Insights into the Biochemistry of Microbial Mercury Methylation

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Microbial mercury methylation is an enzyme-catalyzed process carried out by certain anaerobic bacteria and archaea. The two-gene cluster *hgcAB* common is essential for mercury methylation. The genes encode a cobalamin-dependent protein, HgcA, and a ferredoxin, HgcB, which perform methyl transfer and cofactor reduction, respectively. Determining the interactions of HgcA and HgcB with other cellular components will provide greater understanding of methyl transfer mechanisms and the biochemical pathways involved in methylmercury production. The relationship between HgcA and HgcB and other metabolic pathways, such as one-carbon metabolism and electron transport, is of particular interest. These findings will provide essential data to inform metabolic and reactive transport models that can be used to predict mercury cycling from single organisms to ecosystems. Our studies will help understand the processes that control the fate and transformation of mercury in freshwater streams.

We have expressed HgcA and HgcB heterologously in *E. coli* and reconstituted both proteins with their respective cofactors. Changes in redox states of cobalamin and the 4Fe-4S clusters were characterized by UV-Vis spectroscopy. In collaboration with the Ragsdale lab at the University of Michigan, we are characterizing HgcA by EPR spectroscopy to investigate the coordination environment of the corrinoid cofactor in the Co(II) state. We are also investigating the transfer of electrons from pyruvate to HgcB facilitated by pyruvate ferredoxin oxidoreductase (PFOR). In an ongoing collaboration through a user proposal to EMSL (PNNL), we have collected two-dimensional NMR spectra to elucidate the structure of the cobalamin-binding domain of HgcA in solution. Insights obtained from these studies are complemented by structural bioinformatics to obtain a more complete picture at a systems level relevant to all Hg-methylating bacteria and archaea.