II.C.8 Development of Water Splitting Catalysts Using a Novel Molecular Evolution Approach

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Objectives

- Develop a high-throughput ($>10^4$) system for the synthesis of potential metal-binding peptide catalysts of the water splitting reaction directly on an array of electrodes.
- Quantify the baseline catalysis rate of the system.
- Through iterative rounds of synthesis and analysis, improve the efficiency of catalysis (decrease the observed overpotential for the system) by 15% in each of three years.

Technical Barriers

This project addresses the following technical barriers from the Hydrogen Generation by Water Electrolysis section (3.1.4.2.2) of the Hydrogen, Fuel Cells and Infrastructure Technologies Program Multi-Year Research, Development and Demonstration Plan:

(G) Capital Costs

(H) System Efficiency

Technical Targets

Improved catalytic performance for electrolysis

This project has the goal of generating new, more efficient and more cost-effective catalysts for hydrogen production via electrolysis. Specifically, this work will be applied towards meeting the hydrogen production energy efficiency target of 69% by 2012.

Accomplishments

- Optimized the synthesis of peptides (potential water splitting catalysts) on porous methacrylate surfaces using light-directed synthetic methods, achieving densities as high as 800,000 peptides per square inch.
- Continued to optimize electrode surfaces with polyindole coatings for detailed testing of Mn-peptide catalysis.
- Performed testing of a set of Mn binding peptides showing measurable catalysis (an improvement in power consumption for a given current of about 10%).
- Designed additional peptides that bind multiple metals and established a collaborative effort to incorporate inorganic metal clusters into peptide environments.
- As part of a partnership with CombiMatrix, we have been able to synthesize peptides on electrode arrays with stepwise yields greater than 90%.

Introduction

Direct conversion of water to molecular hydrogen and oxygen via electrolysis followed by regeneration of electrical power in a hydrogen fuel cell would be, in principle, an ideal mechanism for the generation and utilization of hydrogen. However, a number of problems still remain to be solved. One of these stems from the fact that the conversion of water to hydrogen via electrolysis using conventional metal electrodes involves a substantial activation energy, necessitating that the reaction be driven by a considerably higher potential than simple thermodynamics would demand. This overpotential represents a significant energy loss during conversion, impacting the economic practicality of using hydrogen as a fuel in this way.

The biggest part of this overpotential comes from the water splitting reaction at the oxygen evolving electrode (the anode). This is because of the multi-electron nature of the reaction and the high-energy, partially oxidized intermediates that must be formed in order to generate molecular oxygen and protons from water. Fortunately, Nature has developed a catalyst, the oxygen evolving complex (OEC) of photosystem II (a complex found in the photosynthetic apparatus of plants), that works with almost no overpotential for this reaction. The OEC contains four manganese atoms that have a structure and chemical environment defined by the surrounding protein. The manganese cluster is directly involved in the redox process and stabilizes the highly reactive intermediates in the oxidation of water. In recent years, a considerable amount has been learned about the characteristics of this complex, including both the redox properties of the manganese atoms at various stages during the four-electron oxidation of water and the structure of the surrounding protein at moderate resolution.

Approach

A novel combinatorial biochemical approach is used to develop manganese binding peptides for modification of the surface of the electrolysis anode used during hydrogen production. The design of these peptides will include features of the OEC and of a model system developed at ASU in which bacterial reaction centers lacking the OEC have been modified to bind and oxidize manganese. The approach involves the light or electrochemically-mediated production of large libraries of manganese-binding peptides using a process similar to that employed in the photolithographic generation of deoxyribonucleic acid (DNA) chips (for example by Affymetrix or CombiMatrix) [1]. Each member of the library will be attached to a different microelectrode on a fabricated surface. The current/voltage characteristics of each electrode will be measured in series, looking for the peptide/Mn complexes that result in the lowest overpotential for water splitting. These peptide sequences will then be used as the initial guesses for a subsequent round of molecular evolution. Note that once the peptide-based catalysts are developed in this way, the same types of combinatorial approaches can be used to introduce nonnatural chemical features into the peptides, increasing their resistance to degradation by naturally occurring enzymes and other chemical processes. In principle, similar techniques could also be used to develop catalysts for the hydrogen evolving cathode (e.g., using hydrogenase as a model) or for the electrodes in hydrogen fuel cells.

Results

The overall goal of the project is to improve the efficiency of catalysts for water splitting. This year, we have focused on four tasks. These are 1) optimizing light-directed synthesis of peptides; 2) optimizing electrode surfaces for detailed analysis of target proteins; 3) developing electrochemically-directed synthesis of peptides; and 4) designing, analyzing and creating candidate metal-peptide complexes for optimization.

Optimizing light-directed synthesis of peptides. Last year, we developed the basic chemistry for light-directed peptide synthesis using a micromirror array projection system. This year, we have optimized the chemistry further and created a number of peptide arrays with densities as great at 800,000 peptides per square inch (we normally work with areas of about 0.25 square inches though we are trying to move to larger areas now). We decided not to go the next step and use this approach to synthesize peptides on arrays of electrodes at this time. As will be explained below, we have been able to synthesize peptides on arrays of electrodes using electrochemical methods, using instrumentation that facilitates both the synthesis and measurement of catalytic activity.

Optimizing electrode surfaces for detailed analysis of target proteins. Last year, we demonstrated that it is possible to modify commercial electrodes with a conducting polymer and perform accurate electrochemical measurements. However, in order to perform more accurate, comparable measurements of different candidate peptides, we have focused this year on fabrication of stable electrodes within a well defined area. The strategy for fabricating these electrodes is shown in Figure 1. The electrodes are rather large with gold surfaces, but only a very well defined region of the gold is exposed; the rest is covered with SU-8 (a photopolymerizable plastic). It has proven challenging to create sets of electrodes with truly comparable electrolysis activities, but we have been varying the fabrication, conditioning and analysis methods, resulting in considerable improvements. At this point, about half of the electrodes show current vs. voltage curves that can be normalized and compared. We will continue

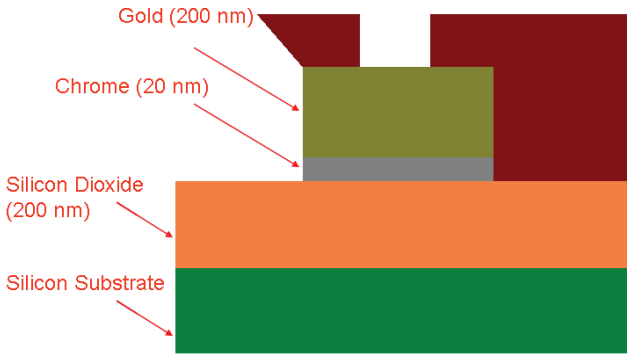

FIGURE 1. The fabrication scheme for the electrodes we are using to carefully characterize candidate peptides. The red region is SU-8, a photopolymerizable polymer.

to optimize the reproducibility of this approach as it is critical for the careful evaluation of the peptide candidates.

Developing electrochemically-directed synthesis of peptides. Last year, we initiated a collaboration with the company CombiMatrix to develop electrochemical synthesis of peptide libraries directly on an array of electrodes. CombiMatrix previously developed this technology for oligonucleotide synthesis on electrodes and we have adapted the process for peptide synthesis. The approach evolves around the localized electrochemical production of acid above the electrode, allowing the deprotection of the terminal amino acid. In this way, a peptide library can be built up layer by layer and the yield can be measured at each step. Currently, we have shown that we can create peptides up to 10 amino acids. We have been making poly glycine as a representative peptide. Though the yield estimates have an error of a few percent, it is clear we are getting more than 90% stepwise yield, with 94% being the estimate from multiple trials. An example is shown in Figure 2 of a series of polyglycine peptides up to 10 amino acids long, with each addition being generated using electrochemically directed synthesis. The peptides were patterned in the shape of numbers, such that 2 means a peptide two glycine residues long and 10 means ten glycine residues in length. At the end of the synthesis, each was terminated with a fluorophore. Note that between steps the chemistry was capped with acetic anhydride so that any amine that was simply left unreacted after coupling of the next amino acid was removed (not available for future steps including the reaction with the final dye). This allowed the determination of stepwise yield. Currently, we are making arrays of peptides that include a five amino acid epitope, YGGFL, for which there is a commercially available antibody. This is done as a check to make sure that we are not only making peptides, but actually making peptides with the correct sequence.

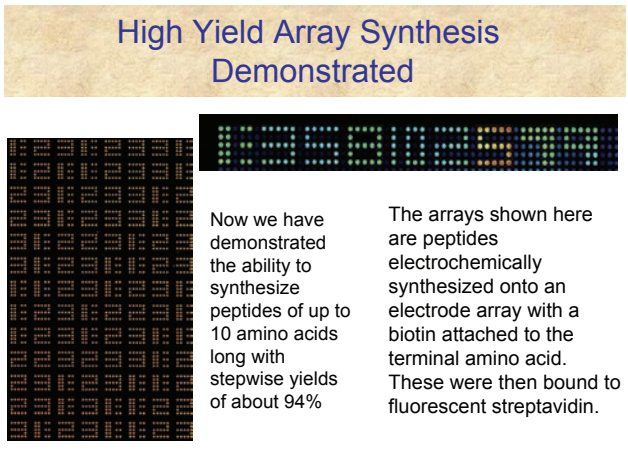

FIGURE 2. A series of peptides produced by electrochemically directed synthesis. The numbers represent the number of glycines in the peptide (it is polyglycine). The amount of fluorescence represents the number of peptides that made it to the terminal step.

Much of the time this year was spent on integrating the CombiMatrix electrosynthesis machine with a commercial peptide synthesizer. This has been a nontrivial task as it involves both a substantial amount of hardware modification and the writing of completely new software for controlling the interaction between the two instruments. The integration is complete for version one.

The other aspect of our interaction with CombiMatrix is in the area of electrochemical readout at each of the electrode positions. We originally purchased an instrument from CombiMatrix for performing this and spent considerable time working with it to measure the current/voltage relationships of the individual electrodes. However, we discovered that this instrument was really not adequate in the end for our electrochemical measurements as its behavior at bias voltages above a few tenths of a volt was not optimal and its ability to perform readout was compromised if we ran it with the polarity needed to measure oxygen evolution. Since that time, CombiMatrix has developed a new instrument with additional capabilities. We have been working with them recently to test that instrument for its ability to perform current voltage measurements over the voltage range of electrolysis. The results have been very promising. They have been able to take our peptide arrays and measure individual currents at a series of voltages with an on-chip reproducibility (once one accounts for a capacitive drift during the serial readout) of greater than 90%. We have now ordered the instrument and hope to receive it in the next two months.

The array synthesis and measurement aspects of the project have certainly taken longer to implement than desired. However, the ability to measure the electrochemical activity of 12,500 different potential electrocatalysts in one experiment is so powerful, both for this application and many others, that getting that capability completely worked out in a robust manner will facilitate the development of electrocatalysts not only for water splitting but hydrogen evolution, carbon dioxide reduction, and then all of the reverse reactions that are involved in fuel cell function.

Designing, analyzing and creating candidate metal-peptide complexes for optimization. During the past year, a number of different metal peptide designs have been individually synthesized and tested. The point of this is two-fold. First, we need to generate the peptide designs to use as starting points in the array-based optimization studies described above. Second, we need to run our electrochemical analysis approaches through their paces, finding out how large an effect one might see on current-voltage relationships just

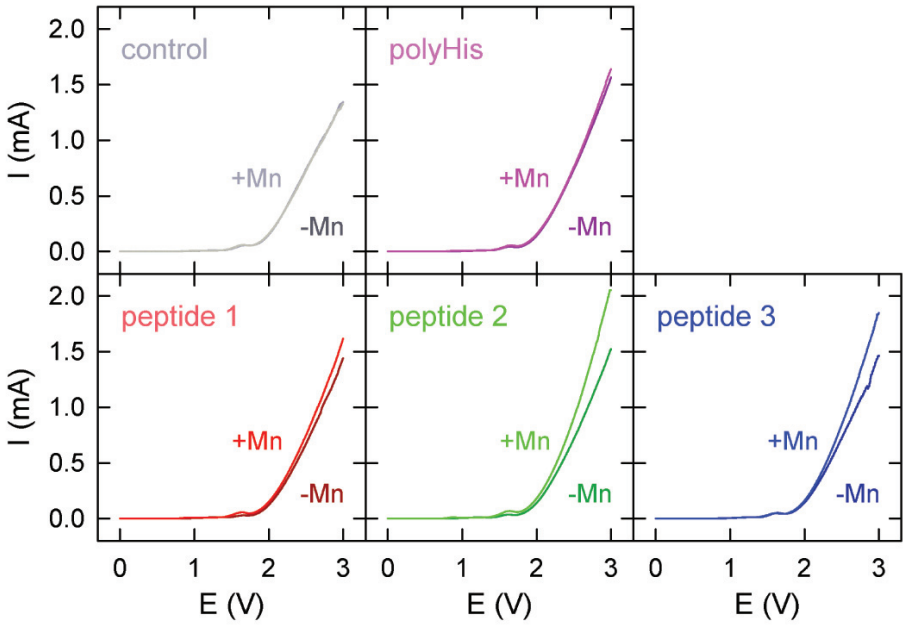

FIGURE 3. Characterization of catalytic activity. Three candidate peptides (1-3) and two controls (a peptide that cannot bind Mn and a polyHistidine peptide known to bind divalent cations but not expected to have catalytic activity) were tested with and without Mn for their activity in enhancing electrolysis. Peptides 1-3 show varying amounts of decreased voltage for a particular current in the presence of Mn, implying an improved ability to catalyze water splitting under these conditions. The control peptides show essentially no Mn effect.

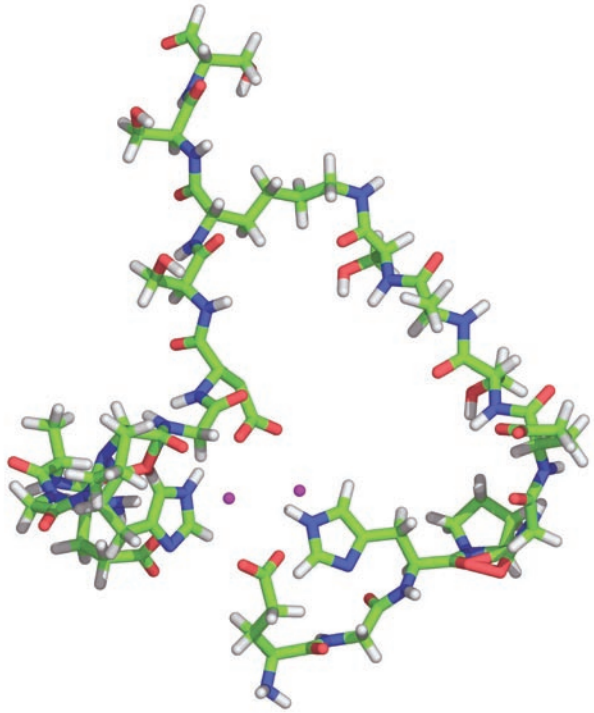

FIGURE 4. Creating systems with Mn clusters. We are taking two approaches to creating systems with multiple Mn clusters. The first is to design peptides with more than one metal binding site (A). In addition, we are collaborating with Prof. William Armstrong of Boston University to incorporate his synthetic Mn clusters into our peptide systems (B).

by attaching peptides to surfaces and then comparing activity with and without metals bound. Last year, we demonstrated that there was apparent catalytic activity of one such peptide. This year, we have expanded these measurements to several peptides. The current-voltage relationships with and without Mn bound are shown in Figure 3. There were two control peptides (one which should not bind Mn and one that may bind Mn but should not have activity) and three potentially catalytic peptides that were designed to bind a single Mn ion. The controls, as expected, showed no change in catalytic activity upon Mn binding. In contrast, the three designed metal-binding peptides each showed some activity, with peptide 2 giving the strongest results. This now provides us with at least two peptide sequences (2 and 3) to use in the optimization experiments. Obviously, one of the first things we need to do is to synthesize these sequences (control sequences and peptides 1-3) on our array system and determine whether we can detect the differences in electrocatalysis between these peptides on the array.

We have now designed and synthesized a series of peptides that should bind two Mn atoms. An example of a branched peptide designed to do this is shown in Figure 4. We have not yet tested these peptides for activity. Note that it should be straightforward to synthesize branched peptides like this on the CombiMatrix instrument and optimize their sequence.

It is almost certainly the case that it will be possible to much more adequately stabilize the intermediates in the water splitting reaction using multinuclear centers. These peptides will be tested in the coming months. Finally, we have developed a collaboration with Dr. William Armstrong at Boston University. His laboratory has synthesized a series of multi-Mn compounds that may mimic the water splitting complex in nature (Figure 4). We are designing peptide systems (mostly branched peptides) that will guide the assembly of multi-Mn structures and then stabilize them. This type of self-assembling multinuclear system should be easy to work with for commercial purposes. We hope to test the first of these later this year.

Conclusions and Future Directions

During the past year we have:

- Achieved light-directed peptide synthesis that is functional at >100,000 peptides/slide.
- Achieved electrochemically patterned synthesis using modified CombiMatrix instrumentation at >10,000 peptides per slide.
- Partially optimized electrodes for performing detailed catalytic testing.
- Demonstrated the ability to design multiple Mn-binding peptides that show apparent electrocatalytic activity relative to control peptides.
- Designed peptide systems to utilize multinuclear metal centers and established a collaboration with an inorganic synthesis lab for the integration of multinuclear complexes into our peptide systems.

Our goals for next year are:

- Test the synthetic accuracy of our electrochemically patterned synthesis.
- Work through the deprotection chemistry needed to create the peptides of interest (we are limited in terms of the deprotection conditions we can use, particularly in terms of strong acids, and we need to work out appropriate blocking groups for the critical amino acids).
- Integrate a new CombiMatrix measurement instrument into our system, allowing the measurement of current-voltage relationships under water splitting conditions for all 12,500 electrodes on the CombiMatrix chips.
- Perform an optimization of one or more of our Mn-binding peptides in terms of catalysis using the modified CombiMatrix instrumentation.
- Perform detailed catalytic measurements on the multinuclear Mn-binding systems we have designed.

Special Recognitions & Awards/Patents Issued

1. Aptamer-Enriched Oligonucleotide Libraries and Methods for Making; Matt Greving, Neal Woodbury; Tech ID#: M6-030, Provisional Filed: 3/21/2006, Serial Number 60/784,496.

2. Porous Acrylate Copolymer Films Optimized for in situ Synthesis and Analyte Detection; Matt Greving, Neal Woodbury; Tech ID# M7-054, Provisional Patent Filed: 01/23/07, Serial Number 60/897,222.

FY 2007 Publications/Presentations

1. T Northen, D Brune & N Woodbury (2006) Synthesis and Characterization of Peptide Grafted Porous Polymer Microstructures. *Biomacromolecules* 7: 750-754.

References Cited

1. Fodor, 1991 Science 251(4995):767.