

Experiment 9: Metal Ion – Protein Interactions¹

There are a number of ways that you can tell if a reaction has proceeded; if a gas has been released, if a precipitate has formed, if a color change is observed or if heat is noted in the reaction. We have seen examples of all of these throughout the term. Sometimes a color change can be subtle and you need instrumentation to notice what is happening (e.g., myoglobin vs. reduced myoglobin). You have now done two experiments using the UV-Vis spectrophotometer and today you will use it again to try and determine what is going on in a competitive reaction of a protein with copper and mercury.

Possible coordination sites for metal ions in proteins are $-\text{CO}_2^-$, $-\text{CONH}-$, NH_2 , $-\text{OH}$ (serine, threonine), $-\text{ArOH}$ (tyrosine), $-\text{S}^-$ (cysteine), and imidazole (histidine). There are also a few coordinating groups other than amino acid residues in certain proteins; e.g., heme group. The different combinations of coordination sites, coordination structure, bond nature, and metal ions, create a wide range of activities for metalloenzymes. The elucidation of the mechanism of metalloenzyme reactions thus requires the identification of the nature of the metal-protein system (coordinating group, etc.). The $-\text{S}^-$ residue of cysteine is particularly important, as it serves as a catalytic site in many enzymes, and is readily susceptible to reaction with heavy metals such as Pb and Hg. This forms the basis for the mechanism of toxicity of the heavy metals at the atomic and molecular level. Imidazole very often acts as a general acid-base catalytic center in enzymes, and also strongly coordinates to certain metal ions.

There are a great number of copper-containing enzymes known: a few of these are ceruloplasmin, hemocyanin, amine oxidase and laccase. Serum albumin, a protein which has one SH group (cysteine), is considered to be the Cu-transporting protein in blood.

In this experiment, the system Cu^{2+} - bovine serum albumin (BSA, MW = 65,000) is studied spectrophotometrically.

Procedure

- The following solutions are already made up and available:
 - 2% BSA solution in 0.33 M $\text{CH}_3\text{CO}_2\text{Na}$ (0.5 g in 25 mL)
 - 0.015 M $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$
 - 0.001 M $\text{Hg}(\text{ClO}_4)_2$ or HgCl_2
- Using these solutions, make up the solutions listed below and record their spectra (330 – 810 nm). Solutions B', C', and D' do not have to be prepared separately. Simply add the solid Na_2EDTA to the respective solutions in the cell after recording their spectra.

Solution	BSA (mL)	Cu (mL)	Hg (mL)	Water (mL)
A	3.00	0	0	2.00
B	0	1.00	0	4.00
C	3.00	1.00	0	1.00
D	3.00	1.00	1.00	0
E	3.00	1.20	0	0.80
B'				
C'	Add 10 mg Na_2EDTA (solid) to solutions B, C, and D			
D'				

- Consider Solution A as your blank.

¹ Adapted from *Laboratory Introduction to Bio-inorganic Chemistry*, E. Ochiai, D.R. Williams, MacMillan, London, 1979.

B. Analysis of the Spectra and Contents of the Lab Report

1. Attach all spectra, with arrows drawn to indicate any peaks. Tabulate the wavelengths of those peaks.
2. Why is it necessary to run Solution B?
3. What does Solution D show you?
4. What does Solution E show?
5. What do the EDTA solutions show?