II.F.3 Use of Biological Materials and Biologically Inspired Materials for H_2 Catalysis*

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*Congressionally directed project

Objectives

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

Photoelectrochemical Hydrogen Production

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 to probe specific metrics for competitive evaluation including:

- Shelf life of the hydrogen catalyst
- Longevity of catalyst under sustained hydrogen 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 degradation.
- Demonstrated that hydrogen production activity of encapsulated hydrogenases is sensitive to pH.
- Demonstrated that encapsulated hydrogenase materials can be reused and recycled.
- Demonstrated that electroactive gel matrices (polyviologen and viologen doped silica gels) can be manipulated to effect hydrogen production activity.
- 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.
- Discovered that hydrogen production can be achieved with pure noble metal and noble metal alloy composite nanoparticles.
- Demonstrated the synthesis of size constrained nanoparticles and demonstrated that nanoparticle size effect catalytic activity.
- 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).

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 H_2ases 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 H_2ases enzymes; and b) the use of H_2ases 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 H_2ases stability and the further stabilization of H_2ases 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

1. **Synthesis of protein encapsulated metallic nanoparticle catalysts.** We have characterized and defined reaction conditions yielding well defined catalytically active alloys of Pt, Pd, Zn, Ni using a library of protein cages as templates for the biomimetic synthesis of active H_2 generating catalysts. These protein-metal nanomaterials are composites in which the protein cage serves to direct the formation of the metal cluster and serves to isolate the active nanoparticle within an open framework. As part of an ongoing effort to move away from Pt as the catalytically active metal center, we have begun exploring alloying platinum with various other transition metals. We have continued to pursue our goals of a) developing H_2ases mimics that are catalytically active, b) immobilizing the active catalyst systems to electrode surfaces, and c) enhancing the direct electron transfer between mediators and active catalyst nanoparticle. We have built on our established synthesis for mineralizing Pt^0 in ferritin and heat shock protein, and determined synthetic conditions for mineralizing palladium as well as alloys of both Pd and Pt. We have successfully alloyed both Pd and Pt with zinc and nickel in ratios of 3Pt/Pd:1M (M = Ni, Zn). We've already known Pt to be a highly active catalyst for H_2 production, but we've also seen very high activities with the Pt/Pd-alloys as well.
2. **Synthesis of discrete Pt nanoclusters, direct determination of cluster size by noncovalent mass spectrometry, and activity-based size dependence.** We have genetically and chemically modified a small protein cage from Listeria innocua Dps (LiDps) for the controlled synthesis of platinum (Pt) nanoclusters, analyzed the Pt^0 nanocluster growth by non-covalent mass spectrometry, and verified core formation by using these nanoclusters as a hydrogen production catalyst. As shown in Figure 1, charge state distributions of both Pt^{2+} ion bound (black) and Pt^0 nanoclustered (red) LiDps were shifted to higher m/z compared to the untreated LiDps in accordance with the increasing numbers of Pt^{2+} loaded. Only one population was observed at each Pt^{2+} loading implying that binding or nanocluster formation events occur homogeneously throughout the cage population rather than in an all-or-nothing manner. We have previously demonstrated efficient hydrogen production using the Pt^0 mineralized Hsp cages using a coupled $Ru(bpy)_3^{2+}$/methyl viologen based photosystem. While the LiDps samples with theoretical loadings equal to or less than 45 Pt^0 (theoretical load of 100 Pt^{2+}/cage) produced hydrogen near at baseline levels, the 75 Pt^0 (theoretical load of 200 Pt^{2+}/cage) containing LiDps generated approximately four times more hydrogen than the background level.

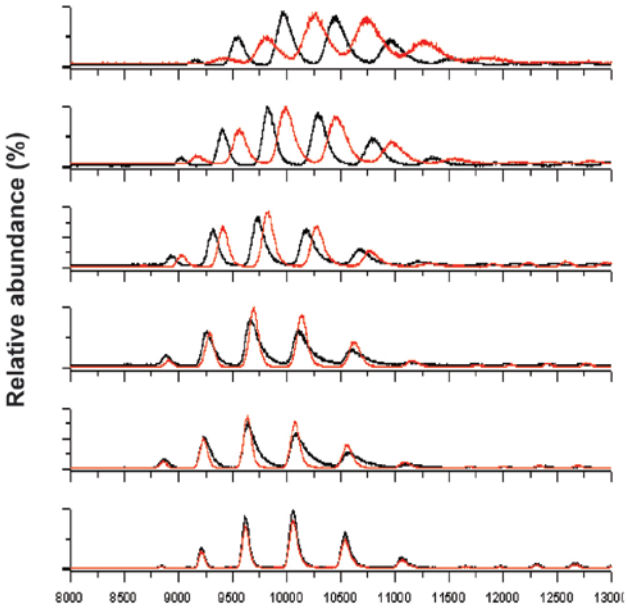

FIGURE 1. Overlaid mass spectra of the Pt^{2+} bound (black) and Pt^{0} mineralized (red) LiDps cages at various loading of Pt^{2+} (0, 12, 24, 48, 100, and 200 Pt/cage, bottom to top). 23+ charged peaks of the cages are indicated.

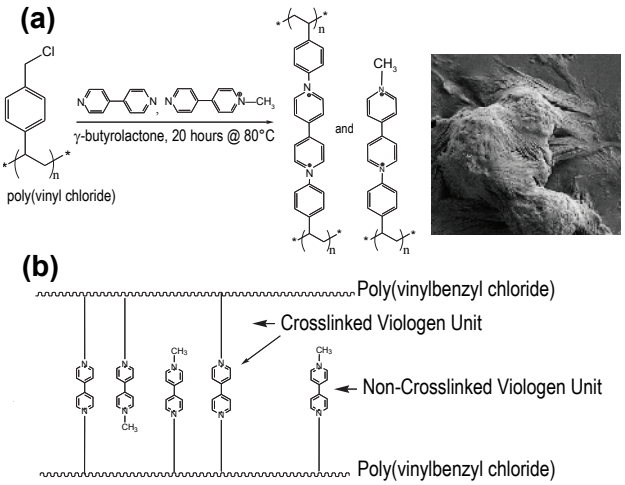

FIGURE 2. Chemical synthesis of a poly(viologen) matrix with controlled porosity. (a) Poly(vinylbenzyl)chloride is reacted with 4,4'-dipyridyl reagents to give (b) a partially crosslinked polymer matrix. A scanning electron microscopy image of the partially crosslinked poly(viologen) matrix is show (upper right).

This data clearly support the idea that the 75 Pt atoms form a single nanocluster rather than multiple nanoclusters in the LiDps. In contrast, the 45 Pt^{0} or lower Pt^{0} containing cages appear to fail to form a sufficiently large cluster to catalyze hydrogen production.

3. **Synthesis of electroactive crosslinked poly(viologen) gels.** Polyvinyl benzyl chloride, used as a back bone structure, has been crosslinked with 4,4' dipyridyl to form a porous gel structure that provides an electroactive matrix for catalyst immobilization. A key advancement is the observation that the density of the crosslinking of these gel structures can be modified by using a small percentage of viologens that are assymetrically methylated at one of the pyridine nitrogens (Figure 2). Inclusion of the methylated dipyridyl limits crosslinking and results in a gel structure that is more open and porous, but which is still cationic, facilitating the binding of the protein based catalyst systems. Varying the extent of methylation in the material will effectively modulate the consistency, pore size, and accessibility of the gel based redox mediators to immobilized catalysts.
4. **Covalent attachment.** To effectively perform protein-film voltammetry with our Fn-noble metal composite materials we have incorporated using a covalent linker between the working electrode and our protein cages. Doing this eliminates the possibility of desorption as well as buffer effects which may introduce artifacts into the voltammogram. To create this covalent bond we have incorporated the use of a carbodimide linkage with carboxylates on the surface of the electrode and terminal amines on the exterior of the protein, such as lysines. We can test the robustness of the linkage by monitoring the covalently attached protein on the electrode electrochemically.

   In order to directly attach redox mediators we have created a unique thiol mutant H14C in the subunit of the ferritin cage, which we predict from the atomic resolution structure, will be presented on the exterior of the assembled cage. The unique thiol allows us to attach a azide moiety to which we can selectively couple a viologen mediator. This will allow us to create a nanoparticle that incorporates an active Pt particle on the inside and a redox mediator on the exterior (coupled to the cage through a covalent linkage to the cysteine thiol). The mutant was generated using a polymerase chain reaction-based site directed mutation and the resulting construct sequenced to confirm the correct deoxyribonucleic acid sequence. Expression of the protein was confirmed by gel electrophoresis and mass spectrometry was used to confirm the subunit mass of the mutant. Our contention is that the hydrogen production efficiency of our system, while good, can be improved by enhancing the electron transfer between the exterior of the protein cage to the active noble metal nanoparticle *inside* the cage. An analogous strategy is also being implemented for H_2ases from various sources.
5. **Incorporation of mimetic and enzyme catalysts into poly(viologen) gels.** Binding of the mimetic and enzyme catalysts to the crosslinked

poly(viologen) gels has been achieved. Binding is pH dependent and the protein does not bind well below pH of 6.5. We have demonstrated qualitatively that the catalysts will communicate electronically with the gels by monitoring the color change associated with hydrogen oxidation coupled to the reduction of the gel matrix indicated by the appearance of blue-violet color of reduced viologens.

6. **Reusability of silica gel encapsulated H_2ases.** We have been examining running reactions with a defined amount of added reductant then harvesting the gas. These catalytic gels are stored and reductant is added later to examine the impact of use on durability. Thus far we can determine multiple additions of reductant are possible and that gels have the promise to be reusable and we are currently addressing in a defined manner the effect of multiple uses on shelf life. In addition, we can see that the accumulation of hydrogen limits the ability to produce hydrogen presumably due to product inhibition and we are currently examining mechanisms to remove hydrogen as it is produced and at the same time measure hydrogen production (Figure 3). This is a component of a second generation prototype device we are designing in which a hydrogen utilizing fuel cell is coupled to the hydrogen producing materials. In this manner hydrogen production can be easily and and reliably measured and we can rapidly address various factors to optimize these systems.
7. **The effect of the addition of carbon nanotubules on hydrogen production by Sol-Gel encapsulated H_2ases.** We are now working on coupling our silica gel encapsulated H_2ases to electrode surfaces thereby eliminating the need for mediators. Since, silica gels themselves are not conducting we incorporate carbon nanotubes to produce a conducting material. The methyl viologen soaked carbon nanotube doped gels produced hydrogen at pH 5.6 and pH 8 once dithionite was added. Methyl viologen soaked nanotube doped gels produced hydrogen at a much higher rate than the gels without carbon nanotubes. This is most likely due to the conductive behavior and increased surface area for electron transfer to the H_2ases. This is the important first step to producing a material that can be coupled directly to an electrode so that reducing equivalents can be provided electrochemically.

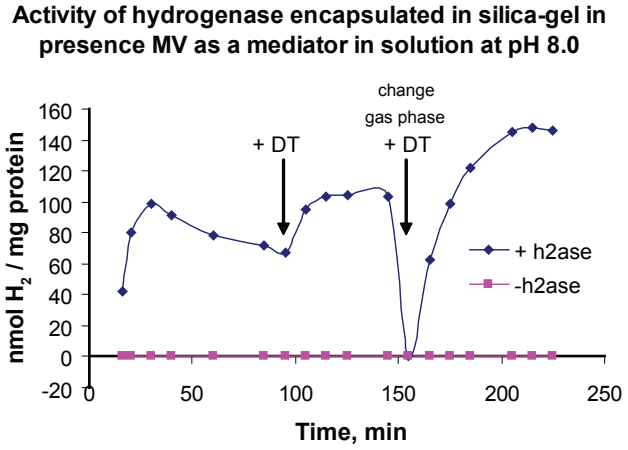

FIGURE 3. Hydrogen production from methyl viologen (MV) soaked sol-gels initiated by addition of only dithionite (DT).

Conclusions and Future Directions

The project has laid a firm groundwork for the use of H_2ases and synthetic mimetics in hydrogen producing materials. The project demonstrates that materials can be generated that have reasonable shelf lives and high catalytic capabilities. We have systematically developed strategies to adapt both H_2ases and synthetic mimetics for use in prototype hydrogen production devices. The salient advancements in the previous year of funding include:

- Major advancements were made in the area of synthesis of noble metal and noble metal alloy catalytic nanoparticles and the incorporation of nanoparticles and H_2ases into electroactive gels and onto surfaces.
- Tools have been developed to advance biomimetic/biohybrid device design and fabrication.
- Twenty two presentations and publications and one patent pending.
- The remainder of the project will focus on preparing manuscripts for publication and as future directions we will focus mainly on the direct attachment of catalysts and the attachment of gel encapsulated catalysts to electrode surfaces to directly assess the efficacy of our novel materials for use in devices.

Special Recognitions & Awards/Patents Issued

1. US patent application: PCT/US06/018,900.

FY 2008 Publications/Presentations

Presentations

John Peters

1. 8th International Conference on Hydrogenase, Breckenridge, CO, Aug. 2007.

2. Department of Chemistry & Astrobiology Program, University of Washington, Oct. 2007.

3. Laboratory for Basic Biological Problems, Pushchino, Russia, Oct. 8, 2007.

4. AFOSR BiosolarH2 Review Meeting, Princeton, NJ, Nov. 2007.

5. Gordon Conference on Protein Cofactors, Radicals and Quinones, Ventura, CA, Jan. 2008.

6. RCN/TBI Research Coordination Workshop, Yellowstone National Park, WY, Jan. 2008.

7. Department of Chemistry and Biochemistry, Arizona State University, Feb. 2008.

8. Department of Chemistry and Biochemistry, Cal. State University, Fullerton, March 2008.

9. Department of Chemistry, University of Montana, March 2008.

10. Department of Chemistry, Hamilton College, April 2008.

11. Astrobiology Science Conference, Santa Clara, CA, April 2008.

12. Gordon Conference on Iron-Sulfur Enzymes, New London, NH, June 2008.

13. Fourth International Symposium on Biorganometallic Chemistry, Missoula, MT, July 2008.

Trevor Douglas

1. Astrobiology Center Madrid, Spain, March 2008.

2. University Seville, Spain, March 2008.

3. University of Barcelona, Spain, 2008.

4. NanoSpain08 Conference, April 2008 (Braga Portugal).

5. University of Rome, April 2008.

6. Gordon Conference on Organic Structures & Properties, Il Ciocco, Lucca, Italy, April/May 2008.

Publications

1. Z. Varpness, C. Shoopman, J. Peters, M. Young, T. Douglas "H_2 catalysis by Pt nanoparticles in Ferritin" *ACS Symposium Series* (2008) 986, 263- 272.

2. M. Uchida, M.T. Klem, M Flenniken, M. Allen, Z. Varpness, E. Gillitzer, P. Suci, M. Young and T. Douglas "Biological Containers: Protein Cages as Multifunctional Nanoplatforms" *Advanced Materials* (2007) 19, 1025-1042.

3. S. Kang, J. Lucon, Z. B. Varpness, L. Liepold, M. Uchida, D. Willits, M. J. Young, and T. Douglas "Monitoring Biomimetic Platinum Nanocluster Formation using Non-covalent Mass Spectrometry and Cluster Dependent H_2 Production" *Angewandte Chemie* (2008) *submitted.*