Structural and Thermodynamic Investigation of Pb^{2+} Binding to Human Cardiac Troponin C

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ABSTRACT

Toxic metal exposure is linked to a variety of health issues including cardiovascular disease and diabetes. It is known that toxic metals are capable of mimicking essential metals for binding sites in proteins. It is also known that both divalent cadmium (Cd^{2+}) and lead (Pb^{2+}) can disrupt Ca^{2+} signaling pathways. However, little else is known about how this happens at the molecular level. Research in the Spuches Lab is geared towards understanding metal toxicity from a structural and thermodynamic perspective. In this project, I will investigate Pb^{2+} binding to human cardiac troponin C (hcTnC), a Ca^{2+} binding protein that is responsible for heart muscle contraction. Isothermal titration calorimetry (ITC) studies of Pb^{2+} binding to wild-type and C35A/C84A N-domain hcTnC will be conducted to determine the stoichiometry of metal binding as well as ΔG, ΔH, and TΔS of the reaction. Circular dichroism (CD) experiments will also be used to probe the structural changes that occur upon Pb^{2+} binding to wild-type and C35A/C84A N-domain of hcTnC. My results will be compared to data obtained for Cd^{2+} in the Spuches lab in an effort to understand lead toxicity at the molecular level.

INTRODUCTION

The recent events in Flint Michigan highlight the persistent problem of Lead (Pb) toxicity in the United States.

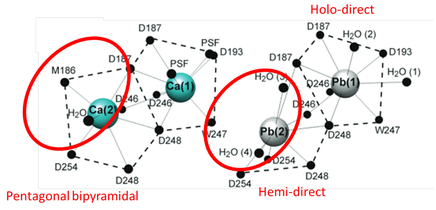
While the use of Pb was banned from paint and gasoline products in the mid 1970s, researchers are finding new areas of possible exposure. Studies conducted by the NC Department of Public Health (2018) and the New York City Department of Health and Mental Hygiene (2019) both suggested that spices can also be a cause of lead exposure. In 2018, researchers at Johns Hopkins first reported the high concentration of toxic metals present in e-cigarettes.

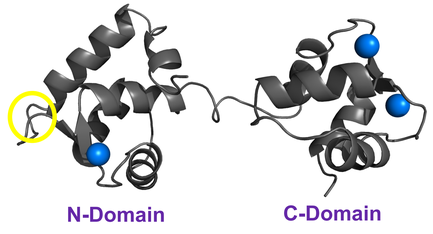
Chronic exposure to Pb can lead to severe neurological, cardiovascular, reproductive, skeletal, renal, and liver dysfunction in addition to developmental disabilities in children.

While it is known that toxic metals are capable of mimicking essential metals for binding sites in proteins, the identity of these protein targets are largely unknown.

MOTIVATION AND METHODS

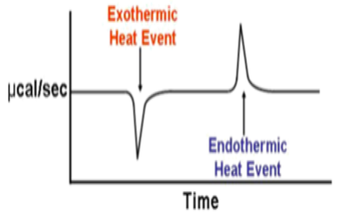
It has been found that both divalent cadmium (Cd^{2+}) and lead (Pb^{2+}) can disrupt Ca^{2+} signaling pathways. However, little else is known about how this happens at the molecular level.
Research in the Spuches Lab is geared towards understanding metal toxicity from a thermodynamic and structural perspective.

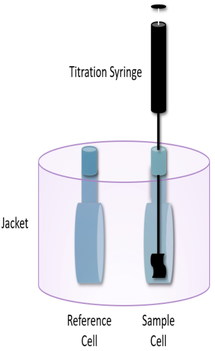


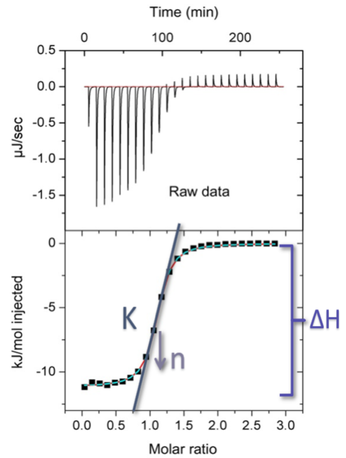


In this project, I will investigate Pb^{2+} binding to the human cardiac troponin C (hcTnC), A Ca^{2+} binding protein, and compare my results to data obtained for Ca^{2+} and Cd^{2+} in the Spuches lab to understand the molecular mechanisms of Pb toxicity.

ISOTHERMAL TITRATION CALORIMETRY (ITC)

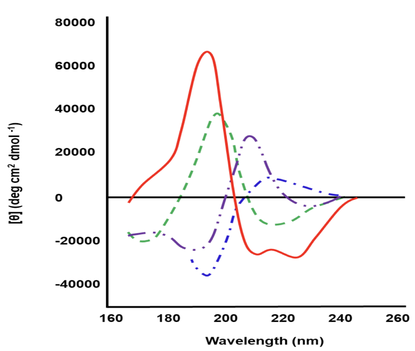






Representation of ITC schematic. ITC analysis provides a full thermodynamic analysis of a ligand-binding system, including the enthalpy, binding constant, and ligand stoichiometry.

CIRCULAR DICHROISM (CD)



Circular dichroism is a spectroscopic technique that uses linearly polarized light to probe the secondary structure of proteins.

Representative CD spectra of the structural curves for protein solutions containing mostly alpha helices (—), anti-parallel beta sheets (------), β-turn (—••—), or random coiled structures (—•—).

EXPECTATIONS

Isothermal Titration Calorimetry Experiments of Pb^{2+} binding to hcTnC will provide the following:

1. Stoichiometry of Pb^{2+} binding to the protein
2. The affinity, K_a, or how strong the interaction is between Pb^{2+} and the protein.
3. Provide us with the important thermodynamic parameters (G, H and TS) that can then be compared to other metals such as Ca^{2+} and Cd^{2+}.

Circular Dichroism will provide us with the following information:

1. Structural changes that occur upon metal binding.
2. Are these changes similar to or very different to Ca^{2+} and Cd^{2+}.

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