Abstract

Mercury is introduced into the environment from either natural occurrences (volcanoes) or from human activities (combustion of fossil fuels). Once mercury is introduced into the environment, mercury undergoes a complex geochemical cycle and is converted to both inorganic and organic forms. The three inorganic forms of mercury are elemental mercury (Hg^0), mercurous ions (Hg^I), and mercuric ions (Hg^{II}), and the most abundant organic form of mercury is methylmercury (MeHg). Organomercurial compounds like MeHg are the most toxic form because of their hydrophobicity and the increased toxicity is believed to be associated with the ability of organomercurials to efficiently permeate membranes and bioaccumulate in organisms. High levels of MeHg have been found in fish in many areas around the world, and therefore human consumption of contaminated seafood represents a serious danger for human health. Bacteria isolated from mercury-contaminated environments have evolved a system that allows them to efficiently convert both ionic and organic mercury compounds to the less toxic elemental mercury. This mercury resistance is due to the acquisition of a transferable genetic element known as the *mer* operon. The *mer* operon encodes for several proteins including two enzymes, the organomercurial lyase MerB and the mercuric ion reductase MerA. MerB catalyzes the protonolysis of the carbon-mercury bond that results in the formation of two products, a reduced-carbon compound and inorganic ionic mercury Hg^{II}. MerA catalyzes the reduction of Hg^{II} to elemental mercury Hg^0, which is volatile and less toxic. Due to their unique ability to breakdown MeHg, MerA and MerB are considered crucial to bioremediation efforts to clean up MeHg from contaminated waterways. The mechanistic details of how MerB and MerA function together at the atomic level to protect bacteria from mercury toxicity is one of the interests of Omichinski group. A clear understanding of these mechanistic details is crucial for maximizing utilization of the *mer* system in bioremediation efforts. In particular, we are interested in understanding the unique mechanism by which MerB cleaves carbon-mercury bond. More specifically, we have been using NMR spectroscopy and X-ray crystallography to structurally and mechanistically characterize MerB. Based on our previous structural studies, three important residues have been identified (Cys96, Asp99 and Cys159). They are required for cleavage of the carbon-Hg bond by MerB and we have determined the structure of the mercury bound product in the active site. As a follow up to the earlier studies, my project involves using X-ray crystallography to more precisely define the roles of Cys96, Asp99 and Cys159 in substrate binding and carbon-Hg bond cleavage. Two different approaches were implemented to fulfil this goal. In the first approach MerB mutants were tested for their activity and to help define the exact role for the three

catalytic residues. In the second approach, MerB inhibitors and other potential non-organomercurial substrates were used to probe the MerB active site.

In the first approach we mutated D99 to serine because in all but four of the known MerB variants, the Cys,-Asp-Cys catalytic triad found in *E.Coli* MerB is conserved. In the other four MerB variants, the two cysteine residues are conserved but the Asp is changed to Ser. To model this, a MerB D99S variant of *E. Coli* MerB was expressed and purified for crystallization to compare with wild-type active site in the free and mercury-bound state. Interestingly, we obtained a protein with a pink color during the purification of the MerB D99S variant and the electron density maps obtained during x-ray crystallography studies indicated the presence of a metal-MerB complex. Analysis by ICP-MS and X-ray fluorescence indicated that the D99S mutant of MerB bound copper in the active site. Further, electron paramagnetic resonance (EPR) and NMR studies identified the copper as Cu (II). In contrast, the wild-type MerB protein containing the serine residue in the active stie did not co-purify with copper and the X-ray structure of this MerB in complex with mercury is virtually identical to the structure of the MerB D99S-Hg complex. Our results provide evidence of how MerB may have evolved from the structurally homologous copper-binding protein NosL. In addition, they suggest that the aspartic acid residue is crucial for the cleavage of carbon-Hg bonds of organomercurials without binding other metals such as copper.

In the second approach, we probed the active site of MerB through testing its binding to additional organometals other than organomercurial. We have tested the interaction of MerB with organotin and organolead compounds. Trimethyltin (TMT) and trimethylead (TEL) showed binding to D99 , whereas diethyltin (DET) and diethylead (DEL) were cleaved by MerB, In addition, DET and DEL show higher binding affinity than even MeHg for MerB. Furthermore, dimethyltin (DMT) inhibits MerB using an alternative mechanism as seen with TMT and TEL. DMT induces a dramatic change in the active site by disrupting a cation-π interaction between Try95 and Arg155 in the active and it appears that this interaction is essential for catalytic activity. These results suggest that organomercurials may not be the sole substrate for MerB. On the other hand, these observations raiss the question of whether or not MerB could function in bioremediation efforts for other organometal contaminations. These results indicate also that the presence of other metals may have important implications when using MerB in bioremediation systems. The information obtained from this study will be crucial for maximizing the applications of MerB in bioremediation efforts.