Assembly and Characterization of Myoglobin-Carbon Nanofiber Electrodes for Biosensing

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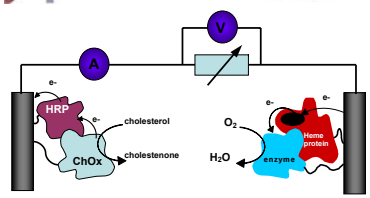
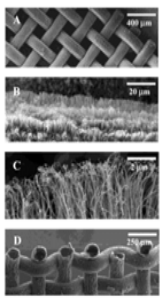
Abstract

The purpose of my experiments was to study the current response of myoglobin solutions at carbon Nanofiber (CNF) electrodes. This relation was studied using cyclic voltammetry (CV), particularly to research the response of the carbon nanofiber electrode to varying Mb concentrations that had been oxidized to Fe(III) with potassium ferricyanide, and dissolved in Tris buffer (pH 6.5). Through these experiments, small but significant peaks were found in the CVs that corresponded to myoglobin redox activity. This correspondence was discovered by comparing Mb CVs to control CVs containing pH 6.5 Tris buffer. The two types of CVs were found to be almost identical except for peaks that most likely indicate myoglobin redox activity. This hypothesis was found to be true as the peaks would diminish if the solution tested had less myoglobin and increase if more myoglobin was added. To attain numerous concentrations of myoglobin, a UV spectrophotometer was used to calculate the present concentration and what measures must be taken to obtain a new concentration. By evaluating these different concentrations of myoglobin with CVs, a limit of detection at which the peaks no longer appear was found. It can be concluded from these experiments that myoglobin's redox reactions can be detected in the presence of carbon nanofiber electrodes.

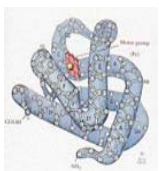
Introduction

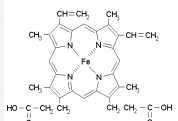
With new compact, lightweight, reliable power sources comes a demand for smaller, more portable or implantable biosensors, even smaller than sensors currently on the market (upper right). Manmade batteries add bulk to the overall sensor, so they will not always suffice. The ideal step is creating practical sensory electronic devices to take advantage of natural fuel sources in human body, such as proteins and enzymes. More research in this area would allow for miniaturized self-sufficient bioelectronic devices. A schematic diagram of such a device to be used for cholesterol sensing is shown at right.





Carbon nanofibers (CNFs, at left) are a type of biocompatible electrode material with many attractive features, such as small size, large surface area and high chemical stability. These features make CNFs ideal for electrochemical biosensing schemes. The Stevenson group specializes in the synthesis and detailed study of CNF electrodes assembled in-house.



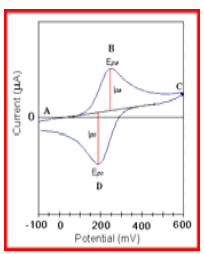


Myoglobin (Mb, shown above) is an extremely compact protein in vertebrates that serves a reserve supply of oxygen and transports oxygen in muscle. Its capacity to bind oxygen depends on the oxidation state of its heme group, a non-polypeptide unit located in crevice of molecule that is composed of four pyrrole rings and an iron atom. This iron atom may be either Fe(II) or Fe(III), but only Mb with Fe(II) can bind oxygen. In its Fe (III) state, Mb shows good redox chemistry at many electrode surfaces, including CNFs. Therefore, Mb is a possible biosensor component that may be studied using a variety of electroanalytical techniques.

Cyclic voltammetry (CV) is one of the most effective electroanalytical techniques, because it's both versatile and easy to use. A CV is derived from current (i) as a function of potential (V). It consists of the potential of an electrode, in a solution, being cycled and current being measured. Redox reactions (rxns) appear in CV as peaks which may be further analyzed.

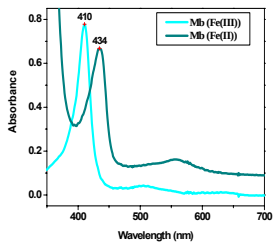
In CV, three electrodes are used:

- **Working Electrode**-source of electrons for rxns
- **Reference Electrode**-zero-current reference point
- **Counter Electrode**-employed to prevent current flow to reference electrode
  - Thin platinum or gold wire for low reactivity



Procedure

Protein Purification and Preparation



UV spectrum of Mb(III) and Mb(II)
Note that peaks are at 410 and 434 nm, respectively.

- **Tris buffer with pH of 6.5**
  - 3.03g of Tris(hydroxymethyl)($C_4H_{11}NO_3$) was diluted to 0.1 M using nanopure H_2O.
  - Adjusted the pH to 6.5 using concentrated sulfuric acid (H_2SO_4) and sodium hydroxide (NaOH)
- **Oxidizing iron atom in myoglobin from Fe(II) to Fe(III)**
  - 40 mg of myoglobin was dissolved in 0.4 mL of buffer
  - A few crystals of potassium ferricyanide ($C_6FeK_3N_6$) were added
  - The solution was dialyzed at 4° C for 24 hours.
  - Using ultraviolet-visible (UV-Vis) spectrophotometer, measurements were taken with Mb(III) at 434 and 410 nm
  - Mb(III) was reduced to Mb(II) by adding a few crystals of sodium hydrosulfite ($Na_2S_2O_4$) and also measured
  - Mb concentration was determined by measuring absorption difference between Fe(III) and Fe(II) at 434 nm.
  - A molar absorptivity of 114,000 $M^{-1}\,cm^{-1}$ was used in Beer's law calculations.
  - Mb solutions were further diluted to working concentrations using Tris buffer

CNF Electrode Preparation

- **Wetting an electrode**
  - Acrylic was applied to upper stem and 2.2 mm square on CNF
  - CNF was attached to copper clamp in electrochemical cell
  - Electrodes were wetted by cycling potential repeatedly between ~1 V and -0.8 V with an Ag/AgCl reference electrode at 300 mV/s in 1 M KNO_3 (potassium nitrate) solution
  - Repeat until electrode surface is no longer shiny
- **Blank CVs and Mb CVs were taken with parameters of 0.4 to -0.4 V at 10 mV/s.**
  - The electrochemical cell was purged with either N_2 or Ar for 10-15 min. prior to CV
  - A blanket of inert gas was maintained in the cell throughout all measurements

Calculations

- Beer's Law
- **$A=\varepsilon bc$**

-where A=absorbance, ε=molar absorptivity, b=path length, and c=sample concentration

- Formal Redox Potential
- **$(E_{pa}+E_{pc})/2$**

-where E_{pa}=anodic peak potential and E_{pc}=cathodic peak potential

- Reaction Reversibility
- **I_{pc}/I_{pa}**

-where I_{pa}=anodic peak current and I_{pc}=cathodic peak current

- Electrons Transferred
- **E_{pc}-E_{pa}**
- Surface Coverage
- **Q/nFA**

-where Q=peak area, n=electrons transferred, F=Faraday's constant, and A=area of electrode

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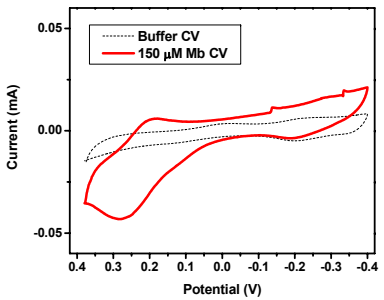
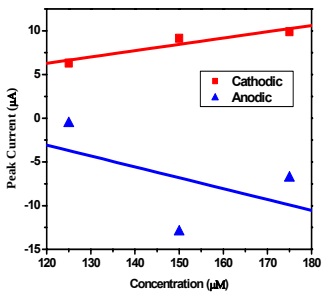
Results and Discussion



Blank CV vs. Mb CV of 150 μM concentration

- **Only 4 good Mb CVs could be analyzed and compared to blank CVs.**
  - Procedures were complex
  - Limited by Time and availability of supplies
- **Observations**
  - CNFs can be used for only one series of Mb CVs
  - Parameters set at .4 to -.4 V potential range and 10 mV/s scan rate to prevent Pt oxidation
  - Mb CVs studied and analyzed in relation to concentration
- **Four Mb CVs were taken at concentrations of 100, 125, 150, and 175 μM**
  - All the CVs produced were exceptional, except for the 100 μM, since it was close to the limit of detection
- **Average redox potential: -0.232 V**
- **All CVs yielded a reaction reversibility other than 1, and are thus categorized as quasireversible**
- **They differ in their peak-to-peak separation for one electron transfer, 59 mV, but it is expected that Mb rxns have one electron transferred**
  - Changes between Fe(II) and Fe(III)

Sweep	Concentration (μM)	Peak Potential (V)	Peak Current (μA)	Peak Area (μC)	Surface coverage ($moles/cm^2$)
Cathodic	125	.205	6.3	.75	7
	150	.18	9.15	1.1	10
	175	.204	9.9	1.35	12
Anodic	125	.253	.6	.043	N/A
	150	.284	13	1.62	
	175	.266	6.83	.71	



Graph of linear trend lines, showing that the limit of detection is somewhere around 100 μM.

- **Limit of detection**
  - Point of concentration at which myoglobin can no longer be substantially identified using CV analysis
  - Found to be around 100 μM
- **Anodic peaks were found to be unstable, possibly because in the cathodic sweep, Fe(III) is reduced to Fe(II), and this may bind oxygen and produce disorderly CVs**

- **Surface Coverage of Cathodic peaks was directly proportional to Mb solution concentration**
  - These values indicate that Mb was covering almost all of the CNF and was showing good redox chemistry

Conclusion

The information that I have presented is all based off of preliminary experimentation and the results obtained from them. Thus, few suppositions can be made without further experimentation and analysis. However, from the data I collected, I can hypothesize that the redox reactions of myoglobin can be detected in the presence of carbon nanofiber electrodes. This means that there may be a possibility of applying this relationship to creating a biosensor that sufficiently identifies different processes in the human body. Further research based off of my experimentation will produce further information about the potential of myoglobin use in biosensor work and the applications of carbon nanofiber electrodes. This research would most likely involve gathering more Mb CVs at more concentrations and doing these experiments repeatedly to identify a definite pattern and the potential for Mb and CNFs to be used for biosensors.

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