



Electro-organic Reactions and Redox Active Biomolecules: A Student Diary*

by James F. Rusling and Albert J. Fry

It is early December, and I have been in chemistry grad school for about 3 months. It is a big transition, and a bit tougher than I expected. The courses are not too hard, but the focus here is very much on research. I have chosen the group in which I will do my thesis research, and I am happy about that. This group does electrochemistry on organic and biological molecules. It was a hard choice to make because there is some cool research going in the labs of professors in this department. But I feel that this group is right for me. It will expand on my previous undergraduate research experience in organic and bioanalytical chemistry, and I will be exposed to modern aspects of electrochemistry, materials science, and biochemistry. Careerwise, this experience could lead to opportunities in academia or in the pharmaceutical or specialty chemicals industries. Also, my new group is active in developing synthetic reactions as well as fundamental studies of protein redox chemistry. This gives me several options for a thesis research project. My professor says that I can start by taking some training in both areas and we will decide together on a specific project sometime in the spring. I'll be hooked up with an advanced grad student or postdoc in each area, and they are going to train me in the basic techniques. I have decided to record my experiences, and below is my diary.

Research in Organic Electrochemical Synthesis

I will have my first training session in organic electrochemistry soon. Our group develops catalytic procedures for synthetic electrochemical reactions, and also investigates their mechanisms. I have learned that a major function of the group is modification of electrode surfaces to make them more selective for a particular synthetic reaction.

This can involve direct attachment of monolayers of molecules to electrode surfaces, or deposition of a suitable polymer or nanoparticles on an electrode. I have noticed instruments in the lab that are used in more conventional organic synthesis, such as gas and liquid chromatographs, and UV-visible and Fourier transform infrared (FTIR) spectrometers. That is good because I know how to use some of

these techniques. Some group members also told me that they use liquid chromatography-mass spectrometry (LC-MS), and it is a great help in analyzing their reaction products. Methods like atomic force microscopy, scanning electron microscopy, and surface FTIR are used in the group for characterization of the electrode surfaces they make.

A week or so later: I have done my first electrochemical synthesis experiment. I did cyclic voltammetry in undergraduate analytical lab, so it was not totally new. The reactor cell I used was like two connected beakers in a water jacket (Fig. 1). One beaker is the working electrode compartment, and the other is the counter electrode compartment, separated by a salt bridge. This is so that products from the two compartments do not combine to give undesirable products. The reactor had a carbon cloth working electrode, which is a cool material, like a conducting black fabric that you cut to the right size with scissors. It has a large surface area to facilitate the catalytic reaction. The reactor has counter and reference electrodes, and is hooked up to a device called a potentiostat to control the voltage at the working electrode. The working electrode voltage is set to produce enough energy to drive the reaction, and you can also measure the charge consumed by the reaction. By comparing the measured yield to the theoretical yield predicted from this charge using Faraday's law, you can estimate the efficiency of the reaction.

For the working electrode, the one that catalyzes the reaction, I learned how to make nanoparticle-polyion films.¹ We were given the 40 nm diameter manganese oxide nanoparticles from a collaborating inorganic chemistry group. Then, we adsorbed a layer of polystyrene sulfonate onto the carbon cloth, washed it, and then adsorbed a layer of a cationic polymer. The manganese oxide nanoparticles are negatively charged, so they were adsorbed on the cationic polymer layer to make the electrode. The reaction was to convert styrene to styrene oxide with this manganese oxide nanoparticle electrode.² I determined the products with GC and then GC-MS, and got my first experience using GC-MS. The first time I ran the reaction, the yield was lousy. But I did it twice more and both times the yield of styrene oxide was good. My mentor said that we could also use cyclic voltammetry to help characterize the system, but we decided to leave that for another time.

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*Ed. Note: The authors of this piece are firmly established professionals; but they enjoyed playing students again to present the diverse and exciting area of organic and biological electrochemistry.

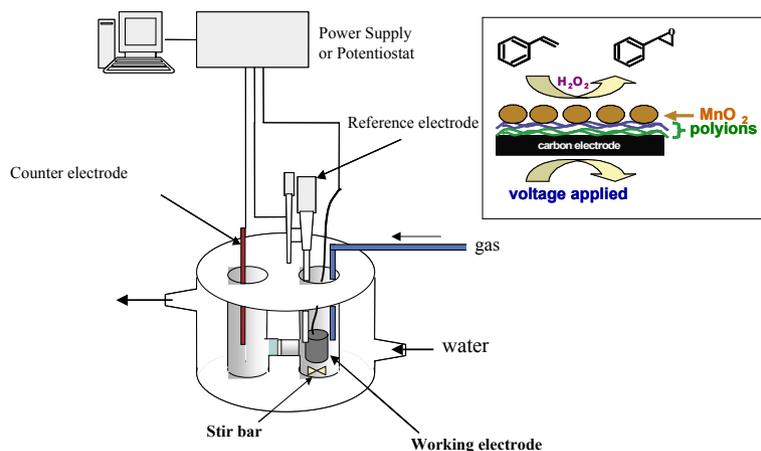


Fig. 1. Typical reactor cell for benchtop electrochemical synthesis. Inset on the right illustrates the epoxidation of styrene on a manganese oxide nanoparticle electrode.

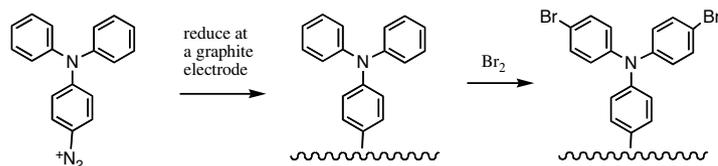


Fig. 2. Construction of an electrocatalytic electrode bearing triphenylamine groups.

Bonding Electrocatalysts to Electrode Surfaces for Synthesis

It is several weeks later, and I am doing a reaction with a different catalytic electrode. In addition to what I have already tried, there are other ways to modify electrode surfaces. Some ways have the disadvantage that the coating is removed readily, so that the electrode must be recoated on a regular basis. Two Frenchmen described a good method to beat this problem.³ They found that reduction of phenyldiazonium ion ($C_6H_5N_2^+$) at a graphite surface forms phenyl radicals, which bind chemically to graphite electrodes, and that it is possible to carry out chemical reactions on the aryl groups of the modified electrode. These ideas were used recently to produce an electrocatalytic electrode by attaching a triphenylamine electrocatalyst to a graphite fiber electrode (Figure 2).⁴ You reduce a diazonium ion containing the triphenylamine nucleus (a well-known electrocatalyst) at a graphite electrode and then brominate the electrode to block undesired reactions at the *para* positions. Voltammetry shows that the electrode catalyzes the oxidation of substrates such as silanes and benzyl ethers. You can make electrodes for catalytic oxidations on a preparative

scale by preparing a larger electrode using the same surface chemistry.

I made the electrode in Fig. 2 on a 3 x 3 in. piece of carbon cloth and used it in controlled potential electrolysis of benzyl methyl ether. The cell was similar to the one in Fig. 1. I got a good yield of the expected product benzaldehyde, that I measured by gas chromatography. I think I am getting the hang of this!

Voltammetric Studies of Protein Films

In preparation for the last phase of my training, I was assigned to read several review articles on protein film voltammetry.^{5,6} I found that protein or enzyme films can be used for biosensors, bioreactors, and electrochemical studies of protein redox properties. However, it is tricky, because adsorption of denatured proteins can make a film that blocks electron transfer on metal electrodes. About 25 years ago, researchers found that control of electrode surface chemistry was a key to good protein electron transfer. Early success stories included the attachment of monolayers of 4,4'-bipyridyl or related thiol derivatives to metal electrodes to promote direct electron exchange between proteins and the electrodes. Before this, it was difficult to get direct protein voltammetry. A little later, edge

plane pyrolytic graphite electrodes containing carboxylate groups were also found effective. So, surface chemistry plays an important role in modern protein electrochemistry.

Also, I found that amperometric biosensors have been developed with mediators to deliver electrons between electrodes and enzymes. While this is not direct electron transfer, immobilized enzymes in films can have high catalytic activity, and the devices can be driven by the mediators. Electrochemical glucose sensors based on this principle with the enzyme glucose oxidase are used routinely by diabetic patients to measure glucose in their blood. I saw one type advertised by the blues guitarist B. B. King on TV last week.

Protein film voltammetry can be done with many types of films. Proteins can be coadsorbed with other chemicals to give monolayers with reversible voltammetry on edge pyrolytic graphite electrodes. Self-assembled monolayers of organothiols on gold electrodes that adsorb or chemically bond with proteins can be used to give reversible electrochemistry. Insoluble surfactants and polyions of various types can be used to make stable films.^{5,6}

Cyclic voltammetry is a major electrochemical tool for studying protein films. A typical setup is shown in Fig. 3. It is the usual cyclic voltammetry setup using a potentiostat to control the applied potential and measure the current in an electrochemical cell. It can be used for all sorts of electroactive materials; but in this case the protein film is attached to the working electrode. I used this setup to obtain cyclic voltammograms for a peroxidase enzyme from the tuberculosis bacterium in a lipid film. We got the enzyme from a collaborator in Brooklyn.

These films are simple to make. I dissolved some lipid (dimyristoylphosphatidyl choline) in water and sonicated the dispersion to make vesicles. Then I added the protein in a neutral buffer, and spread a few microliters of the mixture on a pyrolytic graphite disk electrode. This was dried overnight with a small bottle over the electrode to make the drying slow. Then the protein-film coated working electrode was placed into the electrochemical cell, and cyclic voltammetry was done. This involved a linear scan of voltage to negative values, followed immediately by another linear scan in the positive direction. The results are shown in Fig. 4. This peroxidase is an iron heme enzyme, and the peaks are caused by reduction of the Fe^{III} enzyme to the Fe^{II} form on the forward scan, followed by oxidation of the Fe^{II} enzyme

Equipment for protein film voltammetry

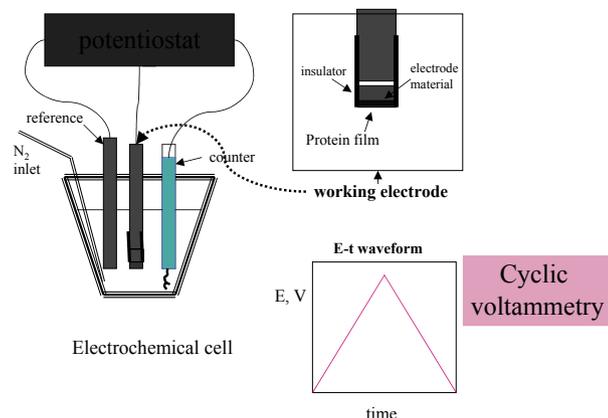


Fig. 3. System used for cyclic voltammetry of protein films.

to Fe^{III} on the reverse scan. The results tell me that the reaction is reversible, because both peaks are nearly equal in height and at about the same voltage. We know that these peaks are caused by the enzyme because lots of characterization studies have been done on these protein-lipid films, such as visible absorbance spectroscopy, circular dichroism, reflectance infrared spectroscopy, spectroelectrochemistry, electron spin resonance (ESR) spectroscopy, and other methods. All these experiments showed results consistent with the native protein and not with the free iron heme group or denatured protein or anything like that.

Another method used to make films in our lab is layer-by-layer deposition of enzymes and polyions of alternate charges.⁶ This is the same method I used to make the manganese oxide films, so it is versatile. Although it is a little more time-consuming than the lipid film method, the films are usually stable and good for biosensors.

Cyclic voltammetry can be used to get all sorts of information about the protein film. The integral under the peak gives the charge used for protein electrolysis, which tells us how much electroactive enzyme is present in the film using Faraday's law. A scan rate study of the separation of the two peaks as a function of the rate of voltage scan can be analyzed with theory to obtain electron transfer rate constants. Addition of hydrogen peroxide to the solution caused the Fe^{III} peak to increase, and the Fe^{II} peak to disappear. This is because the enzyme catalytically reduces hydrogen peroxide. The rate of this reaction can also be obtained by analysis of the catalytic voltammetry peak. Also, this type of catalytic current increase can be used in biosensors, in this case a sensor for hydrogen peroxide. If you want to

do fundamental studies, all the rates I mentioned above can be measured, and also all the spectroscopic methods mentioned above can be used. For optical spectra, the films are usually made on transparent indium-tin oxide electrodes, or you can do reflectance. So it seems that a lot of information about the redox chemistry, redox states, and bioactivity of the enzyme can be obtained with these films.

Outcome

I have finally finished my initial training. I have learned a lot about organic and biological electrochemistry in the past several months. I also found that there is an organization called ECS that holds great research meetings twice a year, and there is an Organic and Biological Electrochemistry Division in that Society. Student membership is cheap; I am definitely going to join!

What did I learn about electrochemical research? In addition to many new electrochemical and characterization techniques, I have been exposed to modern surface modification and film construction methods. Also I learned how electrochemistry is linked to many other areas like organic and inorganic chemistry as well as biology. It also utilizes peripheral techniques such as chromatography, all sorts of spectroscopy, and even quantum mechanical computations and voltammetry simulations. Now I need to decide on the specific area that I will specialize in for my thesis research, and dream up a project of my own. Everything was so interesting; it is going to be a tough decision. ■

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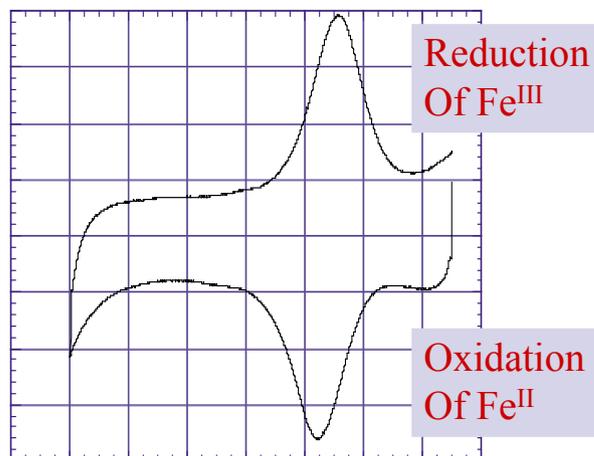


Fig. 4. Background subtracted cyclic voltammogram at 0.1 V/s of *Mycobacterium Tuberculosis* catalase peroxidase in a thin film (~0.5 μm) of the lipid dimyristoylphosphatidyl choline on a pyrolytic graphite electrode in pH 6.1 buffer.

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