Testing Extractants to Study How Protein Interacts with Iron Oxide Minerals

Aylmer Tan[1], Stephany S. Chacon[2,5], Carrie L. Marean-Reardon[4], Patrick N. Reardon[3], Maria C. Franco[4] and Markus Kleber[2]

[1]Department of Biology, Oregon State University, Corvallis OR 97330, [2]Department of Crop and Soil Science, Oregon State University, Corvallis OR 97330, [3]Nuclear Magnetic Resonance Facility, Oregon State University, Corvallis OR 97330 , [4]Department of Biochemistry and Biophysics, Oregon State University, Corvallis OR 97330, [5]Climate and Ecosystems Division, Lawrence Berkeley National Laboratory, Berkeley CA, 94720

Abstract

The goals of this experiment were to find a suitable extractant to extract protein adsorbed onto iron oxide minerals and to determine whether those minerals can modify protein. Data obtained from this experiment provides crucial background information to study the effects hydrogen peroxide has on protein in the presence of an iron oxide mineral. We found in this experiment that Sodium dodecyl sulfate and Guanidine Hydrochloride were able to extract some of the protein off of iron oxide minerals. Additionally, NMR spectroscopy was used to analyze the protein for any modification revealed that the extracted protein remained unchanged.

General Problems

- Microbes in the soil produce an array of exoenzymes that are essential in the cycling of nutrients. Furthermore, soils contain a large variety of proteins that are important as sources of organic nitrogen and for biological function.
- Hydrogen peroxide, which is a by-product of biological activity that can accumulate naturally in soil, can react with iron to form hydroxyl radicals, which are strong oxidants.

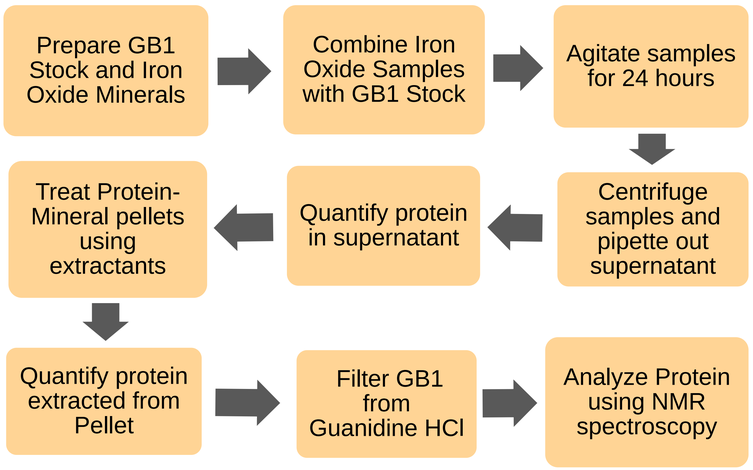
Specific Questions

- What is the best extractant that will allow us to optimize adsorption parameters for future analysis of GB1-Hematite-Peroxide experiments?
- Considering that iron is very abundant in soil within iron oxides, and in solution, how does it interact with soil exoenzymes and other proteins?

Hypothesis

- Guanidine Hydrochloride and Sodium dodecyl sulfate (SDS) are able to extract protein off of the iron oxide minerals by disrupting their bonds.
- Interaction between iron oxide minerals and protein will lead to changes in protein structure and side groups.

Method



- GB1 stands for “Protein G B1 domain”.
- Sodium dodecyl sulfate (SDS) is an anionic detergent consisting of a carbon tail attached to a sulfate group[1].
- Guanidine Hydrochloride (HCl) is a denaturant which can denature and unfold proteins and allow refolding when removed[2].
- Quantification steps were done using Ultraviolet-visible spectroscopy.
- Filtration of GB1 from Guanidine HCl was done using a centrifugal concentrator with a molecular weight cut off of 3.0 kD.

Results

Determine extraction efficiency of SDS and Guanidine HCl:

- GB1 almost completely adsorbed to hematite and goethite within 24 hours. The following graphs represent the peak absorbance of the GB1 stock compared to the supernatant separated after mixing.

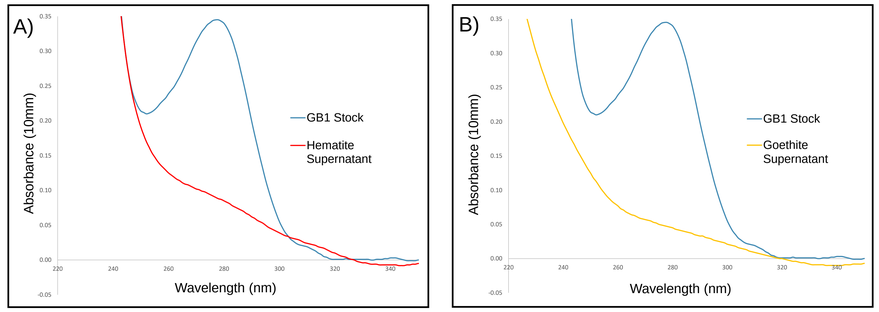


Figure 1. **A**: Absorbance spectrum comparing GB1 concentration in stock solution to Hematite supernatant after mixing. **B**: Absorbance spectrum comparing GB1 concentration in stock solution to Goethite supernatant after mixing.

- SDS buffer and Guanidine HCl were able to extract some of the GB1 that was absorbed to hematite and goethite. The absorbance spectrums below represent the characteristic peaks of GB1 in the extractants after treating the iron oxide minerals with them.

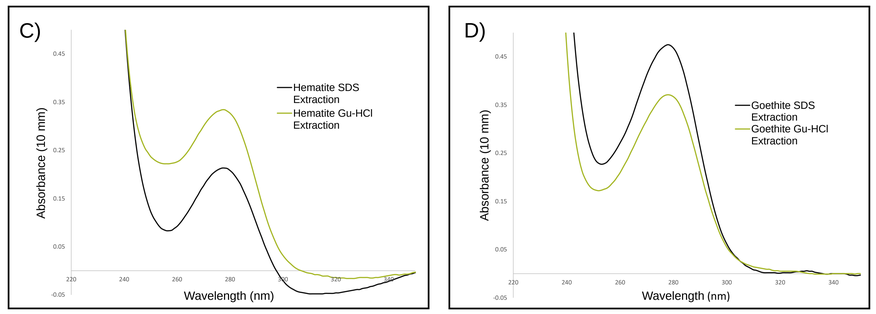


Figure 2. **C**: Absorbance spectrum of the extractants used on Hematite. **D**: Absorbance spectrum of the extractants used on Goethite.

- The extraction efficiency of SDS and Guanidine HCl on Hematite and Goethite were calculated using the data from the absorbance spectrum.

Table 1: Extraction efficiencies of SDS and Guanidine HCl

Extraction Solution	Minerals	
	Hematite	Goethite
SDS	11.8%	22.1%
Guanidine HCl	12.4%	17%

Determining the effects iron oxide minerals have on GB1 that was successfully extracted off the surface:

- Analysis using NMR spectroscopy shows that the GB1 extracted from the iron oxide minerals is no different from GB1 from the protein stock. The following figures represent the HSQC data obtained using NMR for Nitrogen-15 and Carbon-13 which compares the GB1 extracted from Hematite and Goethite to GB1 from the protein stock.

Results Continued

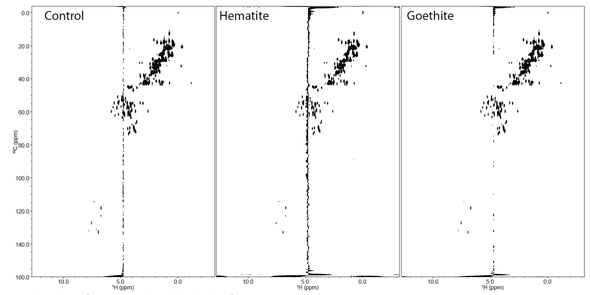


Figure 3. Carbon-13 HSQC data.

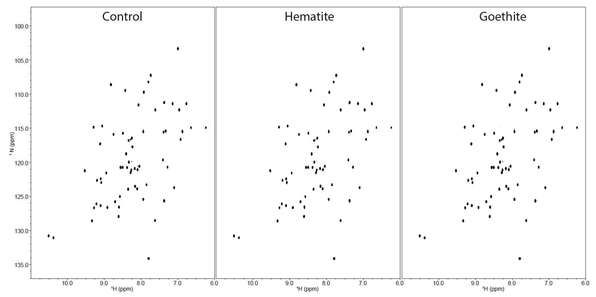


Figure 4. Nitrogen-15 HSQC data.

Conclusion

- Iron Oxide Minerals have a strong affinity for GB1 which makes extracting the protein difficult.
- Both extractants can be used to extract proteins from iron oxide minerals for future experiments involving hydrogen peroxide. However, both of them could only extract a small percentage of protein from the minerals
- The small percentage of GB1 that was successfully extracted from the iron oxide minerals showed no changes when analyzed using NMR spectroscopy.
- The centrifugal concentrator used to filter GB1 from Guanidine HCl has a molecular weight cut off of 3.0 kD. Potential fragments smaller than 3.0 kD could have been lost in the filtrate.

Future Work

The next step of this experiment is to test the effects hydrogen peroxide has on protein in the presence of an iron oxide mineral. We can test this because we have a working method to extract protein off the iron oxide minerals and NMR data that suggests that those minerals do not change protein.

References

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