Arsenate (As(V)) resistance in *Escherichia coli* requires reduction to arsenite (As(III)), which is then extruded from cells. The responsible enzyme is ArsC, an arsenate reductase encoded by *E. coli* plasmid R773. ArsC uses glutathione and glutaredoxin (Grx) as reductants. Grx2 has been shown to be the preferred hydrogen donor in the catalytic cycle. In the present study, we have initiated a series of experiments to identify the sites of interaction of Grx2 with ArsC.

The crystal structure of ArsC has been solved with substrate and product bound. As predicted by mutational studies, the substrate, arsenate was shown to be bound to Cys-12 in a sulfur bound pentacoordinate adduct. During the formation of this covalent enzyme-substrate complex there is a conformational change in which Arg-94 and Arg-60 exchange positions. The ArsC-arsenate complex is stabilized by hydrogen bonding to Arg-60, Arg-107 and the amide of Gly-11. The covalently bound product is a novel thiarsahydroxy adduct with Cys-12. These structures have led to a proposed mechanism for arsenate reduction by R773 ArsC that requires: 1) arsenate binding, 2) glutathione binding, 3) donation of a proton by Grx2 and removal of oxidized glutathione from the ArsC-arsenite adduct, and 4) hydrolysis of the ArsC-arsenite bond. The first and final steps of this proposed mechanism are supported by these crystal structures. However, the nature of the interaction between GSH and Grx2 has not yet been established. In the present study the perturbation of the NMR solution structure of ArsC by Grx2 will be examined to identify the points of contact between these two proteins. In addition, the dynamic properties of ArsC and ligand-induced conformational changes that occur during the catalytic cycle will be analyzed. Previously, the structure of ArsC was studied using NMR spectroscopy, and 128 of the 141 residues were assigned. Some of the unassigned region is the active site. We have collected data on $^{15}$N-labeled ArsC and found that the 2D spectrum has regions of difference from the previous data, suggesting that the protein may exist in multiple conformations. Using $^{15}$N/$^{13}$C/$^2$H-labeled ArsC, we have collected a HNCACB spectrum to assign the backbone. Once all the residues are assigned, we will examine structural changes that occur during the catalytic cycle, including interactions with Grx2. Supported by NIH grant GM52216 and RGM052216C.