II.F.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 production energy efficiency target of 75% by 2010.

Accomplishments

- Demonstrated the ability to synthesize peptides (potential water splitting catalysts) on porous methacrylate surfaces using light-directed synthetic methods at densities as high as 100,000 peptides per square inch and stepwise yields of 92%. In addition, the peptide density on the surface was high enough to perform mass spectroscopy and verify peptide sequence.
- Developed stable electrode surfaces with polyindole coatings (a conducting polymer) and demonstrated the ability to perform amide chemistry on these surfaces with roughly 50% stepwise yields (this is still being optimized).
- Designed a set of Mn binding peptides to use as starting guesses for catalyst development as well as nonbinding controls.
- Using the set of starting Mn binding peptides, showed that it was possible to a) attach the peptides to the electrode surfaces described above, b) measure baseline electrolysis current voltage curves and relate these to known electrochemical relationships, c) measure the binding of Mn to the peptides using infrared (IR) spectroscopy, and d) show that in the presence of Mn the electrolysis rate was faster than in its absence.
- Designed and fabricated test electrode arrays of 100 electrodes.
- Partnered with CombiMatrix to initiate the development of electrochemical peptide synthesis directly on large electrode arrays (12,000 to 90,000) that can be assayed in situ.

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 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, 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

Here we propose to use a novel combinatorial biochemical approach 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 Arizona State University (ASU) in which bacterial reaction centers lacking the OEC have been modified to bind and oxidize manganese. The approach involves the light-mediated production of large libraries of manganese-binding peptides using a process similar to that employed in the photolithographic generation of DNA chips (Affymetrix). 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, etc. Note that once the peptide-based catalysts are developed in this way, the same types of combinatorial approaches can be used to introduce non-natural 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 first year, we have focused on three tasks. These are: 1) optimizing light directed synthesis of peptides, 2) developing electrode surfaces and determining baseline electrochemical values, and 3) developing electrochemically directed synthesis.

Optimizing light directed synthesis of peptides. We have developed a test surface for optimization of peptide synthesis chemistry by using a porous polymer film. The advantage of this film is that enough peptide is made in a region so that it is possible to perform mass spectroscopy as well as simple staining tests (i.e., color tests for amines, etc.). This was essential for the improvements described next. We have succeeded in making much denser arrays of peptides than we had previously. Figure 1 shows both the process (lower panel) and the result of a synthesis (upper panel). We are using light directed synthesis, similar in concept to that described in reference 1. A micromirror array is used to remove a blocking group from the growing chains of peptides only in the regions where a specific amino acid is to be added. In this way it is possible to make essentially any sequence of amino acids on the surface in each position as defined by the light pattern. The upper portion of Figure 1 shows a small region of an array in which all possible combinations of 20 amino acids in two positions of a peptide have been synthesized. These peptides (12 amino acids) are known to bind the protein transferrin and fluorescently labeled transferrin was used to stain the array. Some substitutions resulted in increased binding strength, some decreased binding strength and some had little effect. This demonstrates the ability to optimize molecular recognition using this approach. We can make peptides with an element density of 115,000 spots per square inch, allowing the synthesis of about 30,000 different peptides in the 1 x 1.5 cm region of our patterned surface. We have also performed a quantitative determination of the stepwise yield of synthesis both using the light-directed and standard solid phase synthesis protocols. The per step yield of light-directed synthesis is 92%. The per step yield of standard solid phase synthesis is 99.6%. Thus, we are now able to make quite long peptides (20 amino acids is very reasonable) in which we replace up to about 10 of the amino acids using light-directed steps, performing the remaining steps by standard solid phase synthesis. This allows us to explore the function space of peptides with specific changes at the light directed positions. The ability to vary ten positions in the peptide should be adequate for the proposed work optimizing catalytic activity of metal binding peptides.

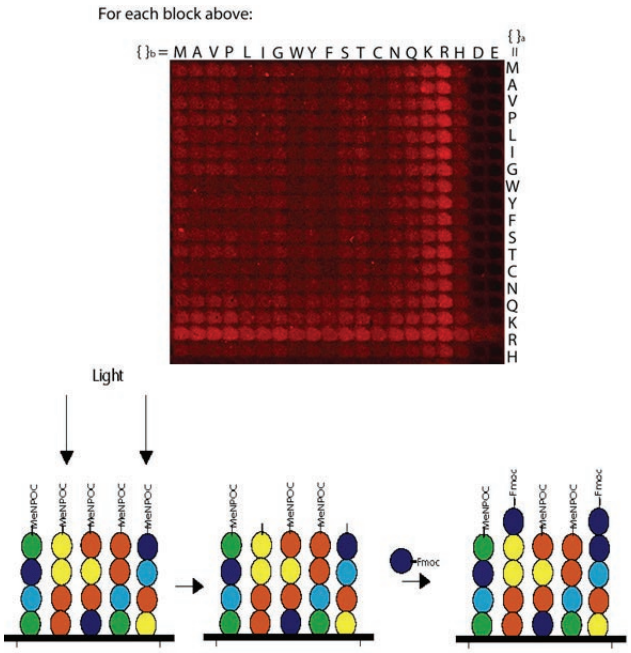

FIGURE 1. Light-Directed Peptide Synthesis. Top: A portion of a peptide array (12-mers) made using our light directed peptide synthesis. The array is 20 x 20 and represents all possible variations of natural amino acids at two positions in the peptide. The signal intensity is due to binding of fluorescently labeled transferrin (a protein). We started with a peptide that binds transferrin and used this approach to optimize the binding. This demonstrates the concept of optimizing peptide activity through high throughput synthesis in array formats of many specific variants. Bottom: A schematic of the optical synthesis procedure used to generate in situ peptides. The arrows show the patterned projection of light onto two elements of an array. This results in removal of a photosensitive blocking group. This results in free amines at the positions the light was applied. Subsequent addition of the next amino acid results in coupling only at those sites. In this way an array of peptides with any desired sequence at each position can be generated.

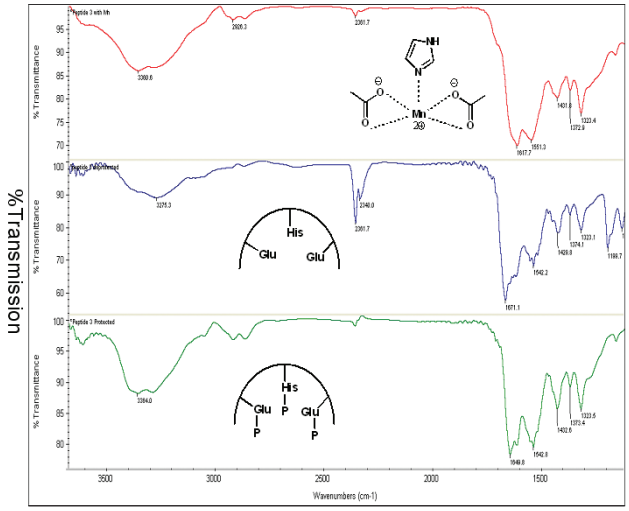

FIGURE 2. IR Spectra of the Bound Peptides. The spectral signatures of the peptide attached to the electrode change depending on whether it is protected (bottom), deprotected (middle) or has Mn bound (top). Of particular interest for this study is the fact that Mn^{2+} binding clearly changes the carbonyl stretch frequencies, as would be expected. This binding can be titrated and has a micromolar binding constant. The arrow shows the position of the carbonyl stretch frequency.

Developing electrode surfaces and determining baseline electrochemical values. We are developing an electrode surface chemistry appropriate for the in situ synthesis and electrochemical measurement of the catalysis of our metal binding peptides. Our current system involves starting with either a gold or platinum electrode and electropolymerizing polyindole on it. Polyindole is a conducting polymer and the monomers used in this polymerization are modified to contain a free amine group to use in attachment or in situ synthesis of peptides. Figure 2 shows IR spectra taken directly from the surface of a polyindole covered gold electrode to which a peptide has been attached. The spectral features are from both the indole film and from the peptide. The lower trace shows an indole surface with a protected peptide attached (side groups still protected after synthesis). The middle trace is the deprotected peptide. The top trace is the deprotected peptide plus Mn^{2+}. Note the substantial shift in the carbonyl stretches near 1650 wavenumbers when the Mn binds to the carboxylic acid groups of the peptide. We have been successful detecting Mn binding to peptides on electrodes both by using IR spectroscopy and by performing electrolysis measurements. In particular, one of our designed peptides looks like an excellent starting point for the optimization process (Figure 3). Figure 4 shows the catalytic behavior of this peptide with and without Mn (one would not expect catalytic activity without Mn). As can be seen, there is a small, but quite significant increase in the slope of the current vs. voltage curve in the presence of Mn that is absent when no Mn is bound. This suggests that the Mn-binding peptide under study should be an excellent starting point for the optimization of catalysis via high throughput synthesis on electrodes of variants and screening.

In parallel, we are designing and fabricating electrode arrays (the above work has thus far been done on single electrodes). The initial arrays with 100 electrodes (10 x 10) are shown in Figure 5. These are nearly ready for use. A problem we are trying to solve at this point is generating a stable electropolymerized indole surface on a small electrode. In its original form, the electropolymerized surface was not chemically attached to the gold electrode. During peptide synthesis, which involves extensive washing steps, the polyindole has a tendency to lift off the surface, particularly if the surface area is small. We are now redesigning the surface to include sites of covalent attachment that will integrate into the polyindole during electropolymerization.

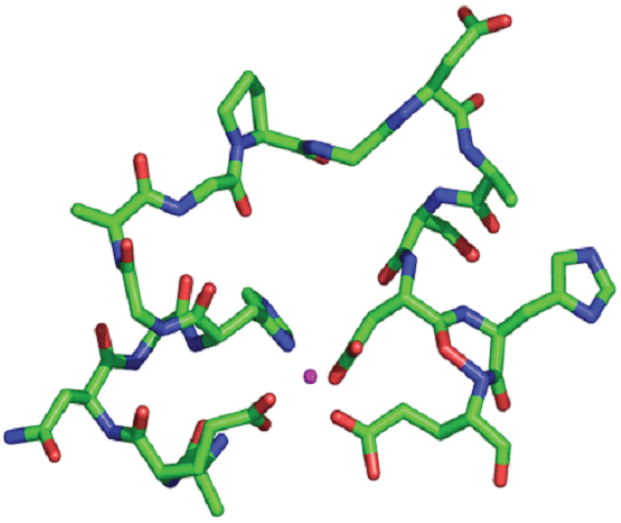

FIGURE 3. Peptide Design. This structure is one of the initial guesses for a Mn^{2+} binding peptide, based on a critical survey of Mn^{2+} binding in proteins and previous work engineering Mn^{2+} binding sites into proteins. This was the peptide used for the work in Figure 4.

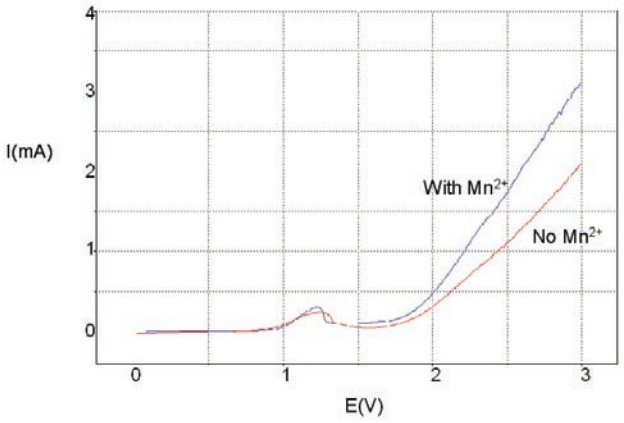

FIGURE 4. Current/Voltage Curve of Peptide Before and After Mn^{2+} Titration. Electrolysis curves for two electrodes" Lower (red) curve: the Mn-binding peptide without Mn, Upper (blue) curve: the Mn-binding peptide with Mn. The bottom axis is voltage in volts. The vertical axis is current. Not that in the presence of Mn^{2+}, the current above about 1.5 V is always somewhat higher than without Mn^{2+}.

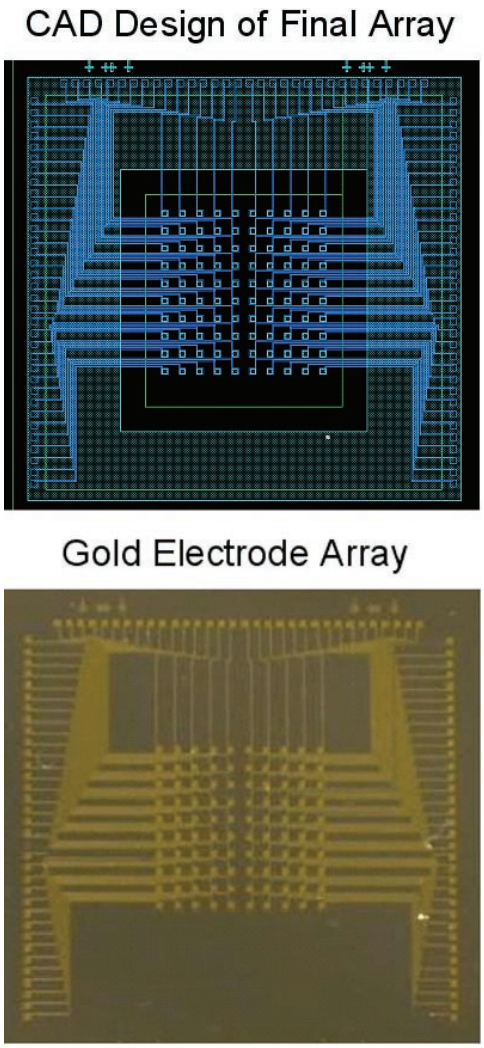

FIGURE 5. Fabricating Electrode Arrays. The top picture shows the electrode design. The bottom figure is an actual micrograph of the fabricated gold electrode array. It is set up as a 10 x 10 array with one lead for each electrode. For larger arrays, we will need to use an electronic addressing system.

Developing electrochemically-directed synthesis of peptides. We have initiated collaboration with the company CombiMatrix to develop electrochemical synthesis of peptide libraries directly on an array of electrodes. CombiMatrix has previously developed this technology for oligonucleotide synthesis on electrodes. The concept evolves around the localized electrochemical production of acid above the electrode, allowing the deprotection of the terminal nucleotide. We are working with them to modify the software and hardware (their contribution) and the chemistry (our contribution) to perform electrochemically directed amide synthesis with their system. We have been able to demonstrate electrochemically directed peptide bond formation, but there have been a number of software problems that have thus far kept us from creating more complex peptide arrays. Working with CombiMatrix, the software issues have apparently been identified and they are redesigning the software. We should be able to resume the synthesis aspect of this project in a few weeks when they return the instrument with the new software. They have also developed an electrochemical measurement system for addressing and performing current/voltage measurements at each electrode in the array. An example of attaching the enzyme horse radish peroxidase to a biotin coupled to specific electrodes and measuring the peroxidase activity electrochemically is

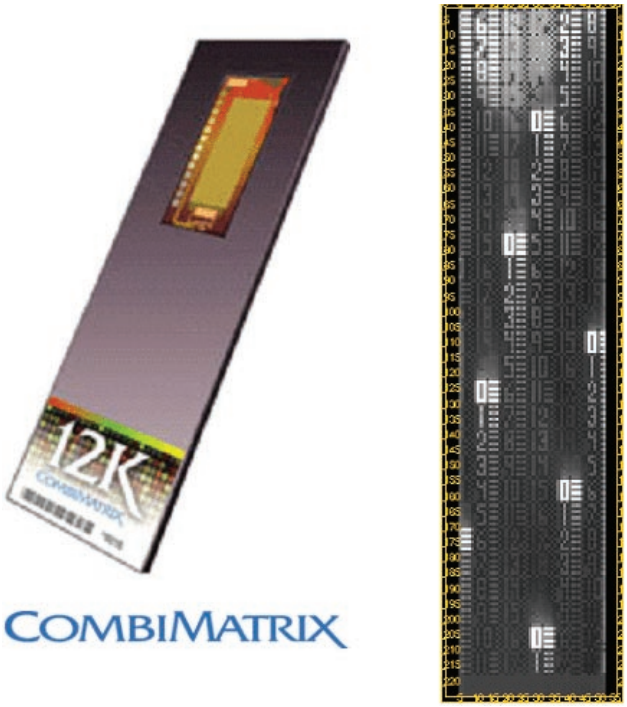

FIGURE 6. Electrochemical Synthesis. CombiMatrix has developed an electrochemical array technology for making nucleic acids. Their machines have been set up in our lab and we are converting them for peptide chemistry. The picture on the left shows one of these chips. The picture on the right is a reconstruction of the array from a series of electrochemical measurements at each electrode. In this case, the patterned attachment of biotin was performed, generating the numbers seen as bright regions on the array. A horse radish peroxidase – streptavidin fusion protein was then added and it bound only where the biotin was attached. The intensity at each point is proportional to the current measured upon application of a voltage due to peroxidase catalyzed oxidation. The different numbers are elements upon which different sized linkers were synthesized prior to biotin attachment. This was done to test the effects of distance on the electrochemical signal. Both synthesis and electrochemical measurements can be performed on each element in the array separately. This array has 12,500 elements. Currently, CombiMatrix is developing a chip with 90,000 elements for release soon.

shown in Figure 6. Clearly, the successful conversion of this system for large scale synthesis of peptides and peptide complexes would be a huge leap forward as they are already at the 12,500 electrode chip stage and will soon introduce chips with 90,000 electrodes.

Conclusions and Future Directions

During the past year we have:

- Achieved light directed peptide synthesis of >10,000 peptides per slide
- Initiated electrochemically directed synthesis in collaboration with CombiMatrix
- Fabricated electrode arrays for testing (up to 10 x 10)
- Electrode surfaces have been modified with polyindole + peptide
- Initial peptide guesses have been used to determine baseline catalytic currents at zero overpotential

Our goals for next year are:

- Light Directed Synthesis: improve yields, implement on electrodes
- Electrochemical Synthesis: optimize synthesis conditions
- Electrochemical Measurements: move to multielectrode systems
- Catalyst Optimization through variation and screening

Special Recognitions & Awards/Patents Issued

1. Direct Maldi-MS characterization of polymer arrays on high site density substrates; Trent Northen, Neal Woodbury; Provisional Filed: 6/15/2005.

2. Microarray of three-dimensional heteropolymer microstructures and method therefor; Trent Northen, Neal Woodbury; US Patent M4-067 Filed 5/4/2005.

3. Aptamer-Enriched Oligonucleotide Libraries and Methods for Making, Matt Greving, Neal Woodbury; Provisional Filed: 3/21/2006.

FY 2006 Publications/Presentations

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

2. T. Northen and N. Woodbury. (2005) Light-Directed Movement of Polymer Microstructures. *Langmuir 21*, 4949 - 4953.

References

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