High-Resolution Mass Spectrometric Analyses of Mercury-DOM Complexation and Cellular Response of *Geobacter sulfurreducens* PCA Following its *hgcAB* Gene Deletion

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Naturally dissolved organic matter (DOM) forms strong complexes with mercury (Hg), affecting Hg chemical speciation, transformation, and bioavailability in aquatic environments. However, exact DOM molecular compounds that react with Hg remain unclear because of the heterogeneous nature and complex composition of DOM. We applied Fourier transform ion cyclotron resonance mass spectrometry coupled with electrospray ionization (ESI-FTICR-MS) to directly probe the formation of Hg-DOM complexes and compositional changes of DOM following its interactions with Hg. Hg-containing DOM molecules were directly identified, with their molecular formulae confirmed by corresponding Hg isotopic peaks present in the mass spectra. A common moiety of $-N_2S_4$ was found in most of the identified Hg-containing DOM formulae, indicating high affinity interactions between Hg and S- and N-containing functional groups in DOM. Most of the "lost" and "new" DOM molecular formulae after reaction with Hg were found to contain S, N, and O, consistent with the view that Hg(II) forms strong complexes through a two-fold coordination involving one reduced S and one O or N in DOM.

Meanwhile, mass spectrometric shotgun proteomics was used to identify differences in the proteome expression between wild-type *Geobacter sulfurreducens* PCA and a Δ*hgcAB* mutant, which is deficient in Hg-methylation genes, *hgcA* and *hgcB*, and between wild type and a Δ*omcBESTZ* mutant, which is deficient in five outer-membrane *c*-type cytochromes OmcB, OmcE, OmcS, OmcT, OmcZ, required for dissimilatory metal reduction. We were able to delineate the global response of *G. sulfurreducens* PCA in both mutants and identify cellular networks and metabolic pathways that were affected by the loss of these genes. Deletion of *hgcAB* increased the expression of most *c*-type cytochromes, consistent with our previously observed increase in Hg reduction in the Δ*hgcAB* mutant. Deletion of *omcBESTZ* was found to increase expression levels of various methyltransferases, indicating that a loss of dissimilatory reduction capacity resulted in elevated activity among one-carbon metabolic pathways. This result also agrees with experimentally determined high rates of Hg methylation by the Δ*omcBESTZ* mutant. Additionally, enzymes associated with the folate branch were found to be under-represented in the Δ*hgcAB* mutant strain relative to the wild type, which supports the hypothesis that the function of HgcA and HgcB may be linked to one carbon metabolism through the folate branch of the Wood-Ljungdahl pathway by providing methyl groups required for Hg methylation.