Solubility optimization and structural characterization of the Hg(II)-specific transcriptional regulator MerR in complex with its operator DNA

Microbes have evolved mechanisms to prevent toxic accumulation of nonessential metal ions or excesses of essential metal ions within the cell. Mercury (Hg) is a heavy metal that serves no known biological function and is toxic to all living organisms. The *mer* operon is found in bacteria from contaminated environments and confers resistance to toxic levels of Hg. The genes in the *mer* operon translate into a complement of proteins which capture and transport Hg species inside the cell, cleave organomercurials such as highly toxic methylmercury, and reduce Hg(II) to relatively benign Hg(0). MerR is a Hg(II)-dependent repressor-activator which controls the transcription of the structural genes of the *mer* operon. The purpose of this study was to describe allosteric conformational changes that dimeric MerR and its operator DNA undergo upon Hg(II) binding.

A complex of MerR with a 23 bp dsDNA construct representing its operator DNA (MerOP) was prepared using excess MerOP. The MerR-MerOP complex was purified using gel filtration chromatography and a comprehensive solubility optimization was performed. After controlled precipitation of the complex using polyethylene glycol, aliquots of the concentrated, precipitated complex were incubated with a variety of salt, buffer, and additive components. The effect of each component on re-solubilization was determined using a Bradford protein concentration assay, and a solubility-optimized buffer was identified, which was used for all subsequent experiments. In order to study conformational changes after binding of Hg(II), half of the purified MerR-MerOP complex was incubated with Hg(II) and both complexes (Hg-MerR-MerOP and MerR-MerOP) were concentrated to ~10mg/mL. Dynamic light scattering (DLS) experiments confirmed monodispersity of MerR-MerOP and Hg-MerR-MerOP at high concentrations. Preliminary analysis of small-angle neutron scattering (SANS) data indicates that binding of Hg(II) results in a structural change of MerOP within the complex. The data are consistent with *in vivo* and *in vitro* footprinting data suggesting that binding of Hg(II) to MerR initiates an allosteric conformational change in the MerR DNA binding domains, which consequently underwind MerOP to initiate transcription by RNA polymerase. Our results may thus represent the first direct observation of DNA underwinding induced by a transcriptional regulator.