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Study of Autoxidation in Hemoglobin in the Presence of Alcohol

McKayla A. Wieczorek
University of Nebraska-Lincoln, mckaylaw3185@gmail.com

Hideaki Moriyama
University of Nebraska-Lincoln, hmoriyama2@unl.edu

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McKayla Wieczorek, Hideaki Moriyama

School of Biological Sciences, University of Nebraska, NE

Background

In order to learn more about the effects alcohol has on the body, we investigated its effects on the circulatory system. Our bodies' circulatory systems are the main way that we transport chemicals to where they are needed. One of the major roles of the blood, is the role of the red blood cells, which bind to and transport oxygen molecules to our cells where they can be used for cellular respiration. Without the transfer of oxygen, cellular respiration would not be possible, and without cellular respiration, our cells cannot produce adequate amounts of energy.

Hemoglobin proteins in the red blood cells are what make oxygen transfer possible. Hemoglobin is made up of four subunits—two alpha subunits and two beta subunits—and each subunit has a site which can bind to a substrate. In our bodies, that substrate is an oxygen molecule, We call this oxyhemoglobin, and our bodies have ways of making sure that our hemoglobin stays in this form. Over time however, hemoglobin will loose its oxygen molecules, and instead bind to water molecules. This form is called aquamet hemoglobin, and it cannot be used by our bodies. Our goal was to explore how the consumption of alcohol affected this transformation of hemoglobin. We measured the amount of oxyhemoglobin by analyzing the protein absorbance peaks, and we hypothesized that the addition of alcohol would result in a faster accumulation of aquamet hemoglobin.

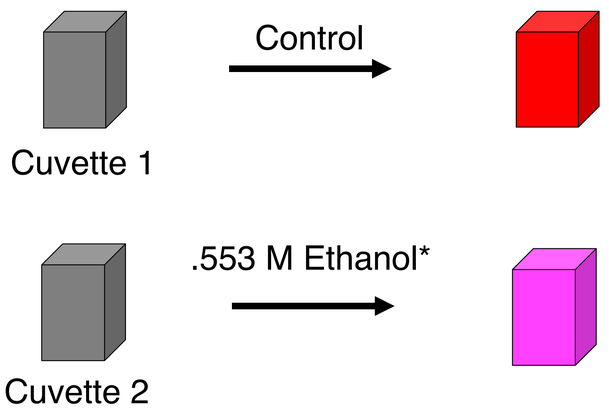

Figure 1. Experimental Set-up
The cuvettes represent the conditions in a red blood cell.
***This concentration is greater than LD_{50} in mice which would be much greater than the legal limit in humans.**

Approach

- We used two cuvettes to mimic the conditions in a red blood cell. One cuvette contained alcohol while the other did not.
- Each cuvette consisted of 120 microliters of 0.176 mM hemoglobin and 100mM HEPES buffer pH7.
- 4 microliters of 100% ethanol was added to 120 microliters of hemoglobin solution to act as the experimental cuvette to achieve a final concentration of .553 M.
- The absorbance of each cuvette was measured every 10 minutes for 6 hours and then again at 20 hours.
- The cuvettes were kept at 37ºC for the duration of the experiment.

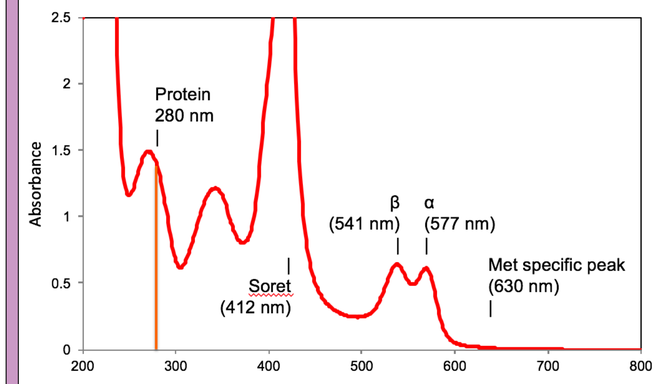

Figure 2. Reference hemoglobin absorbance spectrum

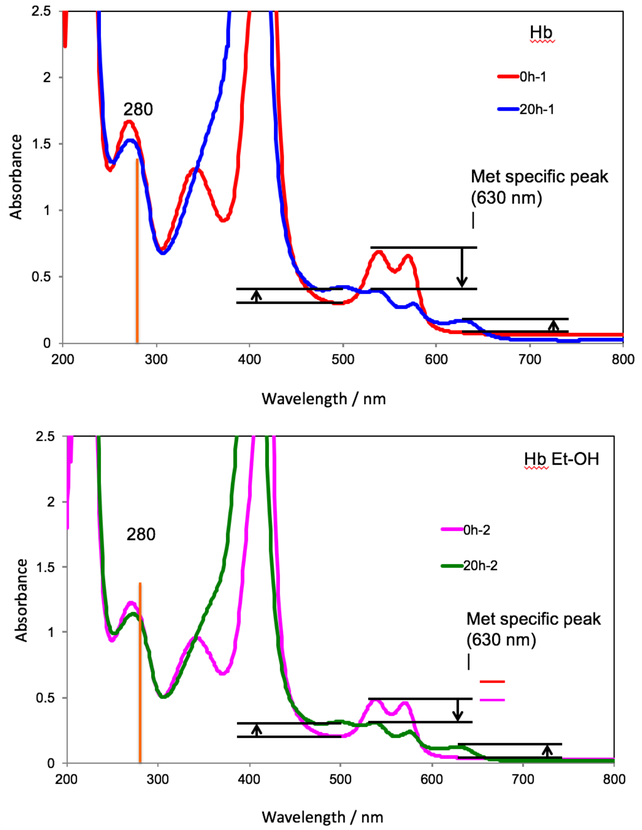

Figure 3.

Initial absorbance compared to absorbance at 20 hours for both the experimental and control conditions. The difference between the two spectra is not distinguishable.

Acknowledgments

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Results

After examining the change in absorbance peaks over time, we saw a decrease in the oxyheme peaks in both the experimental and control cuvettes. This indicates that the amount of oxyhemoglobin gradually decreased. Additionally, we saw an increase in the met specific peak in both cuvettes, which indicates an increase in aquamet hemoglobin.

Next, we looked at the rate at which these changes took place. As shown in Figure 4, the rate of change for both the oxyheme peak and the aquamet peak were the same, regardless of whether or not alcohol had been added to the system. Based on these results, we can conclude that alcohol does not affect hemoglobin function.

It is important to keep in mind that this experiment was performed *in vitro*. There are many additional processes occurring in our red blood cells simultaneously with this reaction, and different effects may occur under these conditions.

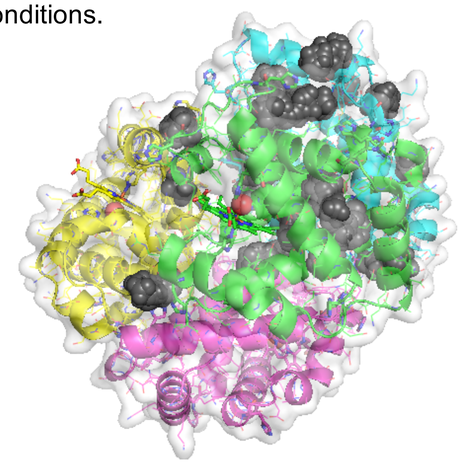

Figure 4.
Ethanol molecule bound to hemoglobin protein

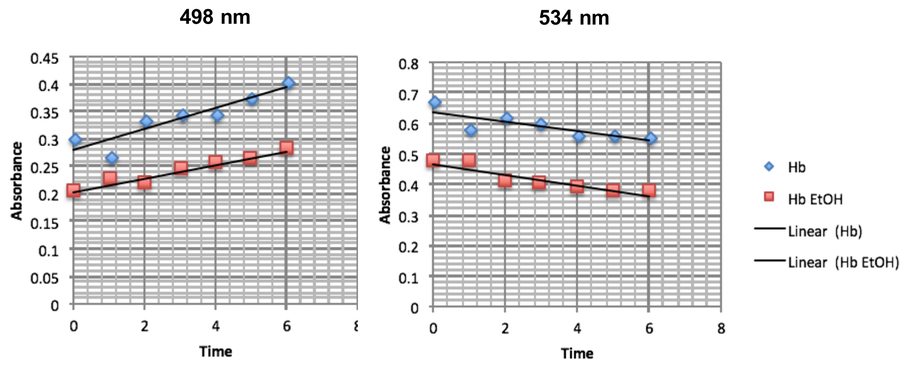

Figure 4. Rate of change in hemoglobin peaks
The 498 nm peak represents the amount of aquamet hemoglobin present while the 534 nm peak represents the oxyhemoglobin.

References

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