59. Mechanism of Mercury Binding by Methanobactin from Methylocystis strain SB2

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Project Goals: Methanotrophs are ubiquitous in the environment, and despite their critical function in many different ecosystems, the biogeochemical factors that affect their activity and community structure are poorly understood. It is known that copper plays a key role in methanotrophic physiology, but the mechanism used by these microbes for copper acquisition was only recently discovered. This compound, methanobactin (mb), is the first example of a "copper-siderophore" or chalkophore. Mb binds many different metals, including mercury. Further, recent data show that different methanotrophs make different forms of mb that have varying metal affinities. The general objectives of this proposal are thus to consider how mb made by different methanotrophs alters the bioavailability of metals of concern to the DOE and how this affects: (1) the physiology, metabolism and gene expression in methanotrophs; (2) the broader microbial community structure and activity in laboratory soil columns, and; (3) the bioavailability of different metals in subsurface environments.

One of the persistent and substantial problems in remediation of hazardous waste sites is the mobilization and uncontrollable transport of radionuclides and heavy metals from these sites to surrounding areas. Some microbially-mediated processes can at least temporarily immobilize and reduce the toxicity of these materials through dissimilatory reduction that leads to precipitation and sorption under anaerobic conditions. As such, microbial-mediated processes can limit the dispersal of these materials and thus also limit the exposure of surrounding areas. Microorganisms, however, have effective and ubiquitous mechanisms to solubilize different metals and that non-specific binding of metals by these biogenic metal chelators may increase their solubility, mobility, and bioavailability. Here we are examining how the metal chelating agents analogous to siderophores in methane-oxidizing bacteria i.e., methanotrophs, binds copper and mercury individually and in mixed metal environments. Such studies will enable us to determine how methanotrophic activity may affect the copper and mercury mobility in subsurface waters, including at DOE sites.

Recent work in our laboratories has identified the genetic basis of mb and that many, but not all methanotrophs can synthesize mb (1-5). Interestingly, mb contains two heterocyclic rings, either imidazole (imi), oxazolone (oxa) or pyrazinedione (pyr) rings with an associated enethiol group, which together are responsible for metal binding (6-8). Given the structure of mb, it is quite possible that some if not all mbs can also bind toxic metals such as mercury and that mb made by one methanotroph may affect the bioavailability of metals to other methanotrophs. Our findings show that mb from *Methylosinus trichosporium* OB3b (mb-OB3b) does indeed bind mercury in addition to copper, and in doing so, reduced toxicity associated with Hg(II) to both α- and γ- Proteobacteria methanotrophs (9. At Hg to mb-OB3b ratios $\leq$ 1.0, Hg is coordinated via the two oxa rings and the associated enethiols groups. At Hg to mb-OB3b ratios $\geq$ 1.0, both oxa ring and associated enethiol group can each bind Hg separately. Interestingly, mercury binding by mb-OB3b was evident both in the presence and absence of copper, despite the fact that mb had a higher affinity for copper due to the rapid and irreversible binding of mercury by mb. Metal analyses indicated that Hg(II), after bound by mb- OB3b, may have been reduced to Hg(0) but was not volatilized. Rather, mercury remained associated with mb, and also was found

associated with methanotrophic biomass. It thus appears, although the mercury-mb complex was cell-associated, mercury was not removed from mb.

In this report, Hg binding by the structurally unique methanobactin from *Methylocystis* strain SB2 (mb-SB2) was examined and compared to mb-OB3b. Mb-SB2 is functionally similar (1,3), but structurally different (7) to mb-OB3b (6). In mb-SB2 one of the oxa rings is replaced by a imi ring and the redox active amino acids in mb-OB3b, Cys, Met, and Try, are replaced with Ala or are missing . Here mb-SB2 is shown to bind the common forms of Hg found in the environment, Hg^{2+} HgCN and CH_3Hg^+ as well as stimulate the solublization of metallic Hg. In general, the binding constrants and spectral; UV-visible absorption, fluorescent and circular dichroism, properties of mb-SB2 differed with different forms of Hg. Hg(II) and HgCN were coordinated by both oxa and imi ring and the associated enethiol groups whereas CH_3Hg^+ was coordinated via the oxa ring and both enethiol groups. The spectral, kinetic and thermodynamic changes following Hg binding by mb-SB2 also differed from the changes associated with mb-OB3b. Like mb- OB3b, copper did not displace Hg bound to mb-SB2 and was not volatilized. However, in constrast to mb-OB3b, mb-SB2 preferentially bound Hg over Cu in mixed metal environments. The perferential binding of Hg over Cu was related to the kinetics of Hg and Cu binding. The results suggest mb can bind Hg, even in environments with low Hg to Cu ratios.

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