# Abstract

Metabolism of D-amino acids is of considerable interest due to their key importance in cellular functions. The enzymes D-serine dehydratase (DSD) and D-cysteine desulfhydrase (DCyD) are involved in the degradation of D-Ser and D-Cys, respectively. We determined the crystal structure of *Salmonella typhimurium* DSD (*St*DSD) by multiple anomalous dispersion method of phasing using selenomethione incorporated protein crystals. The structure revealed a fold typical of fold type II PLP-dependent enzymes. Although holoenzyme was used for crystallization of both wild type *St*DSD (WtDSD) and selenomethionine labeled *St*DSD (SeMetDSD), significant electron density was not observed for the co-factor, indicating that the enzyme has a low affinity for the cofactor under crystallization conditions. Interestingly, unexpected conformational differences were observed between the two structures. The WtDSD was in an open conformation while SeMetDSD, crystallized in the presence of isoserine, was in a closed conformation suggesting that the enzyme is likely to undergo conformational changes upon binding of substrate as observed in other fold type II PLP-dependent enzymes. Electron density corresponding to a plausible sodium ion was found near the active site of the closed but not in the open state of the enzyme. Examination of the active site and substrate modeling suggested that Thr166 may be involved in abstraction of proton from the Cα atom of the substrate. Apart from the physiological reaction, *St*DSD catalyses α, β-elimination of D-Thr, D-Allothr and L-Ser to the corresponding α-keto acids and ammonia. The structure of *St*DSD provides a molecular framework necessary for understanding differences in the rate of reaction with these substrates.

*Salmonella typhimurium* DCyD (*St*DCyD) is a fold type II PLP-dependent enzyme that catalyzes the degradation of D-Cys to $H_2S$ and pyruvate. We determined the crystal structure of *St*DCyD using molecular replacement method in two different crystal forms. The better diffracting crystal form obtained in presence of benzamidine illustrated the influence a small molecule in altering protein interfaces and crystal packing. The polypeptide fold of *St*DCyD consists of a small domain (residues 48-161) and a large domain (residues 1-47 and 162-328) which resemble other fold type II PLP-dependent enzymes. X-ray crystal structures of *St*DCyD were also obtained in the presence of substrates, D-Cys and βCDA, and substrate analogs, ACC, D-Ser, L-Ser, D-cycloserine (DCS) and L-cycloserine (LCS). The structures obtained in the presence of D-Cys and βCDA show the product, pyruvate, bound at a site 4.0-6.0 Å away from the active site. ACC forms an external aldimine complex while D and L-Ser bind non-covalently suggesting that the reaction with these ligands is arrested at Cα proton abstraction and transimination steps, respectively. In the active site of *St*DCyD cocrystallized with DCS or LCS, electron density for a pyridoxamine phosphate (PMP) was observed. Crystals soaked in cocktail containing these ligands show density for PLP-cycloserine. Spectroscopic observations also suggested formation of PMP by the hydrolysis of cycloserines. Mutational studies suggested that Ser78 and Gln77 are key determinants of enzyme specificity and the phenolate of Tyr287 is responsible for Cα proton abstraction from D-Cys. Based on these studies, we proposed a probable mechanism for the degradation of D-Cys by *St*DCyD.

The acid-induced arginine decarboxylase (ADC) is part of an enzymatic system in *Salmonella typhimurium* that contributes to making this organism acid resistant. ADC is a PLP-dependent enzyme that is active at acidic pH. It consumes a proton in the decarboxylation of arginine to agmatine, and by working in tandem with an arginine-agmatine antiporter, this enzymatic cycle protects the organism by preventing the accumulation of protons inside the cell. We have determined the structure of the acid-induced *St*ADC to 3.1 Å resolution. *St*ADC structure revealed an 800 kDa decamer composed as a pentamer of five homodimers. Each homodimer has an abundance of acidic surface residues, which at neutral pH prevent inactive homodimers from associating into active decamers. Conversely, acidic conditions favor the assembly of active decamers. Therefore, the structure of arginine decarboxylase presents a mechanism by which its activity is modulated by external pH.