A Hybrid Biological-Organic Photochemical Half-Cell for Generating Dihydrogen

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Objectives

The goal of this work is to design, fabricate, characterize, and optimize a biological/organic hybrid electrochemical half-cell that couples Photosystem I (PS I), which efficiently captures and stores energy derived from sunlight, with either a Fe-Fe hydrogenase (H_2ase) or a Ni-Fe H_2ase, which is capable of a high rate of H_2 evolution. The central concept is to deliver the electron from PS I to the H_2ase rapidly and efficiently using a covalently bonded molecular wire that connects the active sites of the two enzymes. The PS I-molecular wire-H_2ase complex will be tethered to a gold electrode through a baseplate of cytochrome (Cyt) c_6, which will additionally serve as a conduit of electrons from the gold to PS I. Cyt c_6 will be covalently bonded to the electrode through a self-assembling monolayer of functionalized alkanethiols. The device should be capable of transferring at least 1000 e^- per second from PS I to the H_2ase to carry out the reaction: $2H^+ + 2e^- + 2h\nu \rightarrow H_2$.

Technical Barriers

This project addresses the technical challenge of efficiently coupling solar energy conversion to the reduction of protons for H_2 production. The major challenge is to deliver low-potential electrons from PS I to H_2ase rapidly and efficiently in vitro by a method that does not depend on inefficient diffusion chemistry. The solution to this challenge is a biologically inspired, hybrid biological/organic electrochemical half-cell that couples PS I, which efficiently captures and stores solar energy, with H_2ase, which can efficiently combine protons and electrons to produce H_2 via a molecular wire. This bioinspired, solid-state device will evolve H_2 when illuminated and will have both energy- and research-related applications.

Abstract

This report describes our progress towards the design, assembly, characterization, and optimization of a biological/organic hybrid photochemical half-cell. This device will couple PS I, which efficiently captures and stores energy derived from sunlight, with either an [FeFe]-H_2ase or a [NiFe]-H_2ase, which can generate a high rate of H_2 evolution when provided with a source of electrons (see Figure 1). In the last two years, we have designed and tested the concept of using a covalently bonded, molecular wire

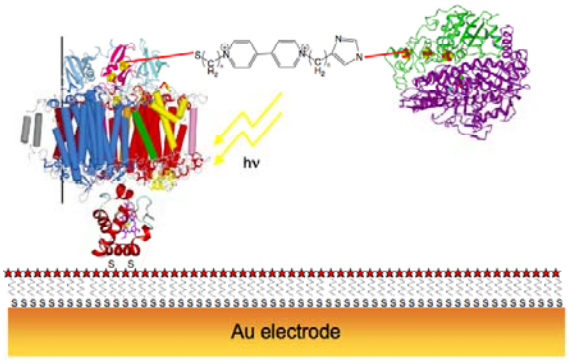

Figure 1. A diagram depicting the hybrid biological/organic photochemical half-cell.

to connect the electron transfer chains of the two enzymes directly. Using metal nanoparticles (NPs) in place of H_2ase, we have validated this design concept and have shown that, when illuminated, PS I can efficiently deliver electrons via such a molecular wire to an inorganic catalyst to potentiate H_2 production. Our results show that low-potential electrons can be transferred without loss and at high rates directly from PS I to an external catalyst. The PS I-molecular wire-H_2ase construct will eventually be tethered to a gold electrode through a baseplate of Cyt c_6, which will additionally act as a conduit of electrons from the gold surface to PS I. Cyt c_6 will be covalently bonded to the electrode either directly or through a self-assembling monolayer of functionalized alkanethiols. The completed device should be capable of transferring ~1000 electrons per second from PS I to the H_2ase to perform the half-cell reaction: $2H^+ + 2e^- + 2h\nu \leftrightarrow H_2$. As illustrated in Figure 1, the device is highly modular, which makes it possible to construct and verify the performance of individual components of the system without requiring the device to function in an all-or-nothing manner.

Progress Report

1) Engineering PsaC with a Gly ligand at Cys13 and attachment to PS I. The PsaC subunit of PS I binds two low-potential $[4Fe\text{-}4S]^{1+,2+}$ clusters, termed F_A and F_B, which serve as the terminal electron acceptors of the PS I reaction center. In the C13G/C33S variant of PsaC, Gly has replaced Cys at position 13; this creates a protein that is missing one of ligating amino acids to Fe/S cluster to F_B. We tested the idea that a rescue ligand, consisting of an external thiolate, is retained when inorganic Fe/S clusters are inserted *in vitro* into the recombinant C13G/C33S variant of PsaC. Using a variety of analytical techniques, including non-heme iron and acid-labile sulfur assays, and EPR, resonance Raman, and Mössbauer spectroscopies, we showed that the C13G/C33S variant of PsaC binds two $[4Fe\text{-}4S]^{1+,2+}$ clusters despite the absence of one of the Cys ligands. ^{19}F NMR spectroscopy showed that an external thiolate indeed replaces Cys13 as a substitute ligand to the F_B cluster. The finding that site-modified $[4Fe\text{-}4S]^{1+,2+}$ clusters can be chemically rescued with external thiolates demonstrates that it is possible to attach an additional electron transfer cofactor to PsaC via an external ligand directly bonded to the F_B Fe/S cluster. Following this logic, a methyl viologen derivative, 1-(3-thiopropyl)-1'-(3-(acetylthio)propyl)-4,4'-bipyridinium, was synthesized and used to rescue chemically the F_B cluster of the C13G/C33S PsaC variant. The chemical coupling was accomplished by loading a Sephadex PD-10 column with the viologen derivative and passing the variant PsaC, at a ratio of 1:1, through the column. The tethered PsaC product was washed by ultrafiltration over a 3-kDa cutoff membrane to remove any unbound viologen. UV/visible absorption and EPR spectroscopies were performed on the chemically reduced, tethered PsaC product, which confirmed the presence of two reduced $[4Fe\text{-}4S]^{1+}$ clusters and a viologen radical. The tethered PsaC was rebound to F_X cores in the presence of PsaD, resulting in a reconstituted PS I-tether complex. To verify the ability of the tethered viologen to accept an electron from P700 upon excitation with light, steady state kinetics at 600 nm and flat cell, room temperature EPR techniques are currently being employed. **Publication: Antonkine et al. 2007.**

2) Photosystem I/metal nanoparticle bioconjugates for the photocatalytic production of H_2. The following-proof of concept experiment shows that electrons can be captured from PS I via the molecular wire and transferred to a Pt catalyst, thereby leading H_2. These studies were guided by the knowledge that catalytic production of H_2 has previously been achieved with the aid of metal nanoparticles (NPs). Photocatalytic production of H_2 has been achieved by means of ethanol reforming on the surface of Au and Pt NPs supported on semiconductor materials such as titania (TiO_2). These semiconductor materials supply the energy for H_2 production in the form of reducing electrons. A major drawback to this type of photocatalytic H_2 production is that photons must have energy greater than the band-gap of titania in order to produce a charge-separated state that is capable of driving the reaction $2H^+ + 2e^- \prod H_2$. This band-gap is 3.2 eV, which corresponds to light wavelengths shorter than ~350 nm. Thus, only a very small fraction of incident solar radiation has sufficient energy to drive H_2 production. Covalent attachment of PS I to Au- or Pt-NPs provides an attractive alternative to these titania-supported particles

for the photocatalytic production of H_2. The antenna pigments of PS I absorb all light wavelengths shorter than 700 nm, which represents 43 to 46% of the total solar emission that reaches the Earth's surface. Nearly all of the photons that are absorbed are converted into the charge-separated state $P_{700}^{+}F_B^{-}$, which means that PS I has a quantum yield of ~1.0. Moreover, the charge-separated state is stable for ~100 ms, and the low potential electrons produced have a redox potential that is thermodynamically favorable for H_2 evolution. Thus, this system should evolve H_2 if electrons from PS I can be transferred to the NP surface in less than 100 ms. The C13G/C33S variant of PsaC can be chemically rescued by thiolate ligands and is fully active when bound to P700-F_X cores in the presence of PsaD. As described above, this construct has the ability to transfer electrons from the F_B cluster to an external acceptor. Facile surface modification of Au- and Pt-NPs, coupled with a capacity to catalyze the reduction of protons to H_2, make these particles attractive candidates for this approach. The functionalization of Au- and Pt-NPs by thiolated molecules is an extensively explored and well-documented field of study. Thus, PS I can be covalently attached to the surface of such NPs by reconstituting PS I with the C13G/C33S variant of PsaC with a dithiol linker molecule. One sulfhydryl group of the molecule acts to modify the surface of the NP while the other functional group serves as the ligand to the F_B cluster. The scheme for this experiment is shown in Figure 2.

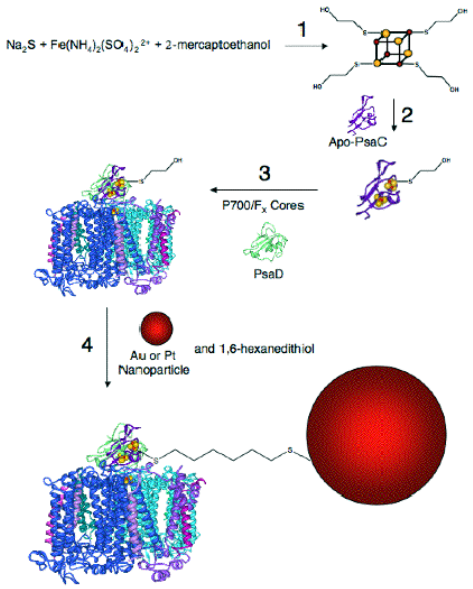

Figure 2. Construction of a PS I/1,6-hexanedithiol/NP bioconjugate began with the formation of [4Fe-4S] clusters in solution by combining sodium sulfide, ferrous ammonium sulfate, and 2-mercaptoethanol. Apo-PsaC was reconstituted *in vitro* with these [4Fe-4S] clusters to yield holo-PsaC. PS I was then rebuilt by combining reconstituted PsaC and P700/F_X cores in the presence of PsaD. The 2-mercaptoethanol ligand to the F_B cluster was displaced and PS I was covalently linked to a Pt or Au nanoparticle by 1,6-hexanedithiol.

Due to its relatively short length (~ 1.2 nm), 1,6-hexanedithiol was selected for the initial studies. According to Marcus theory, it should allow electron transfer to occur from PS I to the NP before the charge recombination between P_{700}^{+} and F_B^{-} occurs. H_2 analysis by gas chromatography (Figure 3) shows that only illuminated PS I coupled to Au-NP and Pt-NP were able to produce H_2. PS I/Au NP bioconjugates produced 3.4 µmol H_2 h^{-1} mg Chl^{-1} while PS I/Pt NP bioconjugates were able to produce 9.6 µmol H_2 h^{-1} mg Chl^{-1}. No H_2 was detected for any of the controls that lacked one or more of the components. These results prove that the electron is being transferred to the NPs only when the rebuilt PS I is covalently attached to the particle surface by the dithiolate linker, and that light produces the charge-separated state in PS I necessary for H_2 evolution. This intermediary step allows for the photocatalytic production of H_2 by a rebuilt PS I that utilizes a bivalent tether molecule. This proof-of-concept experiment experiment therefore shows that an approach, which links PS I to a site-engineered Fe-Fe

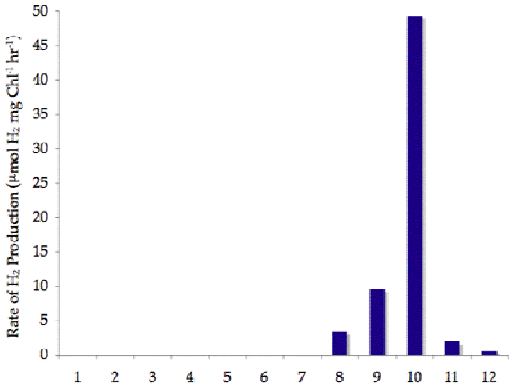

Figure 3. Rates of H_2 production from PS I-Pt NPs. The tether is 1,6-hexanedithiol, and unless otherwise noted, samples were illuminated. No H_2 production was observed for Pt NP (1), WT PS I (2), Pt NP with WT PS I (3), Pt NP with WT PS I and tether (4), Pt NP with F_X cores, C_{13}→G/C_{33}→S variant PsaC, and tether (5), Pt NP with rebuilt PS I (6), and Pt NP with rebuilt PS I, tether, and without light (7). H_2 evolution was observed in Au NPs with rebuilt PS I and tether (8), Pt NPs with rebuilt PS I and tether (9), and Pt NPs with rebuilt PS I, tether, and Cyt c_6 (10). Rates of H_2 photocatalytic production for "Platinized PS I (11) and the PS I-PsaE/H_2ase fusion protein (12) from the literature are also plotted.

or Fe-Ni H_2ase for the efficient photoproduction of H_2, is realistic. **Publication: Grimme *et al.*, 2008.**

In more recent follow-up experiments, the pH optimum for H_2 evolution by PS I/Pt NP bioconjugates was found to be 8.0. By increasing the actinic light intensity, higher rates of H_2 evolution (~75 μmol H_2 mg Chl^{-1} h^{-1}) have been achieved with 1,6-hexanedithiol as linker. Alkane-dithiols with differing numbers of carbons have been tested. 1,3-propanedithiol gave the poorest rates of H_2 evolution (2 μmol H_2 mg Chl^{-1} h^{-1}), and increasing the number of carbons from 6 to 10 caused the rate of H_2 evolution to progressively decrease by 75%. Interestingly, the use of 1,4-benzenedithiol as the molecular wire produced the highest rates of H_2 evolution observed: 159 μmol H_2 h^{-1} mg Chl^{-1}. These data suggest that it will be possible to optimize the length as well as the chemical nature of the molecular wire to maximize the throughput of electrons to either the Pt nanoparticle or the hydrogenase enzyme.

Future Directions. Studies are currently underway: (1) To optimize the rate of H_2 evolution from the PS I/Pt-NP bioconjugate system. Current efforts are aimed at optimizing the rate of electron donation to PS I, and therefore to increase the number of electrons that are available for H_2 reduction at the NP surface. In related studies, we plan to make PS I/NP conjugates with metal catalysts caged in viral coat proteins in collaboration with the Douglas/Peters/Young labs at Montana State University. (2) To modify the small subunit of the [NiFe]-H_2ase of *Ralsonia eutropha.* (3) To modify the [FeFe]- H_2ase of *Clostridium acetobutylicum*. The modified H_2ases will be tested to determine whether these molecules can be linked through their distal [4Fe-4S] clusters to PS I via the molecular wire. A system for the heterologous expression and purification of the [FeFe]-H_2ases has been established, and characterization of the mutant proteins is in progress. Reconstitution studies of the small subunit of the [NiFe]-H_2ase are also underway. (4) To increase the optical cross section of PS I by replacing PS I with IsiA/PS I supercomplexes, which increase the antenna content of PS I by ~225 chlorophylls. (5) To construct G82C/D87C variants of Cyt c_6 from *Synechocystis* sp. PCC 6803. These changes lie on an α-helical portion of the protein that opposes the heme and are designed to orient the Cyt c_6 on the gold electrode for optimal electron transfer to PS I. Crosslinking of Cyt c_6 to PS I via a zero-length cross linker like EDC will provide an additional increase in electron transfer. And (6) To explore the use of the PS I/molecular wire system as a "light-activated, multi-electron delivery device" for the study of enzymes that carry out complex redox chemistry using electrons that typically are delivered by diffusion-limited processes.

Publications

1. Antonkine, M. L., Maes, E. M., Czernuszewicz, R. S., Breitenstein, C., Bill, E., Falzone, C. J., Balasubramanian, R., Lubner, C., Bryant, D. A., and Golbeck, J. H. (2007) Chemical rescue of a site-modified ligand to a [4Fe-4S] cluster in PsaC, a bacterial-like dicluster ferredoxin bound to Photosystem I. *Biochim Biophys Acta* 1267, 712-724.
2. Grimme, R., Lubner, C., Bryant, D. A. and Golbeck, J. H. (2008) Efficient photohydrogen production from Photosystem I and metal nanoparticles joined by a "molecular wire. *J. Am. Chem. Soc.*, submitted for publication.
3. Lubner, C., Grimme, R., Boekema, E., Fromme, P., Golbeck, J. H. (2008) Functional characterization of Photosystem I/IsiA supercomplexes from an *iscA* null mutant of *Synechococcus* sp. PCC 7002. Manuscript in preparation.