Protein Engineering in the Hg(II) Resistance (*mer*) Operon

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NABIR research objectives. The widely found bacterial mercury resistance (*mer*) operons functions in Hg biogeochemistry and bioremediation by converting reactive inorganic [Hg(II)] and organic [RHg(I)] mercurials to relatively inert monoatomic mercury vapor, Hg(0). We study its metalloregulator, MerR, and the two enzymes which mediate these processes, MerA, the mercuric reductase, and MerB, the organomercurial lyase to dissect their basic mechanisms and accelerate their in vivo and in vitro performance.

Results and plans. Cytosolic expression of an engineered single polypeptide (107aa) antiparallel ("hairpin") coiled-coil metal binding domain (MBD) derived from MerR protects *E. coli* (1,2) and *Deinococcus* (2) from Hg(II). Outer-membrane expression of MBD also protects *E. coli* (2). MerR and MBD bind other thiophilic metals with unique coordination geometries distinct from S_3 coordination they use for Hg(II), and they exhibit partly overlapping metal preferences, suggesting enhanced flexibility in MBD. This work defines a novel metallosequestration domain and establishes that MerR's specificity in transcriptional activation does not reside just in metal binding. Near-term work will complete determinations of kinetic and thermodynamic parameters of metal interactions of both proteins. This will be followed by analysis of point mutants with altered metal specificity to define the metal-driven allosteric responses of the MerR coiled-coil and to discern at what steps in transcriptional activation metals are distinguished. We also continue efforts on 3-D structure determination of MerR.

MerB. With UGA crystallographer Cory Momany, we are currently analyzing a data set for MerB-Hg collected to 2 Å (7% Rsym) at the Advanced Photon Source. It has two clear Hg sites, consistent with 2 molecules per asymmetric unit. We expect this data set to provide an initial MerB 3-D structure but will continue growing crystals for both MerB and MerB-Hg (and other metals) as backups.

MerA. The plasmid- and transposon-borne *mer* operon functions in many different cytosolic environments. With Sue Miller (UCSF) we're dissecting its dependence on the cytosolic redox buffering system, which varies widely among prokaryotes. We've found that in *E. coli* lacking its major cytosolic thiol buffer, glutathione (GSH), the specific activity of Hg(II)-reductase (MerA) in cells expressing the complete *mer* operon (MerA plus transport proteins MerTPC) declines by 25%, compared to the parental strain with a consequent 50% decline in the Hg resistance phenotype compared to the wild-type parental strain. In GSH cells expressing only MerA or its catalytic core domain (which lacks the N-terminal metal chaperone domain) the specific activity of both forms of the enzyme declines by ca. 55%, indicating that the metal chaperone domain of MerA cannot compensate for a lack of cytosolic low molecular weight thiols. We have also characterized intracellular reduction of Ag(I) and Au(I/III) by wild-type *E. coli* expressing the complete *mer* operon, and are extending this work to metals of DOE interest, including Co and Cr.

1. Song, L. Caguiat, J., Li, Z., Shokes, J., Scott, R.A., Olliff, L. and Summers, A.O. (2003). Engineered single chain, antiparallel, coiled coil mimics MerR metal binding site. J. Bacteriol., in press. 2. Qin, J., Song, L., Brim, H., Daly, M., and Summers, A.O. (2004). Engineered Tandem MerR Metal Binding Domain Functions on Cell Surface, submitted to AEM.