Photosynthesis

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Electron Transport between Photosystem II and Photosystem I Encapsulated in Sol–Gel Glasses**

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Photosystem I (PSI) and photosystem II (PSII) are the primary solar-energy-converting enzymes of oxygenic photosynthetic organisms.[1] PSI is a robust, potent, and highly efficient photosensitizer capable of providing electrons at a high reduction potential (−0.55 V vs. normal hydrogen electrode, NHE) from its reducing end.[2] These properties have prompted many attempts to integrate PSI into biohybrid systems for solar energy conversion and storage.[3] In a biological setting, water photooxidation by PSII provides a source of electrons for the reductive processes driven by PSI, but in contrast to PSI, integration of PSII into nonbiological systems is very difficult.[4] Both photosystems were successfully "wired" to conductive electrode surfaces, but their integration into a photocatalytic bioelectrochemical device has not been reported to date.[5]

Herein we demonstrate a simple scheme for mediated coupling of PSII and PSI in solution; this coupling enables electron flow from water photooxidized by PSII all the way to the reducing end of PSI. Furthermore, we show that the same scheme can be reconstituted either when both photosystems are coencapsulated in sol–gel glasses, or when one photosystem is encapsulated and the other is in solution. The sol–gel trapping technique is a proven method for encapsulating a wide variety of biological materials, from intact whole cells to functional individual enzymes.[6,7] In addition, sol–gel systems are porous and optically transparent,[8] which makes them ideal scaffolds for photoinduced electron transfer systems such as the photosynthetic machinery.[9] The main difference between the sol–gel and solution samples is that the photosystem complexes are immobilized within the sol–gel cavities instead of diffusing freely in solution. This property suggests interesting possibilities of segregating PSI and PSII in distinct microenvironments while maintaining electron flow between the photosystems.

PSII is not naturally directly coupled to PSI (Figure 1, top). Instead, electrons flow from PSII to a pool of membrane-soluble plastoquinones that diffuse between the acceptor and donor sides of PSII, and cytochrome b_6f (b6f), respectively.[10] Only a fraction of the electrons extracted from water at the oxygen-evolving complex of PSII end up at the acceptor side of PSI. The rest are cycled between PSII and b6f whereby directed diffusion of quinones, the release of protons on the water oxidizing face, and their consumption on the reducing face of the membrane actively pumps protons across the membrane, and generates proton-motive force.[11]

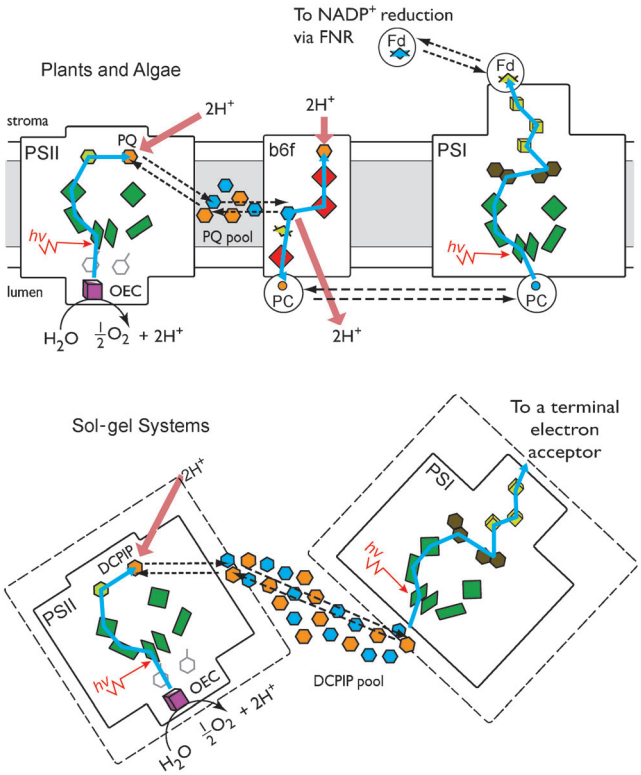

Figure 1. Electron and proton flow in native photosynthetic membranes (top), and mediated electron flow between sol–gel encapsulated PSI and PSII (bottom). Pink arrows indicate proton flow, solid blue and dashed black arrows indicate electron transfer by tunneling and diffusion of soluble carriers, respectively. The oxidized and reduced states of the redox carriers are shown by orange and blue colors, respectively. Fd = ferredoxin, PC = plastocyanin, b6f = cytochrome b_6f, PQ = plastoquinone, OEC = oxygen-evolving complex, FNR = ferredoxin $NADP^+$ reductase.

By using the amphipathic quinone analogue 2,6-dichlorophenolindophenol (DCPIP) as an electron carrier, we were able to bypass b6f and set up an alternative pathway of electron flow from PSII to PSI (Figure 1, bottom). Although it is well established that DCPIP can be reduced by PSII, and the reduced form $DCPIPH_2$ is an electron donor to PSI,[12] DCPIP-mediated electron flow from PSII to PSI was not reported to date, to the best of our knowledge. This

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opens many possibilities for driving reductive chemical processes that use visible light and water as energy and electron sources, respectively. In particular, the capacity of PSI to drive photocatalytic hydrogen production either by coupling to natural hydrogenases or by depositing platinum on its reducing end has been known for many years.[13] O'Neill and Greenbaum have demonstrated photogeneration of molecular hydrogen by sol–gel-encapsulated PSI,[14] and several other hybrid systems were recently reported.[15]

The dynamics of photoinduced charge separation in PSI, with or without DCPIP, and/or PSII was followed by monitoring the specific absorption of the chlorophyll cation radical, $P700^{+\bullet}$, that is formed during the process. A pulse amplitude modulation spectrophotometer (Dual-PAM-100, Walz, Germany) was used in absorption mode to measure the light induced $P700^{+\bullet}$ absorption changes at 810–830 nm.[16] The typical PSI activity pattern characterized by a rapid rise followed by a slow decay of $P700^{+\bullet}$ (Figure 2) was observed exclusively in mixtures of PSI, PSII, and catalytic amounts of DCPIP, but only after the dark-adapted samples were illuminated by actinic light. Notably, measurements of samples containing only PSII with or without DCPIP resulted in no detectable $P700^{+\bullet}$ signals, thereby ruling out contributions from residual PSI in the PSII preparation. These observations strongly suggest that DCPIP is the mediator of electron flow from PSII to PSI. It is photoreduced by PSII, and the product, $DCPIPH_2$, is reoxidized by PSI.

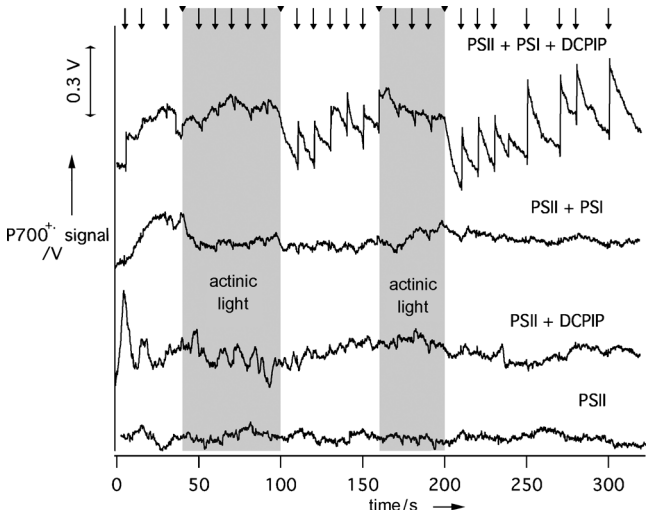

Figure 2. Coupling between PSII and PSI in aqueous solution by DCPIP. A pulse amplitude modulation spectrophotometer triggered specific and selective activation of PSI by a saturating NIR light pulse with microseconds on–off characteristics (designated by arrows). It then measured the changes in absorption of the $P700^{+\bullet}$ cation radical at 810–830 nm that reflect the dynamics of photoinduced charge separation across the PSI reaction center.[16] These absorption changes were translated into voltage and recorded as $P700^{+\bullet}$ signal. Samples were dark-adapted for 10 min prior to measurement, and methyl viologen was added to the solution as a terminal electron acceptor to carry away electrons from the reducing end of PSI.[17] In this setting, reduction of $P700^{+\bullet}$ by an external electron donor is the only way to recycle the photocatalytic activity of PSI. The gray areas represent the periods when both PSI and PSII were excited by actinic light. When PSII and DCPIP are combined with PSI (top trace), DCPIP photoreduction by PSII during these periods leads to accumulation of $DCPIPH_2$ that is an external electron donor to PSI. The electron transfer rate from $DCPIPH_2$ to $P700^{+\bullet}$ is too slow compared to P700 photoxidation during PSI illumination by actinic light. But, as soon as this light is turned off, $P700^{+\bullet}$ is recycled, and the typical PSI activity pattern of a rapid rise and slow decay of the $P700^{+\bullet}$ signal is clearly observed.

Once the scheme for direct, DCPIP-mediated, coupling of PSI and PSII has been established in solution, we set out to reproduce it in sol–gel matrices. Following the method of O'Neil and Greenbaum,[14] we encapsulated cyanobacterial PSI in tetramethyl orthosilicate sol–gels. PSII is more labile to its surrounding pH and chemical conditions;[18] thus, the encapsulation of PSII into sol–gels is much more challenging, and has not been reported to date. The key to successful encapsulation was in tuning the gelling reaction to occur within several minutes after adding the sol–gel precursor to the photosystem solutions, and their rapid and thorough mixing. Sol–gel samples were tested by standard assays of PSI and PSII photochemical activity and integrity, including absorption spectra,[19,20] spectrophotometric assays of DCPIP oxidation and reduction,[12] $P700^{+\bullet}$ dynamics,[16] and oxygen evolution.[21] All tests verified that both photosystems retained their activity and structural integrity throughout the sol–gel encapsulation process (Figure 3). Importantly, we observed the same oxygen evolution profiles for PSII in sol–gels and in buffer solutions (Figure 3d). This result implies that sol–gel encapsulation has no effect on the photostability of PSII and it is possible to encapsulate PSII in sol–gel systems while maintaining its capability to split water and evolve oxygen, which is the hallmark of oxygenic photosynthetic activity.

The successful encapsulation of active PSII and PSI in sol–gel systems has made it possible to test whether the DCPIP-mediated coupling that was demonstrated in solution is also possible when either PSI or PSII, or both, are encapsulated in sol–gels. As shown in Figure 4, the sol–gel samples showed significant activity of PSI under the same illumination conditions as in solution (Figure 2). The $P700^{+\bullet}$ signal intensity is about ten times lower than in solution when PSI and PSII are coencapsulated, but only about five times lower when one photosystem is encapsulated and the other remains in solution. This result is most likely due to the limited diffusion of the redox carriers through the sol–gel pores, which is a well known issue in sol–gel-encapsulated catalytic systems and can be improved by reducing the dimensions of the sol–gel particles.[6] Thus, our results clearly demonstrate the feasibility of the DCPIP-mediated linear electron flow scheme between PSI and PSII in sol–gel systems and in solution, as depicted in Figure 1. In this setting, the difficult task[4] of integrating PSII into a nonbiological photocatalytic system is straightforward. Yet, the scheme is generic. In our case, plant PSII was coupled to cyanobacterial PSI by using an artificial redox mediator, but in principle, any combination of natural, genetically engineered, and rationally designed enzymes, as well as artificial redox catalysts and mediators, can be "wired" in a new context.

Photostability remains a major issue in implementing natural as well as artificial light-driven water-splitting catalysts.[4] PSII is a source of inspiration for highly efficient

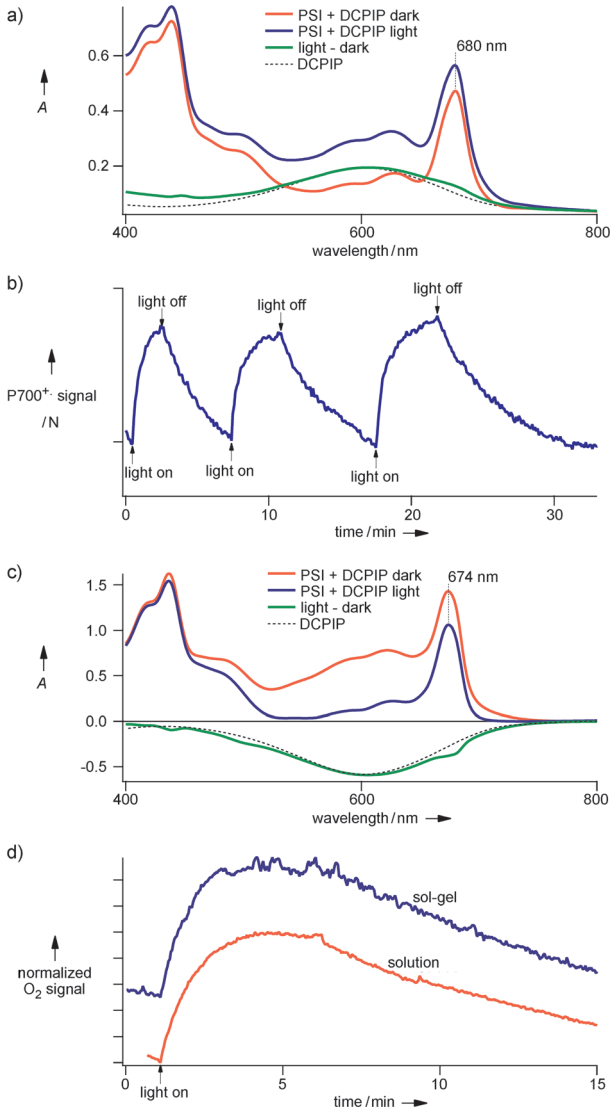

Figure 3. Activity assays of sol–gel-encapsulated PSI and PSII. a) Photooxidation of ascorbate-reduced DCPIP by PSI coencapsulated with methyl viologen, and illuminated at 550 μmole $m^{-2} s^{-1}$. b) Dynamics of photoinduced charge separation across the PSI reaction center reflected in the rise and decay of $P700^{+\bullet}$ cation radical signal, c) Photoreduction of DCPIP by PSII illuminated at 720 μmole $m^{-2} s^{-1}$. d) Evolution of molecular oxygen by PSII illuminated at 720 μmole $m^{-2} s^{-1}$ in sol–gel (blue) and solution (red), with potassium ferricyanide as an electron acceptor. The lowest energy absorption bands of chlorophyll a in PSI (a), and PSII (b) samples at 680, and 674 nm, respectively are typical of unimpaired and functional complexes. Any breakdown of the complex would have resulted in shifting these absorption bands toward the typical protein-free chlorophyll a absorption peak at 660 nm.

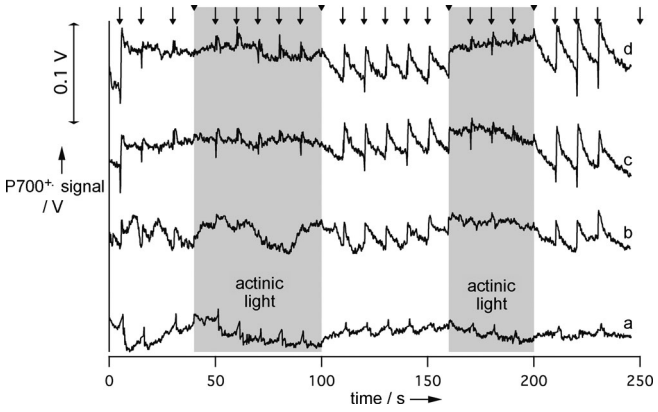

Figure 4. PSI activity in PSI/PSII mixtures encapsulated in sol–gels. Experimental conditions were the same as in Figure 2. The first sample (a) was a control containing PSI and PSII encapsulated in sol–gel without DCPIP, the second sample (b) was the same as (a) but with DCPIP coencapsulated in the sol–gel, the third (c) had PSI encapsulated in the sol–gel and PSII in solution, and the fourth (d) had PSII in sol–gel and PSI in solution. In the last two samples, sol–gels containing the encapsulated photosystem were crushed manually and added to the solution containing the other photosystem. DCPIP was present both in the sol–gels and in solution.

water-splitting systems, but it was not optimized for high stability. Instead, photosynthetic organisms employ an elaborate and dedicated machinery of protein degradation and synthesis that turns over PSII typically every 20 min.[22] Sol–gel encapsulation has been shown to improve the stability and activity of many enzymes,[6] but in this case we find (Figure 3d) that although encapsulation retained the oxygen evolution activity of PSII, it could not sustain it for longer time periods than in solution. This behavior is because the loss of PSII activity is most likely the result of photodegradation of its light-harvesting pigments and redox cofactors, whereas the stabilization effect of sol–gel encapsulation is considered to be the result of confining the protein within a silica cage, which prevents unfolding by restricting the protein mobility.[23] Nonetheless, sol–gel encapsulation offers new possibilities for increasing PSII photostability by immobilizing and segregating it in distinct microenvironments. For example, Larom et al. have demonstrated that an alternative electron transfer pathway from a PSII mutant to cytochrome c significantly increases its photostability in isolated thylakoid membranes;[24] coencapsulation of this mutant with cytochrome c in sol–gel systems may result in the same effect.

In conclusion, beyond the straightforward demonstration of a mediated electron transfer pathway from photooxidized water to the reducing end of PSI, the system presented here is a general medium and a modular scheme that may open new possibilities for novel reaction pathways combining biological and nonbiological elements.

Experimental Section

Highly active PSII particles, known as BBY particles,[25] were isolated from fresh lettuce. PSI particles were isolated from the photosynthetic membranes of the thermophilic cyanobacterium *Thermosynechococcus elongatus*.[20] Details of sample preparation and activity assays are provided as supplementary information.

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