Reconstitution and spectroscopic characterization of the corrinoid protein HgcA

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Mercury methylation is an enzyme-catalyzed process associated with the metabolic activity of anaerobic bacteria and archaea. It has been shown that a two-gene cluster is required for mercury methylation in *Desulfovibrio desulfuricans* ND132 and *Geobacter sulfurreducens* PCA. The genes encode a cobalamin-dependent protein, HgcA, and a 2[4Fe-4S] ferredoxin, HgcB, consistent with roles as a methyl carrier and electron donor required for cofactor reduction, respectively. Among bacteria and archaea with sequenced genomes, gene orthologs are present in all confirmed methylators but are absent in nonmethylators, suggesting a mercury methylation pathway common to all methylating bacteria and archaea.

The current focus of our studies is on the cobalamin binding domain of HgcA (HgcA-CBD), as it may participate in catalyzing the transfer of a methyl group to a Hg(II) species. HgcA-CBD was expressed heterologously in *E. coli* as a maltose-binding protein fusion to enhance solubility. After purification by ion-exchange chromatography and gel filtration under strictly anaerobic conditions, we characterized the binding of cobalamin to HgcA-CBD using UV-Vis spectroscopy. HgcA-CBD binds one equivalent of cobalamin *in vitro* with a typical cofactor occupancy of >90%. The reconstituted protein was characterized by cyclic voltammetry and UV-Vis spectroscopy to investigate redox potentials and redox states relevant for activity. The spectroscopic data are consistent with coordination of a Cys thiolate to the cobalt center of the cofactor in HgcA. Cyclic voltammetry indicates that the midpoint potential for the Co(II)/Co(I) couple is close to -560 mV (vs NHE). The present results suggest that, in addition to a methyl donor, a continuous source of low-potential electrons is required for continued activity of HgcA *in vivo*. To determine the structure of HgcA-CBD at atomic resolution by NMR spectroscopy in collaboration with EMSL, we have collected preliminary data of isotopically labeled (^{13}C, ^{15}N) HgcA-CBD. Complemented by structural bioinformatics and computational studies, the spectroscopic and structural characterization of HgcA will provide unprecedented insights into the biochemical mechanism and metabolic role of HgcA in Hg-methylating bacteria and archaea.