Bismuth Binding in Human Metallothionein

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METALLOTHIONEIN

Metallothionein (MT) is a cysteine rich protein with a molecular weight of 6 to 8 kDa [1]. Mammalian MT is found in the liver, kidneys, brain and central nervous system [2]. There are four isoforms of the metallothionein protein (MT1-MT4), but my project focuses on the MT1 isoform. MT1 has two domains. The beta domain has 9 cysteines, and the alpha domain has 11 cysteines [3].

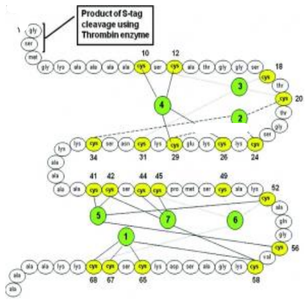

Figure 1. MT1 structure of seven metals bound to four cysteines. Cysteines are highlighted in yellow

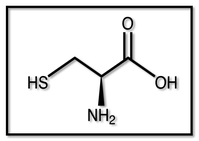

Figure 2. The cysteine amino acid

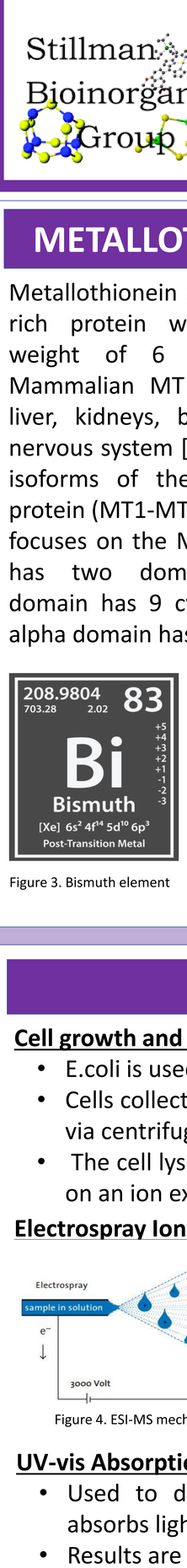

Figure 3. Bismuth element

BISMUTH

Bismuth is found in the 15th group of the periodic table and is classified as a heavy metal with an atomic mass of 208.98 Da. It is a therapeutic metal used in metallodrugs such as Pepto Bismol. Bismuth is one of the least toxic heavy metals [4].

METHODS

Cell growth and purification:

- E.coli is used to overexpress the human MT1 protein.
- Cells collected after they are separated from the broth via centrifuge.
- The cell lysate is collected by gravity filtration and put on an ion exchange column for purification.

Electrospray Ionization Mass Spectrometry:

Figure 4. ESI-MS mechanism

- Measures the charge-to-mass ratio of ions.
- Able to determine structural details of a species from charge states and changes in mass.
- Does not disrupt species.

UV-vis Absorption:

- Used to determine how much a chemical species absorbs light.
- Results are plotted in absorbance vs. wavelength.

METALS

Essential metals like zinc, copper and iron can be obtained through a healthy diet. These metals have significant importance to human health. Other metals, like bismuth, can be used in metallodrugs.

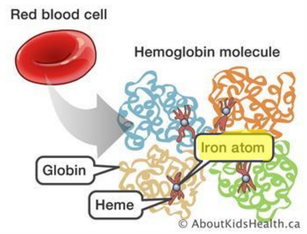

Figure 5. Hemoglobin molecule showing central iron atom

OBJECTIVE

My project investigates the binding pathway of bismuth into MT1 by using mass-spectrometry and UV-vis absorption.

RESULTS – ESI-MS

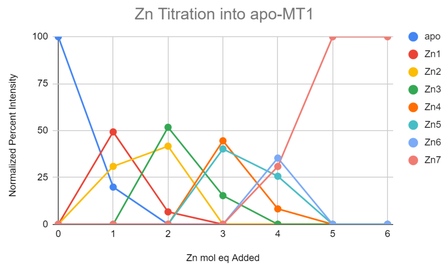

Figure 6. Zinc Titration into apoMT1 (0 – 6 eq)

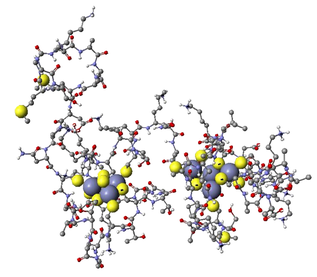

Figure 7. Structure of Zn_7MT

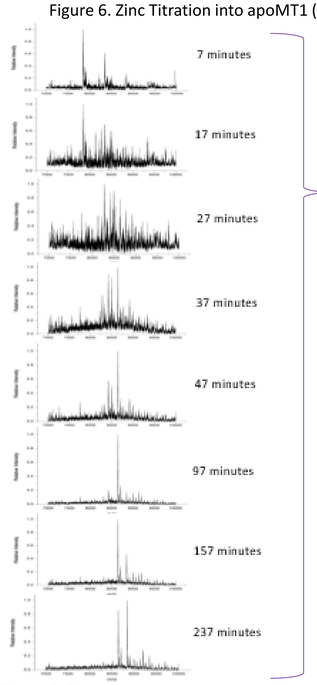

Figure 8. Deconvoluted spectra from the addition of $Bi[EDTA]^-$ to Zn_7MT

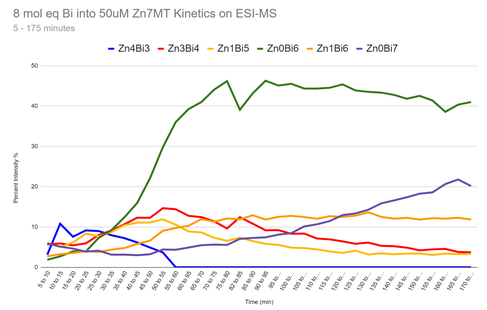

Figure 9. Reaction progression (5 to 165 minutes) when 8 mol eq. $Bi[EDTA]^-$ added to Zn_7MT

When bismuth is added to Zn_7MT it quicky displaces the zinc from the protein. Many species present for first 30 minutes of reaction.

Zn_7MT → Zn_4Bi_3 | Zn_3Bi_4 | Zn_1Bi_5 → Zn_0Bi_6 → Zn_0Bi_7

Step from Zn_0Bi_6 to Zn_0Bi_7 is much slower.

RESULTS – UV-VIS ABSORPTION

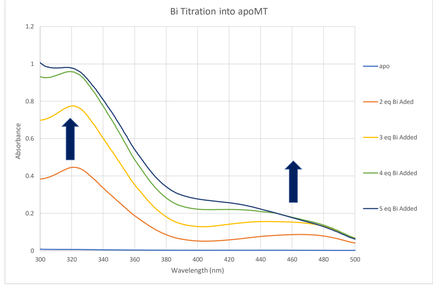

Figure 10. $Bi[EDTA]^-$ Titration into apoMT monitored by UV-vis spectrometry

A peak at 319 nm and at 466nm increase until 4 mol equivalents of $Bi[EDTA]^-$ are added to apoMT. The solution turns yellow as more bismuth is added.

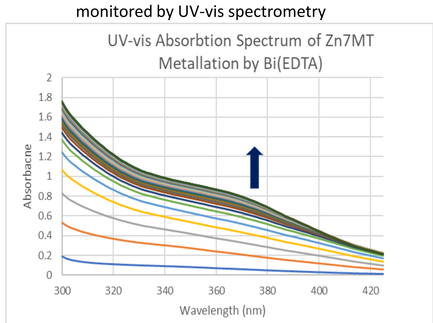

Figure 11. Kinetic absorbance spectrum of Zn_7MT with the addition of 10 mil eq. $Bi[EDTA]^-$

Ligand-to-metal-charge-transfer (LMCT) band confirmed at 380 nm when $Bi[EDTA]^-$ is added to Zn_7MT. This is the sulfur-bismuth band indicating that the bismuth is binding to the protein and displacing the zinc.

SUMMARY

- Pepto Bismol is an example of a metallodrug containing bismuth.
- Bismuth displaces zinc from MT1 stepwise, displacing 1 or 2 zinc at a time.
- LMCT band confirmed at 380 nm for Bi^{3+} – S

Figure 12. Pepto Bismol

REFERENCES

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5. Duncan E K Sutherland & M.J. Stillman. The "magic numbers" of metallothionein. *Metallomics,* 2011, **3,**444-463

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