Bot. Bull. Academia Sinica (1992) **33:** 9-15

A photoelectrochemical cell using electrodes modified by photosystem I particles of spinach

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(*Received June 25, 1991; Accepted November 11, 1991*)

Abstract. Photosystem I particles isolated from spinach were used to modify the electrode of a photoelectrochemical cell. The presence of photosystem I particles on the electrode was essential for photovoltage generation by the cell. This effect is closely related to the properties of electron donors used. The spectrum of monochromatic incident photon to current conversion efficiency (IPCE) was found to be coincident with the absorption spectrum of photosystem I particles in solution. The maximal values of IPCE were 7.7% and 5.0% at wavelengths 420 and 700 nm, respectively. This device could be used as a model system to investigate the photosynthetic electron transport of photosystem I. We also demonstrated it to be a possible means for the direct conversion of solar energy into electricity.

Key words: Allylamine; Photoelectrochemical cell; Photosystem I.

Introduction

It has been shown that photoactive biological materials, such as chlorophyll a (Takahashi and Kikuchi, 1976), chloroplasts (Haehnel *et al.*, 1978), isolated chloroplast particles (Lemieux and Carpentier, 1988; Gross *et al.*, 1978; Pan *et al.*, 1982, 1983; Fan *et al.*, 1982; Bhardwaj *et al.*, 1982, 1981a, b, c; Janzen and Seibert, 1980; Seibert *et al.*, 1982; Rao *et al.*, 1990), bacterial reaction centers (Janzen and Seibert, 1980; Seibert *et al.*, 1982; Seibert and Kendall-Tobias, 1981, 1982) and purple membrane fragments from halobacterium (Lopez and Tien, 1980) could be used in the photoelectrochemical cells to harvest the solar energy. The photoactive biological materials were incorporated into bilayer lipid membrane (Tien, 1982), suspended in a galvanic cell (Haehnel *et al.*, 1978) or strongly adsorbed on the electrode surface (Janzen and Seibert, 1980; Seibert *et al.*, 1982; Takahashi and Kikuchi, 1976; Rao *et al.*, 1990). Lopez and Tien (Lopez and Tien, 1984) incorporated the photosystem I particles isolated from spinach into their bilayer lipid membrane. An open circuit voltage (V_{oc}) of about 10 mV and a short circuit current (I_{sc}) of 30 pA could be achieved. Photosystem I particles were also employed by Bhardwaj *et al.* (1982) in their flavin mononucleotide - photosynthetic photoelectrochemical cells which generated synergistically a V_{oc} of 670 mV and I_{sc} of 1.6 mA. An energy conversion efficiency of 0.2% was obtained for their cell.

Recently, Rao *et al.* (1990) successfully deposited photosystem II partilces from spinach on dye-derivatized TiO_2. They obtained photocurrents up to 35 μA/cm^2 of electrode surface and 10 mA/mg of chlorophyll.

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[4]**Abbreviations:** AA, allylamine; Asc, ascorbate; Chl, chlorophyll; $DAD_{ox\ (or\ red)}$, oxidized or reduced form of diaminodurene; EDC, 1-ethyl-3-(3-dimethylamine propyl)-carbodiimide; HQS, hydroquinone 2-sulfate; IPCE, incident photon to current conversion efficiency; I_{sc}, short circuit current; P_{700}, reaction center of photosystem I; PSI, photosystem I; TMPD, N, N, N′, N′-tetramethylphenyldiamine; V_{oc}, open circuit voltage.

The maximum monochromatic incident photon to current conversion efficiency observed was 12% for their PSII dye-derivatized TiO_2 photoelectrochemical cell. On the other hand, several groups (Janzen and Seibert, 1980; Seibert *et al.*, 1982; Seibert and Kendall-Tobias, 1982) utilized the bacterial photosynthetic reaction centers isolated from *R. sphaeroides* to modify electrodes such as SnO_2 and platinum by direct strong adsorption. This "reaction center-SnO_2" electrode showed light-dependent charge separation between the reaction center and the semiconductor SO_2. Such primary charge separation resulted in a photovoltage of 80 mV under open circuit conditions and a photocurrent as high as 0.5 $\mu A/cm^2$ under short circuit condition. However, their "reaction center-platinum" electrodes did not generate any significant photoelectric response under the same condition.

Cuendet and Gratzel (1984) also immobilized photosystem I or photosystem II on semiconducting powder. A mixture of these two systems yielded only trace amount of H_2 rather than photovoltage. They further immobilized hydrogenase on semiconducting particles such as TiO_2 and CdS, and produced H_2 in the presence of sacrificial electron donors (Cuendet *et al.*, 1984).

In this report, we demonstrated the possibility that photosystem I particles can be used to modify the inert platinum electrodes through linking molecules. This photosystem I-platinum electrode developed photopotential under illumination. Such modified photosystem I-platinum electrode may be used as a new probe for investigating the reaction of photosynthetic electron transport of PSI as well as to serve as a model system for the design of photoelectrochemical devices.

Materials and Methods

Preparation of Photosystem I Particles

Photosystem I particles were isolated from market spinach according to a modified method of Schiozawa *et al.* (Burkey and Gross, 1981). The chlorophyll concentration was measured according to Arnon (1949). The absorption spectrum of PSI particles was obtained using DW2000 spectrophotometer (Aminco).

Modification of the Platinum Electrode with Photosystem I Particles

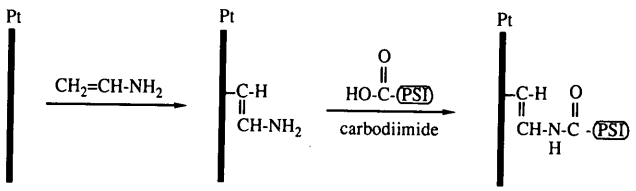

Fig. 1. Modification of a platinum electrode by PSI particles through linking allylamine. The detailed modification procedure was as described in "Materials and Methods".

A bright clean platinum electrode (1 cm^2 in area) was washed with concentrated HNO_3 and HCl alternatively for 1 h. It was then thoroughly rinsed with double distilled water and air dried for immediate use.

The functional groups which serve as linking molecules between photosystem I particles and inert platinum was introduced by immersing the clean platinum into 50% (v/v) allylamine solution (Land and Hubbard, 1973). After 30 min the allylamine treated platinum electrodes (AA-Pt) were thoroughly rinsed with double distilled water. AA-Pt was then incubated for 60 min in a solution containing 0.3 M phosphate buffer (pH 6.0), 0.05% Triton X-100, 200 μg chl/ml PSI (Chl/P_{700} ratio was 75 μg chl/mol P_{700}) and freshly made 0.1 M EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide) as catalyst (Burkey and Gross, 1981). The reaction of this modification is shown in Fig. 1. The PSI-AA-Pt electrode was gently rinsed with double distilled water and air dried for immediate use. The PSI-AA-Pt electrode was stable for several weeks when stored in 50 mM phosphate buffer (pH 7.0) at 4°C in the dark (data not shown).

Cell Design

The two-compartment photoelectrochemical cell as shown in Fig. 2 has been previously described elsewhere with minor modification (Pan *et al.*, 1983). The PSI-AA-Pt electrode was immersed in the light compartment (the compartment facing the light source) which contained an electron donor to PSI (as indicated), 80 mM Tris-HCl (pH 8.2) and 100 mM NaCl. The counter clean platinum electrode was immersed in the dark compartment which contained 80 mM Tris-HCl (pH 8.2), 100 mM NaCl and 1 mM methylviologen as PSI electron acceptor. The photopotentials across the load resistance were measured using a conventional pH meter with high input impedance. The light source was

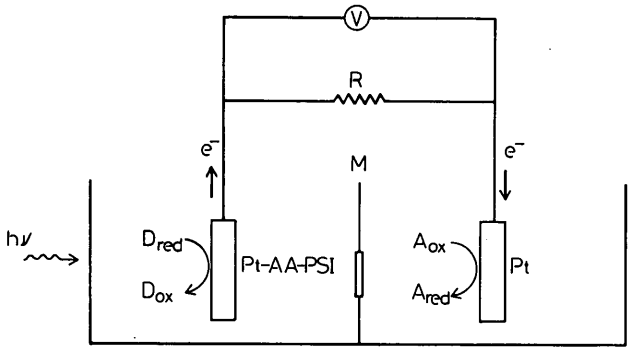

Fig. 2. Cell design. The design of two-compartment photoelectrochemical cell was as described in "Materials and Methods". $A_{ox\ (or\ red)}$: oxidized or reduced form of PSI electron acceptor; $D_{red\ (ox)}$: reduced or oxidized form of electron donor to PSI; M: membrane to separate two compartments; Pt: platinum electrode; PSI-AA-Pt: PSI-allylamine modified platinum electrode; R: external resistance.

provided by a xenon lamp with power supply model 8540 and model 8541 from Oriel Corporation (Stanford, CT). The light intensity on the surface of electrode was determined as 1.7×10^3 W/m² using a model 25 Kettering Radiometer (Yellow Spring, OH) or Oriel Detection System model 7070 (Stanford, CT). The photocurrents generated under different wavelengths were obtained by illuminating the cell at the same light intensity with appropriate optical filter from Detrich Optics Inc. (Hudson, MA). The monochromatic incident photon to current conversion efficiency (IPCE) was calculated according to the equation derived by Rao *et al.* (1990):

$$\text{IPCE}\ (\%) = \frac{1240 \times \text{photocurrent}\ (\mu\text{A/cm}^2)}{\text{wavelength}\ (\text{nm}) \times \text{photon flux}\ (\text{W/m}^2)}$$

Cyclic Voltammetry

The working electrodes for the measurement of cyclic voltammetry were prepared as described above with an area of 1 cm². Platinum and saturated calomel served as counter and reference electrodes, respectively. The supporting electrolyte was 50 mM phosphate buffer (pH 7.0). The measurements were made at the potential sweep rate of 25 mV/s. Light intensity was adjusted to be 1.7×10^3 W/m² as mentioned above. All potentials are given versus the saturated calomel electrode (SCE). Cyclic voltammetry was carried out with CV-1 Cyclic Voltammogragh (Bioanalytical System Inc., IN) online with an X-Y recorder (CX-446, Ono Sokki).

Materials

All chemicals were of analytical grade and used without further purification. Allylamine and hydroquinone 2-sulfonate were purchased from Aldrich. Diaminodurene, 1-ethyl-3-(3-dimethylamino propyl)-carbodiimide, flavin mononucleotide, and N, N, N', N'-tetramethylphenyldiamine were obtained from Sigma.

Results

Development of Photovoltage

The development of open circuit photovoltage is shown in Fig. 3. In the presence of the electron donor to PSI, DAD plus Asc (an electron reservoir), the photovoltage was generated within a few seconds. The steady state photoresponse could last continuously at least for 30 min under this condition (data not shown). As light was turned off, the photovoltage dropped immediately to the initial level. Such light-dark cycle could be carried out without any deteriorated effect on the photoresponse. The electron donor was crucial for

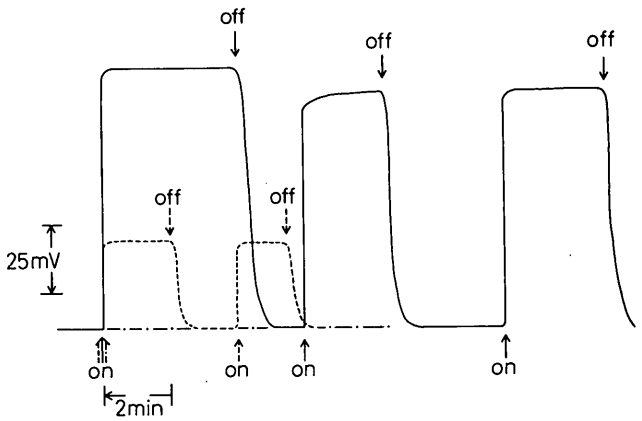

Fig. 3. Time course of the photoresponse of the PSI-allylamine platinum electrode. The PSI-allylamine platinum electrode was prepared as described in "Materials and Methods". The light compartment contained 80 mM Tris-HCl (pH 8.2), 100 mM NaCl, PSI-AA-Pt electrode and PSI electron donors, 1 mM DAD and 2 mM Asc, if present. The dark compartment consisted of 80 mM Tris-HCl (pH 8.2), 100 mM NaCl, 1 mM methylviologen (as PSI electron acceptor) and plain platinum electrode. The light intensity was 1.7×10^3 W/m². Arrows: up, light on; down, light off. ——, DAD/Asc; ---, Asc alone; —·—·— , DAD_{red} alone.

the generation of the photoelectric effect. In the presence of the oxidized form of electron donor such as DAD_{ox} the photovoltage did not develop as expected. On the other hand, ascorbate could support only one third of the photopotential generated by DAD + Asc since Asc alone is a less effective electron donor than DAD_{red} (as shown in the next section).

For unmodified (plain) or allylamine-treated electrode, there was no photovoltage developed at all. If the PSI-AA-Pt electrode was further treated with 5% trichloroacetic acid to denature PSI on electrode, the photoresponse disappeared (Table 1). These results indicated unambiguously that the development of photovoltage was absolutely due to the charge separation of PSI on the surface of electrode.

Table 1. *The photoresponse of a photoelectrochemical cell using the PSI-allylamine platinum*

The modification of platinum by allylamine and PSI particles and the photoresponses were measured as described in "Materials and Methods".

Electrodes	TCA Treatment	V_{oc} (mV)
PSI-AA-Pt	−	80.0±8.7
	+	0.0±0.0
AA-Pt	−	0.0±0.0
	+	0.0±0.0
Pt	−	0.0±0.0
	+	0.0±0.0

V_{oc}, open circuit photovoltage; AA-Pt, allylamine modified platinum; PSI-AA-Pt, PSI-allylamine modified platinum; +, 5% TCA treatment.

Table 2. *The photoresponse of a photoelectrochemical cell using PSI-allylamine platinum electrode and various electron donors*

The preparation of PSI-allylamine platinum electrode and the measurement of photoresponse were as described in "Materials and Methods".

Additions	PSI	V_{oc} (mV)
DAD/Asc	+	80.0±8.5
	−	0.0±0.0
DAD	+	0.0±0.0
	−	0.0±0.0
TMPD/Asc	+	20.0±2.5
	−	0.0±0.0
TMPD	+	0.0±0.0
	−	0.0±0.0
HQS	+	8.0±0.9
	−	0.0±0.0
Asc	+	7.0±1.0
	−	0.0±0.0

The concentrations of additions: DAD, 0.5 mM; TMPD, 0.5 mM; HQS, 8 mM; and Asc, 2 mM. +, active PSI; -, inactivated PSI by 5% TCA; V_{oc}, open circuit photovoltage.

The Effect of Electron Donors

As mentioned previously, the photovoltage developed was dependent on electron donors to PSI (cf. Asc *vs* DAD in Fig. 3). Several electron donors to PSI were scrutinized in our system. Table 2 shows that various electron donors might be used in this photoelectrochemical cell to generate photovoltage. Among the electron donors investigated, DAD was most efficient. This is not surprising since DAD is the best electron donor while HQS or Asc are relatively poor donor to PSI. This might reflect the degree of accessibilities for respective electron donors to PSI reaction center. The denaturation of PSI particles on the surface of electrode (treated with 5% trichloroacetic acid) abolished the photoresponse completely no matter what kind of electron donor was present.

The Incident Photon to Current Conversion Efficiency at Various Wavelengths

The monochromatic incident photon to current conversion efficiency (IPCE) was determined by illuminating the PSI-AA-Pt electrode under appropriate conditions as shown in Fig. 4. The light intensity at the wavelength of interest was adjusted to be 1.7×10^3 W/m^2. The photocurrent was measured by a conventional pH meter with high input impedance. The monochromatic incident photon to current conversion efficiency, as calculated from the equation derived by Rao *et al.* (1990), is plotted as a function of the excitation wavelengths. The IPCE curve shows two prominent peaks at wavelengths around 420 and 700 nm where they reach 7.7 and 5.0%, respectively. The IPCE of our electrochemical cell is slightly smaller than that of Rao *et al.* (cf. 12%, Rao *et al.*, 1990) who immobilized photosystem II particles on dye-derivatized TiO_2 film. The absorption spectrum of PSI was obtained spectrophotometrically. The maxima of IPCE coincide with

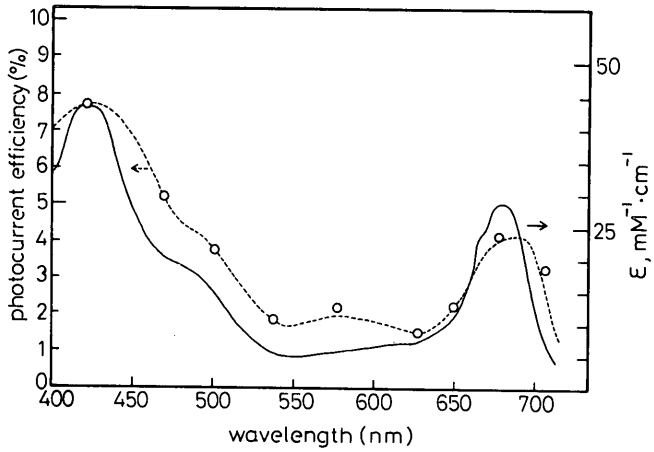

Fig. 4. The incident photon to current conversion efficiency of PSI-allylamine electrode at various wavelengths and absorption spectrum of the PSI particles. The preparation of PSI-AA-Pt electrode and the measurement of photocurrent were as described in "Materials and Methods". The monochromatic incident photon to current conversion efficiency was calculated according to the equation derived by Rao *et al.* (1990). The absorption spectrum of PSI particle was measured in a solution containing 50 mM phosphate (pH 7.0) and 50 μg Chl/ml. - ∘ -, spectrum of monochromatic incident photon to current conversion efficiency; ——, absorption spectrum of PSI particles in solution.

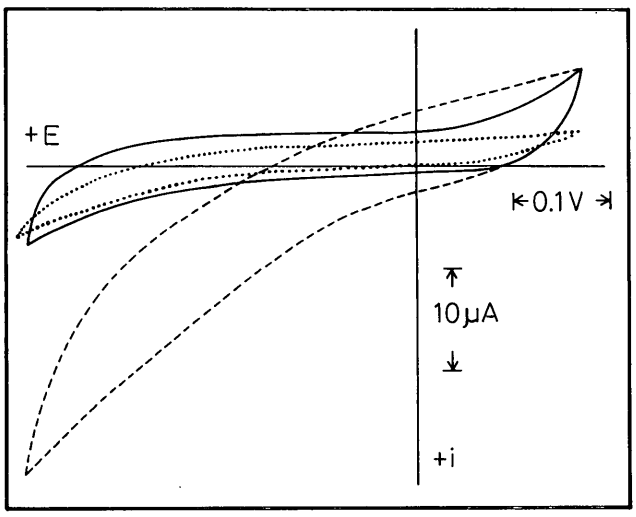

Fig. 5. Cyclic voltammogram of PSI-allylamine platinum electrode. The PSI-allylamine platinum electrode was prepared as described in "Materials and Methods". Cyclic voltammetry was scanned at potential rate of 25 mV/sec with various electrodes in 50 mM phosphate buffer (pH 7.0). ——, plain Pt electrode with or without illumination; ..., PSI-AA-Pt electrode without illumination; ---, PSI-AA-Pt electrode under illumination.

those of the absorption spectrum of PSI particles in solution. A smaller IPCE was observed at the wavelength around 550-630 nm. The origin of this small photocurrent is unknown and deserves further exploitation. Nevertheless, we believe that photocurrent observed was probably the response of PSI particles on the surface of the platinum electrode.

The Cyclic Voltammetry of PSI Modified Electrode

The cyclic voltammetry of PSI-modified electrode under illumination was investigated as depicted in Fig. 5. When plain platinum electrode was tested, the current was almost negligible (Fig. 5, ——). The coating of PSI particles through allylamine even slightly decreased the current in the dark (Fig. 5, ...). However, the current was enhanced significantly under illumination when the PSI-allylamine electrode was used (Fig. 5, ---). The increase of photocurrent was PSI-particle -dependent since the treatment of modified electrode with 5% trichloroacetic acid abolished the photoresponse (Table 1,2). Reductive scanning produced negligible photoresponse. However, oxidative scanning generated PSI-dependent photocurrent. The cathodic photoresponse demonstrates that PSI reaction center donates electrons to the platinum electrode resulting in the electron flow to the external load. The study of cyclic voltammetry showed the feasibility of using the PSI-allylamine electrode as a device for photoconversion.

Discussion

PSI particles have been used to convert the solar energy into electricity (Bhardwaj *et al.*, 1982, 1981a, 1981b, 1981c; Pan *et al.*, 1982, 1983; Fan *et al.*, 1982; Lopez and Tien, 1984; Janzen and Seibert, 1980; Seibert *et al.*, 1982; Rao *et al.*, 1990) and for the production of H_2 gas (Cuendet and Gratzel, 1984) as well. In the former case the PSI particles were either incorporated into lipid bilayer membrane (Lopez and Tien, 1984) and supporting the filter membrane (Bhardwaj *et al.*, 1982, 1981a, 1981b, 1981c; Pan *et al.*, 1982, 1983; Fan *et al.*, 1982), both of which were separated into two compartments, or strongly adsorbed on the surface of electrodes (Janzen and Seibert, 1980; Seibert *et al.*, 1982; Rao *et al.*, 1990). Both processes provide the possibilities of utilizing solar energy and the model systems to study the reaction of photosynthetic electron transport.

It is demonstrated in the present studies that the PSI particles could be immobilized on the surface of platinum electrode through linking molecules. This PSI -immobilized electrode can be used in photoelectrochemical cells. The photovoltage generated by illuminating the PSI-AA platinum was dependent on the presence of PSI on electrode surface. The plain (unmodified control) electrode or the electrode with inactive PSI particles (i. e. treated with 5% trichloroacetic acid) could not develop the photoresponse. Meanwhile, the spectrum of incident photon to current conversion efficiency coincides with the absorption spectrum of PSI particle in solution. The photovoltage depended also on the presence of electron donors to PSI particle. The magnitude of photoresponse was a function of efficiency of the electron donors to PSI. Hydrophobic electron donors such as DAD and TMPD were more efficient than hydrophilic electron donors such as HQS and Asc. This implies that the photovoltage generated depends on the accessibility of electron donors to the reaction center of PSI. Thus, the photoelectric effect was the result of the charge separation on electrode surface under illumination. The charge separation then induced the electron movement across the electrode surface through the external resistance load. The electron donors might prevent the charge recombination by reducing the oxidized PSI reaction center. The photoevents occurred on the electrode surface may be elucidated in the following scheme:

$$P_{700} \xrightarrow{h\nu} P_{700}^{+} + e^{-} \quad (1)$$

$$X + e^{-} \longrightarrow X^{-} \quad (2)$$

$$P_{700}^{+} + D_{red} \longrightarrow P_{700} + D_{ox} \quad (3)$$

where P_{700} is the reaction center of PSI, X the primary electron acceptor of PSI, and $D_{red\ (ox)}$ the reduced (or oxidized) form of electron donors to PSI. X^- then donates electrons to the surface of electrode which leads to an external resistance load. The exact mechanism of interaction of X^- on the electrode surface requires further elucidation.

Janzen and Seibert (1980) failed to generate a photovoltage using bacterial photosynthetic reaction centers which were strongly adsorbed on the surface of platinum electrodes. However, in the present study, a photoelectric effect was observed. The discrepancy between these two laboratories may be due to the different photosynthetic materials employed. Spinach PSI particles were immobilized through linking molecules on the surface of the electrode in this study, while the bacterial reaction centers were strongly adsorbed in their laboratory. The olefinic structure of allylamine may increase the electron transfer from PSI particles to the platinum surface. It is also possible that the covalent immobilization may change the conformation of PSI particles to make X^- interact on the electrode surface in a more feasible way or may increase the accessibility of electron donors to PSI. The exact cause for the discrepancy requires more detailed investigation. Nevertheless, our device can be used as a model system for the study of photosynthetic electron transport. In addition this photoelectrochemical cell can serve as a prototype for the direct conversion of solar energy into electricity.

Acknowledgements. The authors wish to thank Dr. R. Bhardwaj and Professor I.-J. Fan for their helpful suggestions. We also wish to thank Dr. Y. C. Chow for the measurement of cyclic voltammetry and Mr. Chuan Shen Chen and Chi Meng Tzeng for the secretarial assistance. This work was supported by a grant from the National Science Council, Republic of China (NSC79 -0203-B007-08) to RLP.

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利用菠菜光合作用第一光系統蛋白複體製備光電化學電池電極

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本文利用菠菜光合作用第一光系統蛋白質複體來修飾光電化學電池之電極。第一光系統蛋白質複體對於此電池產生光電壓極爲重要，此電池特性與光合作用電子供給之特性有關。單色光入射光子轉化光電流效率之光譜與第一光系統蛋白複體在水溶液中之吸收光譜極爲相似，其最高效率在波長 420 nm 時爲 7.7%，在波長 700 nm 時爲 5.0%，本電池可以用來作研究光合作用第一光系統電子傳遞機制，亦可作爲直接將太陽能轉化成電流之用。