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Investigating the mechanism of thylakoid direct electron transfer for photocurrent generation

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ABSTRACT

Bioanodes incorporating thylakoids from spinach were studied to determine the mechanism of direct electron transfer to carbon electrodes. These electrodes generated a photocurrent of 0.43 ± 0.02 μA/cm^2. The change in this photocurrent was measured when individual components of thylakoids were removed, inhibited, or activated in order to determine which components contributed to the photocurrent. The results indicate that photosystems I and II, plastoquinone, cytochrome b_6f, and plastocyanin are involved in the direct electron transfer mechanism and both electron transport pathways in photosynthesis (cyclic and noncyclic) contribute to the photocurrent. One of the benefits to using an organelle as a biocatalyst is the possibility for multiple electron transfer pathways at the electrode surface. The reported thylakoid electrodes show a contribution of electrons from the first five electron transfer steps in photosynthesis with only the last two steps not participating.

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1. Introduction

As global energy consumption increases, an increasingly large area of research is the development of biological or bio-inspired methods for solar energy conversion [1]. These photovoltaic devices, or solar cells, are divided into a number of different categories including organic thin film, dye sensitized solar cells (DSSC), and silicon based and III–V semiconductor solar cells. However, even the most efficient of these devices have efficiencies only as high as 30% [2]. Nature is capable of much more efficient solar energy conversion. Photosynthesis in plants has a quantum efficiency greater than 90% [3]. To take advantage of that, research groups have developed electrodes capable of photocurrent generation through a number of methods: incorporation of chloroplasts [4,5], thylakoids [6–8], photosystems [9–11], photosynthetic reaction centers of bacteria [12–15], or by mimicking electron transfer pathways in photosynthetic complexes [16].

Previously, we have reported a bio-solar cell incorporating a thylakoid bioanode [7]. When combined with a Pt air-breathing cathode, the bio-solar cell generated a short circuit photocurrent density of 2.14 ± 0.11 μA cm^{-2} with an open circuit voltages of 0.46 V, and with a laccase oxygen reduction biocathode, the cell had a short circuit photocurrent density of 14.0 ± 1.8 μA cm^{-2} and open circuit voltage of 0.72 V [7]. Our system has a number of advantages compared to the previously reported methods. The immobilization method for our electrodes is quite simple. The thylakoids are adsorbed to the carbon surface and a thin layer of silica is used to hold them in place. The use of a mediator can lead to decreased open circuit voltage or greater instability [8,17], but we have removed those possible complications because, unlike previously reported thylakoid bio-solar cells [6], our thylakoid electrodes do not require a mediator. Additionally, because we are using the entire organelle, the proteins will be in their native environment which increases their stability [18]. This also allows for multiple electron transfer pathways at the electrode surface, since there are many redox species and electrocatalysts in the pathway. However, the mechanism of direct electron transfer (DET) for this thylakoid anode was not determined previously. In this work, we use a combination of removal, inhibition, and activation of the various components of the thylakoid membrane in order to determine which contribute to the photocurrent generated by our system.

Thylakoid membranes are found in cyanobacteria and chloroplasts of plants and are responsible for the light-dependent reactions of photosynthesis, which convert solar energy into chemical energy with water as the only input. Electron transport through the thylakoid membrane occurs via two different pathways, as shown in Fig. 1. The noncyclic pathway begins with the absorption of light by photosystem II (PSII) to oxidize water to O_2. The electrons from PSII are passed to plastoquinone (PQ) to form plastoquinol, which then binds to the cytochrome b_6f complex (b_6f) where plastoquinol is oxidized back to PQ and the protons are released into the lumen. The electrons are transferred from b_6f to plastocyanin (Pc), a Cu-containing protein loosely bound to the

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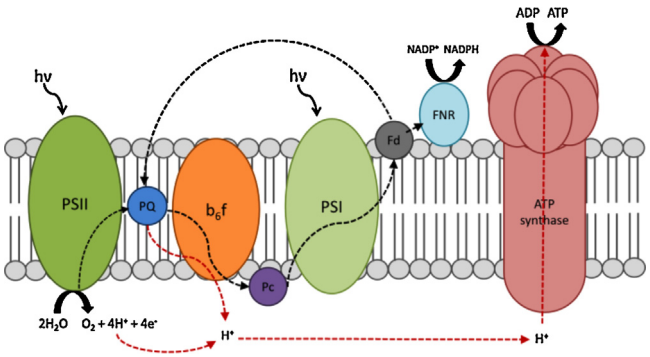

Fig. 1. Schematic of thylakoid membrane showing the components involved in the light-dependent reactions of photosynthesis. Electron flow is shown with dashed black arrows while proton flow is shown with dash red arrows. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

lumen side of the thylakoid membrane [19]. The reduced form of Pc passes its electron to photosystem I (PSI) which then transfers the electron to ferredoxin. From ferredoxin, the electron flow can proceed by two different pathways. In the noncyclic pathway, electrons from the reduced ferredoxin are used to reduce $NADP^+$ to nicotinamide adenine dinucleotide phosphate (NADPH) by the enzyme ferredoxin-$NADP^+$ reductase [20]. In the cyclic pathway, electrons from reduced ferredoxin are transferred back to PQ to generate plastoquinol, thereby completing the loop for the pathway. The protons generated during photosynthesis create a proton gradient which is used to produce adenosine triphosphate (ATP) by ATP synthase. The cyclic pathway allows for increased generation of protons by b_6f, because protons from plastoquinol are being pumped into the lumen, increasing the proton gradient and increasing ATP production. The noncyclic pathway leads to increased production of NADPH. The two pathways allow for the chloroplast to regulate production of these two compounds [21].

2. Experimental

2.1. Materials

Organic spinach was purchased from a local supermarket. Tetramethyl orthosilicate (TMOS) and diuron were purchased from Fluka (USA). Toray carbon paper (TGP-H-60, non-wet-proof) was purchased from Fuel Cell Earth (USA). Zinc chloride was purchased from Macron Chemicals. Magnesium chloride was purchased from Mallinckrodt Chemicals. D-Sorbitol was purchased from Acros Organics. DE52 preswollen microgranular DEAE cellulose was purchased from Whatman. All other reagents used were purchased from Sigma (USA). Solutions were prepared with 18 MΩ cm deionized water from a Milli-Q system.

2.2. Thylakoid membrane isolation

Thylakoid membranes were isolated from organic spinach according to our previously published procedure [7]. Isolated thylakoids were suspended in a solution containing 330 mM sorbitol, 2 mM $MgCl_2$, and 50 mM HEPES, pH 7.8 (thylakoid isolation buffer). Chlorophyll content was determined spectroscopically and the suspensions were diluted to 2.5 mg chlorophyll/mL before being stored at −20 °C until use.

2.3. Electrode fabrication

In order to prevent a loss of thylakoid activity due to reactive oxygen species production, 3 μL of catalase from *Aspergillus niger* (Sigma, 9.47 kU/mg, 28.73 mg/mL) is added to 100 μL of 2.5 mg/mL thylakoid suspension (intact or depleted) prior to electrode modification. A 50 μL aliquot of the thylakoid/catalase mixture was physically adsorbed onto Toray® carbon paper electrodes and allowed to dry. A thin silica layer was vapor deposited onto the thylakoid layer by hydrolysis of tetramethyl orthosilicate [7,22]. The modified electrodes were stored overnight at 4 °C and used the next day.

2.4. Plastoquinone removal from thylakoids

Plastoquinone was removed from the thylakoids following a procedure by Siegenthaler [23]. In summary, dimethyl β-cyclodextrin (45 mM) was added to intact thylakoid suspension with a chlorophyll concentration of 75 μg/mL. After incubation for 10 min, the mixture was centrifuged at 8000 × *g* for 5 min at 0 °C. The pellet was resuspended in thylakoid isolation buffer at the same concentration as the initial intact thylakoid suspension and stored at −20 °C until use.

2.5. Ferredoxin removal from thylakoids

Ferredoxin was removed from intact thylakoids using a procedure adapted from Rao et al. [24] In summary, Whatman DE52 anion exchange cellulose equilibrated with 20 mM pH 7.5 phosphate buffer and sodium chloride (6 g/L) were added to the intact thylakoid suspension. The negatively charged ferredoxin binds to the cellulose which is separated by centrifugation at 500 × *g* for 5 min. This supernatant is centrifuged again at 12,000 × *g* for 10 min and the pellet, which contains the ferredoxin depleted thylakoids, is resuspended in thylakoid isolation buffer at the same concentration as the initial intact suspension and stored at −20 °C until use.

2.6. Ferredoxin-$NADP^+$ reductase removal from thylakoids

FNR was removed from thylakoids following a procedure previously reported by Hind [25]. In summary, intact thylakoids were suspended in a solution containing 10 mM CHAPS, 10 mM $MgCl_2$, and 10 mM pH 7.2 MOPS. After incubation on ice for 10 min, the mixture is centrifuged at 12,000 × *g* for 10 min. This process is repeated three times and the final pellet is resuspended in thylakoid isolation buffer at the same concentration of the initial intact suspension.

2.7. Photoelectrochemical analysis

Electrodes were tested in the absence and presence of light provided by a 250 W Halogen lamp at 5200 lumens in a lab fabricated dark box. Electrochemical experiments were performed using a Digi-IVY DY2023 potentiostat. All potentials are reported versus an Ag/AgCl (saturated KCl) reference electrode. Amperometric measurements were performed by applying a voltage of 0.45 V and measuring the current as the light was turned on and off. The difference between the photocurrent and dark current was calculated for each set of experimental conditions (intact, depleted, or inhibited thylakoids). Because the activities of different batches of isolated intact thylakoids vary, the photocurrent from each set of depleted or inhibited thylakoids is compared to the photocurrent of intact thylakoids from that same batch. This is reported as the percent of intact thylakoid photocurrent and allows for comparison of photocurrent response between different batches of intact thylakoids. Control experiments were performed in the exact same experimental setup but with electrodes containing no thylakoids. All experiments were performed in triplicate and reported uncertainties correspond to the standard deviation of those triplicate measurements.

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