Light-Driven Hydrogen Production from Photosystem I-Catalyst Hybrids

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Abstract

Solar energy conversion of water into environmentally clean fuels, such as hydrogen, offers one of the best long-term solutions for meeting future global energy needs. In photosynthesis, high quantum yield charge separation is achieved by a series of rapid, photoinitiated electron transfer steps that take place in proteins called reaction centers (RCs). Of current interest are new strategies that couple RC photochemistry to the direct synthesis of energy-rich molecules, offering opportunities to more directly tune the products of photosynthesis and potentially to increase solar energy conversion capacity. Innovative designs link RC photochemistry with synthetic molecular catalysts to create earth abundant biohybrid complexes that use light to rapidly produce hydrogen from water.

Introduction

Solar energy is an important source of renewable, non-carbon energy.[1,2] An abundant resource, the sun provides more energy to the earth in one hour than humans consume in an entire year. Sunlight, however, is highly diffuse and intermittent, and thus the large scale utilization of solar energy requires its cost-effective, long-term storage. To address these needs, current solar energy research involves developing practical schemes for capturing, converting, and storing the sun's energy in the form of high-energy chemical bonds of molecules as a reduced fuel, or "solar fuel", such as hydrogen, methanol, or methane. The direct production of hydrogen using sunlight and water is a particularly attractive approach to sustainable energy production. Unlike carbon-based fossil fuels that produce environmentally harmful CO₂, hydrogen is a clean fuel because, upon burning to release the energy in its chemical bond (140 MJ/kg), water is produced. Recent advances in solar fuel research involve using Nature's photosynthetic chemistry for direct solar hydrogen production.

For over a billion years, Nature has optimized the photosynthetic machinery that converts light energy into chemical energy. [3,4] The primary energy conversion reactions occur in integral membrane proteins called reaction centers (RCs) and involve rapid, sequential electron transfers resulting in efficient charge separation across the membrane, establishing an electrochemical potential. Photosynthesis occurs in two main classes of organisms: photosynthetic bacteria that do not evolve oxygen, and organisms such as green plants that perform oxygen evolution. The first X-ray crystal structures of the RC from purple photosynthetic bacteria were determined in the mid-1980's.[5,6] The bacterial RC structure reveals key design features for photosynthetic photochemistry and remains an excellent "model" system for spectroscopic exploration of basic RC structure and function relationships.[7] Light-induced electron transfer in the bacterial RC

results in directional proton transport across the membrane, establishing a pH gradient that drives ATP synthesis.[3] Higher plants, green algae, and cyanobacteria use two types of large RCs in tandem to convert and store solar energy as chemical free energy. Photosystem II (PSII) [8] carries out light-driven oxidation of water whereas Photosystem I (PSI) [9] catalyzes transmembrane light-driven electron transfer between small electron carrier proteins. These electrons are then used to produce a more stable reductant NADPH, the biological equivalent of H_2 , which is a source of reducing equivalents for Calvin cycle CO_2 fixation and final chemical energy storage. However, kinetic limitations for CO_2 fixation and electron transfer through the photosynthetic electron transfer chain causes saturation of solar energy capture with insolation that is approximately 20% of mid-day sun. [10•,11] This excess light-harvesting capacity is one of the sources for loss of solar energy conversion efficiency in photosynthesis.[10•-13]

PSI's built in light-harvesting and charge separation capabilities make it an attractive candidate for solar fuel production. [14] Multiple carotenoids and chlorophylls encircle the protein periphery, together acting as an efficient antenna system for light capture. The captured excitation energy is transferred to the center of the protein complex where the primary electron donor of PSI, P700, becomes oxidized with concurrent electron transfers through a cascade of donor/acceptor molecules terminating in electron transfer to a $[4Fe-4S]$ cluster called F_B . The quantum yield of PSI approaches 1, thus nearly every photon captured is converted to the charge-separated state $P700^+ F_B^-$. The $P700^+ F_B^-$ state is long-lived, ~60 ms, and the F_B cluster has a standard midpoint potential of -580 mV (vs NHE, pH independent) that provides sufficient driving force to power alternative reduction chemistry, including the reduction of protons to H_2 . In contrast, both bacterial RC and PSII have terminal quinone acceptors ($E_m \sim 0$ mV) that provide no driving force for proton reduction.

Engineering PSI to produce H_2 either as a replacement or in addition to NADPH requires redirection of PSI electron transfer to a catalytic component for reductive proton-coupled chemistry (Figure 1). Photosynthetic hydrogen would provide a fuels product with higher energy storage (E_m , pH 6.3 = -370 mV) than NADPH (E_m , pH 6.3 = -300 mV) and one that potentially could be developed to avoid down-regulation that occurs at high light intensities. PSI's photogenerated electrons have been successfully coupled to hydrogenase enzymes and platinum catalyst systems. The use of abiotic catalysts for photosynthetic hydrogen production is of interest since they provide an opportunity to avoid the oxygen liability of hydrogenases. The overall focus of this review highlights recent strategies inspired by artificial photosynthetic systems that link PSI photochemical charge separation to H_2 -evolution via synthetic molecular complexes. These new biohybrid designs are composed of earth-abundant materials, work in water, and have high catalytic activity thereby directly addressing several key challenges to making solar fuels a viable energy source.

Photosystem I-Hydrogenase Hybrids

One earth-abundant PSI-hybrid strategy includes using hydrogenases, enzymes that catalyze the reversible interconversion of H_2 to protons and electrons. Two types of hydrogenases (classified by the metal composition of their respective active site metal centers) have been linked to PSI, each with inherent advantages and disadvantages. [15,16] [FeFe]-hydrogenases preferentially catalyze proton reduction and can evolve H_2 at high rates ($10,000\text{ s}^{-1}$), but these enzymes are highly sensitive to O_2 , which can irreversibly damage the active site. [NiFe]-hydrogenases are less sensitive to O_2 than [FeFe]-hydrogenase, but are more commonly associated with H_2 oxidation under physiological conditions.[17•] Linked PSI-[NiFe]-hydrogenase constructs have been made by gene fusion and chemical crosslinking, but these constructs are hampered with low

electron transfer efficiency.[18] Higher rates of turnover (Table 1) have been observed for covalently linking [FeFe]-hydrogenase systems via a molecular wire to the F_B cluster[19] or via gene fusion to ferredoxin.[20] A concern with hydrogenase-based hybrids is that O_2 sensitivity will make it difficult to generate H_2 at the same time as O_2 in future water splitting systems unless O_2 evolution and H_2 production can be physically divided. Advances in understanding a subclass of O_2 tolerant [NiFe]-hydrogenases may extend current PSI H_2 -based technologies.[17•]

Photosystem I-Platinum Systems

In 1985 photocatalytic H_2 evolution from platinized chloroplasts was first reported.[21] Notably, a recent report demonstrated the extreme durability (85 consecutive days of photocatalysis) of a platinized PSI hybrid created by photodeposition of metallic Pt onto PSI isolated from a thermophilic cyanobacterium.[22] A great improvement in rate of H_2 production (Table 1) was achieved with a novel PSI bioconjugate design that directly tethers a Pt-nanoparticle via a dithiol molecular wire to a mutated [4Fe-4S] F_B cluster.[23] Another highly active hybrid is a self-assembled, noncovalent, electrostatically-associated PSI-Pt nanoparticle complex.[24] Importantly, this is the first hybrid to be spectroscopically characterized, providing mechanistic insight into key protein-catalyst interactions and electron transfer reactions related to catalytic function. Although Pt-based systems produce high, sustained photocatalysis rates, future widespread usage will be limited by the scarcity and high cost of precious metals.

Homogeneous Artificial Photosynthetic Systems

Synthetic transition metal catalysts containing Co, Fe, and Ni have been extensively studied and provide scalable alternatives to the benchmark H_2 evolution catalysts, platinum and hydrogenases. [25-29] The successful use of earth-abundant catalysts in artificial photosynthetic

systems provides motivation for linking synthetic complexes to PSI chemistry. Multi-molecular homogeneous systems perform photo-induced hydrogen production with freely-diffusing photosensitizer, catalyst, and sacrificial electron donor mixtures. A popular class of catalyst used in these experiments is the cobaloximes; low cost, O₂ insensitive, synthetically facile pseudomacrocyclic bis(dimethylglyoxamato) cobalt complexes.[30] Good progress continues in creating suitable earth-abundant catalysts that, when coupled with photosensitizers, exhibit long-lived and high catalytic turnover numbers (Figure 2).[31-38] Linked photosensitizer-cobaloxime supramolecular assemblies provide an opportunity to hardwire electron and proton transfer pathways, but, in fact, show poorer H₂ generation capabilities than unlinked multi-molecular systems.[39-41] Structure-function studies show that supramolecular structures provide efficient, ultrafast electron transfer to the catalyst from the photosensitizer excited state, but concurrently enable fast charge recombination pathways.[42,43] New linked photosensitizer-cobaloxime designs are aimed at stabilizing the Co(I) catalytic intermediate state and limiting charge recombination.[44•]

Coupling Nature's Photosynthetic Chemistry with Synthetic Catalytic Function

Interfacing molecular catalysts with PSI provides the opportunity to create a novel class of biohybrid complexes and gain otherwise unobtainable insight about catalytic function in an aqueous protein environment. Importantly, H₂ generation via the catalyst will be tied into the long-lived charge separated state provided by PSI, thus directly addressing the major limitations of uncontrolled or fast back charge recombination reactions encountered in multi- and supramolecular artificial systems. An ongoing synthetic challenge is to discover durable molecular catalysts that are highly active in purely aqueous solution for both electro- and photocatalysis. Embedding catalysts within a protein environment eliminates the artificial

systems' requirement of organic solvent. The key to creating a functional hybrid system is the effective coupling of rapidly formed single electron photo-excited charge-separated states of PSI to the slower multiple proton-coupled electron transfer reactions at the transition metal catalyst sites. Two successive electrons from PSI must reach the molecular catalyst, thus optimized donor side reactions are required to rapidly re-reduce oxidized donor of PSI, $P700^+$. Because most systems to date have only looked at half of the water-splitting reaction (herein, proton reduction), sacrificial donors such as sodium ascorbate and electron mediators such as cytochrome c_6 are often employed.

Since they have been extensively studied in electro- and photocatalysis, cobaloximes serve as useful starting points for investigating fundamental mechanisms for coupling excited-state RC photochemistry to catalytic bond formation. The PSI-Pt nanoparticle hybrid work established a biomimetic, self-assembly method for coupling PSI to a surface-modified nanoparticle catalyst that did not require covalent linkage for rapid photocatalysis; [24] suggesting that molecular recognition can direct catalysts to the [4Fe-4S] cofactor/acceptor side of PSI and establish the geometry necessary for intra-molecular electron transfer. Hybrid protein-molecular catalyst complexes form following stoichiometric addition of a molecular catalyst to buffered protein solution; unbound catalyst is removed by size exclusion methodologies (Figure 3). PSI and the cobaloxime derivative $\text{Co}(\text{dmgH})_2\text{pyCl}$ (where dmgH = dimethylgloximate, py = pyridine) readily self-assemble in buffer and the resultant complexes rapidly produce H_2 in aqueous solution upon exposure to visible light (Table 1).[45••] The successful generation of H_2 from the PSI-cobaloxime hybrid is a clear demonstration of Nature's optimized RC photon capture and charge-separation chemistry driving photocatalysis with observed turnover rates that are faster

than those reported for related synthetic photosensitizer-cobaloxime complexes[30,46] and that approach the rates observed for the best PSI-Pt nanoparticle (cyt c₆) system to date.[24]

A related PSI-molecular catalyst hybrid uses a Ni(II) diphosphine catalyst developed by DuBois and coworkers[47] as an efficient electrocatalyst for H₂ production.[48] $[\text{Ni}(\text{P}_2^{\text{Ph}}\text{N}_2^{\text{Ph}})_2](\text{BF}_4)_2$ readily binds to native PSI in ratios dependent on the initial amount of $[\text{Ni}(\text{P}_2^{\text{Ph}}\text{N}_2^{\text{Ph}})_2](\text{BF}_4)_2$ added to the protein, similar to $\text{Co}(\text{dmgH})_2\text{pyCl}$ complex formation with PSI. Self-assembly of the $[\text{Ni}(\text{P}_2^{\text{Ph}}\text{N}_2^{\text{Ph}})_2](\text{BF}_4)_2$ with isolated PSI resulted in an active photochemical H₂-forming biohybrid complex that functions in aqueous solution at near-neutral pH.[49••] (Table 1) Notably, the PSI hybrid generates H₂ at a rate two orders of magnitude greater than rates reported for a molecular photosensitizer and $[\text{Ni}(\text{P}_2^{\text{Ph}}\text{N}_2^{\text{Ph}})_2](\text{BF}_4)_2$ system, although this catalyst was quite durable in the artificial system.[31] The protein environment enables photocatalysis in completely aqueous conditions at pH 6.3, distinct from the strong acid requirement for rapid electrocatalytic and artificial photocatalytic H₂ production of $[\text{Ni}(\text{P}_2^{\text{Ph}}\text{N}_2^{\text{Ph}})_2](\text{BF}_4)_2$. [50]

An issue with PSI-molecular catalyst biohybrids is that catalysis is short-lived (<1.5 h for $\text{Co}(\text{dmgH})_2\text{pyCl}$; < 3 h for $[\text{Ni}(\text{P}_2^{\text{Ph}}\text{N}_2^{\text{Ph}})_2](\text{BF}_4)_2$). Although rapid catalysis is observed immediately after light exposure, the high reactivity quickly tapers off. Degradation of protein is not likely a reason for activity loss, as PSI is functionally robust with activity nearly unchanged after months of continuous testing.[51••] Instability of the molecular catalyst is the most likely issue. Homogeneous artificial systems for light driven hydrogen production have displayed significant problems with photodecomposition of cobaloxime catalysts within a few hours, with ligands sometimes added to the reaction mixture throughout the experiment to prolong catalytic activity.[46] Metal analysis indicates that >90 % of the molecular catalyst ($\text{Co}(\text{dmgH})_2\text{pyCl}$ or $[\text{Ni}(\text{P}_2^{\text{Ph}}\text{N}_2^{\text{Ph}})_2](\text{BF}_4)_2$) has dissociated from the protein following

photocatalysis. Whether the catalyst degrades first, then is released from the protein or vice versa is not known. Nevertheless, the high turnover numbers achieved within a short time by biohybrids are promising, and provide a benchmark that can be improved upon with more durable earth abundant molecular catalysts linked to PSI.

Protein Directed Delivery of Catalysts

Hydrophobic interactions dominate the binding of molecular catalysts to PSI, with the catalyst tucking itself in hydrophobic pockets provided by the large PSI protein matrix (~ 350 kDa).[9] Thus, different strategies are needed to achieve directed binding of molecular catalysts to PSI. A promising method involves using a catalyst carrying protein to assemble PSI-molecular catalyst hybrids. This method uses inherent protein-protein electrostatic interactions between PSI and its acceptor protein flavodoxin, a small acidic protein that docks to a basic patch provided by PSI and in close proximity to the F_B cluster.[52] Controlled incorporation of $[\text{Ni}(\text{P}_2^{\text{Ph}}\text{N}_2^{\text{Ph}})_2](\text{BF}_4)_2$ with the flavodoxin protein was demonstrated by catalyst integration within the native cofactor binding pocket provided by the apo-protein.[49••] Photocatalysis experiments with this modified flavodoxin demonstrated a new mechanism for biohybrid creation that involves protein-directed delivery of a molecular catalyst via apo-flavodoxin to the reducing side of PSI for light-driven catalysis. The rate of photocatalysis nearly doubled and the length of catalysis was extended 30% compared to catalysis of the directly self-assembled Ni-PSI complex. The catalyst delivery approach provides the potential for self-repair of the biohybrid system with a mechanism for introducing fresh catalyst to the acceptor end of PSI, and studies with the second acceptor protein of PSI, ferredoxin, impart additional experimental possibilities for creating novel solar fuel hybrids.

Concluding Remarks

The molecular catalyst-protein hybrids overcome the solubility barrier, with the protein bringing the chemistry of the molecular catalysts into aqueous conditions. These systems thus provide the opportunity to understand basic structure-function relationships of non-native inorganic complexes within protein environments. The transition metal sites not only add catalytic function to PSI, but provide direct spectroscopic probes of localized catalyst-protein binding sites and opportunities to monitor intermediates in light-induced catalytic reaction mechanisms using transient EPR and optical spectroscopies. The first spectroscopic data of the Ni(I) sites in PSI and apo-flavodoxin[49••] and the Co(II) site of cobaloxime-substituted apo-myoglobin[53] have been reported. Other related hybrids include heterogeneous bioinorganic systems for photochemical conversion that link proteins with inorganic nanomaterials[54••] and photovoltaic devices.[51••]

In summary, by interfacing biology and chemistry, novel PSI biohybrids were created that provide a new approach to solar fuels research. Innovative biohybrid designs accomplish practical O₂ tolerant, earth abundant photo-generation of H₂ by combining Nature's optimized RC light capture and conversion capabilities with the creativity offered by synthetic chemistry. This research is in its early stages. We believe the weak link in current molecular catalyst-hybrid architectures is the chemistry (concerns include E_m, pK_a, and stability of molecular catalysts) and not the biology, which helps suggest targets towards the development of an optimized system. Opportunities for future hybrid motifs include developing ligand designs that promote interactions, such as electrostatic or covalent, between synthetic molecular catalysts and proteins.[55-57] Another opportunity suggested by the initial steps discussed here is to redirect PSI reducing equivalents to drive catalytic reactions *in vivo* by genetic modifications.[58••] Re-engineered photosynthetic systems may be able to incorporate molecular catalysts *in vivo* to

overcome the inherent inefficiencies of photosynthesis and produce practical solar fuel strategies for wide-scale use.

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Figure 1. Photosystem I-catalyst hybrid systems for light-driven H₂ production. The center panel depicts a diagram of the overall photocatalytic scheme of H₂ production from Photosystem I-catalyst systems wherein a photogenerated electron from PSI is transferred to a bound catalyst. The catalyst must accept two successive photogenerated electrons from PSI to generate one H₂ molecule. Electron donors, plastocyanin or cytochrome c₆ in the presence of excess sodium ascorbate, reduce the photon-oxidized primary donor of PSI. This is necessary for rapid turnovers of PSI that enable multi-electron transfer to the catalyst site(s) for H₂ production. Outer groupings include three types of catalysts that have been successfully incorporated with PSI's photochemistry to make functional H₂-producing hybrids. Earth-abundant molecular catalysts include cobaloxime,[45••] Ni diphosphine,[49••] and Ni-apoflavodoxin.[49••] Examples of noble metal catalysts include Pt nanoparticles bound to PSI via electrostatic binding, [24] Pt nanoparticles linked via 1,6-hexanedithiol to the F_B cluster of PSI, [23] and Pt nanoclusters formed by photodeposition onto PSI.[22] The last grouping highlights some PSI-hydrogenase hybrids including a cyt c-PSI linked to a [FeFe]-hydrogenase by octanedithiol, [19] an [FeFe]-hydrogenase:ferredoxin fusion protein, [20] and [NiFe]-hydrogenase genetically fused to PSI. [18]

Figure 2. Structures of molecular catalysts for H₂ production. Cobalt catalysts are shown the green box. On the left is the cobaloxime linked to PSI (**1**) [45••] and in supramolecular complexes (**2**).[39] The right side illustrates variations of pentadentate nitrogen-Co catalysts used in synthetic systems for photocatalysis (**3-5**) [33,34,37] which are suggested to be more robust and water soluble. The bottom panel shows a Co dithiolene catalyst (**6**) which can perform rapid catalysis in acetonitrile-water mixtures.[35] In the blue box nickel catalysts are shown. Extensive variations on nickel diphosphine catalysts have been developed to increase the

solubility and functionality in aqueous solution (**7-9**).[31,36,38] Catalyst (**7**) has been linked to PSI and Ni-apoflavodoxin for H₂ production.[49••] Catalyst (**10**) shows a nickel pyridine thiolate catalyst intended to mimic the [NiFe]-hydrogenase active site.[32] The purple box shows an iron containing catalyst intended to mimic the [FeFe]-hydrogenase active site which has been appended into a peptide photocatalytic system (**11**). [57]

Figure 3. Preparation of Photosystem I-molecular catalyst hybrids. Dark-adapted PSI (~ 5 μM PSI monomer in a buffer pH 7.8 – 8.0) is incubated with 2-10 mole equivalents of molecular catalyst (diluted from a 5 mM catalyst stock solution prepared in DMSO). The PSI and molecular catalyst are incubated for 2 h at room temperature in the dark to allow complex formation. Unbound catalyst is removed from PSI-catalyst complexes by size exclusion filtration (50000 MWCO devices) or chromatography followed by several washing steps. Metal analysis is used to determine the amount of catalyst bound to PSI. For the photocatalysis experiment, a reaction mixture containing 80 nM PSI-catalyst hybrid, 100 mM sodium ascorbate (sacrificial electron donor), and 4 – 8 μM cytochrome c₆ (electron mediator) is illuminated with a xenon lamp. H₂ bubbles produced during illumination of a PSI-cobaloxime hybrid solution are shown.[45••] The amount of H₂ generated is determined by sampling and analyzing the headspace every 10 – 30 min for H₂ by gas chromatography.

Figure 1

Figure 2.

Figure 3.

Table 1. Rates of light-driven hydrogen production and turnover numbers for PSI-Catalyst hybrid systems

PSI-Catalyst System	Rate of H ₂ Production [mol H ₂ (mol PSI) ⁻¹ s ⁻¹]	TON (time)
PSI-nanoclusters photoprecipitated, long-lived ^{a, [22]}	0.002	nd [*] (2000 h)
PSI-[NiFe]-hydrogenase, genetic fusion ^[18]	0.01	nd [*] (3 h)
PSI-nanoclusters photoprecipitated, short-lived ^{a, [22]}	0.13	nd [*] (2 h)
PSI-[FeFe]-hydrogenase-PetF <i>in vitro</i> complex ^{b, [20]}	0.31	nd [*] (0.5 h)
PSI-Ni diphosphine ^{a, [49]}	0.73	1870 (3 h)
PSI-[FeFe]-hydrogenase-Fd protein complex ^{b, [20]}	1.07	nd [*] (1 h)
PSI-molecular wire-Pt nanoparticle ^{a, [23]}	1.17	4210 (12 h)
PSI-NiApoFld protein delivery ^{a, [49]}	1.25	2825 (4 h)
PSI-cobaloxime ^{a, [45]}	2.83	5900 (1.5 h)
PSI-Pt nanoparticle ^{a, [24]}	5.83	80000 (4 h)
PSI-molecular wire-[FeFe]-hydrogenase ^{a, [19]}	52.4	nd [*] (3 h)
[*] <i>nd, not determined, redox mediators: ^aCyt c₆, ^bPC</i>		