Biomimetic Synthesis of a H₂ Catalyst Using a Protein Cage Architecture

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ABSTRACT

A biomimetic approach has been used to develop an artificial hydrogenase that catalyses the efficient reduction of protons producing hydrogen gas. Analogous to the unique biological metal clusters found in hydrogenase enzymes, the engineered active sites are small, well-defined Pt clusters deposited on the interior of a heat shock protein cage architecture with stoichiometries of 150 to 1000 Pt per protein cage. The proton reduction reaction is driven by visible light through a coupled reaction with Ru(bpy)$_3^{2+}$ and methyl viologen as an electron-transfer mediator. Hydrogen production rates are comparable to those of hydrogenase on a per protein basis and exceed production rates of other reported Pt-based catalysts. These results demonstrate the utility of a biomimetic approach toward addressing the needs of hydrogen production.

There is significant interest in the use of hydrogen gas as an alternative fuel. The practicality of the increased use of hydrogen fuel cell technologies is dependent on the ability to produce stores of hydrogen gas in an efficient, economically feasible, and environmentally sound manner. The majority of hydrogen produced for energy yielding applications is generated by the process of reforming methane or fossil fuels, and thus a hydrogen energy economy based on these approaches does little to reduce the dependence on nonrenewable fossil fuels. Therefore, the development of catalysts for the production of hydrogen gas efficiently from a renewable source is of paramount importance.

Biological production of hydrogen is likely to play a key role in the emerging hydrogen economy. In particular, a group of enzymes, hydrogenases (H₂ases), have attracted a great deal of attention because of their ability to efficiently catalyze the reversible reduction of protons to form H₂. While rates of up to 9000 H₂ per enzyme per second have been observed from these enzymes, the extreme sensitivity of these enzymes to oxygen, limited expression, and difficult isolation have hindered their use as a practical means of hydrogen production.

Hydrogenase enzymes are produced by a variety of microorganisms where they function in either hydrogen oxidation or proton reduction. Hydrogen oxidation is coupled to the generation of reducing equivalents to drive energy yielding or biosynthetic processes. During anaerobic fermentation, some microorganisms are capable of coupling the oxidization of sugars to the regeneration of electron carriers, which are used for proton reduction and the production of hydrogen gas. Hydrogenases, like enzymes in general, are assembled to display precise organizational motifs that use their protein architecture to position and chemically poise an active site in which pathways for substrate access and product removal are key “design” features. Substrate, reductant, and product must have access to and from the catalytic site. Furthermore, continuous cycling of the catalyst requires ongoing addition of reactants and removal of products. The catalytic site of hydrogenase enzymes consists of unique biological metal clusters (Fe or NiFe) with carbon monoxide and cyanide ligands.

A major goal of biomimetic chemistry is to couple enzyme design principles with synthetic capacity to create new functional materials showing dramatic property enhancements. Artificial enzyme systems can combine the advantages of biology with the directed synthesis capacity of chemistry. Through this approach, hard inorganic materials (metals) can be interfaced with soft biological materials (proteins) to create new composite functionalities.

With the high cost and limited supply of platinum, it is necessary to explore ways of maximizing the catalytic efficiency of Pt on a per atom basis in order to develop economically feasible catalysts. In a particle based approach toward developing a Pt catalyst, it is necessary to minimize the diameter of the particle and thus increase the surface area (i.e., the number of exposed Pt atoms per particle). A number of different synthetic approaches have been used to
synthesize platinum nanoparticles with different passivating layers. The passivating layer generally interferes with the exposed Pt atoms and reduces efficiency. In this study, we have employed a protein cage as a synthetic platform which, unlike a passivating layer, does not coat the entire surface of the nanoparticles but still isolates the particle in solution and prevents aggregation.

Protein cage architectures have been used as biotemplates to create interfaces between proteins and metals. Protein cage architectures are self-assembled from a limited number of protein subunits to create well-defined, container-like morphologies in which the interior and exterior surfaces can be chemically distinct. In addition, molecular access to the interior can be controlled by pores at the subunit interfaces. We have previously shown that protein cage architectures can be utilized as size- and shape-constrained reaction environments for nanomaterials synthesis. These cages have been shown to stabilize inorganic nanoparticles in defined sizes and crystal forms. In addition, through genetic and chemical modifications, active sites can be created at precise locations within the cage architecture to create dramatically new functionality.

We have adopted a biomimetic approach to create a protein-based catalyst that functions as a hydrogenase mimic. The self-assembled cage-like architecture of the small heat shock protein (Hsp) from Methanococcus jannaschii has been used to encapsulate metal clusters with a defined spatial arrangement. Hsp assembles from 24 subunits into a 12 nm cage defining a 6.5 nm interior cavity with pores through the cage architecture, by which molecules can shuttle between the inside and outside environment (Figure 1). Cage-like architectures have previously been shown to act as a molecular container for the encapsulation of both organic and inorganic materials. By using the interior of the cage for spatially selective mineralization, the protein architecture of the Hsp mimics the controlled molecular access to the active site displayed in H2ase.

In the present work, we describe the synthesis of Pt nanoparticles encapsulated within the Hsp cage. Briefly outlined, purified Hsp was incubated with PtCl4− at 65 °C for 15 min. The protein cage was incubated with either 150, 250, or 1000 Pt per protein cage. Subsequent reduction with dimethylamine borane complex (CH3)2NBH3 resulted in the formation of a brown solution. Characterization of the reaction product by size exclusion chromatography revealed retention volumes identical with untreated Hsp and showed coelution of protein (280 nm) and Pt (350 nm) components (Figure 2). Dynamic light scattering indicated no change in the particle diameter after the reaction. Visualization of the Pt-treated Hsp (Pt−Hsp) by transmission electron microscopy (TEM) revealed electron dense cores identified as Pt metal by electron diffraction (Figure 3A inset) and intact 12 nm protein cages when negatively stained (Figure 3B). In the stained samples, the Pt particles can clearly be seen above the background stain and are localized within the cage structure. For average loadings of 1000 Pt/cage, metal particles of 2.2 ± 0.7 nm were observed (Figure 3C). At theoretical loadings of 250 Pt/cage, particles of 1 ± 0.2 nm were observed (Figure 4), while at loadings of 150 Pt/cage, no particles could be distinguished due to the limitation of the electron microscope. Hsp-free control reactions resulted in the formation of aggregated Pt colloids, which rapidly precipitated from solution. Control reactions using bovine serum albumin (BSA), at the same total protein concentration, also resulted in bulk precipitation and only a fraction of the Pt remained in solution with a wide distribution of particle sizes (3−120 nm) when observed by TEM (Supporting Information).

The Pt−Hsp protein cage composites are highly active artificial catalysts able to reduce H+ to form H2 at rates...
reported literature values (20 H₂/Pt/min,19 16 H₂/Pt/min,25 and 6.5 H₂/Pt/min27), where comparisons are possible. In addition, initial H₂ production rates for Pt–Hsp are approximately 20-fold greater than those obtained for the Pt particles produced in protein-free control reactions. The long-term stability of the coupled photochemical reaction to produce H₂ has not been optimized, and a significant slowing down of the reaction is observed after the first 20 min (Figure 6). The H₂ production decay is mainly due to the degradation of the photocatalyst (Ru(bpy)₃²⁺, Supporting Information), and the electron mediator (MV²⁺), which is subject to Pt-catalyzed hydrogenation.25

Unlike the hydrogenase enzymes, the artificial Pt–Hsp systems are not sensitive to O₂ and show no significant inhibition of H₂ production by CO but are poisoned by thiols. The Pt–Hsp catalyzed reaction was driven by the presence of the reduced viologen (MV⁺). The MV⁺ could be generated either by the photoreduction described above or by using the Jones reductor38 (Zn amalgam), which yielded rates for H₂ production approximately 40% slower than the coupled photocatalytic processes. Also, the Pt–Hsp is able to catalyze the reverse reaction (H₂ → 2H⁺ + 2e⁻) as monitored by the in situ reduction and bleaching of methylene blue.39 Importantly for the utility of this artificial system, the Pt–Hsp construct is remarkably stable and can be heated to 85 °C without precipitation of the composite or loss of the catalytic activity.

We have used a well-defined thermally stable protein cage architecture to generate an artificial hydrogenase having many of the features common to those biological catalysts. We have introduced small metal clusters in a spatially selective manner to the interior of the cage-like structure of Hsp that act as active sites for the reduction of H₂. The specific activities of these artificial enzymes are comparable to known hydrogenase enzymes and significantly better than previously described Pt nanoparticles. The protein cage architecture of Hsp acts to maintain the integrity of the small clusters, preventing agglomeration, and controlling access to these “active sites”. The Pt–Hsp composite is stable up to 85 °C illustrating the utility of using protein architectures for the design and implementation of functional nanomaterials.
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Supporting Information Available: Text of experimental details and figures of a TEM photomicrograph of BSA control with 1000 Pt per protein and UV-vis spectra of Ru(bpy)$_3^{2+}$ photodegradation. This material is available free of charge via the Internet at http://pubs.acs.org.

References

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