Molecular Structure and Dynamics of Mercury Biotransformations

ORNL SFA (Laboratory Research Manager: Liyuan Liang)

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As a part of the ORNL SFA, this study emphasizes subcellular processes, including transfer of Hg(II) between specific enzyme components as well as enzyme-catalyzed reactions involved in bacterial Hg resistance and methylation. The initial focus has been on the biomolecular structure and solution conformations of several key proteins and enzymes, which confer mercury resistance in bacteria that impact mercury speciation and bioavailability in the environment. The expression of mercury resistance genes in the *mer* operon is controlled by the metalloregulator MerR at the level of transcription. Previous *in vivo* and *in vitro* biochemical work showed that binding of Hg(II) by MerR induces a significant conformational change that ultimately results in the transcription of the structural genes. Although structures exist for several activated MerR-family regulators, no structure has been determined for MerR or any non-activated MerR-family protein. Small-angle X-ray scattering (SAXS) on apo- and Hg(II)-bound forms of MerR in aqueous solution revealed that specific binding of Hg(II) transforms apo-MerR from a flattened, compact state into an elongated conformation that ultimately initiates transcription by RNA polymerase. Molecular dynamics (MD) simulations were performed on a homology model of MerR-Hg(II) to characterize the conformational dynamics of Hg(II)-MerR. MD revealed large amplitude interdomain motions involving fluctuations on the nanosecond time scale. The nature of these motions suggests a role in propagating allosteric changes from the metal-binding site to the DNA-binding site.

The mercuric reductase MerA catalyzes the reduction of Hg(II) to Hg(0). Each monomer of the MerA homodimer consists of a catalytic core domain, a flexible linker, and an N-terminal domain, NmerA, which acquires Hg(II) and transfers it to an active site in the core homodimer for reduction. Using SAXS and molecular dynamics simulations, we have studied the structure and dynamics of MerA to elucidate the role of its N-terminal domain.

Bacterial reduction of Hg(II) can also occur distinct from *mer* operon activity by a dissimilatory reduction mechanism in metal-reducing bacteria such as *Shewanella* and *Geobacter*. We hypothesize that this process is 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 determined its solution structure by SAXS. Neutron reflectometry revealed its interaction with hematite surfaces, where it assembles into a monomolecular layer. We have also obtained x-ray diffraction data of OmcA to a resolution of 2.6 Angstrom. Reduction experiments *in vitro* have shown evidence for a direct electron transfer between Hg(II) and OmcA. Ongoing studies focus on the Hg(II) reduction capacity of OmcA *in vivo*.

Future efforts of this SFA Task will address atomic and subcellular mechanisms relevant to mercury biotransformation using structural biology and computer simulations. This work will probe macromolecular dynamics, enzyme reaction pathways, and mechanisms of intracellular mercury transfer to gain a fundamental understanding of biomolecular processes impacting mercury speciation.