II.B.3 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 (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 69% (lower heating value) by 2012.

Accomplishments for Fiscal Year 2008

- Developed a method for performing matrix-assisted laser desorption/ionization (MALDI) mass spectroscopy directly on the surfaces of our CombiMatrix electrode array to efficiently and reliably evaluate peptide attachment and proper deprotection.
- Developed a successful methodology for coupling peptides or segments of peptides directly to CombiMatrix electrodes.
- Optimized the yield of peptide synthesis, both electrochemically and using standard Fmoc synthesis, on CombiMatrix chips by implementation of appropriate protective groups and by developing other methods to reduce synthesis of side products. Currently stepwise yield is at 70% which is sufficient for initial peptide arrays.
- Demonstrated the ability with control patterns on CombiMatrix chips to perform current/voltage (I/V) measurements for different regions with high reproducibility (about 2%).

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

Here we 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 includes 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-mediated production of large libraries of manganese-binding peptides using a process similar to that employed in the photolithographic generation of deoxyribonucleic acid chips (for example by Affymetrix or CombiMatrix). 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 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 three tasks. These are: 1) coupling peptides or peptide segments to CombiMatrix electrodes, 2) optimizing synthesis of peptides on CombiMatrix chips, 3) optimizing measurement of electrocatalytic activity on CombiMatrix surfaces. As explained below, our original strategy was to develop the measurement system on individual gold electrodes and then transfer the system to the array platform that was being optimized concurrently. However, the progress on the CombiMatrix electrochemical chips outpaced the development of the test system on individual gold electrodes, so we are now focusing all of our effort on synthesizing peptides and making measurements on the CombiMatrix electrode arrays.

MALDI Mass Spectroscopy on Surfaces

We spent considerable effort developing and modifying an approach for performing matrix assisted laser desorption ionization mass spectroscopy directly from electrode surfaces so that we could determine exactly what peptides had been synthesized or attached to those surfaces. An example of this will be given below. This has been an essential tool in moving forward. Briefly, when we want to perform chemical product analysis on the surface, peptides are coupled or synthesized directly on top of photolabile linkers, previously coupled to the electrodes. After peptides are synthesized or coupled to this surface, the photolabile linkers are cleaved using ultraviolet (UV) light under dry conditions (to avoid diffusion), an appropriate matrix is added to the region of interest and MALDI mass spectroscopy is performed directly from the surface.

Initial Work with Individual Gold Electrodes

In order to characterize catalytic activity, we initially sought to both couple pre-synthesized peptides to individual gold electrodes via indole surfaces and to synthesize them in situ on these surfaces. At one point early on, we managed to fabricate a set of surfaces with our coupled peptides that showed that two of our rationally designed Mn-binding peptides showed significant enhancement of electrolysis. These results were reproducible on the original set of electrode/peptide systems. Unfortunately, it turned out that our fabrication methods were not robust. As we tried to expand this effort, making more surfaces and coupling peptides, we found that the indole attachment layers we had been using were less stable than desired and frequently being damaged during deprotection of the peptide. A number of different approaches were attempted to solve this problem, including thicker indole layers, different types of electrode construction (we were fabricating our own electrodes by lithography), going to different attachment schemes (thiol coupling to gold) and many variations on the chemical conditions. However, we were unable to reproducibly fabricate

stable surfaces that allowed us to observe the catalytic activity (or even verify the attachment and deprotection) of the Mn-binding peptides. While the early studies had shown us we were on the right track with the peptides, it was clear that our individual electrode system was inadequate. At the same time, however, we had been working on peptide synthesis and attachment to commercial CombiMatrix 12,500 electrode chips, which were ultimately the platform we intended to use for creating and searching catalyst libraries, and had made enough progress with that platform that we decided to focus our efforts directly on creating peptide arrays with the CombiMatrix system.

Coupling Peptides or Peptide Segments to CombiMatrix Electrodes

One aspect of the work with the CombiMatrix electrode arrays involved learning how to best couple peptides to surface in high yield. It was important to be able to couple peptides (or peptide fragments), as opposed to always performing in situ synthesis, for two reasons. First, this provides a useful means of rapidly placing controls at particular positions on the chip. In addition, this also provides a means of adding part of a peptide in a single step (it is often the case that we would like to add a piece of a peptide to the growing chain rather than one amino acid at a time). We developed and characterized an appropriate coupling chemistry and verified attachment of the peptide to the surface, however, our initial coupling yields were low. A typical reaction mixture contained 5 mg of a particular peptide, 1 equivalent of catalyst o-Benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU, which varies by peptide because of the different molecular masses of each peptide), and 100 uL of di-isopropylethylamine, dissolved in 1.5 mL of dimethyl formamide. Our efforts now focus on optimizing this. To increase coupling yields, we are now testing the use of dimethyl sulfoxide to enhance solubility of the peptide. We are also evaluating the effects of reducing the HBTU to 0.8 equivalents to reduce the chance of capping free amines on the surface and we are testing a variety of other solvents such as dichloromethane and acetonitrile, again to enhance peptide solubility. Adding more peptide to the reaction mixture might also increase the coupling yield, though the amount added is somewhat limited by the amounts of peptides produced and by peptide solubility in different solvents. We are testing these conditions by first coupling a photolabile linker to the slide surface, and then coupling a peptide using a particular condition to the photolabile linker. This permits MALDI mass spectroscopy measurements to determine if the peptide is present on the surface after cleaving the photolabile linker with UV light.

Optimizing Electrosynthesis of Peptides on CombiMatrix Chips

A major goal of the project has been to optimize the in situ synthesis of test peptides on the CombiMatrix chip. We first integrated a rather old peptide synthesizer with electrosynthesis equipment provided by CombiMatrix, because that is what we had available that was programmable. We initially used (t)ert-(B)ut(o)xy(c)arbonyl (Boc) chemistry to grow our peptides on the electrodes. However, Boc deprotection requires a rather high acid concentration for complete deprotection, and detailed MALDI analysis revealed that we were having considerable difficulty achieving complete deprotection using our electrochemically patterned production of acid. We then investigated the electrochemical deprotection efficiency using trityl-protected amino acids which require much milder acidic conditions compared to the Boc protected amino acids. Because trityl-protected amino acids are not generally available commercially, we devised a method in which we added Fmoc-protected amino acids (the standard in normal solid phase synthesis) and then replaced the Fmoc group with a trityl group in situ on the chip before performing the electrochemically patterned deprotection. Thus far, we have been using up to 10 minutes of electrochemical deprotection time and the yield of the final product (in this case added two amino acids electrochemically to a peptide made by normal solid phase Fmoc synthesis over the whole chip) is about 50%. This corresponds to a stepwise yield of 70% (Figure 1). This is high enough for our initial array production, which is now ongoing.

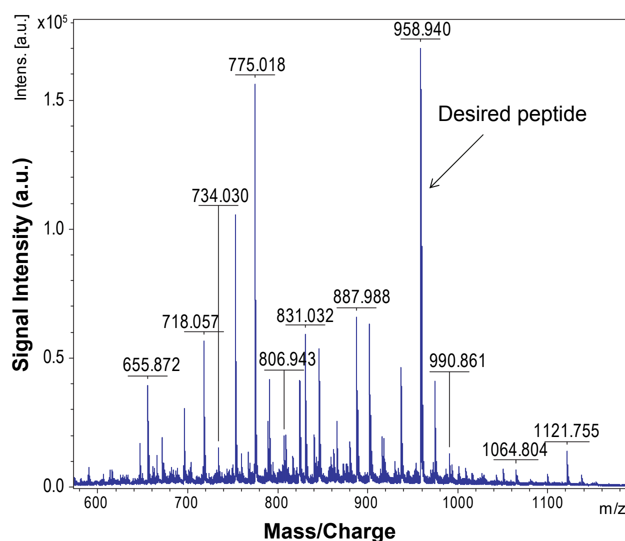

FIGURE 1. 70% Stepwise Yield and MALDI-Based Detection (A MALDI mass spectrum taken directly from the surface of a CombiMatrix chip after a two step electrochemical synthesis procedure. Analysis of the spectrum indicates that our step-wise yields of the desired product are approximately 70%.)

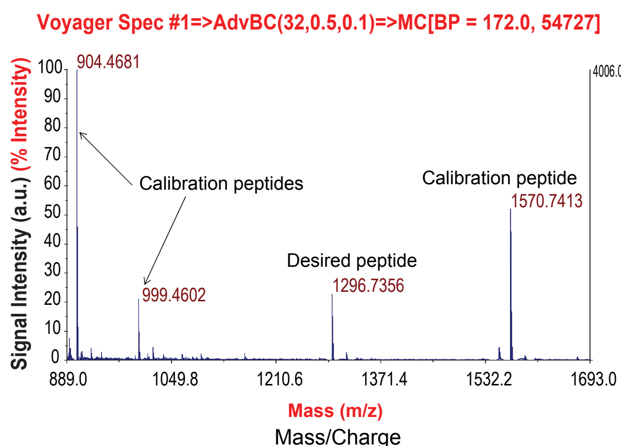

FIGURE 2. MALDI Spectrum of In Situ Synthesized Control Peptide 4c (Shown is a spectrum of control peptide 4A [does not bind Mn] which has been synthesized in situ using standard Fmoc chemistry on the CombiMatrix chip. Note that it is essentially pure – most of the other peaks are calibration peptides.)

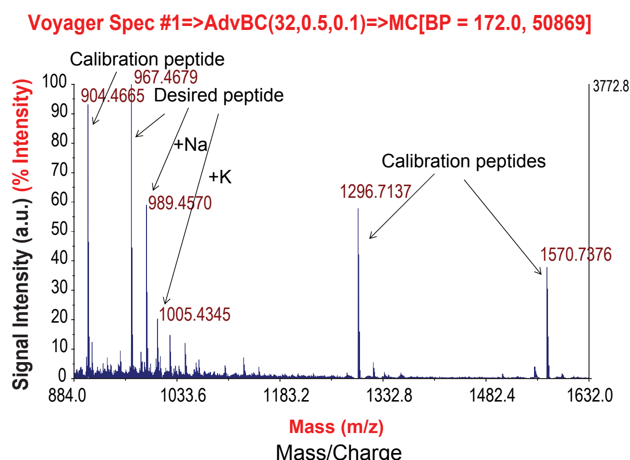

FIGURE 3. MALDI Spectrum Of In Situ Synthesized Control Peptide 2a (Shown is a spectrum of Mn-binding peptide 2A which has been synthesized in situ using standard Fmoc chemistry on the CombiMatrix chip. As with 4A, it is essentially pure – most of the other peaks are calibration peptides. This is important because this peptide, unlike 4A, contains asp and glu residues that have previously given us difficulty in these syntheses due to side-chain protection problems.)

We also shifted to a different peptide synthesizer to avoid problems with incomplete reactions that we had observed with the older synthesizer. This resulted in nearly pure in situ synthesized peptides using standard Fmoc chemistry as measured by MALDI mass spectroscopy (Figure 2). We are starting array-based analyses now by synthesizing an in situ a checkerboard pattern of two different peptides from a designed library, control peptide 4 and Mn-binding test peptide 3, on the same slide and performing water electrolysis measurements to examine the influence of the peptides on the electrodes during electrolysis. On individual gold electrodes, peptide 3 was previously shown to increase current values in the presence of Mn ion in solution while control peptide 4, a non-manganese binding peptide, showed no increase. In general, our MALDI mass spectroscopy results have demonstrated that we achieve a high percent yield of peptide when we perform synthesis on the CombiMatrix chip, with relatively low ratios of side products in most cases. Without further opitimization, the synthesis of peptides 3 and 4 are efficient enough to study their influence on water electrolysis. We have prepared a slide for the electrolysis measurements and are in the process of analyzing it. We have also characterized the in situ synthesis of peptide 2 which is another peptide that has shown improvement in electrolysis on gold electrodes (Figure 3). This peptide appears to be essentially pure. We are also planning to make checkerboard patterns of this peptide with the control peptide for analysis. From there we will start to make much larger libraries of peptides that are variants of these initial peptides, looking for optimization of the electrocatalytic activity.

Optimizing Measurement of Catalytic Activity on CombiMatrix Electrode Arrays

One key issue for the success of these studies is the detailed analysis of the candidate peptides that come from screening the arrays. Our initial tests were with unmodified CombiMatrix chips and we found that the electrode to electrode variation was only a couple percent when systematic errors were removed. Next we turned to modified electrodes for which attachment chemistry had been performed. Here we began to see that some electrodes would give large offsets, perhaps due to chemical damage, but the vast majority gave low noise measurements. We are able to generate I/V measurements that, after averaging and normalizing to local controls, are the same between different positions in a checkerboard pattern to within a small percentage (roughly 1-2%, Figure 4).

Either through synthesis (which is now of sufficient yield) or direct coupling of peptides (where we are still working on improving the yield), we are creating arrays of a few peptides by electrochemically deprotecting regions of the slide and either synthesizing or coupling different peptides in each region. In this way we can begin to characterize peptide arrays in terms of their catalytic activity. We have employed the CombiMatrix platform to attach both control and catalytic peptides, either on separate arrays or in blocks on the same array, using electrochemical deprotection of the arrays to pattern the coupling. The I-V curve for electrodes on the array are being measured before and after exposure to Mn, and the relative catalytic activity will be compared between electrodes.

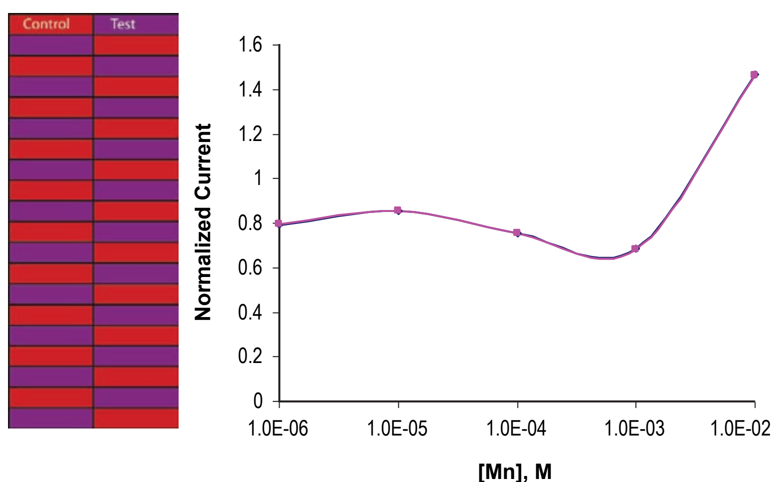

FIGURE 4. Determining the Accuracy of I/V Measurements on the CombiMatrix Platform (This is an example of a measurement series from a CombiMatrix chip in which we have gone through the process of coupling two different peptides in a checkerboard pattern to different regions of the chip [left panel]. In this case, the different regions of the chip turned out to be functionally identical. Current was measured at a series of voltages. In the plot to the right, the current at 2.4 V is shown. There are two curves shown, one for the control regions and one for the test regions, and they overlay each other to better than 2% accuracy. This sets the limit of our ability to discriminate between the electocatalytic function of different regions of the chip.)

Conclusions and Future Directions

During the past year we have:

- Achieved a usable yield of electrochemically patterned synthesis using modified CombiMatrix instrumentation.
- Achieved coupling of peptides or peptide segments to CombiMatrix electrodes.
- Partially optimized electrodes for performing detailed catalytic testing.
- Demonstrated the ability to measure I/V curves on multiple positions in the CombiMatrix array with high accuracy.

Our goals for next year are:

- Continue optimization of the yield and accuracy of our electrochemically patterned synthesis.
- Optimize the coupling conditions for peptide segments.
- Optimize the measurement conditions and accuracy for the 12,000 electrode arrays.
- 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.

FY 2008 Publications/Presentations

1. Northen, TR, Greving, MP, Woodbury, NW “Combinatorial Screening of Biomimetic Protein Affinity Materials” *in press* 2008, *Advanced Materials.*

2. Woodbury, N “A Rapid Approach to Synthetic Antibody Production” Conference; Quebec, Canada, April 3, 2008.

References

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