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Unraveling the Cellular Biochemistry of Mercury Methylation

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Microbial mercury methylation is an enzyme-catalyzed process carried out by certain anaerobic bacteria and archaea. A two-gene cluster *hgcAB* is essential for mercury methylation and encodes a cobalamin-dependent membrane protein, HgcA, and a ferredoxin, HgcB, which are predicted to facilitate methyl transfer and cofactor reduction, respectively. The primary focus of our research is to determine the kinetics of the intracellular methylation reaction and rate-limiting steps. Additionally, we aim to identify metabolites and protein-protein interactions essential for the function of HgcA and HgcB. The metabolic pathways necessary for methylmercury (MeHg) formation by HgcA and HgcB are currently not well understood. To address this knowledge gap, we investigated one carbon metabolism, transport mechanisms and electron donors/acceptors in a series of methylating bacteria and archaea to gain insights into the cellular machinery associated with MeHg formation and to develop experimentally testable hypotheses. Furthermore, we developed a mercury methylation assay, which allows us to measure MeHg production in cell lysates of *Desulfovibrio desulfuricans* ND132 independent of cellular uptake processes. Our experimental results demonstrate that the competing abiotic Hg methylation reaction with methylcobalamin is negligible at neutral pH values (i.e., physiological conditions), which further illustrates that the catalytic effect of HgcAB is required in the biotic reaction. We have also determined the pH and temperature dependence of the biotic methylation reaction and calculated kinetic parameters and rates for the conversion of Hg(II) to MeHg using the Michaelis-Menten formalism. The concentrations of several intracellular components were varied to identify potential rate-limiting metabolites. Our collaborators at the University of Michigan expressed HgcB heterologously as a maltose binding protein fusion construct for characterization by spectroscopic methods and Hg(II) binding experiments. An ongoing Environmental Molecular Sciences Laboratory user proposal aims to identify conditions for HgcB structure determination by solution nuclear magnetic resonance spectroscopy. A detailed understanding of factors controlling the production of MeHg will improve the accuracy of predictive models and facilitate the development of strategies to reduce exposure to this pervasive neurotoxin.