Title: Structural and redox requirements for dioxygen generation coupled to metal reduction by methanobactins: implications for greenhouse gas emissions

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Project Goals: It is the general goal of this project to determine how microbial competition for copper affects net greenhouse gas emissions, specifically methane and nitrous oxide

Abstract Text: Methanobactins (MBs) are low molecular mass (<1,300Da), high-potential (E_m of 483 – 745mV), ribosomally synthesized posttranslationally modified peptides (RiPPs) and represent the extracellular component of a copper acquisition system in a variety of methanotrophs. MBs are characterized by a unique pair of heterocyclic groups with an associated thioamides and coordinate Cu via a N from each heterocyclic group and a S from each thioamide in an N_2S_2 ligand set. Structurally, MBs are divided into two groups with Group I MBs, represented by the MB from *Methylosinus trichosporium* OB3b (MB-OB3b), have a dicyclic structure while Group II MBs, represented by the MB from *Methylocystis* strain SB2 (MB-SB2), has a hairpin-like structure.

In addition to copper, MBs will bind a variety of metals and reduce some but not all following binding. In MB-OB3b, metals such as Cu^{2+} are bond and reduced to Cu^{+} using both oxazolone groups with associated thioamides. In the presence of a molar excess of Cu^{2+} MB-OB3b will also catalytically reduce Cu^{2+} to Cu^{1+}. MB-OB3b binds other metals, such as Fe^{3+}, as a dimer using the N-terminal oxazolone group and thioamide from each MB-OB3b. Both Cu^{2+} and Fe^{3+} bound by MB-OB3b show a similar N_2S_2 coordinated, however, only metals bound as monomers are reduced. In contrast to MB-OB3b, MB-SB2 coordinates all metals as a monomers and oxidized metals, including Fe^{3+}, are reduced following binding. As observed with MB-OB3b. in the presence of a molar excess of metal, MB-SB2 will catalytically reduce metals at a rate of approximately 1 electron • min^{-1} • MB-SB2. Following the loss of 4-5 electrons to metal reduction, MBs will oxidize $2H_2\mathbf{O}$ to $4H^+ + \mathbf{O_2}$ demonstrating a mechanism by which methanotrophs expressing MB can oxidize methane under anoxic conditions through "self-generation" of dioxygen required for the initial oxidation of methane to methanol ($CH_4 + \mathbf{O_2} \rightarrow CH_3\mathbf{O}H + H_2\mathbf{O}$). To initiate studies on the mechanism of water oxidation by MBs, generation of dioxygen following metal reduction is examined in holo-MBs as well as MBs minus the N-terminal or C-terminal heterocyclic groups. The results provide insights into the structural elements necessary to catalyze water oxidation by MBs.

Publications Resulting from this Project

1. Dershwiz P, Bandow NL, Yang J, Semaru JD, McEllistrem MT, Heinze RA, Fonseca M, Ledesma JC, Jennett JR, DiSpirito AM, Athwal NS, Hargrove MS, Bobik TA, Zischka H, DiSpirito AA. 2021. Oxygen generation via water splitting by a novel biogenic metal ion binding compound. Appl Environ Microbiol 87:in press.

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