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Bioelectronics and Biometallocatalysis for Production of Fuels and Chemicals by Photosynthetic Water Splitting†

JAMES WEIFU LEE AND ELIAS GREENBAUM*

Oak Ridge National Laboratory,
P. O. Box 2008, Oak Ridge, TN 37831-6194

ABSTRACT

By inserting metallocatalysts (such as platinum, osmium, or ruthenium) at the reducing site of photosystem I (PSI), electrons that emerge from PSI can be channeled to various redox reactions that could potentially produce fuels and chemicals (such as H_2 and CH_4, and so forth) instead of $NADP^+$ reduction, as in natural photosynthesis. We have recently developed a technique to photoprecipitate metallocatalysts *in situ* at the reducing site of PSI in thylakoid membranes, using water-soluble hexachloroplatinate, hexachloroosmiate, and hexachlororuthenate at biological temperature and neutral pH. This technique combined with "rewiring" of photosynthesis is a potentially important new field of biometallocatalysis. Potential applications of biometallocatalysis will be addressed in this article.

Index Entries: Photosynthetic cell; bioelectronics; metallocatalysis; hydrogen production; product separation.

INTRODUCTION

The primary processes of photosynthesis occur in thylakoid membranes, which contain the key photosynthetic apparatus: Photosystems I and II (PSI and PSII). During natural photosynthesis, water is split to

*Author to whom all correspondence and reprint requests should be addressed.

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molecular oxygen, protons, and electrons by PSII. Electrons from water splitting are transported through a series of carriers to PSI, where they are further energized by photochemical charge separation and used for the enzymatic reduction of $NADP^+$ to NADPH. This is natural photosynthesis.

Natural photosynthetic systems can be modified to make new products. In 1985, it was shown that by inserting colloidal metallic platinum at the reducing site of PSI in thylakoid membranes, the photosynthetic systems were transformed to do a very different photosynthesis—production of molecular hydrogen and oxygen by photosynthetic water splitting (*1*). Hydrogen (H_2) is an important fuel and chemical feed stock. In platinized thylakoids, molecular hydrogen is synthesized through reduction of protons, a reaction that is catalyzed by platinum colloidal particles adjacent to the reducing site of PSI on the stromal side of the thylakoid membrane. The protons and electrons are ultimately derived from water splitting by PSII, whereby molecular oxygen is produced (*1–3*). The net result, therefore, is the conversion of light energy into chemical energy stored as molecular hydrogen:

$$2\,H_2O + \text{light} \rightarrow 2\,H_2\uparrow + O_2\uparrow \qquad (1)$$

In chemically platinized thylakoids, insertion of the metallocatalyst was achieved by chemically precipitating metallic platinum onto the stromal side of PSI through the redox reaction of water-soluble hexachloroplatinate with molecular hydrogen:

$$[PtCl_6]^{2-} + 2\,H_2 \rightarrow Pt\downarrow + 6\,Cl^- + 4\,H^+ \qquad (2)$$

With essentially the same redox reaction, platinization has been also achieved by photoreduction of $[PtCl_6]^{2-}$ *in situ* by PSI instead of H_2 (*4*). Metallic (insoluble) platinum has been identified in photosynthetically platinized thylakoids by X-ray fluorescence analysis (*5*).

Using the technique of *in situ* photoprecipitation of metallocatalysts, it was further discovered that hexachloroosmiate ($[OsCl_6]^{2-}$) can also be photoreduced to metallic osmium at the reducing site of PSI, presumably with a redox reaction similar to that of hexachloroplatinate:

$$[OsCl_6]^{2-} + 4\,e^- \rightarrow Os\downarrow + 6\,Cl^- \qquad (3)$$

Studies have shown that catalytic activity of metallic osmium for hydrogen production is three times as high as that of metallic platinum (*5*).

These prior studies suggest that biometallocatalysis has potential in developing new bioreactors for production of fuels and chemicals. If such reactors are made directly of thylakoids, however, the byproduct (O_2) from water splitting is mixed with H_2, since PSI and PSII are both located in thylakoid membranes. Since the mixture of O_2 and H_2 is explosive, H_2 must be separated from O_2 before storage, transportation, and utilization. The currently available gas-separation techniques, such as cryogenic separation, are energy-consuming. Separation of the H_2 and O_2 mixture might

require more energy that the energy value of the two gases. Haehnel and Hochheimer proposed a two-stage photosynthetic cell to separate O_2 and H_2 evolution (*6*). Their design, however, requires diffusible redox mediators that limit the efficiency of the cell. In this article, we discuss a strategy that can overcome the separation problem based on biometallocatalysis and the vectorial molecular mechanism of photosynthesis. The key idea is to construct separated PSI and PSII reactors using ordered arrays of isolated PSI or PSII particles in direct electrical contact with appropriate metallocatalysts, so that H_2 and O_2 can be synthesized in two separated compartments, thereby avoiding gas mixing. The reactor system is designed to have versatile capabilities. With an appropriate choice of metallocatalysts, it can potentially synthesize new products, such as methane (CH_4), through reduction of CO_2. Furthermore, the PSI and PSII reactors are also potentially good photoelectric cells, which can have application in bioelectronics.

MOLECULAR MECHANISM FOR PHOTOSYSTEM I PHOTOELECTRIC CELL

Structure and Function of Photosystem I Reaction Center/Core Antenna Complex

Photosystem I reaction center/core antenna complexes containing about 40 chlorophylls (chls)/photoactive reaction center pigment (P700) can now be isolated efficiently from thylakoids of plant (such as spinach) leaves using the technique of detergent solubilization and hydroxylapatite column purification (*7,8*). According to recent studies, isolated PSI complexes are elliptical in shape with major and minor axis of about 5 and 6 nm, respectively (*9*). The structure and function of PSI reaction centers are illustrated schematically in Fig. 1. The chlorophylls (represented as bars) serve as an antennae to pick up photons and transfer the photon energy to P700, where the light energy is captured by photochemical reaction. An isolated PSI complex contains electron acceptors (A_o, A_1, F_x, and F_{AB}) in addition to P700 and the antenna chls.

The primary events in photosynthesis are absorption of light and creation of a singlet excited state, transfer of the excitation between pigment molecules, and photochemical charge separation in the reaction center. The photochemistry of P700 generates a primary charge separation ($P700^+ A_o^-$) within about 3 ps. Owing to efficient excitation transfer and trapping, the entire photophysical chemistry can be completed in 10–40 ps (*8,10*). The electron released from P700 by the charge separation is transferred to the terminal acceptor F_{AB} at the reducing side of PSI through intermediate acceptors A_o, A_1, and F_x (*11,12*). PSI is essentially a biological photovoltaic device. The quantum yield of PSI photochemistry is close to

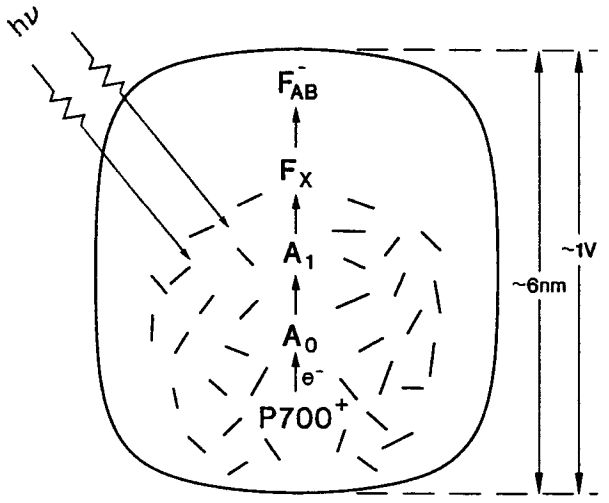

Fig. 1. Schematic illustration of the structure and function of an isolated photosystem I reaction center.

unity (*8,13*). Charge separation in this natural photovoltaic device generates a voltage difference of ~1 V across ~6 nm between the reduced (F_{AB}^-) and oxidized ($P700^+$) sides of the PSI complex, resulting in a very strong electric field (on the order of 10^8 V/m). An isolated PSI particle is arguably the smallest (≈6 nm), fastest, and most efficient photovoltaic cell. These properties of PSI particles may be especially valuable in photodetection, optoelectronic communication, and high-resolution video-imaging devices.

Another important aspect of PSI is its diode property. It is known that the forward electron transfer from P700 to F_{AB} is much faster than its reverse transfer from F_{AB}^- to $P700^+$ (*14*). Based on data from electron spin echo measurements (*15,16*), the electron transfer from P700* to F_{AB}^- is estimated to be 6×10^6/s, which is over 80,000 times faster than the rate (24/s) of the reverse transfer from F_{AB}^- to $P700^+$ (*8*). This result indicates that PSI is also a good photodiode with its forward conductance (from P700* to F_{AB}) much higher than that in the reverse direction. Recent studies with scanning tunneling microscopy have shown that the tunneling conductance of PSI displays typical characteristics of a semiconductor (*9,17*).

Photosystem I Reaction Centers Useful for Construction of Electro-Optical Cell

An important challenge is how to use the electromotive force generated by PSI particles. There have been previous efforts to generate photocurrents with Photosystem II and bacterial reaction centers (*18–24*). Photodependent electrical current was detected with electrodes that were coupled to or directly covered with photosynthetic reaction centers. However, in these prior studies, there was minimal direct electrical contact between

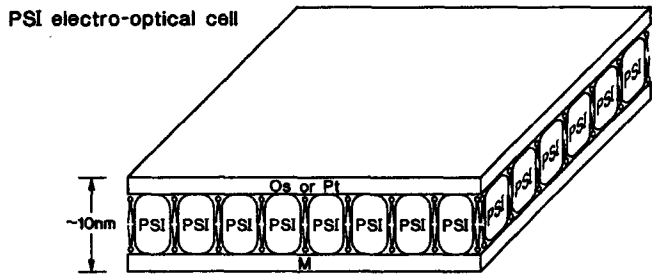

Fig. 2. A proposed Pt(or Os)-photosystem I-metal electro-optical cell.

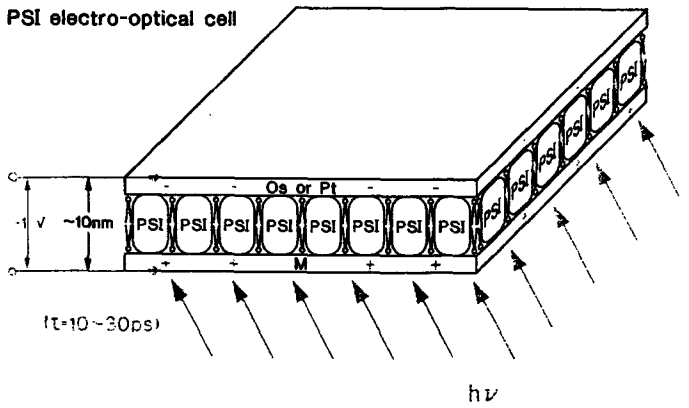

Fig. 3. Action of the proposed PSI electro-optical cell: Activation by light for photocurrent generation.

electrodes and reaction centers. Diffusible redox mediators, such as quinones and *N*-tetramethyl-*p*-phenylenediamine, were used to couple the photochemical reaction to the external electrodes. Owing to the relatively slow diffusive motion of the redox mediators, all of these early studies apparently suffered a common problem of limited efficiency, as clearly pointed out in a study using bacterial reaction centers by Katz et al. (24).

To harness the photocurrent generated by PSI effectively, we have recently designed a PSI photo-electric cell (Fig. 2). It consists of a two-dimensional spatial array of PSI particles sandwiched in between two thin metal films. Platinum (or osmium) film is in contact with the reducing side of PSI, whereas the film on the other side can be any conductor. When metal films are thin enough (< 33 nm), they become essentially transparent to visible light based on the studies by Heller et al. (25). Since metallic platinum is a much better conductor than organic mediators, such as quinones, the PSI photoelectric cell can potentially serve as an efficient photocurrent generator (Fig. 3). As mentioned above, PSI photophysical chemistry can be completed in 10–40 ps after photon absorption with a quantum yield

close to unity. Thus, this PSI photoelectric cell could be the thinnest, fastest, and most efficient photovoltaic cell, which could be useful not only in power generation, but also in fast optoelectronic signal processing.

REACTOR SYSTEM COMBINING PHOTOSYSTEM I AND II PHOTOELECTRICAL CELLS FOR PRODUCTION AND SEPARATION OF H_2 AND O_2

The PSI electro-optical cell also has the capability for hydrogen production, since metallic platinum (or osmium) is also an active catalyst for hydrogen evolution (*1–3,5*). More importantly, the PSI cell can be integrated with a compatible PSII cell to construct a photosynthetic reactor system that is capable of producing H_2 and O_2 with automatic product separation.

Separation of H_2 and O_2 is a problem in the field of solar-hydrogen and oxygen production. Any new separation technology that is energy-saving, efficient, and environmentally safe is a good solution to the problem. Our newly designed photosynthetic reactor system can, in principle, efficiently and safely separate hydrogen from oxygen without additional energy cost. This reaction system, therefore, is a potentially good solution to the gas-separation problem. The reactor system is made of the PSI electro-optical cell as a PSI reactor joined with a compatible Photosystem II (PSII) reactor (Fig. 4). The two reactors are in separate compartments coupled together through an external wire and a proton-conducting water channel.

In this reactor system, energy-saving and efficient separation is guaranteed by the design. As illustrated in Fig. 4, water is photosynthetically split by PSII as usual, producing protons (H^+) and molecular oxygen (O_2). The protons produced in the PSII compartment can be transferred through the proton-conducting channel to the PSI compartment, where H^+ can be reduced by electrons from PSI to produce H_2. Electrons acquired from water splitting are wired to the oxidizing side of PSI, where the electrons are further energized by the PSI photochemical reaction and transported through PSI complexes to the Pt film, where they are used in the reduction of protons for H_2 production. Note: the number of electrons and protons generated and consumed in the two compartments is balanced. Therefore, this reactor system can be operated continuously. The net result is splitting water into pure hydrogen and oxygen. Hydrogen is automatically separated from oxygen without any energy cost, since the two gases accumulate separately in the two (PSI and PSII) compartments. Therefore, this reactor system can provide an efficient and energy-saving gas separation as water is photosynthetically split into hydrogen and oxygen gases in two separate compartments.

This solar energy-conversion system does not produce carbon dioxide (CO_2). The only substrate needed here are water (H_2O) and sunlight. After conversion, solar energy is stored as chemical energy in H_2 and O_2. Use of

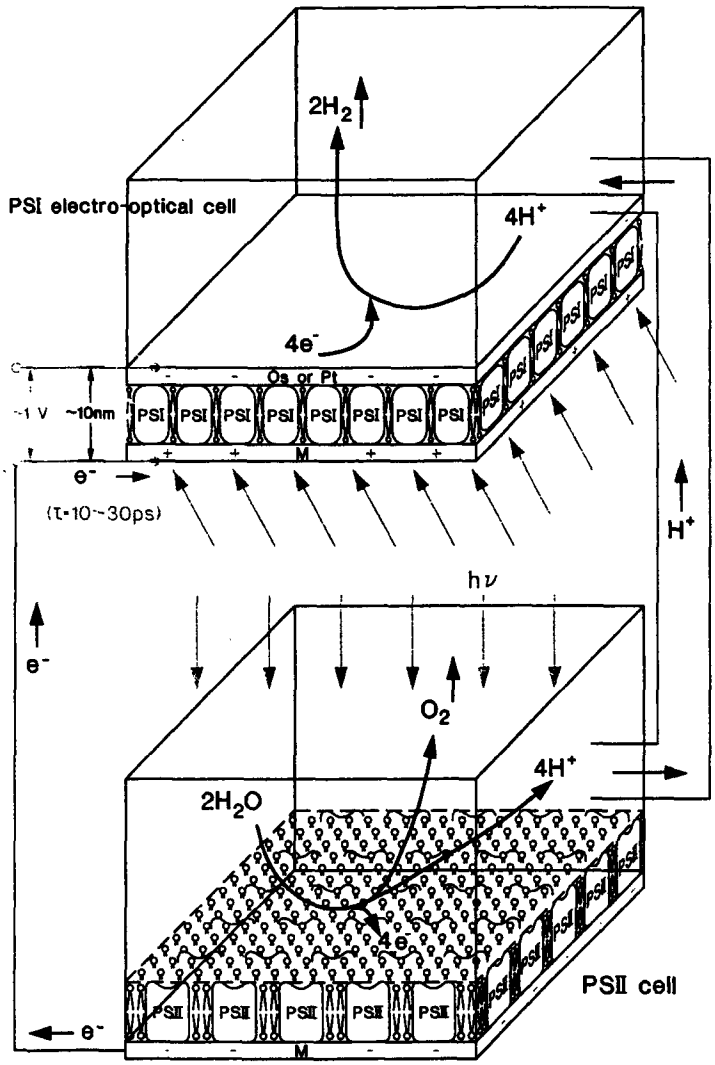

Fig. 4. A photosynthetic reactor system made of PSI and PSII electro-optical cells for production of hydrogen and oxygen in separate compartments.

this energy by burning H_2 with O_2 or in a fuel cell produces pure water without any CO_2 emission, resulting in no alleged CO_2-greenhouse effect. It could, therefore, be the cleanest energy resource on the earth.

Furthermore, the photosynthetic reactor system (Fig. 4) could also be valuable in the field of biometallocatalysis for production of not only hydrogen, but also other chemicals, even drugs and foods depending on the choice of specific catalysts. As an example, this system can potentially produce methane (CH_4) through reduction of CO_2 after the platinum film is replaced by a metallic ruthenium film. It is known that metallic ruthenium is an active catalyst for CO_2 fixation to make CH_4 (*26*).

Table 1
Excitation Decay Kinetics Measured with the Technique of Picosecond Time-Resolved Single-Photon Counting in Platinized and Control PSI-40[a]

PSI-40 control		Platinized PSI-40	
Amplitude	Lifetime, ps	Amplitude	Lifetime, ps
0.981 ± 0.003	15.8 ± 1.2	0.975 ± 0.003	15.9 ± 1.2
0.009 ± 0.002	510 ± 60	0.014 ± 0.002	400 ± 60
0.010 ± 0.002	3200 ± 350	0.011 ± 0.002	2700 ± 350

[a]Excitation was at 660 nm, and fluorescence emission was collected at 680 nm. The reported standard deviations, which are owing to instrument instability, are the maxima from either the control or platinized samples (data cited from ref. 27).

EXPERIMENTAL WORK TOWARD THE CONSTRUCTION OF PHOTOSYSTEM I CELL

We have recently isolated PSI reaction center/core antenna complexes containing about 40 chlorophylls/photoactive P700 from spinach leaves. These PSI particles can be platinized using hexachloroplatinate according to reaction (2). Studies with scanning tunneling microscopy (STM) showed that the platinized PSI particles were about 45% larger than the control PSIs. The result clearly indicated that the isolated PSI particles have a binding affinity for the metallic platinum formed during the platinization reaction at ambient temperature. We further found that PSI particles can be anchored onto a metal (gold) surface by the metallic platinum formed from chemical platinization reaction, resulting in a two-dimensional spatial array of PSI particles "welded" by PSI-Pt-metal binding on the metal plate (*9*). This is a first step in construction of the PSI cell.

Furthermore, we have recently examined the effect of chemical platinization on the photosynthetic function of PSI (*27*). The excitation lifetime within a coupled PSI reaction center/core antenna complex is limited by the photochemistry in the reaction center (*28,29*). If platinization caused release of antenna chls from PSI complexes or if it damaged P700, we would expect an increase in the amplitude of the nanosecond component resulting from the presence of chls that are functionally uncoupled from P700. These potential effects of platinization can be easily detected by measuring PSI excitation lifetimes using the technique of picosecond single-photon counting (*28–30*). The result of the measurements demonstrated that the platinization process does not impede the intrinsic photosynthetic activity of PSI particles. The excitation transfer dynamics measured in both platinized and control PSI particles can be described as the sum of three exponential decay components (Table 1). The fast (about 16 ps) component with a dominant amplitude (0.98) is known to reflect the

photochemically limited core excitation decay (*8,31-33*). No significant change in both the lifetime and amplitude of the fast component was observed before and after platinization. Platinization, therefore, caused essentially no damage to the function of both P700 and antenna pigments in PSI complexes. The excitation transfer and trapping in platinized PSI were as efficient as in the control PSI.

This conclusion is further supported by the measurements of the intermediate (400–500 ps) and the long (>2500 ps) lifetime components, which are likely related to the electron transport within PSI complexes and to the functionally uncoupled chls, respectively (*8,33*). These components were very small in both the platinized and control PSI samples. The extremely small amplitude (0.01) of the long-lifetime (>2500 ps) component indicated that the amount of functionally uncoupled chls was small in both platinized and control PSI particles. About 99% of the chls are functionally connected to the reaction center P700. Neither the PSI isolation procedure nor the chemical platinization process caused any significant uncoupling of the chls in the PSI complexes (*27*). These are signficant results, since they demonstrate that chemical platinization is compatible with PSI function and that the platinization "welding" technique can be used for construction of a PSI photosynthetic reactor discussed above.

In addition to PSI, highly active PSII particles can also be isolated (*34*). The platinization "welding" technique may also be applicable to PSII particles, since their protein subunits have similarity with those of PSI.

For an efficient reactor system (Fig. 4), the photosynthetic reaction centers in each cell must be vectorially oriented. Potentially, there are many ways to orient these reaction centers. Oriented multilayers of PSI particles have already been obtained by drying on a thin polyester (mylar) film (*35*). The reaction center complexes contain charged residues and pigments with specific orientation. Therefore, it is possible to orient them by electric or magnetic fields. This may be a successful strategy for assembly of an oriented reaction center array.

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