Molecular Mechanisms of the *mer* Operon and Hg(II)-Ligand Interactions: Combined Experimental and Computational Studies

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Mercury resistant bacteria exert a strong influence on lowering toxic methylmercury levels at contaminated sites, with the proteins and enzymes of the *mer* operon functioning as an efficient detoxification system. The *mer* operon confers resistance to mercury by encoding specific genes that facilitate uptake of mercuric species, Hg-C cleavage of organomercurials, and reduction of Hg(II) to Hg(0). Combined experimental and computational approaches are used to investigate molecular mechanisms of the *mer* operon, including intramolecular transfer and reduction of Hg(II) by the mercuric reductase MerA, the roles of specific amino acids in the conformational transitions in MerR triggered by Hg(II) binding, and the energetics of Hg(II)-ligand binding interactions.

MerR responds specifically to nanomolar levels of Hg(II) by activating the transcription of *mer* genes. Binding of Hg(II) to MerR was found previously to induce a significant reorientation of the two DNA-binding domains relative to each other, which leads to underwinding of operator DNA followed by transcription. Four MerR mutants, Y27F, Y46F, K99T, and M106I, have been expressed and purified, and SAXS experiments are being performed to characterize the effects of these point mutations on the conformation of MerR. The results will reveal the roles of these key amino acids in propagating allosteric changes at the Hg(II) binding site to the DNA binding domains. MerA converts Hg(II) to less toxic Hg(0), and a pair of cysteines, C464 and C465, is involved in delivering Hg(II) to another pair of cysteines, C42 and C47, at the active site prior to reduction. The mechanisms of intramolecular Hg(II) transfer in MerA are not fully understood, as they are likely to be coupled to specific protonation/deprotonation events. A multi-step quantum mechanical/molecular mechanical (QM/MM) approach is applied to explore and compare the energetics of several possible Hg(II) transfer pathways. Multiple protonation states are considered, and the roles of various active site residues and solvent molecules are hypothesized. Solvation is expected to contribute significantly to the interaction energies involving Hg(II) and affect the relative stabilities of different states during the allosteric transition in MerR and the intramolecular Hg(II) transfer in MerA. However, such information has been lacking. To further determine the effects of solvation on the interactions involving Hg(II), density functional theory and polarizable continuum models are used to compute aqueous binding free energies for a number of complexes containing Hg(II). Excellent agreement with relative experimental data is achieved for both hydration and ligand binding free energies. The standard deviation of the average error (STDEV) in the binding free energies is around 1 kcal mol^{-1} using Solvent Model D (SMD) with two explicit water molecules for hydration of anions and Hg(II)-ligand complexes. The binding free energies are analyzed in terms of local and long-range contributions. It is demonstrated that solvation provides a key driving force for effectively enhancing the interactions between Hg(II) and functional groups in low-dielectric media.