Short communication

Mediator-free solar energy conversion by the artificially installed thylakoid membrane on the functionalized electrode

Jinhwan Lee, Sunghyun Kim *

Department of Bioscience and Biotechnology, Konkuk University, Seoul 143-701, Republic of Korea

A B S T R A C T

Here we report an effective solar energy conversion using a whole thylakoid membrane chemically installed on the anthraquinone-modified glassy carbon (GC) electrode. A mixed monolayer of anthraquinone and carboxyphenyl on the GC surface was formed prior to the thylakoid bioconjugation via reductive C–C coupling. Thus prepared electrodes showed higher photocurrent than those without an anthraquinone layer. Two main electron transfer pathways have been identified from the blocking experiments with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), an inhibitor of Q_B site in photosystem II (PSII) and with paraquat, a possible electron acceptor of F_A/F_B site in photosystem I (PSI). 41% photocurrent follows the normal electron transfer pathway from F_A/F_B to the electrode via anthraquinone. The remaining 59% directly flows from Q_A (or Q_B) to the electrode also via anthraquinone. From the complete solar cell that was constructed in combination with an oxygen reduction cathode, the maximum power density of 0.27 μW/cm^2 with current density of 0.63 μA/cm^2 under one sun illumination was obtained. The turnover frequency was calculated to be 10 water molecule oxidation per PSII unit per second, indicating that the thylakoid membrane on the surface maintained its activity. This work provides a simple and reliable method to photoenergy conversion using thylakoid membranes.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

It has been estimated that the solar energy impinging on the earth for an hour (4.3×10^{20} J) is approximately equivalent to the total energy consumed by humans in a year (4.1×10^{20} J) [1]. Much attention has been paid on harnessing solar energy in the form of electricity and hydrogen as the demand for clean and renewable energy is increasing. One of the best ways to harvesting light is to use photosystems found in higher plants and microalgae since all the subunits have been maximally optimized for photoconversion [2]. Therefore numerous attempts have been made to develop photoelectrochemical cells by installing PSI [3–5] or PSII [6–8] onto the electrode.

For PSI-driven solar cells, the works by Jennings and coworkers are especially notable. They have tested various materials and modification methods in an effort to increase photocathodic current [9–11] and recently reported exceptionally large current of 875 μA cm^{-2} with a p-doped silicon using methyl viologen as an electron acceptor [12]. When an electron donor is present, photoanodic current could flow [13]. Willner and coworkers [14] demonstrated that the PSI multi-layer system using ascorbic acid as an electron donor generated photoanodic current. Despite appreciable photocurrent, the fact that PSI needs sacrificial electron donors or acceptors to maintain

photocurrent is problematic from a practical point of view as securing easily available fuels is important. In this sense, using PSII as a light-conversion unit is advantageous over PSI because water can be used as fuel. Oxygen evolving complex attached to PSII effectively oxidizes water to oxygen molecules producing protons and electrons. Following the natural electron transfer pathway under illumination, electrons can be directed to the anode generating photoanodic current in combination with a suitable cathodic reaction. Willner and coworkers [8] devised sophisticated photoelectrochemical systems where PSII and bilirubin oxidase are integrated onto the anode and cathode, respectively, where produced oxygen molecules at the anode can be used as a fuel at the cathode. Recently they also developed a cytochrome c-coupled PSI/PSII anode for efficient electron transfer [15].

Despite many advantages of PSI or PSII for light conversion, time-consuming procedure of isolation and purification may prevent them from being readily applied to the real world. Better choice is to directly use the thylakoid membrane as a conversion unit, in which PSI and PSII are safely preserved. Lam et al. [16] reported solar energy conversion from suspended thylakoid in a microelectromechanical system using a redox mediator. However, very small power density of 5.7 pW renders it virtually impractical. In the meantime, Calkins et al. [17] demonstrated that the performance could be greatly improved when the anode was modified with thylakoid-carbon nanotube composites and a $Fe(CN)_6^{3-/4-}$ couple was used as a soluble mediator. In combination with laccase as an oxygen reduction catalyst on the cathode, the maximum power density of 5.3 μW cm^{-2} was obtained.

Here we report our results of photocurrent generation from immobilized thylakoid membrane on the anthraquinone-functionalized electrodes without any mediators. Electrochemical behavior of anthraquinone has been well studied [18,19] and its formal potential ($E^{0'} = -0.38$ V vs SHE) is suited to accept electrons from F_A (-0.52 V) or F_B (-0.58 V) of PSI. Two possible electron transfer pathways were identified from blocking experiments. The performance of the cell was tested after constructing a complete solar cell with a suitable cathode.

2. Experimental

2.1. Surface modification and characterization

Three modified surfaces were prepared on glassy carbon electrodes (0.0314 cm^2) diazotization and reductive C–C coupling (Fig. 1) [20]. To prepare GC/CP and GC/AQ, 4-aminobenzoic acid (5 mM) and 2-aminoanthraquinone (0.5 mM) were reacted respectively with 50 mM and 20 mM of NaNO$_2$ in 1 M HCl at 4 °C to form diazonium cations. These solutions were then transferred to the three-electrode setup in which a glassy carbon was used as working electrode. By single potential cycling between 0.6 and -0.4 V vs. Ag/AgCl at scan rate of 50 mV s^{-1}, 4-carboxyphenyl and anthraquinone groups were covalently bonded on the surface, respectively. The GC/AQ-CP was prepared in the same way. 4-Carboxyphenyl diazonium cation solution was transferred to the pre-prepared GC/AQ surface. By potential cycling in the same range, 4-carboxyphenyl groups were grafted to the anthraquinone as well as to the empty part of the GC surface [21,22]. The exposed carboxyl groups in GC/CP and GC/AQ-CP were used to bioconjugate thylakoid via peptide bond formation with amine groups of thylakoid using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and N-hydroxysuccinimide as coupling agents (GC/AQ-CP/Thyl). Thylakoid was isolated from spinach according to Danielsson et al. [23,24]. Chlorophyll concentration was spectrophotometrically determined [25].

Each surface was characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) using Fe(CN)$_6^{3-/4-}$ couple (1 mM) as a probe in pH 7 buffer. The frequency range varied from 10 kHz to 10 mHz with a 5 mV sinusoidal perturbation. Assuming a Randles equivalent circuit, charge transfer resistances (R_{ct}) were calculated from Nyquist plots. Surface morphologies were observed with atomic force microscope (AFM, Park Systems XE-100, Korea) and scanning electron microscope (SEM, JSM 6380, Jeol, Japan).

2.2. Photocurrent measurement and construction of photoelectrochemical cells

A typical three-electrode system has been employed to measure photocurrent at 0.4 V bias potential under one sun illumination (100 mW cm^{-2}) using a solar simulator (McScience, Korea). To analyze electron transfer pathways, thylakoid suspension was treated with DCMU (0.1 mM) and paraquat (1 mM) before bioconjugating to GC/AQ-CP as DCMU blocks Q$_B$ site in PSII [6] and paraquat competes ferredoxin for accepting electrons from the F_A/F_B site in PSI [17]. The complete photoelectrochemical cell was constructed in combination with a Pt/C oxygen reduction cathode. Polarization curve was obtained by scanning potential from open-circuit voltage, from which the power density was calculated as a function of current density. All the measurements were done in 15 mM MES (2-(N-morpholino)ethanesulfonic acid) buffered at pH 6.5 containing 15 mM NaCl and 300 mM sucrose.

3. Results and discussion

3.1. Voltammetric and EIS characterization

Cyclic voltammetry (CV) was employed to examine the monolayer formation by using Fe(CN)$_6^{3-/4-}$ as a probe (Fig. 2A). The prominent redox peaks on a bare GC become smaller on a GC/AQ surface. CP and AQ-CP layers almost completely blocked the surface because negatively charged surfaces expelled also negatively charged Fe(CN)$_6^{3-/4-}$. From three independent experiments under the same conditions except Fe(CN)$_6^{3-/4-}$, the average surface coverages of anthraquinone from the reduction wave were 1.8×10^{-9} and 1.5×10^{-9} mol cm^{-2} for GC/AQ and GC/AQ-CP, respectively.

To get more insight into the charge transfer through the film, EIS characterization has been done (Fig. 2B). Base on a Randles equivalent circuit, ohmic (R_Ω) and charge transfer (R_{ct}) resistances were evaluated. For the better fitting we introduced a constant phase element for double layer capacitance instead of a pure capacitor [26]. The Nyquist plots show very different R_{ct} values with constant R_Ω. R_{ct} of 309 Ω for the bare GC greatly increases to 58 kΩ, 180 kΩ, and 1.9 MΩ for GC/AQ, GC/AQ-CP, and GC/CP, respectively. This trend is consistent with CV results.

3.2. Photocurrent generation and electron transfer pathway

Photocurrent was observed only when the surface was coated with thylakoid membrane (Fig. 3A). The largest current of 0.49 µA cm^{-2} for GC/AQ-CP/Thyl decreased to 0.16 and 0.095 µA cm^{-2} for GC/AQ/Thyl and GC/CP/Thyl, respectively. This confirms the importance of bioconjugation of thylakoid and the role of the anthraquinone monolayer as a molecular conduit through which electrons are effectively transferred to the electrode. Since the formal potential of anthraquinone (-0.38 V vs. SHE) is suitable for accepting electrons from F_A ($E^{0'} = -0.52$ V) or F_B (-0.58 V) in PSI, $F_A/F_B \rightarrow$ electrode is inferred as a primary electron transfer (ET) pathway. When thylakoid suspension was treated with DCMU, which blocks ET from Q$_A$ to Q$_B$,
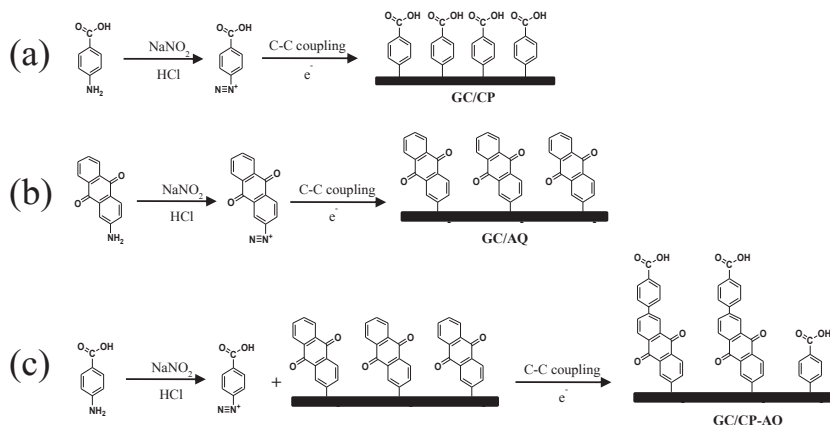

Fig. 1. Schematic representation of surface modification steps to prepare GC/CP, GC/AQ, and GC/AQ-CP.