Communication

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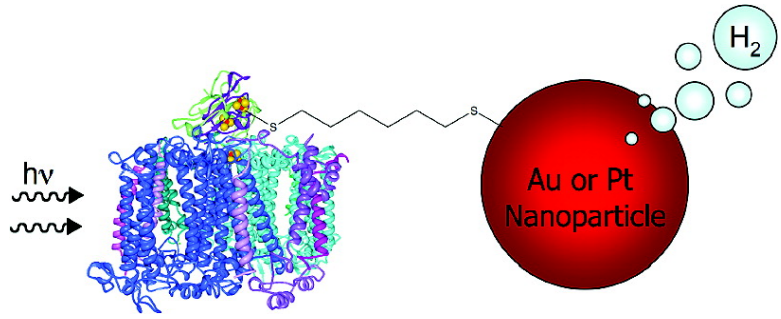

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Photosystem I/Molecular Wire/Metal Nanoparticle Bioconjugates for the Photocatalytic Production of H_2

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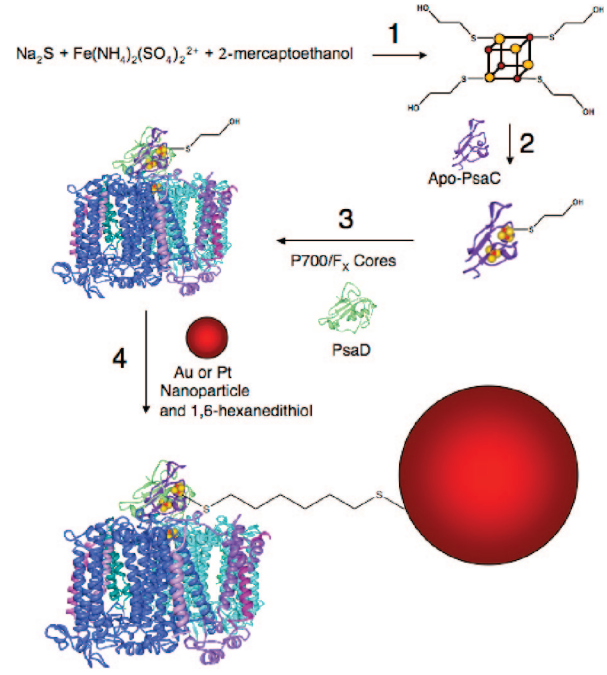
In recent years, gold and platinum nanoparticles have become attractive platforms for the photocatalytic production of hydrogen gas.[1,2] Photocatalysis of H_2 has been achieved by means of alcohol re-forming on the surface of Au and Pt nanoparticles supported on semiconductor materials such as titania.[3–6] These semiconductor materials supply an input of energy in the form of electrons. A major drawback to this type of H_2 photocatalysis is that the photons must have an energy greater than the band gap of the semiconductor material in order to produce a charge-separated state that is able to sustain the reaction: $2H^+ + 2e^- \rightarrow H_2$. For titania, the band gap is ~3.2 eV and corresponds to light with wavelengths shorter than ~350 nm.[7] On the basis of this requirement, only a very small fraction of incident solar radiation has sufficient energy to produce this state. The photosynthetic complex, Photosystem I (PS I), produces a light-induced, charge-separated state that could be used to generate reducing equivalents for H_2 production. The covalent attachment of PS I to Au and Pt nanoparticles provides an attractive alternative to the titania-supported particles for the photocatalytic production of H_2.

PS I has highly favorable properties that favor its use in such applications. The pigments that comprise the antenna complex of PS I absorb all wavelengths of visible light shorter than ~700 nm, which represents 43−46% of the total solar radiation that reaches the surface of the earth.[8] PS I has a quantum yield that approaches 1.0; hence nearly all of the photons that are absorbed are converted into the charge-separated state $P700^+-F_B^-$. This charge-separated state is stable for ~100 ms, and the low potential reductant that is produced is poised at a redox potential favorable for H_2 evolution. The challenge is to transfer the electron from PS I to the nanoparticle surface within this 100 ms time frame. In this communication, we describe the covalent linkage between PS I and the nanoparticle via a molecular wire which enables electron transfer and subsequent H_2 production.

A covalent link between the nanoparticle and the terminal electron transfer cofactor of PS I, F_B, can be fabricated. Recent work in our laboratory highlights the ability of the C13G/C33S variant of PsaC that lacks a native cysteine ligand at a solvent-exposed position on the F_B cluster to be chemically rescued by a thiolated organic molecule.[9] This PsaC variant has the unique ability to transfer electrons from the F_B cluster to a covalently bound external acceptor. The functionalization of Au and Pt surfaces by thiolated molecules is an extensively explored and well-documented field of study.[10–14] It was therefore considered feasible to link Au and Pt nanoparticles covalently to PS I via a bifunctional organic dithiol which serves as the molecular wire.

One functional group of the dithiol molecule modified the surface of the nanoparticle, while the other functional group served as the rescue ligand to the F_B cluster. Due to its relatively short length (~1.2 nm), which enables electron transfer from PS I to the nanoparticle before the otherwise inevitable charge recombination between $P700^+$ and F_B^- occurs, 1,6-hexanedithiol was chosen as a molecular wire for these experiments.

Scheme 1[a]



[a] Construction of the Photosystem I/1,6-hexanedithiol/nanoparticle bioconjugate began with the formation of [4Fe−4S] clusters in solution by combining sodium sulfide, ferrous ammonium sulfate, and 2-mercaptoethanol (1). Apo-C13G/C33S variant PsaC was reconstituted in vitro with these [4Fe−4S] clusters (2) to yield holo-C13G/C33S variant PsaC. PS I was rebuilt by combining reconstituted PsaC and P700/F_X cores in the presence of PsaD (3). 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 (4).

For all experiments, nanoparticles were synthesized according to previously published methods. As determined by TEM analysis of 224 and 208 particles, respectively, these methods resulted in 12.37 ± 1.12 nm citrate stabilized Au nanoparticles[15] or 2.89 ± 0.41 nm mercaptosuccinic acid stabilized Pt nanoparticles.[16] The iron−sulfur clusters in the C13G/C33S variant of PsaC were reconstituted with ferrous ammonium sulfate, sodium sulfide, and 2-mercaptoethanol, which resulted in its rebinding to P700/F_X cores in the presence of PsaD (Scheme 1, 1−3).[17] The rebuilt PS I was then introduced into solutions containing either 12 nm Au nanoparticles or 3 nm Pt nanoparticles at a ratio of 1:1 PS I/nanoparticle (Scheme 1, 4). The final concentration of PS I was 5.0 μg/mL Chl *a* (5.8 nM PS I). 1,6-Hexanedithiol was added to a final concentra-

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Table 1. Rates of Hydrogen Production from PS I/Nanoparticle Bioconjugates As Analyzed by Gas Chromatography

sample	rate of H_2 production (μmol H_2 mg Chl^{-1} h^{-1})	rate of H_2 production (mol H_2 mol PS I^{-1} s^{-1})
Au nanoparticle, rebuilt PS I, 1,6-hexanedithiol	3.4	0.08
Pt nanoparticle, rebuilt PS I, 1,6-hexanedithiol	9.6	0.23
Pt nanoparticle, rebuilt PS I, 1.6-hexanedithiol, Cyt c_6	49.3	1.17

tion of 200 nM to displace the 2-mercaptoethanol ligand that had been retained at the open coordination site of the F_B cluster and to link PS I to the nanoparticle surface. Samples were treated in the dark for ~2 h with slight agitation, which allowed the PS I/nanoparticle bioconjugates to assemble. Dicholoro(phenol)indophenol (DCPIP), at 10 μM final concentration, which was reduced by the sacrificial donor sodium ascorbate, at a concentration of 100 mM, served as the electron donor to $P700^+$. To evaluate whether the reaction was donor-side limited, cytochrome c_6 (Cyt c_6) was added to a PS I/molecular wire/Pt nanoparticle bioconjugate sample due to the fact that Cyt c_6 is a much faster donor to $P700^+$ than is reduced DCPIP.

The PS I/nanoparticle bioconjugates were added to closed vessels with a path length of 2.0 cm that had been purged with N_2 gas, and the samples were illuminated continuously with saturating white light from a Xe arc lamp at an intensity of 2500 μE for 12−16 h. Samples were taken from the headspace gas every 4 h and were analyzed for H_2 by gas chromatography (see Supporting Information for linear plots of H_2 production). Both PS I/molecular wire/Au nanoparticle and PS I/molecular wire/Pt nanoparticle bioconjugates were able to produce H_2 upon illumination (for rates, see Table 1). PS I/molecular wire/Au nanoparticle bioconjugates generated 3.4 μmol H_2 mg Chl^{-1} h^{-1}, while PS I/molecular wire/Pt nanoparticle bioconjugates generated 9.6 μmol H_2 mg Chl^{-1} h^{-1}. Addition of 5 μM Cyt c_6 increased the rate of H_2 production by the Pt nanoparticles to 49.3 μmol H_2 mg Chl^{-1} h^{-1}, which indicates that the H_2 evolution is not limited by electron transfer through the molecular wire, but rather by the donor-side reduction of $P700^+$. Appropriate controls were carried out to verify that all components were required for H_2 evolution (for details, see Supporting Information).

No H_2 production was observed for any of the controls that lacked one or more of the components or when wild-type PS I was used in lieu of the rebuilt PS I. These results indicate that light produces the charge-separated state necessary for H_2 evolution. It also demonstrates that the electron can be transferred to the nanoparticle surface only when the reconstituted PS I is covalently attached to the particle surface by 1,6-hexanedithiol. The bifunctional dithiol molecular wire both rescues the iron−sulfur cluster, F_B, and links the protein to the Au or Pt nanoparticle surface, thereby poising PS I at a distance capable of electron transfer at rates faster than the charge recombination time between $P700^+$ and F_B^-.

Photosystem I has previously been utilized in studies that explore its viability in the photocatalytic production of H_2. Low rates of H_2 evolution have been achieved by the platinization of spinach chloroplasts and PS I.[18−21] In those studies, the rate of H_2 evolution was on the order of 0.2 μmol H_2 mg Chl^{-1} h^{-1}, although a rate of 2.0 μmol H_2 mg Chl^{-1} h^{-1} was achieved in one instance.[19−21] Those studies do not address the method by which the Pt is connected to PS I. Direct covalent attachment of PS I to the Pt via the F_B cluster was not possible in those experiments. In addition, a recent study has appeared in which a PS I−H_2ase construct was engineered by fusing the gene encoding the [NiFe]−H_2ase from *Ralstonia eutropha* H16 with the *psaE* gene (which encodes PsaE, a stromal protein of PS I). Subsequent rebinding of the H_2ase−PsaE fusion product onto a PsaE deletion mutant of PS I produced only 0.2 μmol H_2 mg Chl^{-1} h^{-1}.[22] Clearly, the ~100-fold difference in H_2 production rates between our PS I/molecular wire/nanoparticle bioconjugates and these of previous studies demonstrates that these alternative approaches are suboptimal for the construction of a H_2-producing PS I adduct. Hence, we conclude that we have developed an improved approach by introducing covalent bonds that direct reducing electrons from the electron transfer cofactor, F_B, to the catalytic nanoparticle surface, which enables the observed high rates of H_2 evolution.

The strategy for the attachment of PS I to a nanoparticle surface via a covalent linkage between an electron transfer cofactor and a molecular wire presented in this communication represents an innovative approach for the binding of proteins to surfaces or other proteins. We therefore consider it feasible to use this approach to link PS I to other external catalysts capable of rapid H_2 generation, such as [FeFe]−H_2ase, [NiFe]−H_2ase, or N_2 reduction.

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Supporting Information Available: Experimental and analysis details. This material is available free of charge via the Internet at http://pubs.acs.org.

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