Methanobactin Complexation of Group 12 Transition Metals

Global freshwater resources are under threat by pollution from nutrients and trace metals due to population growth, agriculture and industry. In particular, anthropogenic mercury (Hg) release greatly exceeds natural sources and is the second leading cause of impaired waterways in the United States (US EPA, 2011, 2013). Additionally, mercury can form a highly toxic compound known as methylmercury (MeHg) that bioaccumulates in aquatic food webs and can ultimately impact human and other wildlife. As part of the Oak Ridge National Laboratory (ORNL) Science Focus Area 'Biogeochemical Transformations at Critical Interfaces', research activity includes understanding the biogeochemical and microbial processes that control Hg speciation, transformation and availability in the environment, and the potential for microbial activity in bioremediation of sites high in toxic MeHg (as well as other heavy metal contaminants).

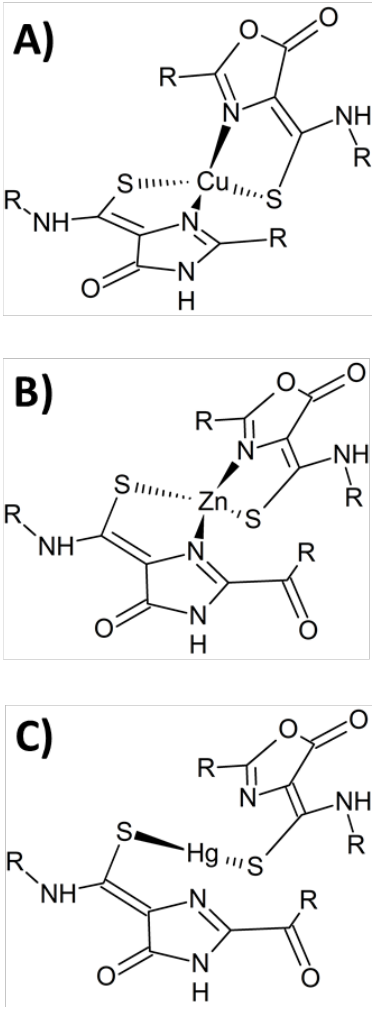

Fig 1. A) Coordination geometry of Cu:mb-SB2 observed in crystal structures. B) Proposed structure of Zn:mb-SB2 from spectroscopic data. C) Proposed structure of Hg:mb-SB2 from spectroscopic and EXAFS data.

The environmental speciation of Hg is modulated by interactions with strong ligands in dissolved organic matter and by interactions with bacteria. One common form of bacteria are methanotrophs, which metabolize methane. The activation of methane involves a methane monooxygenase enzyme, one form of which contains copper (Cu) (Fig. 1A), and methanotrophs facilitate the uptake of Cu from their environment by excreting a post-translationally modified Cu-binding metallophore known as a methanobactin (mb). Several forms of methanobactin are known, two of which are mb-OB3b and mb-SB2. Methanobactins interact strongly with most late transition metals, and recent laboratory studies have shown that methanobactins can effectively detoxify Hg-contaminated substrates and both inhibit and enhance the bacterial production of MeHg, depending upon the specific form of methanobactin studied.

A team of researchers led by Dr. Eric Pierce at ORNL, in collaboration with DOE-BER outreach staff at SSRL, investigated the complexation of late transition metals Zn^{2+}, Cd^{2+} and Hg^{2+} by mb-SB2. The approach combined absorbance, fluorescence and Extended X-ray Absorption Fine Structure (EXAFS) spectroscopies, with time-dependent density functional theory (TD-DFT) calculations for a detailed understanding of the organometallic complexation. At SSRL, Hg L3-edge EXAFS spectroscopy (at beamline 7-3) showed that Hg binds to available S ligands, but not to available N, in a linear 2-coordination site (Fig. 1C). Upon increasing the ratio of methanobactin:Hg, the co-ordination increases to a 2 and 3 mixed site. Importantly, the EXAFS data do not suggest the formation of a tetrahedral geometry that is present in Cu-methanobactin complexes. These findings are in line with the preference of Hg for thiol coordination, but raise

additional questions regarding the possibility of mixed-ligand complexes and their biochemical implications.

This study, published in the *J. Inorg. Biochem.*, is the first detailed spectroscopic and computational study of the complexation of Group 12 transition metals by methanobactin from *Methylocystis sp.* strain SB2. Not only does the team assess geometry and electronic structure changes under various ratios of methanobatin:metal, but also under various experimental timescales. Absorption and fluorescence spectroscopy indicate that when methanobactin is present in excess, dimeric complexes form between the transition metal and methanobactin which changes to monomeric complexation as the ratio of methanobactin decreases to equal that of the transition metal in solution (Fig 2B,C), in agreement with Hg EXAFS spectroscopy. The data also suggest a second, slower phase of complexation occurs over hours to days, which could be related to isomerization. The addition of TD-DFT calculations is imperative to interpreting the spectroscopic features observed here, since they were not yet characterized in the literature. This lays the groundwork for future studies on trace metal complexation with methanobactin from *Methylocystis sp.* strain SB2.

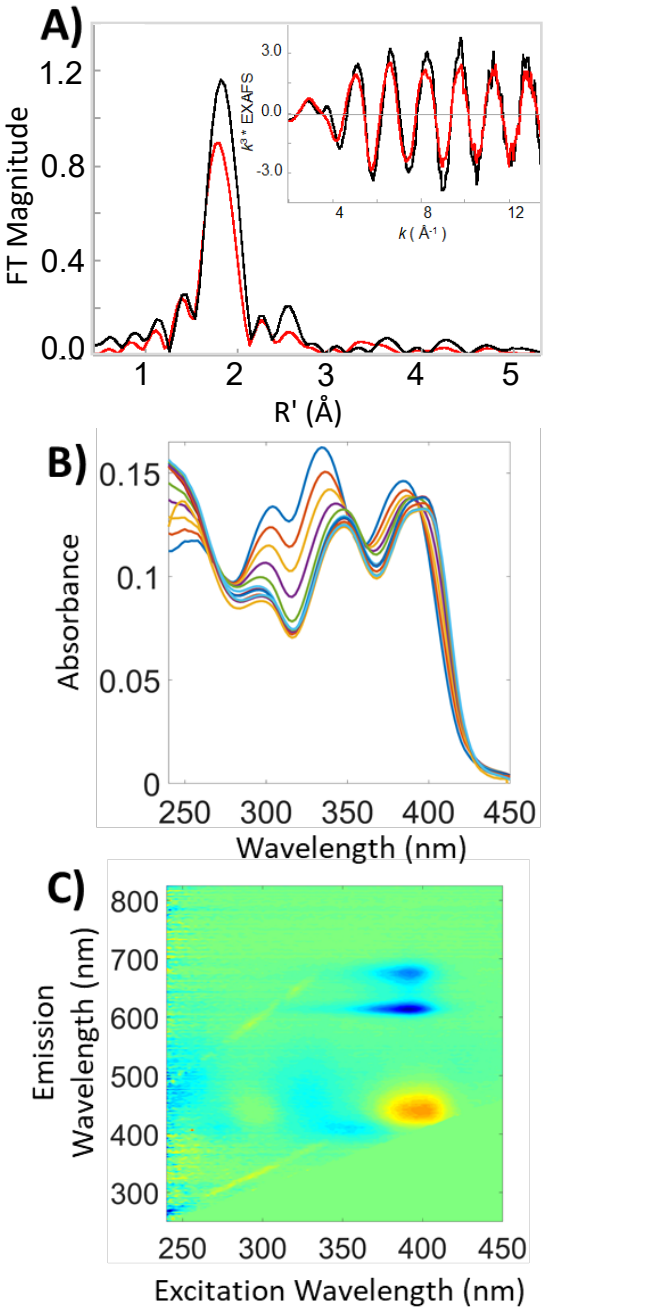

Figure 2: A) A comparison of the non-phase shift corrected Fourier Transforms of the Hg L3-edge EXAFS data for 1:1 (—) and 2:1 (—) mixtures of mb-SB2:Hg^{2+}. The inset shows the corresponding EXAFS comparison. B) Absorbance spectra of mb-SB2 titrated with 10 0.1 equivalents of Hg^{2+}. C) Difference fluorescence excitation- emission matrix of mb-SB2 and a 1:1 mixture of mb-SB2 and Hg^{2+}.

Primary Citation

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