Biophysical characterization of HgcA, a protein required for the biosynthesis of methylmercury

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The biosynthesis of methylmercury has been associated with *hgcA* and *hgcB*, two genes unique to methylating bacteria in anaerobic environments. To date, homologs of these two genes have been identified in 72 bacterial species. The genes encode a corrinoid protein, HgcA, and a 2[4Fe-4S] ferredoxin, HgcB, consistent with roles as a methyl carrier and an electron donor required for corrinoid cofactor reduction, respectively. However, the role of HgcA and HgcB in the context of carbon and energy metabolism of anaerobic bacteria remains unclear. Early studies suggested that mercury methylation in sulfate reducing bacteria is linked to the reductive acetyl-CoA pathway and methyltetrahydrofolate (CH_3THF) as a methyl donor. Here, we investigate correlations between biochemical pathways relevant to carbon and energy metabolism in sequenced genomes of known methylators. Furthermore, multiple sequence alignments and homology modeling suggest an unprecedented coordination of a cysteine thiolate to the Co center of the corrinoid cofactor of HgcA. Heterologous expression of the cobalamin binding domain (CBD) of HgcA using fusion tags was performed to enhance solubility. Reconstitution of these fusion constructs with a cobalamin cofactor *in vitro* enables structural characterization by NMR and X-ray crystallography. Axial ligand coordination and changes in the oxidation state of the corrinoid cofactor are reflected in characteristic changes in UV-Vis absorption spectra. In concert with computational studies, biophysical and structural characterization of HgcA will enable a mechanistic description of methylmercury biosynthesis in methylating bacteria.