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

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NABIR Research Objectives. Proteins of the widely found bacterial mercury resistance (*mer*) operons convert reactive inorganic [Hg(II)] and organic [RHg(I)] mercurials to relatively inert monoatomic mercury vapor, Hg(0). We study the metalloregulator MerR, and the two enzymes that mediate this process: MerA, the mercuric reductase; and MerB, the organomercurial lyase.

Results and Plans. We have generated a single polypeptide (107aa), which contains the coiled-coil metal binding domain (MBD) of the MerR homodimer. In whole cells, and also in purified in vitro cells, MerR and MBD bind several thiophilic metals and metalloids (1). XAFS data show that MBD forms a HgS_3 structure like MerR, and both form a CdS_4 structure; the source of the 4th S may be a solvent thiol. ICP-MS stoichiometry is 1 for both proteins. We have fused the MBD protein to Lpp-OmpA; fluorescent antibody, protease sensitivity, and antibody pull-downs indicate that MBD is surface displayed at ~20,000 copies per cell, providing >600% increase in Hg(II) binding over uninduced cells. Near-term plans include kinetic and thermodynamic studies of MerR and MBD interactions with Hg, Cd, Co, Pb, and U using equilibrium and nonequilibrium dialysis, isothermal titration calorimetry, and various spectroscopies with colleagues in the UGA Center for Metalloenzyme Studies. We can also now regularly get MerR to form small crystals and will use various strategies to get them large enough for a run at APS.

MerB. The 4 cysteines of the 212-amino-acid organomercurial lyase MerB have distinct roles in protonolysis; and the protein itself, not the solvent, is the immediate source of the proton (2). Formation of a Hg(II)-trapped state of MerB is the basis for its requirement of a 2-fold molar excess of thiol (2). We have cryostabilized crystals of MerB, collected 3.3 Å data at UGA, and will be collecting data at APS in February with UGA crystallography collaborator Cory Momany.

MerA. With Sue Miller (UCSF), we are defining the role of the mobile N-terminal domain of MerA (NmerA) by expressing full-length MerA and the catalytic Core with or without an independently expressed NmerA domain in wild-type (WT) *E. coli* and in strains lacking the ability to make glutathione (GSH), the major cellular thiol buffer. These constructs are stable and express well in GSH^- strains. We are now comparing Core and Core+NmerA with WT MerA in Hg resistance and in ^{203}Hg volatilization in WT *E. coli* and in GSH^- strains. We hypothesize that Core alone cannot compete for Hg(II) coordinated to other thiols, but that it requires the mobile NmerA domain to pluck Hg(II) from GSH or cytosolic proteins and to present it to the active site. So we expect that in GSH^- cells, Core will be slow in Hg volatilization compared to WT MerA; and that NmerA, even as a separate protein, will repair Core's defect. Understanding this "bucket brigade" mechanism in MerA will illuminate efforts to broaden MerA's substrate range to radionuclides of interest to DOE and the role(s) of these common N-terminal domains in general.

1. Song, L. (ASM Student Travel Awardee), Caguiat, J., and Summers, A.O. (2002). Poster Q180, 102nd General Meeting of the ASM, Salt Lake City, Utah, May 2002.
2. Pitts, K.E. and Summers, A.O. (2002). The roles of thiols in the bacterial organomercurial lyase (MerB). *Biochemistry*, **41**:10287–10296.