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Bio-inorganic hybrid photoanodes of photosystem II and ferricyanide-intercalated layered double hydroxide for visible-light-driven water oxidation

Masaru Kato [a, b, *], Hisako Sato [c], Ichizo Yagi [a], Miwa Sugiura [b, d, **]

[a] *Section of Environmental Materials Science, Faculty of Environmental Earth Science, Hokkaido University, N10W5, Kita-ku, Sapporo 060-0810, Japan*
[b] *Proteo-Science Center, Ehime University, 3 Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan*
[c] *Department of Chemistry, Graduate School of Science and Engineering, Ehime University, 2-5 Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan*
[d] *PRESTO, Japan Science Technology Agency (JST), 4-1-8, Honcho, Kawaguchi, Saitama 332-0012, Japan*

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ABSTRACT

Photosynthesis converts solar energy into chemical energy. Photosystem II (PSII) oxidizes water to produce oxygen, electrons and protons under solar light irradiation. This light-driven water oxidation initiates a series of reactions in photosynthesis. Basic photoelectrochemical studies on PSII are directed toward the enzymatic applications of PSII for sustainable production of electricity or solar fuels. To maximize the photoelectrochemical catalytic activity of PSII on electrode substrates, interfacial designs between PSII and electrode substrates are important. Herein, we report bio-inorganic photoanodes of PSII and ferricyanide-intercalated layered double hydroxide (LDH) for visible-light-driven water oxidation. PSII is simply drop-cast onto a ferricyanide-intercalated cobalt–aluminum LDH and then shows a turnover frequency of $0.5 \pm 0.1\ s^{-1}$ and a turnover number of 920 ± 40 for 1 h at pH 6.5 at +0.5 V vs. NHE under visible light irradiation. Photoelectrochemical experiments using a PSII inhibitor or a bio-engineered PSII suggest that interfacial electron transfer from the plastoquinone Q_A site of PSII to ferricyanide may play an important role in improving the photo-electrocatalytic activity and stability of PSII. Our studies will open up new possibilities in fundamental or advanced photoelectrochemical studies of PSII.

1. Introduction

Photosystem II (PSII) is a photosynthetic enzyme and found in oxygenic photosynthetic organisms such as plants, algae and cyanobacteria. This enzyme is able to absorb solar light and then to split water molecules into oxygen molecules, protons and electrons. Since PSII is the only known water oxidation enzyme, understanding its protein structure [1,2] and reaction mechanism [3] inspires us to develop PSII-based photochemical devices [4–6] or PSII-inspired artificial water oxidation catalysts [7–12] for the sustainable production of solar fuels.

To study PSII on its efficient photocatalytic water oxidation or complicated internal electron transfer relay [13], PSII is isolated from oxygenic photosynthetic organisms such as thermophilic cyanobacteria. The isolated PSII, however, is quite unstable under light irradiation because it has neither repairing machinery nor electron acceptors for photo-excited electrons, which cause the degradation of PSII [14,15]. Thus, the isolated PSII must be carefully handled in the dark until photochemical experiments, and exogenous electron acceptors such as 1,4-quinone derivatives [16,17], transition metal complexes [6,17–19] and redox proteins [20,21] are necessary during photochemical experiments, where electron acceptors extract electrons from PSII and maintain photocatalytic cycles of PSII.

For photoelectrochemical studies on PSII, electrode substrates work as electron acceptors and extract photo-excited electrons generated in PSII on electrode substrates, resulting in photocurrent generation involving oxygen evolution catalyzed by PSII. The slow

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[*] Corresponding author. Section of Environmental Materials Science, Faculty of Environmental Earth Science, Hokkaido University, N10W5, Kita-ku, Sapporo 060-0810, Japan.
[**] Corresponding author. Proteo-Science Center, Ehime University, 3 Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan.
E-mail addresses: masaru.kato@ees.hokudai.ac.jp (M. Kato), miwa.sugiura@ehime-u.ac.jp (M. Sugiura).

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electron transfer kinetics from PSII to the electrode substrate is known to cause photo-degradation of PSII. Thus, electrode substrates used are important to achieve high photocatalytic activity and stability of PSII photoanodes. Many electrode materials such as indium-tin oxide [5,22–24], titanium dioxide [25,26], Ti-doped Fe_2O_3 [27] and redox polymer [4,24,28–30] have been studied on PSII photoanodes. Two interfacial electron transfer pathways to the electrode substrate are known from the primary plastoquinone Q_A and from the secondary plastoquinone Q_B [22] (Fig. 1). The internal electron transfer from Q_A to Q_B in PSII is a slow electron transfer process and is known to cause photodamage to PSII [13,31]. The use of electrode substrates that are able to efficiently pick up photoexcited electrons from the Q_A site of PSII might improve photoactivity and stability of PSII photoanodes, possibly opening up new possibilities in enzymatic photovoltaic or water-splitting devices with PSII [4,5].

Herein, we report photocatalytic activity and stability of bioinorganic photoanodes of PSII for visible-light-driven water oxidation (Fig. 1). PSIIs were isolated from *Thermosynecococcus elongatus (T. elongatus)*, and immobilized on ferricyanide ($[Fe^{III}(CN)_6]^{3-}$)-intercalated layered double hydroxides (LDHs) on indium–tin oxide (ITO) substrates. Ferricyanide is negatively charged and works as an electron acceptor from the Q_A site [17]. LDHs consist of cationic double hydroxide nanosheets and exchangeable anions, and have been studied for applications such as electrode materials or catalysts [32–36]. In this work, we have prepared LDH-modified ITO substrates, intercalated ferricyanide into the LDH cationic nanosheets and then simply drop-cast PSII on the ferricyanide-intercalated LDH substrates to record photocurrent responses of them under visible light irradiation.

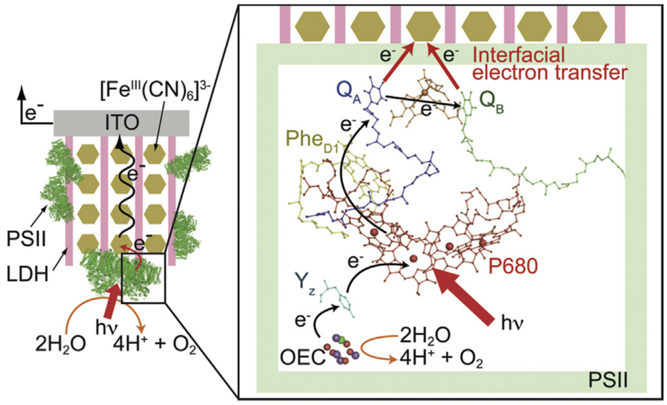

Fig. 1. Schematic representation of the oxygen evolution driven by MAl–[Fe(CN)$_6$]|PSII (M = Mg or Co) photoanodes under visible light irradiation.

2. Experimental

2.1. Materials

Solvents and metal nitrates including $Al(NO_3)_3 \cdot 9H_2O$, $Mg(NO_3)_2 \cdot 6H_2O$ and $Co(NO_3)_2 \cdot 6H_2O$ were purchased from Nacalai Tesque, Inc. or Wako Pure Chemical Industries, Ltd., and used without further purification. Potassium ferricyanide ($K_3[Fe(CN)_6]$), 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and 2,6-dichloro-1,4-benzoquinone (DCBQ) were purchased from Aldrich. ITO-coated glass slides (IN-100, 10 Ω cm^{-2}) were purchased from Furuuchi Chemical Corporation. Mn-depleted PSII was prepared using tris(hydroxymethyl)aminomethane according to the literature [37].

2.2. Isolation of PSII complexes from T. elongatus mutants

PSII core complexes were isolated from the *T. elongatus* strain cells by a Ni^{2+}-affinity chromatography after solubilization of thylakoid membranes with *n*-dodecyl *β*-D-maltoside as described in the literature [17]. Two types of PSII were isolated from *T. elongatus* mutants. One type of PSII has PsbA1 as the D1 protein encoded by the *psbA₁* gene [38] and used for our standard photoelectrochemical experiments. Another type of PSII has PsbA3 as the D1 protein encoded by the *psbA₃* gene ($PSII_{PsbA3}$) [16]. Both types have a His_6-tag on the C-terminus of the CP43 protein of the PSII complex.

O_2 evolution activities of isolated PSII and $PSII_{PsbA3}$ in solution were determined using a Clark-type electrode (CB1-D, HANSATECH). Oxygen evolution of PSII (4 μg Chl mL^{-1}) was measured in a buffered solution containing 40 mM 2-(*N*-morpholino)ethanesulfonic acid (MES), 15 mM $CaCl_2$, 15 mM $MgCl_2$ and 100 mM NaCl at pH 6.5 at 25 °C under saturating white light. A projector (PROCABIN 67-Z, CABIN) equipped with water and IR filters was used as a light source. $K_3[Fe^{III}(CN)_6]$ (the final concentration: 2 mM) or 2,6-dichloro-1,4-benzoquinone (DCBQ) (dissolved in dimethyl sulfoxide; the final concentration: 1 mM) was used as an electron acceptor in the buffered solution. PSII and $PSII_{PsbA3}$ produced oxygen under light irradiation at $(3.8 \pm 0.2) \times 10^3$ μmol O_2 mg Chl^{-1} h^{-1} and $(2.6 \pm 0.2) \times 10^3$ μmol O_2 mg Chl^{-1} h^{-1}, respectively, in the presence of 1 mM DCBQ, while PSII and $PSII_{PsbA3}$ evolved oxygen at $(0.84 \pm 0.06) \times 10^3$ μmol O_2 mg Chl^{-1} h^{-1} and $(0.31 \pm 0.04) \times 10^3$ μmol O_2 mg Chl^{-1} h^{-1}, respectively, in the presence of 2 mM $K_3[Fe^{III}(CN)_6]$.

2.3. Preparation of MgAl–CO_3 LDH on ITO

MgAl–CO_3 LDHs was directly grown on ITO-coated glass slides using a slightly modified method described in the literature [39]. $Mg(NO_3)_2 \cdot 6H_2O$ (0.86 g, 3.3 mmol), $Al(NO_3)_3 \cdot 9H_2O$ (0.62 g, 1.7 mmol) and urea (2.8 g, 47 mmol) were dissolved in 10 mL of water and the mixture was stirred for at least 30 min. ITO-coated glass slides (1 × 2 cm^2) were sequentially sonicated in soapy water, water, acetone, ethanol and water for 10 min each and dried under air before use. The solution was transferred to a Teflon-lined autoclave, and the clean ITO slides were vertically placed in the solution. The autoclave was sealed and heated in an oven at 90 °C for 9 h. After that, the autoclave was naturally cooled to room temperature in the oven. The resulting MgAl–CO_3 LDH/ITO slides were taken out from the autoclave, rinsed with water and then dried under air.

2.4. Preparation of CoAl–CO_3 LDH on ITO

CoAl–CO_3 LDHs were directly grown on ITO-coated glass slides according to the literature [33]. ITO-coated glass slides (1 × 2 cm^2) were sequentially cleaned by sonicating with soapy water, water, acetone, ethanol and then water for 10 min each before use. $Co(NO_3)_2 \cdot 6H_2O$ (0.29 g, 1.0 mmol), $Al(NO_3)_3 \cdot 9H_2O$ (0.19 g, 0.5 mmol), NH_4F (0.18 g, 5.0 mmol) and urea (2.10 g, 35 mmol) were dissolved in 50 mL of water and the solution was stirred for at least 30 min at room temperature. The mixture (20 mL) was transferred to a Teflon-lined autoclave and the cleaned ITO slides were vertically placed in the solution. The autoclave was sealed and heated at 95 °C for 6 h. After that, the autoclave was naturally cooled to room temperature in the oven. The resulting CoAl–CO_3 LDH/ITO slides were removed from the autoclave, rinsed with water and dried at room temperature under air.