

Biomolecular Aspects of Mercury Transformations

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Bacteria participate significantly in mercury transformation in natural and contaminated industrial environments. Bacterial mercury resistance is mediated by the *mer* operon, typically located on transposons or plasmids. It encodes specific genes that facilitate uptake of mercury species, cleavage of organomercurials (MerB), and reduction of Hg(II) to Hg(0). Bacterial reduction of Hg(II) can also occur at concentrations too low to induce *mer* operon function by a dissimilatory metal-reduction mechanism.

Expression of *mer* genes is mediated by MerR, a metal-responsive transcriptional regulator. *In vitro* studies have shown that MerR forms a non-transcribing pre-initiation complex with RNA polymerase and the promoter DNA. Binding of Hg(II) induces conformational changes in MerR and other components of the complex, resulting in the transcription of *mer* operon genes. We have used small-angle scattering techniques to study the regulatory mechanism of MerR in the presence and absence of Hg(II). Our results show that in the presence of Hg(II) the MerR dimer undergoes a significant reorientation from a compact state to a conformation revealing two distinct domains. Molecular dynamics simulations on MerR homology models can be combined with molecular envelope shapes obtained from small-angle scattering experiments to yield detailed structural information. Molecular mechanics (Charmm22) force field parameters have been developed for simulating the trigonal planar Hg(II) coordination geometry observed in Hg(II)-bound MerR.

The bacterial organomercurial lyase, MerB, catalyzes the demethylation of a wide range of organomercurials via Hg-C protonolysis. We have investigated the two major proposed reaction mechanisms of MerB using quantum chemical density functional theory calculations. A model of the active site was constructed from an X-ray crystal structure of the Hg(II)-bound MerB complex. The calculations support a mechanism in which Cys159 forms an initial covalent adduct with MeHg. Cys96 donates a proton to Asp99 and coordinates with Hg(II). Asp99 then protonates the organic leaving group to cleave the Hg-C bond and release the hydrocarbon

reaction product. Two other substrates, vinylmercury and *cis*-2-butenyl-2-mercury, were also tested, and the computed activation barriers for the three organomercurial substrates reproduces the trend in experimentally determined reaction rates.

Dissimilatory metal-reducing bacteria, such as *Shewanella* and *Geobacter* are able to reduce Hg(II) in the presence of mineral oxides. This process has been linked to the activity of outer-membrane multiheme cytochromes. We have isolated and purified the decaheme outer-membrane cytochrome OmcA from *Shewanella oneidensis* MR-1 and characterized its envelope shape in solution by small-angle X-ray scattering (SAXS). Low-resolution structural features were identified and indicate an elongated shape. X-ray crystallography trials are currently underway in an effort to identify potential molecular interaction sites for Hg(II) reduction.

Future efforts of this Science Focus Area task at ORNL will continue to gain fundamental understanding of subcellular processes that profoundly influence mercury speciation and to determine mechanisms at atomic detail concerning mercury transformation using structural biology and high-performance computer simulation.