Measuring the Affinity of Lipoic Acid and Copper

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Introduction

Copper (Cu) is a transition metal vital to several biological processes, including for iron metabolism, as a catalytic agent of enzymes and proteins, and for oxygen transport. Because of the unique chemistry of Cu, evolution has selected stringent mechanisms to control labile Cu. Cu-chelating small molecule ionophores, like the molecule (4-(2′-Pyridylazo)resorcinol) (PAR) can bind to Cu inside cells. One phenomenon that may occur is the accumulation of Cu in the body. This causes certain proteins to undergo a specific post-translational modification, where Lipoic Acid (La) is incorporated into proteins to form Lipoylated Proteins, where a covalent attachment of lipoamide forms to a lysine residue via an amide bond.[1] The effects of excess copper on lipoylated proteins were measured through Ultraviolet-visible spectroscopy (UV-Vis), which was used to investigate La moieties binding to Cu.

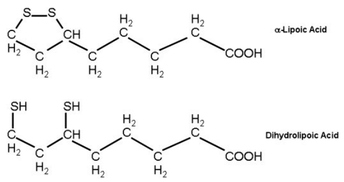


Figure 1: Lipoic Acid and Dihydrolipoic acid Diagram. Lipoic Acid (La) is the component that gets incorporated into proteins and causes them to be Lipoylated. The goal of this project is to obtain Dihydrolipoic Acid, the reduced form of Lipoic Acid, and determine how tightly it binds with copper in this form. [2]

Ultraviolet-Visible Spectroscopy Analysis on the La-Cu Complex

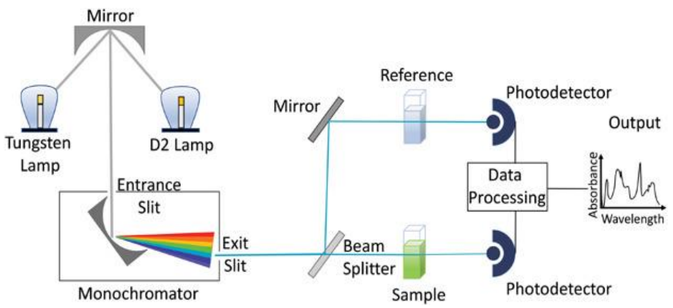


Figure 2: Ultraviolent-Visible Spectroscopy Diagram.. Light in the wavelength range of 200-800 nm is passed through a sample. The detector in the instrument measures the amount of light that is transmitted or absorbed by a sample. UV-Vis can be used to monitor the breaking and forming of new bonds within a chemical reaction.[3]

UV-Vis measures the amount of distinct wavelengths of UV or visible light that get absorbed or transmitted through a sample in comparison to a “blank sample", which in this set of experiments was 7.4 pH and 1 mL of Phosphate-buffered saline (PBS).[4] The range of wavelengths measured was between 200-800 nm.

Dithiothreitol (DTT) and TCEP (Tris-(2-Carboxyethyl)phosphine) are reducing agents for La. Absorbances for the PAR solution alone were peaked at ~400 nm, while the PAR-Cu complex peaked at ~512 nm.

Absorbances of Lipoic Acid and Copper Complex

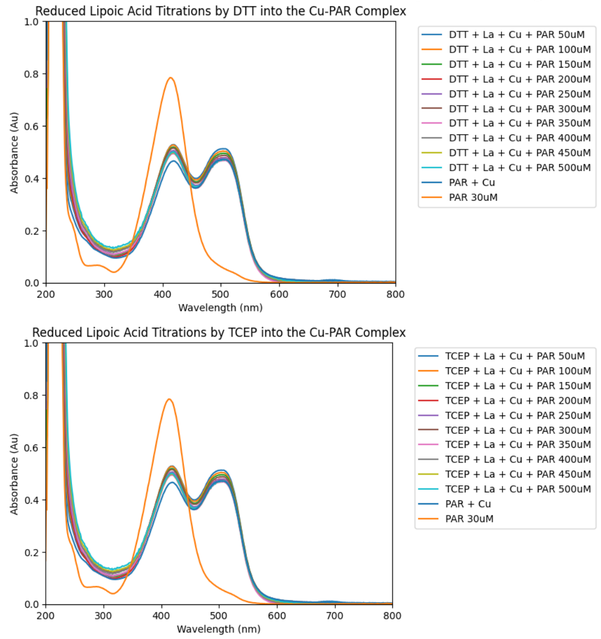
- After the seventh vial was scanned on the UV-Vis, titrations of La in increments of 50 µM were mixed for one minute and then scanned in the UV-Vis. This would proceed until the amount of Lipoic acid in the solution reached 500 µM.
- 7 vials of solution in 1 mL PBS Buffer were prepared for analysis on the UV-Vis:

Preparation for solutions in Uv-Vis Sample

Vial Number (#)	PAR	Cu	La	DTT/TCEP
1	30 µM			
2		10 µM		
3				100 µM
4			50 µM	
5	30 µM	10 µM		
6			50 µM	100 µM
7	30 µM	10 µM	50 µM	100 µM

Figure 3: Table of Different solutions Prepared for Each Run of the UV-Vis

- Each additional titration of La-DTT/La-TCEP lowers the peak of the Cu-PAR complex. Consequently, it is inferred that the La-Cu complex is forming.



Figures 4 and 5: Titrations of Reduced Lipoic Acid from DTT/TCEP into the Cu-PAR complex

Acknowledgements

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Conclusion

In addition, we are interested in determining how differently Cu binds to La when attached to a peptide. After the initial creation of La attached to a peptide via a **Solid Phase Peptide Synthesizer** (SPPS), the hope is to be able to recreate some of these findings in a modeled biological setting. This will help gain a deeper understanding of the protein´s modification and its effects on binding with a hyperaccumulation of Copper in the body.

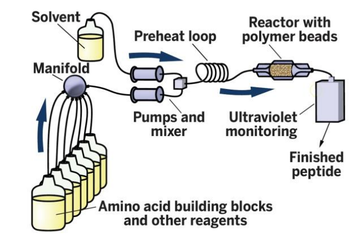

Figure 5: Process to Synthesizing a Peptide.[5]

With the initial testing of the bonding of La and Cu, the next key investigation is how La can bind to Copper in a biological setting.

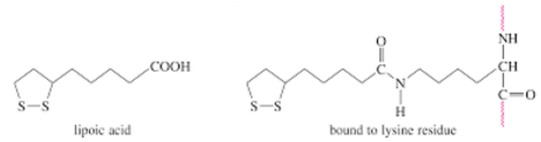

Figure 6: Addition of Lipoic Acid onto Lysine residue. The next steps to replicating a biological environment is to attach lipoic acid to the end of amino acid on a peptide, one example being lysine residue. Although the specific peptide has not been synthesized yet, this is just one example for the direction of this project. [6]

Future Directions

- Testing with reducing agents Ascorbate (Asc) and Nitrilotriacetic acid (NTA) were conducted and showed no indication that the DHLA-Cu Complex formed.
- La should be titrated only when already reduced by a reducing agent. Previous experiments show that La forms a stronger bond with Cu with these conditions.
- Smaller concentrations of Lipoic Acid should be used in order to investigate whether the peak in the La-Cu complex increases as it is hypothesized.

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