Solubility Optimization of the Hg(II)-Specific Transcriptional Regulator MerR for the Study of Molecular Structure and Dynamics

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Mercury (Hg) is a widely found contaminant in the environment and is of particular interest as methyl mercury can enter the food chain and bioaccumulate in higher organisms. Heavy metals such as Hg have no known biological function and are toxic to all living organisms. However, microbes have evolved naturally to deal with heavy metal toxicity resulting in elaborate heavy metal resistance mechanisms. Select bacteria possess specific genes that allow cells to resist poisoning of toxic metals by using multiprotein resistance systems under the control of metal-responsive transcriptional regulators. The transcriptional repressor-activator, MerR, is the archetype of the MerR family of metalloregulators that controls the transcription of a set of genes (the *mer* operon) providing Hg resistance in many genera of bacteria isolated from Hg-exposed ecosystems. The *mer* operon encodes specific genes that facilitate transport of Hg species, cleavage of organomercurials and reduction of ionic Hg(II) to volatile, elemental Hg(0). The 144-residue MerR binds to its operator DNA and functions as a homodimer. It represses transcription of the *mer* operon in the absence of Hg(II) and activates transcription upon Hg(II) binding. Although a wealth of genetic, biochemical, and biophysical data exist for MerR and several structures are known for paralogous members of the MerR family specific for other metals, to date the three dimensional structure of MerR is unknown. Previous attempts at structure determination of MerR using X-ray crystallography and Small Angle Neutron Scattering (SANS) have been hampered by low protein solubility. Here we present our strategy for overcoming these solubility issues by screening a range of discrete conditions including pH, cations, anions and other additives, to optimize the solubility of MerR. We have successfully obtained a customized formulation that enhances the solubility of Hg(II)-MerR and Hgfree-MerR by factors of 20 and 15, respectively. Dynamic Light Scattering (DLS) showed that samples prepared in the respective optimized buffer conditions are monodisperse. Initial sparse matrix vapor diffusion crystallization screening of both Hg(II)-MerR and Hg-free-MerR using the optimized buffer formulation revealed multiple crystallization conditions that are currently being optimized to produce diffraction quality crystals for X-ray studies. We anticipate that this solubility optimization strategy will facilitate our current efforts to solve the first X-ray crystallographic structure of MerR. The increased solubility will also enable use of neutron scattering to study MerR dynamics and conformational changes that are essential for understanding its unique mechanism of transcriptional regulation.