Key elements of proteins involved in the bacterial production of the toxin methylmercury

Mercury is a prevalent global pollutant that has adverse effects on human health. This toxin is present in the environment not only as a result of releases during natural events, such as volcanic eruptions, forest fires and coal seam fires, but also as a result of anthropogenic mercury releases including fossil fuel based power plants, artisanal gold mining, nuclear weapons production, and cement production. Mercury in the environment can be converted to methylmercury, the most toxic form of mercury that people are exposed to through their diets. Methylmercury accumulates in the aquatic food chain and can be present in relatively high levels in fish products that humans consume.

For over 50 years it has been known that anaerobic bacteria are a major source of methylmercury, converting the mercury in their surroundings to this potent neurotoxin. Although the role of anaerobic bacteria as the source of methylmercury present in the environment has long been known, the enzymatic components of the mercury methylation reaction performed in the bacteria remained unknown until recently. It has been hypothesized that the methylation reaction proceeds through a mechanism that uses a cobalamin cofactor where the cobalt atom cycles between the Co(I) and Co(III) charged states, different than other known corrinoid dependent enzymes that transfer methyl groups. Now, the presence of two genes whose protein products appear to support this mechanism, *hgcA* and *hgcB*, has been shown to be directly correlated with the ability of a microorganism to convert mercury to methylmercury. The *hgcA* gene encodes a corrinoid protein hypothesized to be involved in the methyl transfer while the *hgcB* gene encodes a ferredoxin-like protein thought to be necessary for supplying reducing power to regenerate the Co(I) state of the cobalt in the corrinoid cofactor.

In this new work, we investigated key regions and structures of HgcA and HgcB proteins thought to be involved in the mercury methylation reaction. The HgcA protein contains a highly conserved “cap helix” sequence element that is hypothesized to interact with a corrinoid cofactor. Here we show that by disrupting this region of the HgcA protein with mutations that change the structure and/or chemistry, thereby inhibiting interaction with a corrinoid cofactor, the ability of the bacteria to produce methylmercury is lost. These results support the mechanism of a methyl transfer via interaction of the cap helix with a corrinoid cofactor.

We also probed the role of regions of HgcB predicted to be involved by introducing strategic mutations of selected cysteines in the peptide sequence. In addition to two iron-sulfur centers containing cysteines organized in a traditional [Fe-S] center motif, the ferredoxin-like HgcB protein sequence contains three other highly conserved cysteines; two at the extreme C-terminus (Cys95 and Cys96), and another slightly upstream from these two (Cys73). When Cys95 and Cys96 were mutated together to alanine the ability of the bacteria to form methylmercury was diminished. There was also a loss of methylmercury production when Cys73 alone was mutated to alanine.

These results support the role of these cysteines of HgcB in the methylation reaction, perhaps by helping HgcB deliver electrons to reduce the Co(III) to Co(I) in the reaction cycle.

These data and the mutants constructed for the experiments described here can be used in further research on the methylation pathway. Next steps will be to determine the source of the methyl group that is transferred to mercury and the nature of the corrinoid cofactor involved in the reaction. This new work begins to characterize the enzymatic mechanism of formation of toxic methylmercury found in the environment.

Steve D. Smith *and* ***Judy D. Wall***
Biochemistry Division, University of Missouri, Columbia, Missouri, USA

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