Structure/Function Studies and Protein Engineering of ATP-Dependent Peptide Ligases

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Cadmium, mercury, and lead pollute many industrial sites, such as refineries and natural gas plants, and are persistent bioaccumulative toxic compounds associated with a range of human health problems, including effects on the nervous system, reproductive and developmental problems, and carcinogenic effects. Understanding the enzymes involved in the biosynthesis of metal chelating peptides is essential for using plants as tools in the remediation of toxic metal contaminated soils and waters. In response to heavy metal toxicity, plants synthesize metal-chelating peptides (i.e., phytochelatins) derived from glutathione and related molecules as protection. Glutathione is found in mammals, plants, and bacteria and is synthesized by glutathione synthetase (GS), an ATP-dependent peptide ligase. Interestingly, some plants respond to heavy metal stress by synthesizing glutathione analogs in which β-alanine, serine, or glutamic acid replace glycine in the peptide. The specific aims of the proposal are as follows: (1) to determine the structural basis for the synthesis of glutathione analogs; (2) to probe the functional role of the substrate interaction loop; and (3) to diversify substrate specificity.

During the third year of this project, we have determined the 1.9-2.1 Å resolution x-ray crystal structure of GmhGS in three forms: 1) apoenzyme/'open' active site, 2) the 'open' form in complex with γ-glutamylcysteine, and 3) a 'closed' active site form in complex with homoglutathione and ADP. These structures shed light on domain movements occurring within GmhGS during its catalytic cycle. Comparison with GS suggests that two amino acid differences allow for accommodation of a larger substrate in hGS than GS. Site-directed mutagenesis of Leu466 and Pro467 within a conserved active site loop provides insight into the determinants of substrate specificity for β-alanine (hGS) versus glycine (GS) in these related enzymes. The structural studies of GmhGS and the protein engineering of substrate specificity described in Aim 2 are now being prepared for submission of a manuscript.

three-dimensional structure of homo-glutathione synthetase