ELECTRO CHEMICAL UTILIZATION OF THYLAKOID MEMBRANE AND CYANOBACTERIA FOR THE SOLAR ENERGY CONVERSION TO ELECTRICITY

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Abstract - Biological utilization of solar energy to produce electricity and hydrogen has drawn much attention during the past decades as the demand for the sustainable energy sources is ever increasing. Though many artificially designed integrated chemical systems have been developed, the practical application of those systems has difficulties due to high production cost and the short lifetime. Light reactions in photosynthesis are the very effective system nature has developed for the solar energy utilization. Here we report how biomaterials such as thylakoid membranes (TMs) and cyano bacteria can be electrochemically utilized for the electricity and hydrogen production.

Keywords – Solar Energy, Thylakoid, Cyanobacteria, Electricity, Surface Modification, Light Reactions.

I. INTRODUCTION

Establishing a sustainable society largely depends on renewable energy sources that do not emit greenhouse gases. Solar energy in this sense is ideal in that it is absolutely clean and impinging energy of 1.2 x 10^5 TW is much greater than the world's total energy consumption of 18.5 TW.[1,2] Considering eventual depletion of fossil fuels and strict environmental regulations, it is imperative to utilize solar energy. Intensive research therefore has been poured and various types of solar cells have been developed.[3,4]Another types of solar energy utilization called photo-bioelectrochemical systems have also been devised during the past several decades. Various biological materials are used as photocatalysts to convert light to electricity. Many attempts have been made to mimic photosynthetic light reactions that take place in thylakoid membranes (TMs) of green plants and cyanobacteria. Absorbed photons are harvested and used to excite electrons in photosystems I and II (denoted PSI and PSII, respectively), whose quantum efficiency is nearly unity for PSI.[5]It is, therefore, no wonder that many ingenious PBESs have been developed using PSI and PSII as conversion units.[6-8]

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 photo cathodic current.[9-11] When an electron donor is present, photo anodic current could flow[12]. Willner and coworkers[13] demonstrated that the PSI multi-layer system using ascorbic acid as an electron donor generated photo anodic 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.

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. TMs are a huge machinery in which all the subunits such as light harvesting and protein complexes, and oxygen evolving center are embedded in the bilayer membrane, providing an ideal environment for the solar light utilization. Easy extraction, purification, and structural stability offer additional advantages to TMs over PSI or PSII. Lam et al.[14] reported solar energy conversion from suspended thylakoid in a micro electromechanical system using a redox mediator. However, very small power renders it virtually impractical. In the meantime, Calkins et al.[15] 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 $\mu W\ cm^{-2}$ was obtained.

Recently there is growing much interest in utilizing cyano bacteria as photo-biocatalysts to generate electrical current.[16,17] The use of live whole cells in PBEC could overcome those drawbacks mentioned above because there is no need to isolate photosynthetic components from cells, which greatly saves time and effort, and also long-term stability is expected since live microorganisms try to maintain

homeostasis resisting environmental changes.Here we present our ongoing research using TMs and *Anabaena variabilis*, cyano bacterium for an effective solar energy conversion aiming at practical application.

II. EXPERIMENTAL

2.1 Isolation of TMs and Activity Measurement

TM, a subunit of chloroplast, was isolated from spinach according to the established method by Danielsson.[18] Chlorophyll concentration in the isolated TM was spectro photo metrically determined. The isolated TM samples were diluted to a chlorophyll concentration of approximately 3 mg Chl mL^{-1} an dstored at −80°C.TM activity was evaluated in terms of oxygen evolving activity of photosystem II which was measured by a Clark-type O_2sensorat 20°C at one sun illumination using 15 mM MES (2-(N-morpholino) ethanesulfonic acid) at pH 6.5 with 2 mM ferricyanide and 0.5 mM phenyl-p-benzoquinone as an electron accepter.[18]

2.2 Surface Alteration and TM Immobilization

For an effective electron transfer, glassy carbon surface was modified before the TM immobilization. Three surfaces were prepared through diazotization and reductive C-C coupling on which TMs were immobilized.[19]To prepare GC/CP and GC/AQ, 4-aminobenzoic acidand 2-aminoanthraquinone were reacted respectively with $NaNO_2$ in 1 M HCl to form diazonium cations, and then a single potential cycling induced covalent bonding of 4-carboxyphenyl and anthraquinone groups on the surface. Other surfaces were prepared in the same way. 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.

2.3 Cell Culture of *A. variabilis*

A wild type strain of *A. variabilis* was purchased from KCTC (Korean Collection for Type Cultures). Cells were grown at 28°C by shaking at 140 rpm in a BG11(1X) medium containing 20 mM HEPES buffer (pH 7.5) under the 3200 Lux fluorescent light which was operated in a 24 h cycle (12 h light/12 h dark) for a week. Cells wereperiodically grown on agar plates containing 1.2% agar, BG11(1X) and HEPES buffer, and the grown colony was inoculated into liquid medium until the concentration did not exceed 7 μg Chla mL.

2.4 Preparation of Working Electrodes

Reduced graphene oxide-coated indium tin oxide was used as a working electrode. The ITO surface was prepared through the following procedure. First, ethanol-washed ITO pieces (1.0 cm×0.50 cm) were cautiously pretreated with a mixture solution (H_2O:H_2O_2:NH_4OH=5:1:1 by volume) at 70°C for an hour to develop hydroxyl group on the ITO surface and then rinsed with distilled water. The pretreated electrodes were then modified with amino group using a general silanization method (1% 3-aminoprophyl trimethoxysilane for 1.5 h) to improve the affinity to rGO. The silanized ITO electrode was stored in pure anhydrous ethyl alcohol solution before use. rGO was prepared by adding Al foil and HCl into graphene oxide-dispersed water solution. After gently mixing, the sample has stayed still until the aggregated particles were settled down at the bottom. The supernatant analysis showed that GO was reduced to rGO. Thus reduced graphene oxide solution was diluted so that the amount of rGO was 35 μg per mL. To prepare a uniformly coated electrode, first 60 μL of rGO solution was introduced to the ITO surface and dried on a plate at 40°C. This procedure was sequentially repeated with 50 μL and 40 μL rGO solutions. Thus prepared rGO-coated ITO electrodes were carefully rinsed with ethanol and deionized water several times. A freshly prepared rGO-coated ITO electrode was used every time when a new measurement was done.

2.4 Construction of a Dual Functioning Electro-chemical Cell

A three-electrode electrochemical cell that simultaneously measures oxygen evolution rate and photocurrent was constructed. A cylindrical chamber where the working-, counter-, and reference electrodes were located was positioned on the oxygen sensor. The chamber was surrounded by a water jacket to maintain solution temperature at a specified value by a circulator. *A. variabilis* was dispersed inside the chamber. Electrochemical behavior of the working electrode was tested by cyclic voltammetry in a three-electrode setup.

III. RESULTS AND DISCUSSION

3.1Installing TMs on the Modified Surface and Photocurrent Measurements[20]

Figure 1 shows three different surfaces on which TMs are chemically installed.

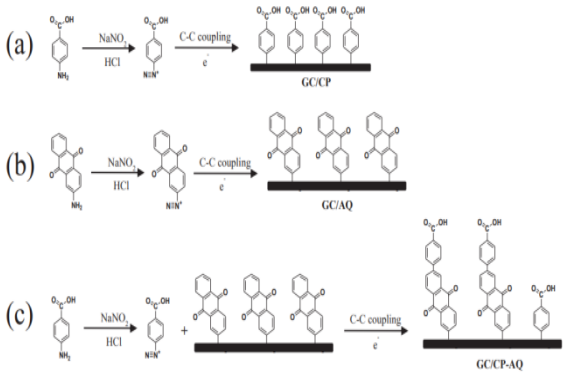


Fig. 1. Schematic representation of surface modification steps to prepare GC/CP, GC/AQ, and GC/AQ-CP.

Cyclic voltammetry and electrochemical impedance spectroscopy using Ferri/ferrocyanide as a probe revealed that the surface was well covered with a monolayer of carboxyphenyl, anthraquinone and CP/AQ mixed monolayer. TMs were chemically installed on each surface and photocurrent was measured (Figure 2).

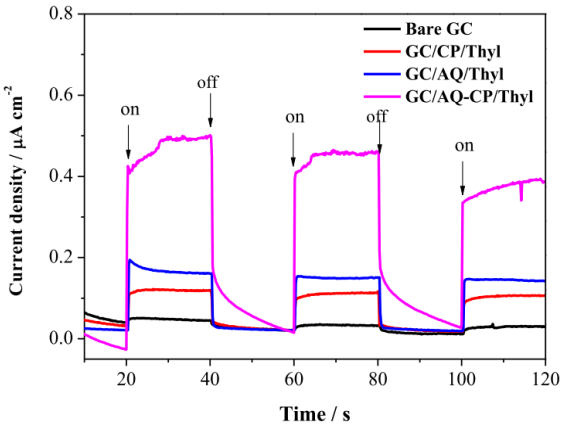


Fig. 2. Photocurrent responses from a bare GC, thylakoid modified GC/CP, GC/AQ, and GC/AQ-CP at an applied potential of 0.4 V vs. Ag/AgCl.

Appreciable photocurrent was observed only when the surface was coated with thylakoid membrane. 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 FA ($E^{0'}$ = −0.52 V) or FB (−0.58 V) in PSI, FA/FB → electrode is inferred as a primary electron transfer pathway. When thylakoid suspension was treated with DCMU, an inhibiter, electron transfer was blocked.

3.2Power Generation

The complete solar cell consisted of a GC/AQ-CP/Thyl anode and a Pt/C cathode was constructed. The polarization curve and thereby power density plot as a function of current density were constructed by scanning potential from open circuit voltage toward zero value (Figure 3). The maximum power density was 0.27 μW cm^{-2} at 0.63 μA cm^{-2}. The quantum yield is about 0.0032%. This power density is much smaller than the reported value of 5.3 μW cm^{-2}, in which thylakoid multilayers were formed onto the multi-walled carbon nanotube-modified electrode and soluble mediators were used to carry electrons from the interior of the thylakoid.[13] However, this case is distinguished from it in that the thylakoid membrane was installed on a pre-functionalized well-polished electrode surface and no redox mediators were used. Direct ET therefore takes place from thylakoid to the electrode via the anthraquinone monolayer.

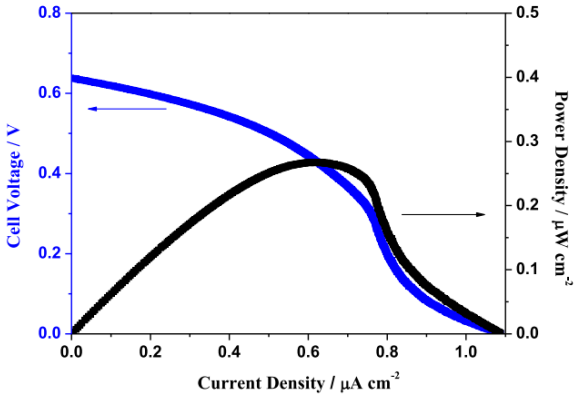


Fig. 3. Photocurrent responses from a bare GC, thylakoid modified GC/CP, GC/AQ, and GC/AQ-CP at an applied potential of 0.4 V vs. Ag/AgCl.

3.3 Solar Energy Conversion Mediated by Osmium Redox Polymer[21]

A remarkable photocurrent enhancement has been observed when Os-redox polymer (Os-RP) was employed. This polymer has been widely used especially in glucose enzymatic fuel cells in which it electrically wires glucose oxidase (GOx) and the electrode. Electrons generated from glucose oxidation by GOx are efficiently transferred to the anode. A similar concept was adopted in this study. As shown in Figure 4, TMs were well mixed with Os-RP and then with indium tin oxide nanoparticles (ITOnp). Thus prepare TMs/Os-RP/ITOnp was spread on the graphite surface.

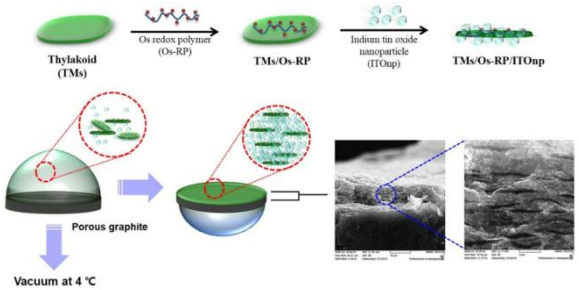


Fig. 4. Formation procedure of a thin film of TM/Os-RP/ITOnp composite material on the porous graphite and SEM images (scale bars: 10 and 2 μm, respectively) of the cross-sectional area of the film and the electrode

Photocurrent as high as 0.5 mA cm^{-2} was achieved at 0.4 V. This is the highest value to our knowledge. Lack of a single component caused a serious current decrease. ET mechanism is TMs → Os-Rp → ITOnp → electrode.

Inhibition experiments with DCMU showed that no oxygen gas was detected after treatment while a fresh TM solution keeps producing oxygen. Although oxygen was not evolved, ca. 41% photocurrent was still measured even afterDCMU treatment. This means that water oxidation still occurs in the oxygen evolving center attached to PSII. Although the

electron pathway from Q_A to Q_B is blocked, another pathway from Q_A to the electrode is available via Os-RP and ITOnp. The remaining 59% photocurrent must come from other components in the Z-scheme. Possible candidates are plastoquinone pool and ferredoxin in PSI, whose redox potentials are more negative than that of Os-RP.

3.4 Construction of the Complete Solar Cell and Power Generation

A single-chamber photoelectrochemical cell was constructed with an TM/Os-RP/ITOnp anode and an air cathode where the oxygen reduction reaction took place (Figure 5A). Upon illumination of visible light, photoelectrons, protons, and oxygen molecules are produced at the anode by photolysis. Electrons move to the cathode through the external load across which voltage is developed. The polarization curve (E vs. I) was constructed by recording voltage drop across the external load whose value changes from 7.1 MΩ to 565 Ω in decreasing order. Power density curve (P vs. I) was then calculated from the polarization curve by P=E× I. Figure 5B shows typical shapes of these plots. The maximum power density (P_{max}) of 122μWcm^{-2}.

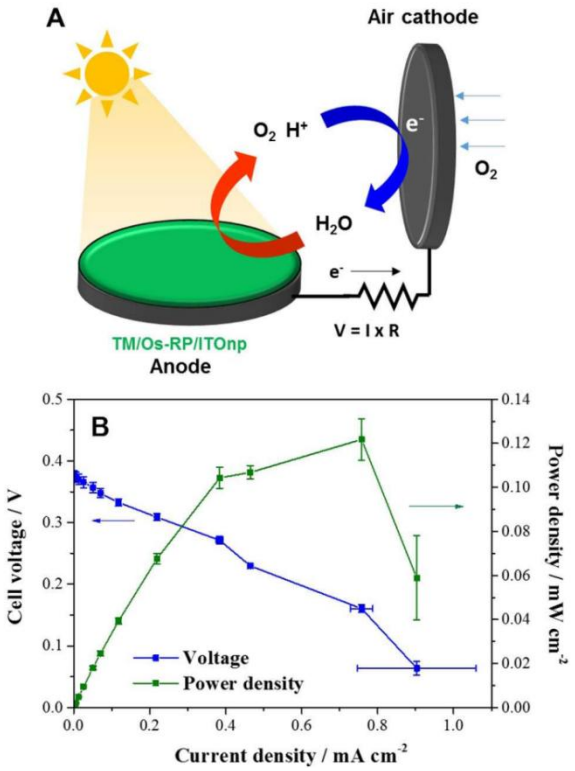


Fig. 5. (A) Schematic representation of a complete PBEC composed of a TM/Os-RP/ITOnp photoanode and a Pt/C air-cathode. (B) Plot of polarization and power density curves as a function of current density.

3.5Solar Energy Conversion Using Live Bacterium *Anabaena variabilis*

One ofgreat disadvantages of using TMs is a short lifetime. Initial large photocurrent does not last long time. After several minutes, it usually decays to half of the initial value. Photo damage or the structural alteration with time could be the reason. However, when live cyanobacterium such as *A. variabilis* was employed, we found that higher photocurrent and much longer lifetime were achieved. The problem lies in how to accommodate a large number of cyanobacterium on the surface and how to effectively direct electrons to the electrode since TMs are situated deep inside the cell. For the first problem, we dispersed the cells in the solution rather than immobilizing them on the surface. For the second problem, we employed a redox mediator that possess properties of freely penetrating cell membranes to take electrons from TMs and deliver to the electrode, and of having suitable redox potential. Benzoquinone was found a suitable mediator. Figure 6 shows the scheme and the whole cell performance.

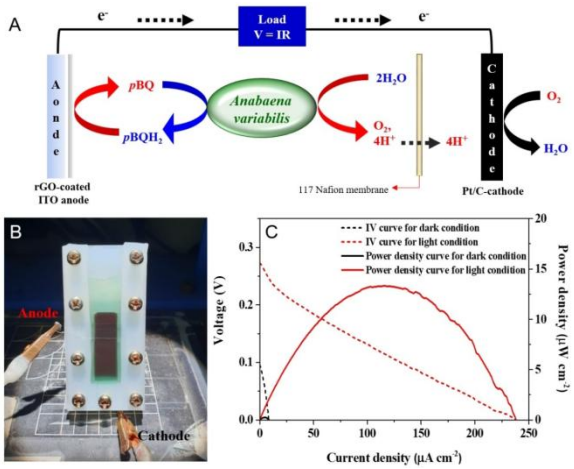


Fig. 6. A Schematic diagram (A) and a photo image (B) of the complete photo-bioelectrochemical cell with dispersed *A.variabilis* (10 μg Chla mL^{-1}) in 10 mM HEPES buffer (pH 7.5) solution containing BG-11(1X) medium. Panel C shows polarization and power density curves as a function of current density. The cell was irradiated with 1 sun light intensity for 15 min before measurements. 1 mM pBQ was used as a mediator. Temperature was maintained at 28°C.

The maximum power density of 13 μWcm^{-2} and the current density of 115 μAcm^{-2}are much higher than those reported. Although some power generation is possible in a mediatorless PBEC,[22]mediated electron transfer systems from immobilized cyanobacteria are usually employed for the efficient solar energy conversion. For example, Gorton and coworkers increased maximum current density to 48.2 μAcm^{-2}from *Leptolyngbya* sp. when they used Os redox polymer and ferricyanide and electron mediators.[23] Ramasamy and his group achieved an enhanced power density of 10 μWcm^{-2}from *Nostoc* sp. when a mediator was added. Without a mediator, only 3.5 μWcm^{-2}was obtained.[24] The same group also showed that genetic engineering to express a redox protein such as cytochrome S on the outer membrane is a promising method to increase photocurrent.[25] Comparing these results, our strategy of dispersing cyanobacteria with a suitable redox mediator could be a powerful means to enhance the PBEC performance. Turnover frequency of water oxidation per unit PSII

was calculated by estimating the number of PSII which could be calculated from the number of chlorophyll *a* molecules in PSII. Since each PSII and PSI has 35 and 95 chlorophyll *a* molecules, respectively and they exist in the ratio of 1:6 (PSII/PSI) in *A. variabilis*.[18] Our obtained value of 10.7 for current density of 115 μAcm^{-2}at the maximum power is much less than the theoretical value of ca. 1000,[6] but greater than those from immobilized PSII or cyanobacteria biofilms,[26] indicating that mediated electron transfer is very promising for the solar energy conversion.

Much higher current has been achieved using a double-mediator system, in which the first mediator such as quinone takes electrons from TMs and deliver them to the second mediator such ferricyanide. Ferricyanide has a property of very fast electrochemical ET. It accepts electrons from reduced quinone and deliver them to the electrode. Although the cell potential is a little lowered than the case of a single-mediator system, the fast ET makes the cell performance better (Figure 7).

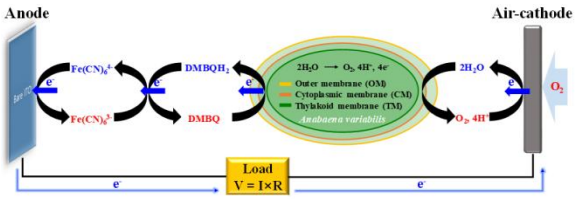


Fig. 7. The schematics of principle of a double-mediated photobioelectrochemical cell.

Our preliminary results show more than 2 mA cm^{-2} photocurrent if sufficient amount of *A. variabilis* and higher mediator concentrations are warranted. These results throw some hope of implementing this technology in a real world. Research is under way and will be reported in due course.

IV. CONCLUSION

1. Nature's biomaterials such as TMs and cyanobacteria can be electrochemically utilized as they are for the solar energy conversion.
2. TMs are good biological materials and can convert solar light to electricity particularly they are installed on the electrode surface. Higher current is generated when a suitable means to wire TMs and the electrode is available. However, a short lifetime and a low current density are problems to be solved.
3. Live cyanobacteria such as *Anabaena variabilis*can be used as a photo-biocatalyst that oxidizes water by solar light. When dispersed in the solution, *A. variabilis*with benzoquinone as a redox mediator produced photocurrent of 223 μAcm^{-2}. Since thylakoid membrane still maintains its activity on the surface, much higher photocurrent would be expected if conducting mesoporous materials that can accommodate higher number of PSIIcytochrome *b6f*-PSI units are used. A double-mediated system may provide a future renewable energy source.

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