Efficient Photoelectrochemical Energy Conversion using Spinach Photosystem II (PSII) in Lipid Multilayer Films

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The need for clean, renewable energy has fostered research into photovoltaic alternatives to silicon solar cells. Pigment–protein complexes in green plants convert light energy into chemical potential using redox processes that produce molecular oxygen. Here, we report the first use of spinach protein photosystem II (PSII) core complex in lipid films in photoelectrochemical devices. Photocurrents were generated from PSII in a ~2 μm biomimetic dimyristoylphosphatidylcholine (DMPC) film on a pyrolytic graphite (PG) anode with PSII embedded in multiple lipid bilayers. The photocurrent was ~20 μA cm^{-2} under light intensity 40 mW cm^{-2}. The PSII–DMPC anode was used in a photobiofuel cell with a platinum black mesh cathode in perchloric acid solution to give an output voltage of 0.6 V and a maximum output power of 14 μW cm^{-2}. Part of this large output is related to a five-unit anode–cathode pH gradient. With catholytes at higher pH or no perchlorate, or using an MnO$_2$ oxygen-reduction cathode, the power output was smaller. The results described raise the possibility of using PSII–DMPC films in small portable power conversion devices.

Photosystem II (PSII) in green plants is one of the key components in the photochemical electron-transfer scheme that effectively converts light energy into chemical potential.[1] The process begins when the primary electron donor P680 in the PSII reaction center is excited either by excitation energy transfer from associated light-harvesting pigment–protein complexes or by direct absorption of light. The excited P680 transfers an electron to a reaction center-bound pheophytin. Subsequently, the electron is transferred in sequence to two quinone acceptors denoted Q$_A$ and Q$_B$.[1] The oxidized P680$^+$ is returned

to its neutral state by extracting an electron from a nearby Mn$_4$Ca cluster where four oxidizing equivalents are built up, ultimately resulting in the oxidation of water to molecular oxygen.

In a previous study, we reported direct voltammetry of the spinach PSII core complex embedded in lipid and polyion films, and elucidated the mechanisms of the resulting electrochemical redox reactions.[2] The PSII–DMPC films used in those studies and in the present work require only tiny amounts of protein, eliminate inefficient diffusion processes, and preserve the PSII native structure.[3] The PSII core complex is embedded within multiple bilayers of dimyristoyl-phosphatidylcholine (DMPC) arranged similarly to stacked lipid bilayer membranes.[1, 2] These films are liquid crystalline at ambient temperature, and water layers separate the lipid bilayers.[3]

Previous reports have described photoelectrochemical devices based on bacterial PSII reaction centers.[4–6] His-tag-engineered cyanobacterial PSII[7] on a nanostructured gold electrode gave a photocurrent of 2.4 μA cm^{-2} at 680 nm (3.3 mW) with 0.2 V versus Ag/AgCl, and PSII entrapped by osmium redox polymers[8] on gold produced a photocurrent density of 14 μA cm^{-2} at 675 nm (100 μmol photons m^{-2} s^{-1}) with 0.3 V versus Ag/AgCl. Reisner et al.[9] used cyanobacterial PSII on a mesoporous indium tin oxide (*meso*-ITO) electrode to oxidize water and produce a photocurrent. They improved the photocurrent by covalently binding PSII to the negatively charged ITO surface.[10] Cyanobacterial PSII/cytochrome *c* (Cyt *c*)/photosystem I (PSI) with poly(vinylpyridine) crosslinking and implanted platinum nanoclusters also generated a photocurrent maximum of 0.22 μA cm^{-2} at 680 nm.[11] A photobiofuel cell featuring a cyanobacterial PSII photoanode and a bilirubin oxidase/carbon nanotube cathode that produced electricity without a sacrificial reagent was reported by Willner et al.[12] The largest output potential was 0.42 V, and the maximum output power was 17 μW cm^{-2}. A spinach thylakoid–multiwall carbon nanotube anode and laccase–multiwall carbon nanotube cathode were combined in a similar cell design[13] to provide energy generation. Willner et al.[14] also fabricated PSI/PSII layer-by-layer films linked by redox polymers polybenzyl viologen/polylysine benzoquinone on an ITO electrode to increase anodic photocurrent sixfold compared with PSII alone.

Here, we report the first use of spinach PSII core complex in cast lipid films that convert light to electrical potential. Films are approximately 2 μm thick and contain about 30 μg of PSII.[2, 3] These films are much easier to prepare than any of the reported PSII photoanodes described above, requiring only drop casting a dispersion of PSII and DMPC vesicles onto the electrode and drying. Photocurrents of PSII–DMPC films were first obtained to demonstrate the possibility of driving the

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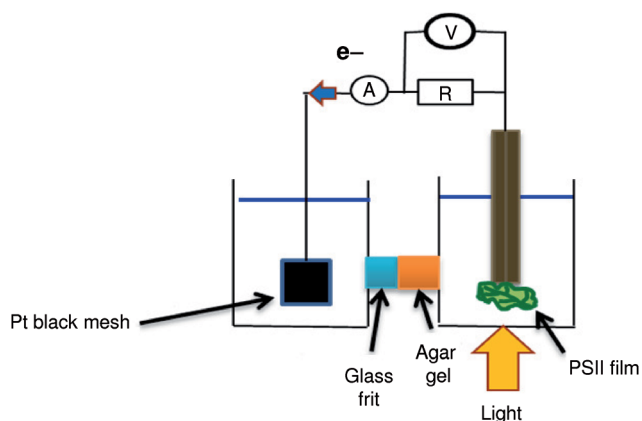
Scheme 1. Photovoltaic cell featuring a PSII–DMPC film photoanode and a platinum black cathode.

electron-transfer processes upon illumination. Subsequently, we fabricated a simple photobiofuel cell using a platinum black mesh cathode and PSII–DMPC on pyrolytic graphite (PG) as a photoanode (Scheme 1), which produced a maximum cell potential of 0.6 V and a power output of 14 µW cm^{-2}. The output potential was larger and the power comparable to the best previously described PSII photocell employing a cyanobacterial PSII photoanode and a bilirubin oxidase/carbon nanotube cathode.[12]

When the PSII photoanode was outfitted with a counter and reference electrode in an electrochemical cell, significant differences in photocurrent for light–dark cycles confirmed that photoexcitation of the PSII core complex injects electrons into the underlying PG electrode. Use of 2,5-dichloro-benzoquinone (DCBQ) as an electron mediator increased the photocurrent by a factor of 100 to 20 µA cm^{-2}. Without the mediator in the cell, direct interfacial electron transfer provided minimal current. Apparently, only a very small amount of PSII in the film is able to transfer photon-induced current directly to PG, so the mediator is needed to deliver electrons efficiently. The formal redox potential of DCBQ in a buffer at pH 6 was 0.13 V versus a saturated calomel electrode (SCE) as measured by cyclic voltammetry. Because the applied potential was more positive than the redox potential, DCBQ near the photoelectrode is maintained in the oxidized state. Photo-induced electrons from PSII are then rapidly shuttled by DCBQ to the electrode with concomitant oxidation of water to molecular oxygen to produce the stable current. Control experiments on DMPC films not containing PSII gave negligible current changes during illumination. The photocurrents of all films were reproducible for consecutive scans with relative standard deviation < 10%. Figure 1b shows that photocurrents increase as the incident light intensity was increased.

The potential applied to the PSII–DMPC PG electrode influenced the magnitude of the photocurrent (Figure 2a). When the applied potential was larger than 0 V versus SCE, the photocurrent increased sharply and achieved steady-state levels at

~0.26 V versus SCE (Figure 2b). Because the standard redox potential of DCBQ is 0.13 V versus SCE, at 0.08 V, DCBQ begins to oxidize and immediately drives electrons into the PG anode, as observed previously for a cyanobacterial PSII system.[12] Increasing the applied potential leads to faster delivery of the electrons, so photocurrent increases. At potentials greater than 0.26 V, the photocurrent reached a limiting steady-state value that is most likely controlled by mass transport of DCBQ. When the applied potential is negative versus SCE, DCBQ remains in the reduced state and cannot accept electrons from PSII. Thus, the photocurrent is negligible.

The designed PSII-based photoelectrochemical cell paired the PSII photoanode and a platinum black mesh cathode separated by a salt bridge (Scheme 1). Different pH catholyte and anolyte were used to maintain appropriate working environments for each half-cell. The platinum black mesh cathodes had an open circuit potential of 0.72 ± 0.07 V versus SCE controlled by a pH 1 electrolyte solution.[15] Anode solutions of pH 6 were used to optimize water splitting by PSII.[16]
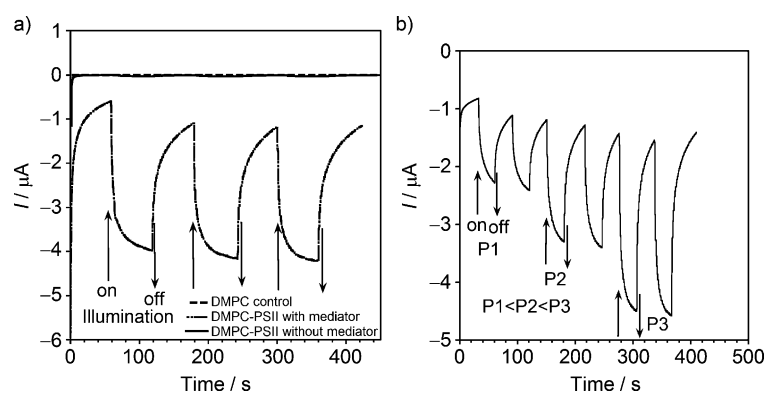

Figure 1. Photocurrent of cast PSII–DMPC film on PG anode in 20 mM 2-(N-morpholino)-ethanesulfonic acid (MES) buffer (pH 6.0), 15 mM CaCl$_2$, 15 mM MgCl$_2$, 100 mM NaCl at 25 °C with 0.1 mM DCBQ as mediator, applied potential 0.26 V versus SCE: a) under an incandescent $\lambda > 400$ nm light at 40 mW cm^{-2} with light–dark cycles every 60 s, photocurrent of PSII–DMPC film with mediator, without mediator, only DMPC film with or without mediator; oxidation photocurrents are downward. b) Influence of visible light illumination on the photocurrent using 30 s pulses at three different intensities at $\lambda > 400$ nm (P3 = 40 mW cm^{-2}, P2 = 27 mW cm^{-2}, P1 = 13 mW cm^{-2}).
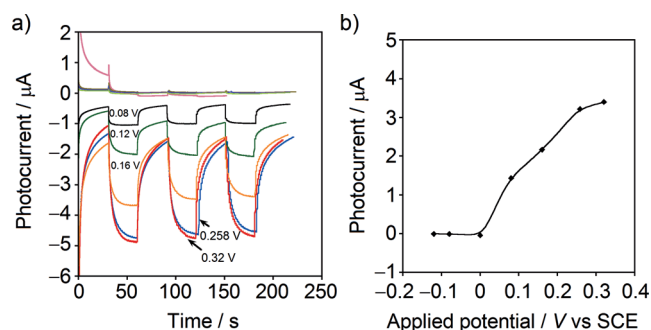

Figure 2. Influence of applied potential on the photocurrent of PSII–DMPC film on PG electrode in 20 mM 2-(N-morpholino)ethanesulfonic acid (MES) buffer (pH 6.0), 15 mM CaCl$_2$, 15 mM MgCl$_2$, 100 mM NaCl at 25 °C with 0.1 mM DCBQ mediator. a) Photocurrent for different potentials in repetitive 30 s light–dark cycles; b) Influence of potential on average photocurrent.
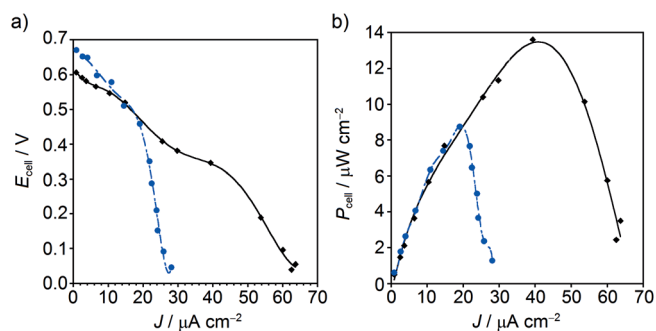
Figure 3. Comparison of discharge of PSII photoelectrochemical cells with salt bridges. Cathode was platinum black mesh in HCl/NaCl (pH 1) electrolyte (■) or in NaClO$_4$/HClO$_4$ (pH 1) electrolyte (◆). a) Cell discharges or polarization curves; b) Dependence of the cell power on current density using illumination at light intensity 40 mW cm^{-2}.

oxide film formation.[17–20] A possible explanation for the current-supporting cathodic reduction in acidic solutions derives from studies by Gilroy and Conway[17] on high-surface-area platinum black. Perchlorate in acid is a strong oxidant ($E^{o'} =$ 1.42 V) that most likely oxidizes the platinum black surface to set up a catalytic cycle involving [Equations (1) and (2)] in the cell.

$$PtO + H^+ + e^- \rightarrow PtOH \tag{1}$$

$$PtOH + H^+ + e^- \rightarrow Pt + H_2O \tag{2}$$

Hydrous oxide films on platinum have been reported after treatment with perchloric acid.[18] In this device, we have an open circuit potential of 0.72 ± 0.07 V versus SCE at platinum

The cell potential versus current density discharge of the cell with perchlorate shows a maximum voltage of 0.6 V and a maximum output power of 14 µW cm^{-2} (Figure 3). The anode oxidizes water to molecular oxygen by light excitation. Consecutive measurements decreased the potential and power output by approximately 5 %. The PSII–DMPC photoanode was stable during one full day of experiments, but lost activity after experiments followed by overnight storage at 4 °C. We also evaluated a cell with platinum black cathode at pH 1 containing HCl/NaCl but no perchlorate. The output voltage was slightly larger than for the perchlorate pH 1 catholyte (Table 1 and Figure 3), but cell power and current decreased, suggesting perchlorate plays an important role in oxidizing platinum in the cathode reaction.
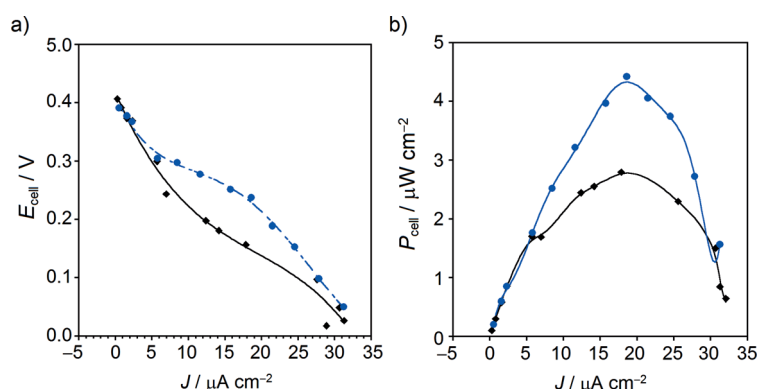

Figure 4. Comparison of discharge of PSII photoelectrochemical cells in undivided cells. Platinum black mesh (■) or α-MnO$_2$/C on PG electrode (◆) as cathodes in pH 6 electrolyte as for PSII–DMPC (anode). a) Cell discharge or polarization curve; b) Dependence of cell power on the current density using illumination at light intensity 40 mW cm^{-2}

Table 1. Comparison of bacterial PSII with spinach PSII/DMPC photocells.

Photobiofuel cell[a]	E_{cell} [V]	P_{cell} max [µW cm^{-2}]	J [µA cm^{-2}] @P_{cell} max
Au/pMBQ/PSII ∣ BOD/CNT/GC[12]	0.42	17	60
PG/PSII–DMPC ∣∣ Pt black/pH 1, salt bridge in HClO$_4$[b]	0.60	14	40
PSII–DMPC ∣∣ Pt black/pH 1 salt bridge in HCl[b]	0.67	8.7	19
PSII–DMPC ∣ Pt black, pH 6, no salt bridge[b]	0.39	4.5	19
PSII–DMPC ∣ α-MnO$_2$-HT/C/PG catalyst, no salt bridge[b]	0.41	3	18
Au/thylakoid-MWNT ∣ Lc-MWNT/Au[13]	0.35	5	25

[a] Abbreviations: BOD: bilirubin oxidase; CNT:carbon nanotube; GC: glassy carbon; PG: pyrolytic graphite; α-MnO$_2$-HT/C: α-MnO$_2$[21] synthesized by hydrothermal method mixed with carbon powder; Lc: laccase; Gox: glucose oxidase; MBH: membrane-bound hydrogenase; MWNT: multiwall carbon nanotube. [b] This work.

Platinum black and oxygen reduction cathode α-MnO$_2$[21] were paired in a common pH 6 electrolyte with PSII–DMPC anodes in a cell with no salt bridge. The output voltage, maximum power, and current density decreased compared with the pH-gradient perchlorate system (Table 1 and Figure 4).

Platinum black cathodes did not reduce water or oxygen in acidic catholytes, and the reaction probably involves platinum

black in perchloric acid, so that an oxide film could be formed similarly to that generated under potentiostatic anodization.[18]

In summary, the natural zwitterionic phospholipid DMPC was used to make biomembrane-like, stacked bilayer films of DMPC and PSII. This film provides a biomimetic environment for the PSII core complex, which retains near-native properties in these films.[2, 3] On electrodes, PSII–DMPC films showed high photoactivity in photocurrent experiments (Figures 1 and 2). Photocells featuring the first reported PSII–DMPC photoanodes and platinum black cathodes with a five-unit pH gradient gave a maximum potential of 0.6 V and a maximum power output of 14 µW cm^{-2}. Distinct advantages of PSII–DMPC films include design simplicity and ease of preparation. Our results raise the possibility of small portable power conversion devices using PSII–DMPC films. For example, we can envision a renewable device for intermittent or emergency low-power generation using a graphite anode base onto which PSII–DMPC is painted for daily use, then washed off and freshly repainted for subsequent use. We presented here several cathodes that

could be used in such a cell (Table 1), depending on performance requirements.

Experimental Section

Anode preparation: The dimyristoyl-phosphatidylcholine (DMPC) film solution was prepared by sonicating 1 mM DMPC in H_2O, then adding PSII (3.69 mg mL^{-1}). An aliquot of this solution (10 μL) was placed on the pyrolytic graphite (PG) surface and allowed to dry overnight. Isolation, purification and characterization of PSII core complex from spinach was reported in our previous paper.[2]

Photocurrent measurements: Data were collected at 25 °C using a thermostated three-electrode cell and a CH Instruments 660A electrochemical analyzer. The light source was a Leica incandescent illustrator (model # 13410311) with three different power settings (P1 < P2 < P3). An LI-250A light meter (LI-COR Inc.) was used to measure light intensity.

Photobiofuel cell construction: The cells were constructed in two-compartment glass cells with cathode and anode compartments, connected by a glass frit with agar gel to measure photobiofuel cell activity (Scheme 1) or in undivided cells. The light source was a quarts halogen illumination system (Dolan–Jenner Fiber Lite, model 190) operating at $P \sim 0.1$ W.

A 1×1 cm platinum mesh made from 0.25 mm diameter wire (Fuel Cell Materials Inc.) was deposited with platinum black by electrolyzing at −5 V versus Ag/AgCl in 30 mM H_2PtCl_6 with 1.5 mM $Pb(CH_3COO)_2$ for 5 min. α-MnO_2 electrodes were constructed as described previously.[21]

Supporting Information: Additional experimental details are provided in the Supporting Information available via http://dx.doi.org/10.1002/open.201402080.

[1] S. I. Allakhverdiev, *J. Photochem. Photobiol. B* **2011**, *104*, 1–8.
[2] Y. Zhang, N. Magdaong, H. Frank, J. Rusling, *Photosynth. Res.* **2014**, *120*, 153–167.
[3] K. Alcantara, B. Munge, Z. Pendon, H. A. Frank, J. F. Rusling, *J. Am. Chem. Soc.* **2006**, *128*, 14930–14937.
[4] O. Yehezkeli, R. Tel-Vered, D. Michaeli, I. Willner, R. Nechushtai, *Photosynth. Res.* **2014**, *120*, 71–85.
[5] F. Wang, X. Liu, I. Willner, *Adv. Mater.* **2013**, *25*, 349–377.
[6] A. Badura, T. Kothe, W. Schuhmann, M. Rogner, *Energy Environ. Sci.* **2011**, *4*, 3263–3274.
[7] N. Terasaki, N. Iwai, N. Yamamoto, T. Hiraga, S. Yamada, Y. Inoue, *Thin Solid Films* **2008**, *516*, 2553–2557.
[8] A. Badura, B. Esper, K. Ataka, C. Grunwald, C. Woell, J. Kuhlmann, J. Heberle, M. Roegner, *Photochem. Photobiol.* **2006**, *82*, 1385–1390.
[9] M. Kato, T. Cardona, A. W. Rutherford, E. Reisner, *J. Am. Chem. Soc.* **2012**, *134*, 8332–8335.
[10] M. Kato, T. Cardona, A. W. Rutherford, E. Reisner, *J. Am. Chem. Soc.* **2013**, *135*, 10610–10613.
[11] A. Efrati, R. Tel-Vered, D. Michaeli, R. Nechushtai, I. Willner, *Energy Environ. Sci.* **2013**, *6*, 2950–2956.
[12] O. Yehezkeli, R. Tel-Vered, J. Wasserman, A. Trifonov, D. Michaeli, R. Nechushtai, I. Willner, *Nat. Commun.* **2012**, *3*, 742.
[13] J. O. Calkins, Y. Umasankar, H. O'Neill, R. P. Ramasamy, *Energy Environ. Sci.* **2013**, *6*, 1891–1900.
[14] O. Yehezkeli, R. Tel-Vered, D. Michaeli, R. Nechushtai, I. Willner, *Small* **2013**, *9*, 2970–2978.
[15] K. Cheng, N. Ashraf, G. Wei, *Sensors* **2006**, *6*, 1187–1198.
[16] M. Völker, T. Ono, Y. Inoue, G. Renger, *Biochim. Biophys. Acta Bioenerg.* **1985**, *806*, 25–34.
[17] D. Gilroy, B. Conway, *Can. J. Chem.* **1968**, *46*, 875–890.
[18] B. E. Conway, *Prog. Surf. Sci.* **1995**, *49*, 331–452.
[19] A. M. Gómez-Marín, J. Clavilier, J. M. Feliu, *J. Electroanal. Chem.* **2013**, *688*, 360–370.
[20] A. A. Topalov, S. Cherevko, A. R. Zeradjanin, J. C. Meier, I. Katsounaros, K. J. J. Mayrhofer, *Chem. Sci.* **2014**, *5*, 631–638.
[21] Y. Meng, W. Song, H. Huang, Z. Ren, S.-Y. Chen, S. L. Suib, *J. Am. Chem. Soc.* **2014**, *136*, 11452–11464.