mainly due to the photoexcitation of PSII and probably also due to the subsequent electron generation via the water-splitting reaction. Figure 4(b) shows the applied potential dependence of the photocurrent in **PSII/A**. The photocurrent values increased with the applied positive potential and the photocurrent direction was reversed at around 50 mV vs. Ag/AgCl (sat.KCl), which is close to the redox potential of Q_B (quinone compound, located at the end of the PSII electron relay system) [3–4, 28]. The Histag, which provides the linkage to the gold electrode, was introduced on the surface of PSII at the point close to the Q_B molecule (Fig. 1); therefore, electron injection from Q_B to the electrode seemed to be easy. Based on these facts, the photocurrent must be generated using the following 4 steps: (i) photoexcitation of PSII, (ii) cascades of electron transfer

reactions in PSII from P680 to Q_B , (iii) electron injection from Q_B to gold electrode, and (iv) re-redox of $P680^{++}$ with the electron generated via the water-splitting reaction at the Mn cluster in PSII.

In the paragraph below, the effects of the nanostructured gold layer on the photocurrent generation properties are described. First, cross-sectional SEM images of nanostructured gold electrodes are shown in Fig. 5(a). As can be recognized from the images, most of the gold nanoparticles are agglomerated and form highly nanoporous multistructures. Further, the thickness of the nanostructured layer of PSII/C was almost twice that in the case of PSII/B, which seemed to depend on the volume of the colloidal solution [20]. In order to evaluate the surface area of the planar and nanostructured gold electrodes, cyclic voltammetric (CV) measurements were carried out in 0.1 M H_2SO_4 aqueous solution [26]. In all the cases, a redox peak was observed at +0.7~+1.0 V vs. Ag/AgCl (Fig. 5(b)) due to the reduction of the gold surface. From the integration of these peaks, the surface areas of each of the electrodes were estimated. The results of the relative values are summarized in Table 1. The table clearly indicates that the effective (electrochemically active) surface area of the electrodes is enlarged by more than one order of magnitude by the use of gold nanoparticles, and the relative ratio depends on the volume of the gold colloidal solution used in the salting-out process in preparation.

Figure 5(c) shows the photocurrent action spectra of the Histag-PSII-immobilized

planar (**PSII/A**: ●) and nanostructured gold electrodes (**PSII/B**: ○, **PSII/C**: □). In the case of all PSII-immobilized electrodes, a clear peak at around 670~680 nm that is consistent with the absorption spectrum of PSII can be seen, and these results show that the photocurrent was mainly due to the photoexcitation of PSII. As summarized in Table1, the relative photocurrent ratios for **PSII/B** and **PSII/C** against **PSII/A** are 6 and 19, respectively; these ratios increased with the number of immobilized PSII and gold nanoparticles. These results are ascribed to the increased electrode area resulting from the use of gold nanoparticles. In addition, the degree of photocurrent enhancement is almost identical to the value that expected from the increased active area of electrode surface. This indicates that even a big protein complex such as PSII (~20 nm [4]) could interpenetrate the nanospace of the gold nanostructures and the expanded electrochemical active surface with gold nanoparticles sufficiently contributed to the increase in the photocurrent values.

4. Conclusions

We have succeeded in chemical immobilization of PSII on the gold surface by an appropriate linker system, and clear photocurrent response from photoexcitation of PSII and subsequent water-splitting reaction. The use of gold nanoparticles substantially enhanced the photocurrents, ascribing to a large increase in the number of PSII immobilization. The present method, for the enhancement actual output signal by using gold nanoparticle multistructures, must be essentially useful for practical application of the other

biocomponents [26]. To make the most of the enhancement effect, smart fabrication of bionano material is required and in progress on this line.

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FIGURE CAPTIONS

Figure. 1 Illustration of PSII [29] (a) and the amino acid sequences of Histag-PSII (b).

Inset figure is an illustration of Histag-PSII dimer. The broken circles show the point for the Histag location, close to the quinone compounds (Q_A , Q_B) located at the end of the electron relay system of PSII.

Figure 2. Oxygen-evolving reaction of Histag-PSII (inset: the result on 0 day) and the time course of activity at a few different temperatures ($\bullet$: -80°C , $\circ$: 4°C and Δ : 25°C). The value are normalized by the concentration of chlorophyll (Chl) and reaction time (hour).

For example, the value on 0 day was $2102 \mu\text{mol} [\text{O}_2] \text{ mgChl}^{-1} \text{ h}^{-1}$. Solution: Histag-PSII (5 μg of Chl/ml), 0.4M sucrose, 15 mM CaCl_2 , and 15 mM MgCl_2 , 15 mM NaCl in 40 mM MES-NaOH (pH6.4) buffer solution with ferricyanide and phenyl-p-benzoquinone as electron

acceptor . Irradiation light: $10000 \mu\text{photon m}^{-2} \text{s}^{-1}$ (400–700 nm).

Figure 3. Preparation procedures of the HisTag-PSII immobilized (a) planar (**PSII/A**) and (b) nanostructured gold electrodes (**PSII/B**, **PSII/C**).

Figure 4. (a) Photocurrent responses on Histag-PSII immobilized gold electrode (**PSII/A**) and reference electrode without PSII (**D**). (b) Applied potential dependence on photocurrent values of **PSII/A**. Irradiated light: 680 nm (3.3 mW). Electrolyte solution: 0.4M sucrose, 15 mM CaCl_2 , 15 mM MgCl_2 , and 0.1 M NaCl in 40 mM MES-NaOH (pH6.4) buffer aqueous solution. Applied potential in Fig. 4(a): 0.2 V vs. Ag/AgCl (sat. KCl).

Figure 5. (a) Cross-sectional SEM images of the nanostructured gold electrodes (**PSII/B**, **PSII/C**). (b) Cyclicvoltammograms of planar (**A**) and nanostructured gold electrodes (**B**, **C**). Electrolyte solution: 0.1 M H_2SO_4 . Scan rate: 0.05 V/s. (c) Action spectra of Histag-PSII immobilized planar (**PSII/A**) and nanostructured (**PSII/B**, **PSII/C**) gold electrodes. Irradiated light: 680 nm (3.3 mW), electrolyte solution: 0.4M sucrose, 15 mM CaCl_2 , 15 mM MgCl_2 , and 0.1 M NaCl in 40 mM MES-NaOH buffer aqueous solution (pH6.4). Applied potential: 0.2 V vs. Ag/AgCl (sat. KCl).

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(b)

MKTLSSQKRYSPVVTLSNSIFATNRDQESSGFAWWAGNARLIN
 LSGKLLGAHVAHAGLIVFWAGAMTLFELAHFIPEKPMYEQGLILIP
 HIATLGGWVGPGGGEVVDTFPFFVGVVHLISSAVLGFGGVYHAIR
 GPETLEEYSSFFGYDWKDKNKMNTILGFHLIVLGIGALLLVAKAM
 FFGGLYDTWAPGGGDVRITNPTLDPRVIFGYLLKSPFGGEGWIV
 SVNNLEDVVGHHIWLICIAAGGIWHILTPFGWARRAFIWSGEAY
 LSYSLGALSMMGFATCFVWFNNTVYPSEFYGPTGPEASQAQAM
 TFLIRDQKLGANVGSAQGPTGLGKYLMSPTGEIIFGGETMRFW
 DFRGPWLEPLRGPNGLDLNKIKNDIQPWQERRAAEYMTAPLG
 SLNSVGGVATEINSVNFVSPRSWLATSHFVLAFFFLVGHLWHAG
 RARAAAAGFEKGIDRESEPVLSMPSLDLVPRGSHHHHHH
 Histag

Figure 1. N. Terasaki et al.

Figure 2. N. Terasaki et al

Figure 3. N. Terasaki et al.

Figure 4. N. Terasaki et al.

Figure 5. N. Terasaki et al.

Table 1. Comparison of Histag-PSII-immobilized planar (**PSII/A**) and nanostructured (**PSII/B**, **PSII/C**) gold electrodes.

Electrode	thickness of nanostructured gold layer / μm ^{a)}	Relative surface area ^{b)}	Photocurrent ^{c)} nA cm^{-2}
PSII/A	0	1	130 (1)
PSII/B	1	7	760 (6)
PSII/C	2	17	2440 (19)

a) Evaluated from the SEM images of side view of each electrodes.

b) Evaluated from the integration of the CV peak in 0.1M H_2SO_4 aqueous solutions. The values of each area was normalized by the values of **PSII/A**.

c) Measured at +0.2 V vs. Ag/AgCl (sat. KCl). $\lambda=680\pm6.5$ nm (intensity: 3.3 mW). Electrolyte solution: 0.4 M sucrose, 15 mM CaCl_2 , 15 mM MgCl_2 , and 0.1 M NaCl, and 40 mM MES-NaOH buffer solution (pH 6.4). The numbers in parentheses are the relative values of photocurrent.