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ARTICLE

Investigation on Electron Transfer from Isolated Spinach Thylakoids to Indium Tin Oxide

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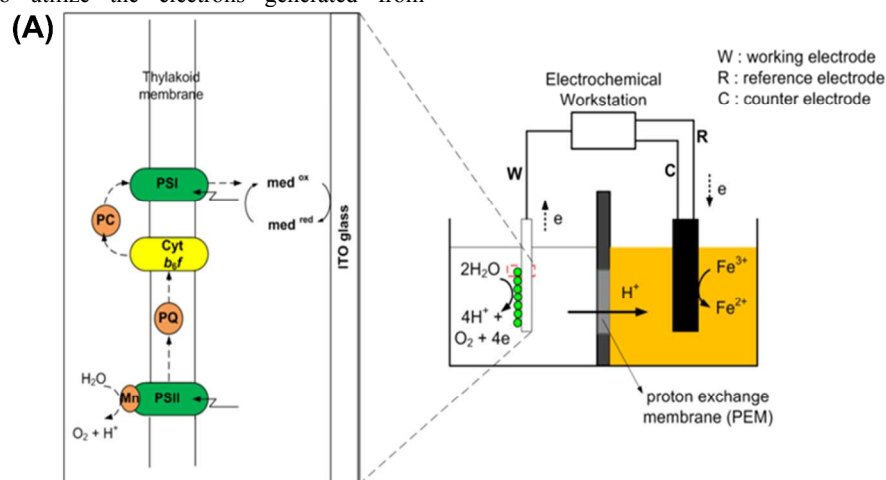
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The electrons generated by the photosynthetic water splitting have been studied for direct electron transfer under light irradiation. Isolated thylakoids are incorporated as biocatalyst with indium tin oxide (ITO) as the electrode in a two-chamber photosynthetic electrochemical cell (PEC). The generated photocurrent is compared between deposited and dispersed thylakoids. The physical properties of deposited thylakoids are observed using field emission scanning electron microscopy (FESEM) and absorbance spectroscopy techniques. The results show the presence of thylakoids with bound photosystems including light harvesting antennas and other protein complexes. Further investigations reveal that the direction of electron transfer can be tuned by varying the applied potentials to promote bi-directional photocurrent. The maximum cathodic photocurrent is obtained at 50 mV vs. standard calomel electrode (SCE), while the maximum anodic photocurrent is enhanced with increasing positive potential applied. Our observation indicates the possibility of either reduction of O_2 or H_2O_2 as the prominent cathodic photocurrent source, while electron transfer from thylakoids to ITO yields the anodic photocurrent.

Introduction

The photosynthetic water splitting that is driven by light harvesting antenna excitation and reaction center charge separation has drawn many interests for its potential applications in the development of biological photovoltaic devices. Approaches to exploit the natural photosynthesis process have been reported on light harvesting antenna, isolated chloroplast or thylakoid¹, and whole photosynthetic organisms such as algae, cyanobacteria, and green or purple bacteria²⁻⁶. In order to utilize the electrons generated from

photosynthesis reactions, efficient contacts between photosynthetic material and electrode as the electron collector is necessary. One issue in surface-immobilized protein is conformational change or denaturation leading to its inactivation⁷. In this study, we focus on investigating the electron transfer between photosynthetic material and electrode in a two-chamber photosynthetic electrochemical cell (PEC) (Figure 1A).



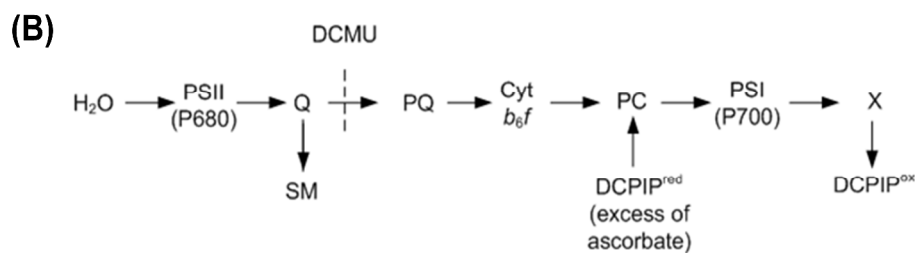


Figure 1. (A) Schematic of two-chamber PEC with dotted red lines showing the deposited thylakoids on ITO. Enlarged view: Electron transport pathway is shown in dotted lines, while thunderbolts indicate light energy excitation. (B) Electron transport chain (ETC) in thylakoid membranes. PS: photosystem, Q and X: primary electron acceptor, PQ: plastoquinone pool, Cyt *b₆f*: cytochrome *b₆f*, PC: plastocyanin. Arrow toward outside the ETC indicates the shuttled electron from ETC. Dotted lines show the electron blockage.

Herein, isolated thylakoids and ITO are used as photosynthetic material and electrode, respectively. To study the effects of proximity and contact between thylakoids and ITO, we compare the photocurrent generated by deposited and dispersed preparations. In addition, the effects of mediator (2,6-dichlorophenol-indophenol oxidized form; DCPIP^{ox}) and herbicide (3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCMU) (Figure 1B)^{8, 9} toward the generated photocurrent are further explored. The function of DCPIP is to intercept generated electrons at the end of PSI, while DCMU is used to block the electron transfer at plastoquinone pool. Aside from mediator and herbicide, O₂ or H₂O₂ generated during photosynthetic water splitting have been reported to influence the generated photocurrent¹⁰ and is also investigated.

Material and Method

Chloroplasts and thylakoids isolation

Chloroplasts were isolated from spinach leaves through several centrifugation steps following SIGMA chloroplast isolation kit protocol (Cat#CP-ISO). Spinach leaves (5 g) were ground in 20 mL chloroplast isolation buffer (CIB) containing 300 mM sorbitol (Aldrich), 100 mM Tris (Bio-Rad), 10 mM sodium chloride (Aldrich), 5 mM magnesium chloride (MP Biomedics), and 1% (w/v) bovine serum albumin (BSA) (Aldrich) as stabilizer at pH 7.8. Leave extract was filtered using 100- μ m filter mesh (Aldrich) and centrifuged at 200 \times g for 3 minutes to remove the cell debris. The supernatant was further centrifuged at 1000 \times g for 7 minutes and the isolated chloroplasts were collected as green pellet.

The chloroplasts were subsequently dispersed in a sugar-free environment to induce osmotic shock for isolation of the thylakoids¹¹. The concentration was determined as the total chlorophyll mass using acetone extraction method¹². Briefly, isolated thylakoids were diluted 100 times in 80% (v/v) acetone solution followed by centrifugation at 3000 \times g for 2 minutes to retain the supernatant. The concentration, expressed as unit chlorophyll basis, was calculated by measuring the supernatant optical density (OD) at 652 nm ($\epsilon_{\text{chlorophyll}} = 36 \text{ L}\cdot\text{g}^{-1}\cdot\text{cm}^{-1}$).

Electrode preparation

ITO (~1.1 mm ITO thickness and <10 Ω/cm^2 resistance) glass was purchased from Wintek Technology Singapore Pte. Ltd. To remove any contaminants, the ITO glass was sonicated twice in acetone followed by water for 10 minutes. The electrode active area was ~1 cm².

The thylakoids were deposited on electrode surface using physical adsorption by dropping ~10 μ L of the preparations spread evenly onto the ITO surface. The modified electrode was dried at room temperature for 30 minutes in the dark. All electrodes were prepared fresh right before use. The investigation on loose interaction was done by dispersing the same thylakoid amount in the PEC chamber with bare ITO as the electrode.

The morphology of the adsorbed thylakoids was observed using field emission scanning electron microscope (FESEM, JEOL JSM-6700F). The dried thylakoids were sputtered with platinum prior to observation. Optical absorbance was observed from 400 nm to 700 nm to monitor the bound protein complexes and light harvesting antennas in the thylakoids.

Electron transfer observation

The electron transfer between thylakoids and electrodes was observed by monitoring the photocurrent generation using two-chamber PEC configuration separated by a proton exchange membrane (PEM) (GasHub). The anode chamber contained 10 mM potassium phosphate buffer (pH 7.2) (Merck/Fluka), while the cathode chamber was filled with 50 mM potassium ferricyanide (Aldrich) in water. Both chambers were supplemented with 100 mM potassium chloride (Merck). Carbon cloth (GasHub) at ~5 cm² was used as both reference and counter electrode in the cathode chamber, while ITO electrode was used as the working electrode in the anode chamber.

The generated photocurrent was measured using electrochemical workstation 660D Potentiostat/Galvanostat (CH Instruments, Inc.) with no applied bias. A tungsten halogen lamp (100 W Philips A1/215 light bulb) without any additional filter was used as the light source. Light intensity was kept constant at approximately 350 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ inside the anode chamber as quantified using QRT1 Quantitherm (Hansatech). A metal shield was used as a shutter to simulate dark condition at predetermined time points. The mediator and inhibitor were added into the anode chamber to investigate their effects on the generated photocurrent. DCPIP^{ox} (2,6-dichlorophenol-indophenol; 60 μM) was used to intercept electrons from electron transport chain while saturating DCMU (100 μM) was used to block electron transfer from PSII to PSI^{8, 9}.

To study the catalytic reduction of O₂ or H₂O₂ on ITO, cyclic voltammetry (CV) was performed using a standard three-electrode configuration with a scan rate of 50 mV/s. ITO glass was used as working electrode while platinum foil and standard calomel electrode (SCE) served as the counter and reference electrode. As-stated anolyte phosphate buffer was used as the

electrolyte. The effect of external applied potential to the ITO electrode in the presence of thylakoids was studied.

Results and Discussion

Protein complexes and light harvesting antennas retain their integrities upon deposition

The isolated thylakoids containing photosynthetic complexes are deposited onto the surface of the ITO. The phospholipid molecules that self-assemble into lipid bilayer of the thylakoid consist of hydrophilic heads oriented outside exposed to the aqueous environment and hydrophobic tails buried between the two layers. Lipid bilayer promotes hydrophilic interaction with ITO¹³. Thus, when adsorption of the isolated thylakoids is performed, direct interaction occurs between the exposed hydrophilic head of the thylakoids and the ITO surface. However, direct deposition of the photosynthetic complexes embedded in the lipid bilayer onto solid surface, such as ITO, may compromise their biological structure and function. To demonstrate that the light harvesting complexes are still bound to the thylakoid and electrode after the deposition protocol, we performed absorbance scan in the visible light range on the ITO-deposited thylakoids.

Distinct absorbance peaks corresponding to chlorophyll *a* (680 nm), chlorophyll *b* (shoulder at 650 nm), as well as Soret band containing peaks for both chlorophylls *a*, *b*, and carotenoids are identified in the spectrum obtained from the electrode with deposited thylakoids (Figure 2A).

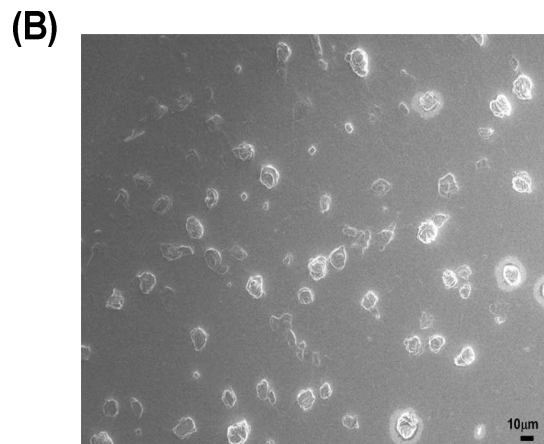
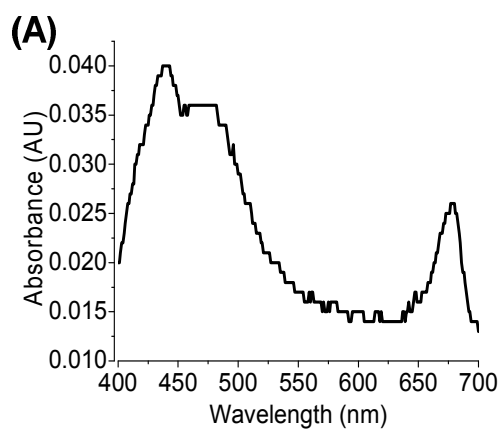


Figure 2. (A) UV-Visible absorbance spectrum of physically deposited thylakoids showing absorbance peak at 680 nm. (B) FESEM image of physically deposited thylakoids on ITO, scale bar is 10 μm .

The presence of these absorbance peaks suggests that the protein complexes and light harvesting antennas remain embedded in the thylakoid membrane with tight contact to the electrode^{14, 15}. The electron micrograph shows well distribution of thylakoids without any aggregation upon adsorption onto ITO (Figure 2B). Each of the adsorbed thylakoids may act as an independent micro-electrode and thus contribute to the collective photocurrent.

The effect of interaction distance, DCPIP^{ox}, and DCMU

Deposition of thylakoids on the electrode is hypothesized to promote close interaction and facilitate electron transfer that result in enhancement of photocurrent. The correlation between interaction distance and electron transfer is studied using deposited (physical adsorption) and dispersed thylakoids in the presence of DCPIP^{ox} as an artificial electron acceptor. It is well-reported that DCPIP^{ox} interrupts ETC at PSI primary electron acceptor (Figure 1)^{9, 16}. The $\sim 12 \mu\text{A}$ photocurrent change for electrode prepared through physical adsorption indicates preserved thylakoid activities (Figure 3). In contrast, measurement using blank and control electrodes in PEC containing dispersed preparations show negligible photocurrent. The observations indicate that close interaction is a crucial factor for the generated photocurrent. Based on this observation, the subsequent experiments are performed using ITO electrode with deposited thylakoids.

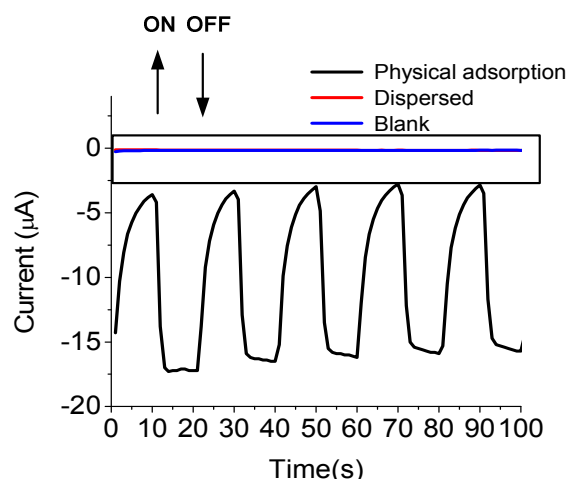


Figure 3. Photocurrent of thylakoids/ITO in the presence of DCPIP^{ox}, inset shows dispersed and blank preparations.

Investigation of the site of electron transfer that contributes to the photocurrent generation is conducted by intercepting or blocking of electron transfer between the moieties in the ETC. Figure 1B illustrates the sites of DCPIP and DCMU interception and blockage. In the absence of DCPIP^{ox}, a small photocurrent of 200 nA is generated indicating the possibility of direct electron transfer (Figure 4). The photocurrent increases to $\sim 15 \mu\text{A}$ in the presence of the mediator DCPIP^{ox} demonstrates a process with more efficient electron transfer. Upon addition of DCMU, which functions to block the electron transfer from PSII to PSI, the generated photocurrent

diminished to 50 nA despite the presence of DCPIP^{ox}. The observation indicates that PSI may be the site of electron transfer from ETC to DCPIP. By contrast, in the presence of DCMU and absence of DCPIP^{ox}, no photocurrent is observed as expected.

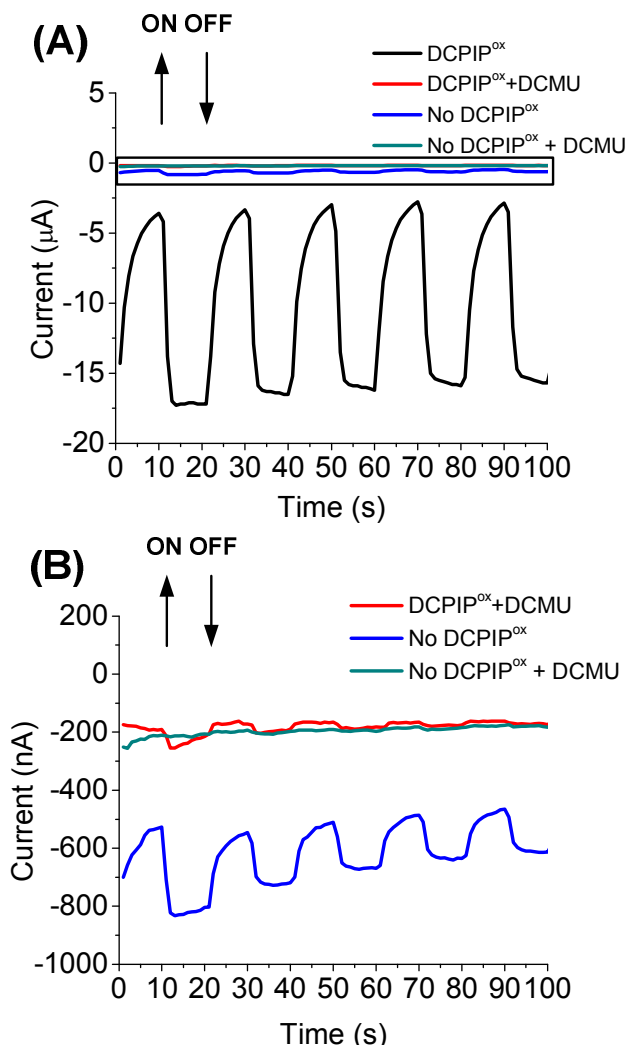


Figure 4. (A) Photocurrent of thylakoids/ITO in the presence of DCPIP^{ox}, DCPIP^{ox} and DCMU, no mediator, and no mediator and DCMU; inset shows photocurrent in the presence of DCPIP^{ox} and DCMU, no mediator, and no mediator and DCMU. (B) Enlarged inset revealing the nanoscale photocurrent.

The effect of applied potentials

To study the effect of applied potentials, a standard three-electrode configuration system is used in a one-chamber electrochemical cell. When different potentials are applied in the absence of mediator, photocurrent can be generated in two directions. Anodic photocurrent (positive direction) indicates electron transfer from thylakoids to ITO, while cathodic photocurrent (negative direction) represents electron transfer from the ITO to electron acceptors in the anode chamber. The bi-directional photocurrent under different working potentials has been previously reported in other systems, such as DNA/CdS and CdS quantum dots^{17,18}.

Cathodic photocurrent is observed upon applying negative bias potentials, and the highest cathodic photocurrent is observed upon application of approximately -50 mV vs. SCE (Figure 5A). The reversed photocurrent polarity from cathodic to anodic may be a result of direct electron transfer due to plastocyanin diffusion to electrode upon applying $\sim +125$ mV vs. SCE¹⁹ (Figure 5B). The observations prompted us to further investigate the source of the cathodic photocurrent.

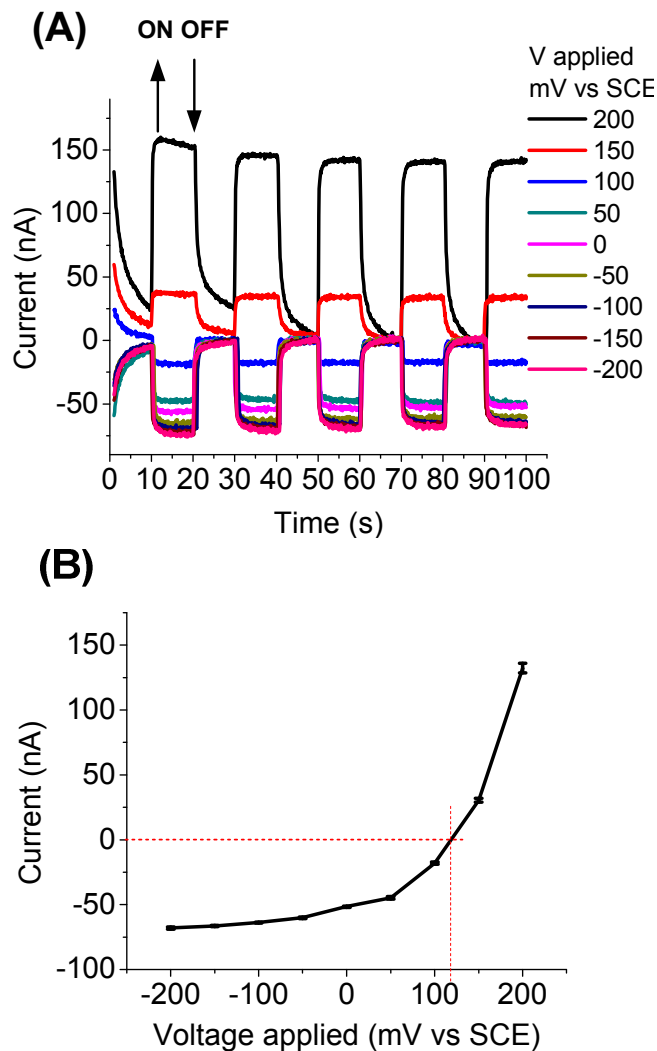


Figure 5. (A) Anodic and cathodic photocurrent generation at different applied potentials in the absence of mediator. Experiment was performed in a single chamber. ITO electrode size is 1 cm². (B) A plot of generated photocurrent vs. applied potential showing reversed photocurrent polarity at $\sim +125$ mV vs. SCE.

The presence of photosynthetic products, O₂ or H₂O₂, is hypothesized to be potential sources for cathodic photocurrent. In intact chloroplasts, the H₂O₂ generated during photosynthesis is neutralized by ascorbate peroxidase (APX) in the stroma. However, isolated thylakoids are devoid of the H₂O₂ scavenging mechanism due to the removal of stroma and stromal proteins²⁰. Since electron acceptors are limited in the isolated thylakoids, O₂ generated during water splitting serves as electron acceptor at PSI and forms the reactive oxygen species (ROS) H₂O₂¹⁰. To demonstrate that the

generation of cathodic photocurrent originates from the reduction of O_2 or H_2O_2 , cyclic voltammetry is performed in the absence or presence of thylakoids.

In the absence of thylakoids, the gradual increase of cathodic current and peak shift upon H_2O_2 addition (Figure 6A) are consistent with previous reports^{21, 22}. In addition to H_2O_2 reduction, the presence of O_2 has been suggested to contribute to the observed cathodic current²¹. To remove residual O_2 , the electrolyte is bubbled with N_2 . An obvious decrease in cathodic current background is observed following the removal of O_2 from the electrolyte. In the presence of thylakoids, redox peaks at -0.5 V and $+0.5$ V vs. SCE are identified, and an increase of 300 nA in cathodic photocurrent is detected upon illumination at ~ -0.5 V vs. SCE (Figure 6B). The CV curves presented here support the hypothesis that O_2 and H_2O_2 generated from the photosynthetic light reaction serve as possible sources of the observed cathodic photocurrent.

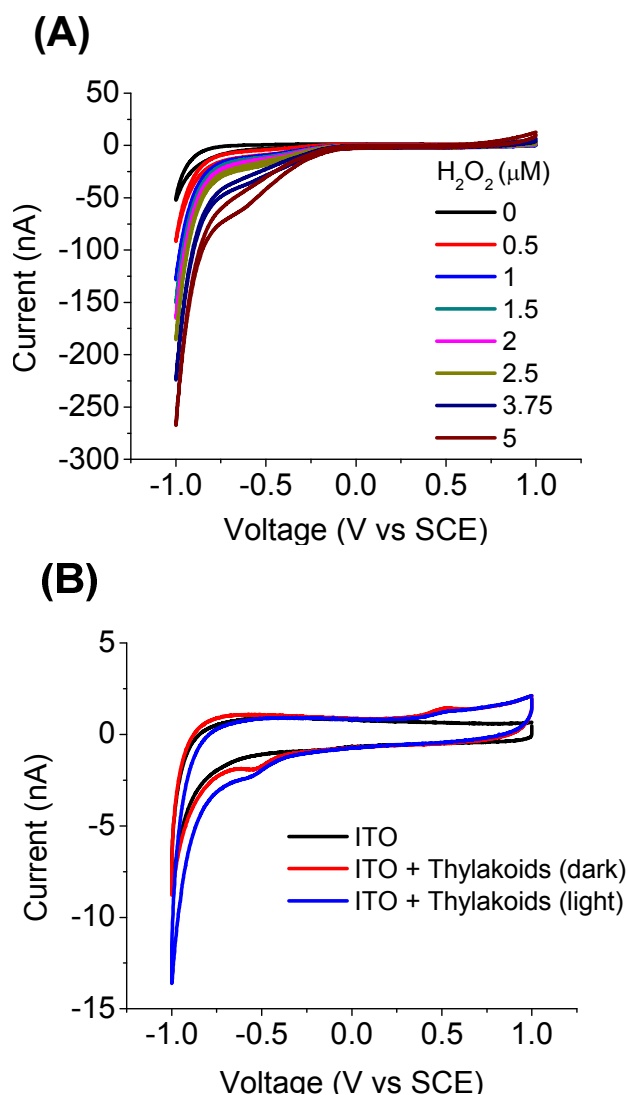


Figure 6. CV of ITO (A) in the absence of thylakoids at various H_2O_2 concentrations without N_2 bubbling and (B) in the presence of thylakoids with N_2 bubbling.

Conclusions

In this work, we investigated electron transfer in isolated thylakoids during photosynthetic reaction by comparing the photocurrents under different conditions. The photoelectrochemical study reveals that significant photocurrent is generated by the physically deposited thylakoids on the ITO surface, especially in the presence of DCPIP^{ox}. More interestingly, application of varying potentials results in a bi-directional photocurrent. Reduction of H_2O_2 or O_2 on the electrode surface is speculated to be possible sources of the observed cathodic photocurrent, while the anodic photocurrent is a result of direct electron transfer from the thylakoid to the ITO.

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