II.H.4 Use of Biological Materials and Biologically Inspired Materials for H2 Catalysis*

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*Congressionally directed project

Objectives

- Optimize the hydrogenase stability and electron transfer.
- Optimize the semiconductor nano-particle photocatalysis, oxygen scavenging, and electron transfer properties of protein nano-cages.
- Gel/matrix immobilization and composite formulation of nano-materials and hydrogenase.
- Device fabrication for H_2 production.

Technical Barriers

This project addresses the following technical barriers from the Hydrogen Production section of the Hydrogen, Fuel Cells and Infrastructure Technologies Program Multi-Year Research, Development and Demonstration Plan:

(AL) Light Utilization Efficiency
(AM) Rate of Hydrogen Production
(AN) Hydrogen Re-oxidation
(AP) Systems Engineering

Technical Targets

Protein-Based Hydrogen Production Materials/Devices:

This project is conducting fundamental studies of hydrogenase enzymes and biomimetic nanoparticle mimics and their incorporation into durable materials. Insights gained from these studies will be applied toward the design and synthesis of hydrogen production materials/devices. Established a set of metrics for competitive evaluation including:

- Shelf life of the H_2 catalyst
- Longevity of catalyst under sustained H_2 production
- Thermal stability

Accomplishments

- Demonstrated that hydrogenase enzymes can be encapsulated within silica gel matrices for enhanced ease of fabrication, without loss of activity, and with some stabilization towards degredation.
- Discovered that hydrogen production can be achieved with synthetic noble metal-protein composite nanoparticles with large reactive surface area. Preliminary (un-optimized) hydrogen production rates are competitive with enzyme catalyzed (hydrogenase) reactions.
- Demonstrated thermal stability of up to 90ºC with H_2 production.
- Demonstrated sustained H_2 production for up to 2 hrs (synthetic catalyst) when coupled to light harvesting system which meets the 2013 target for continuous photoproduction.
- Demonstrated increased O_2 tolerance for hydrogenase enzymes that meet the 2013 target (10 min).

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Introduction

There is significant interest in the use of hydrogen gas as an alternative fuel for the rapidly evolving hydrogen fuel cell technology. 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 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 (H_2ases), have attracted a great deal of attention because of their ability to efficiently catalyze the reversible reduction of protons to form H_2. The catalytic rates observed for these enzymes are far superior to existing hydrogen production catalysts. The catalytic site of hydrogenase enzymes consists of unique biological metal clusters (Fe or NiFe) stabilized by carbon monoxide and cyanide ligands.

Approach

We have adopted a two-pronged approach: a) the development biomimetic catalyst systems combining reduced metal nanoparticles (Pd, Pt, Fe, Co, and Ni) within engineered protein architectures to mimic the activity of hydrogenase enzymes, and b) the use of hydrogenase enzymes themselves incorporated into stabilizing polymer matrices for efficient H_2 production. We are developing composite materials that consist of hydrogen-generating catalytic materials (either hydrogen enzymes or Pd, Pt, Fe, Co, Ni or mixed metal mimics) coupled to photocatalytic materials (light energy) and hydrogen storage materials such as Pd-containing nanoparticles. These composite materials could represent a practical sustainable means of generating hydrogen gas from fully renewable sources. Our most recent efforts have focused on the characterization of hydrogenase stability and the further stabilization of hydrogenase enzymes to promote increased durability for their use in materials and hydrogen production devices. In addition, using biomimetic approaches enzyme mimics have been synthesized using Pt and Pd that mimic the catalytic properties of the enzyme but can be mass produced more readily and have desirable features in the context of durability and oxygen tolerance.

Results

During the first quarter of the project we have focused on the orientation of personnel into the hydrogenase-based materials and the biomimetic materials aspects of the projects and have coordinated the integration of the activities proposed in the project. We are working from a firm platform of the proof-of-concept work that has been demonstrated by the project lead investigators (John Peters and Trevor Douglas) and others including: 1) stable hydrogenases have relatively long shelf lives, 2) hydrogenases can be incorporated in solid gel materials and retain activity, 3) hydrogenase activity can be controlled on conducting surfaces, 4) hydrogenase mimics with high specific activities can be synthesized using biological materials, and 5) hydogenase mimics can be physical linked to photocatalysts to effect light dependent hydrogen production from organic sacrificial electron donors.

In the first reporting period we have already made significant progress on the project and have focused on: a) optimizing the design of mimetic catalysts, and b) coupling hydrogenases and mimetic catalysts to conducting surfaces.

We have adopted a biomimetic synthetic approach to the formation of nanomaterials utilizing protein architectures as constrained reaction environments for fabrication of nanomaterials with controlled size, shape, and surface activity. We have used the structurally defined protein nano-cages (Hsp from *Methanococcus jannaschii* and horse spleen ferritin) to synthesize and encapsulate small metal clusters of Pt and Pd. These extremely small metal particles are protected by the protein cage without passivating the surface of the catalyst. This results in Pt nanoparticles that are more active than Pt particles previously reported in the literature and are comparable in activity to the most efficient hydrogenase enzymes. We have explored the proton reduction activity of these catalysts by coupling them to a range of reduction sources including photogenerated mediators (methyl viologen radical cation MV^+) as well as electrode surfaces (Figure 1). The longevity of the biomimetic catalyst immobilized on an electrode surface far exceeds the coupled photogenerated mediator system under H_2 producing conditions. One limitation on the longevity of the coupled photocatalyst system lies in the instability of the $Ru(bpy)_3^{2+}$ photocatalyst which degrades over the

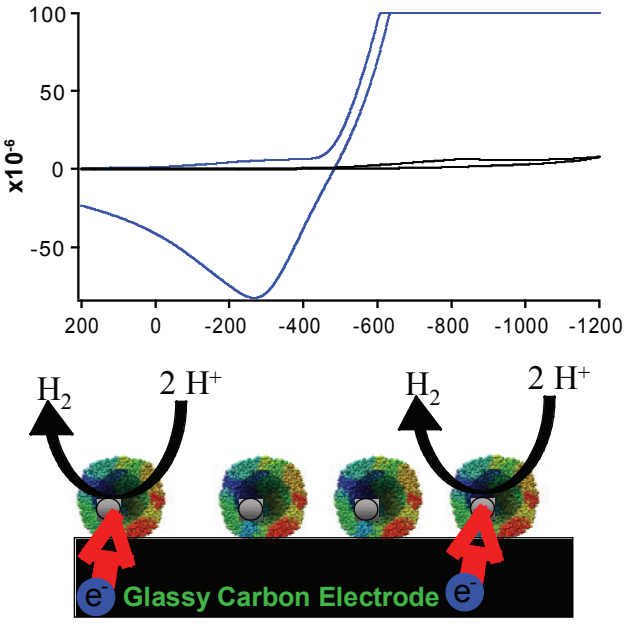

FIGURE 1. Cyclic Voltammetry of Hsp_Pt Nanoparticles showing a Catalytic Reduction Wave indicating H^+ Reduction to form H_2

course of an hour under illumination (Xe-arc lamp 175 W, edta as sacrificial reductant). The electrochemical system however, provides a much more stable reducing environment to evaluate the longevity of the protein encapsulated metal nanoparticles. We are currently investigating the activity and longevity of these biomimetic systems as a function of Pt particle size, protein cage composition, and chemical environment.

The NiFe hydrogenase enzyme from *Thiocapsa roseopersicina*, has also been adsorbed onto a glassy carbon electrode surface and the cyclic voltammetry shows a clear reduction of the catalytic metal cluster (Figure 2). We are currently optimizing the attachment of the hydrogenase to the electrode and exploring the attachment of the hydrogenase and mimics to the carbon electrodes. In addition, we are extending this work to the attachment of other conducting surfaces in advance of the fabrication of a hydrogen production device. We have begun to examine the stability of hydrogenases in solution and immobilized in electroactive gels. We have demonstrated that hydrogenases can be incorporated into electroactive gels such as methyl viologen doped slica gels and polyviologen gels. Hydrogenases encapsulated in both of these gel matrices retain activity and are protected from protease degradation of the enzyme illustrating the real potential for incorporating these biological catalysts into robust materials. In methyl viologen saturated silica gels (Figure 3), hydrogenases can exhibit sustained hydrogen production for more than sixty minutes and we are currently addressing hydrogenase specific activities over this time period to better address half life and efficiency. Thus far, the polyviologen gels examined only support hydrogen oxidation activity and thus we are examining mechanisms to produce polyviologen gels that operate at more negative oxidation-reduction potentials to promote increased driving force toward hydrogen production.

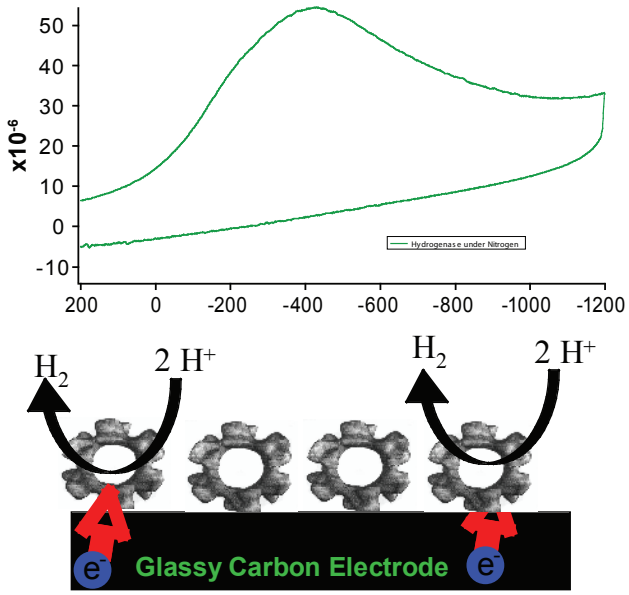

FIGURE 2. Cyclic Voltammetry of the Stable NiFe Hydrogenase from *Thiocapsa roseopersicina*

Conclusions and Future Directions

We have demonstrated that both the hydrogenase and synthetic mimics can be incorporated into polymeric materials and shown to be active with enhanced stability and durability. The future direction will include optimizing covalent attachment strategies for attachment of photocatalysts to hydrogenases (and mimics) as well as the covalent attachment of hydrogenases (and mimics) to conducting surfaces and their encapsulation into electroactive polymers. Second, we will expand the synthesis of our metal nano-clusters to incorporate other metals (Cu, Ni, Zn, Fe, Co) to investigate their effect on particle morphology, size, and activity. Since our ability to evaluate our materials in the context of DOE-defined target metrics is dependent largely on incorporating these materials into hydrogen production devices, we will focus on strengthening the engineering component of the project by recruiting an investigator with engineering experience to work on the project.

Special Recognitions & Awards/Patents Issued

1. Patent Application - PCT/US06/018,900.

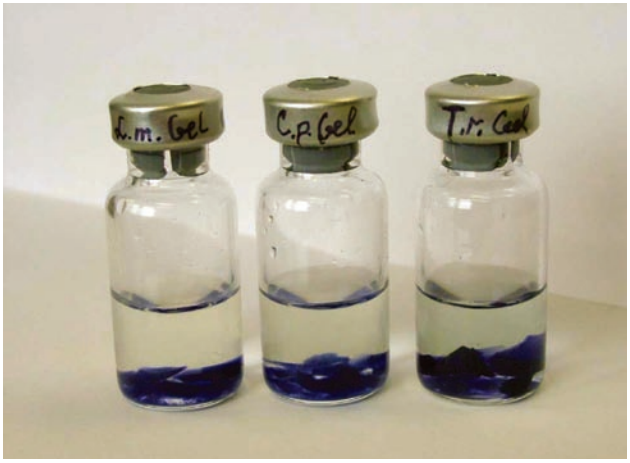

FIGURE 3. Hydrogen Production Assays Vials Containing Solid Slica Gel Encapsulated Hydrogenase Saturated With Methyl Viologen (Blue in Color in the Reduced State)

FY 2007 Publications/Presentations

John Peters

1. "H cluster biosynthesis and FeFe-hydrogenase maturation" European $BioH_2$ Workshop, Berlin, Harnack-Haus, Free University of Berlin, Berlin.

Pending Invitations

1. "Hydrogenase, Structure, Function, and Biosynthesis" *Invited Plenary Speaker* - 8th International Conference on Hydrogenase, Breckenridge, CO, 8/5/07-8/10/07.

2. "Hydrogenase, Structure, Function, and Biosynthesis" Conference on the Biogenesis in Iron Sulfur Proteins: Cluster assembly and Regulation, Villard-de-Lans, France, 6/9/07-6/12/07.

Trevor Douglas

1. "Using the interfaces in self-assembled protein cage architectures for materials synthesis", *Invited Speaker,* American Physical Society, Denver, March 2007.

2. "Self-assembled protein cage architectures for materials synthesis", *Invited Speaker,* American Chemical Society, Chicago, April 2007.

3. "Viruses – dynamic, responsive nanostructures with materials applications", *Invited Speaker,* Materials Research Society, San Francisco, April 2007.