The Molecular Basis of Mercury Methylation: Expression, Purification and Characterization of HgcA

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Mercury (Hg) is a pervasive global pollutant which, as methylmercury (MeHg), bioaccumulates in the food web and is highly toxic to humans and other organisms. Unlike inorganic forms of mercury, which originate from atmospheric deposition and point discharges, MeHg is generated in the environment predominantly by anaerobic microorganisms. The biosynthesis of methylmercury has been associated with hgcA and hgcB, two genes unique to methylating bacteria in anaerobic environments. Homologs of these two genes have been identified in 72 bacterial species with sequenced genomes. The genes encode a corrinoid protein, HgcA, and a 2[4Fe-4S] ferredoxin, HgcB, consistent with roles as methyl carrier and electron donor required for corrinoid cofactor reduction, respectively. However, the specific role of HgcA and HgcB in the context of carbon and energy metabolism of anaerobic bacteria remains unclear. Homology modeling suggests an unprecedented coordination of a cysteine thiolate to the Co center of the corrinoid cofactor of HgcA.

The HgcA cobalamin binding domain (CBD) was obtained by heterologous expression in E. coli using four separate fusion tags to identify a construct with enhanced solubility. After purification by ion exchange chromatography and gel filtration under strictly anaerobic conditions, the HgcA-CBD was reconstituted with its cobalamin cofactor in vitro. The reconstituted protein is subject to biophysical characterization, including UV-Vis, CD, EXAFS and EPR, to investigate its overall structure, identify axial Co coordination ligands and redox chemistry. Recently, we have obtained first samples of isotopically labeled (13C, 15N) HgcA-CBD for structure determination by NMR spectroscopy at EMSL. In concert with computational studies, the biophysical, functional and structural characterization of HgcA will advance our understanding of the molecular mechanisms leading to the biosynthesis of toxic MeHg by methylating bacteria.