The small subunit of carbamoyl phosphate synthetase: snapshots along the reaction pathway

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Carbamoyl phosphate synthetase (CPS) plays a key role in both arginine and. The enzyme from *Escherichi coli* consists of two polypeptide chains referred to as the small and large subunits. The large subunit sontains 1073 amino acid residues and the small subunit contains 382. CPS is active as a tetramer of these heterodimers and has a total molecular weight of approximately 620kDa. Figure 1 shows a ribbon drawing on one of the individual heterodimers. On the basis of both amino acid sequence analyses and x-ray structural studies, it is known that the small subunit belongs to the Triad or Type I class of amidotransferases, all of which contain a cysteine-histidine couple required for activity.

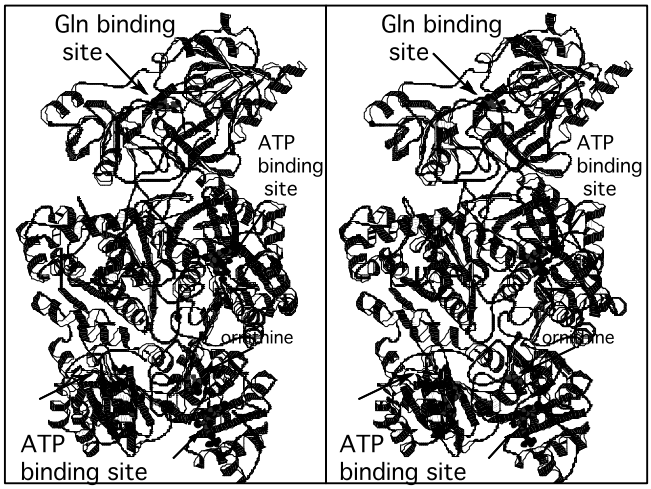

Figure 1: Ribbon drawing of one of the individual heterodimers.

There are two general classes of amidotransferase enzymes that utilize glutamine as a precursor for the *in situ* generation of ammonia. These enzymes have evolved to capture the ammonia by an acceptor substrate prior to the release of this intermediate into the bulk solution. CPS is the best-characterized example of the Triad or trpG-type class of amidotransferases. In this family of enzymes, which also includes GMP synthetase, imidazole glycerol phosphate synthase, anthranilate synthase, and NAD synthetase, among others, there is a conserved trio of residues that is critical for the catalytic hydrolysis of glutamine and the production of ammonia. In CPS from *Escherichia coli*, these residues have been identified as Cys 269, His 353, and Glu 355, and the roles of these amino acids during the catalytic cycle have been addressed by site-directed mutagenesis. A working model for the chemical mechanism exhibited by the Triad-type amidotransferases is presented in Figure 2.

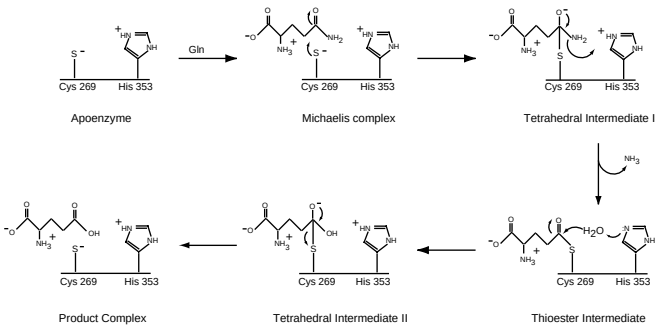

Figure 2: A working model for the chemical mechanism exhibited by the Triad-type amidotransferases.

The hydrolysis of glutamine by the small subunit has been proposed to occur via two tetrahedral intermediates and a glutamyl-thioester moiety. We have determined the three-dimensional structures of the C269S/glutamine and CPS/glutamate g-semialdehyde complexes, A working model for the chemical mechanism exhibited by the Triad-type amidotransferases A working model for the chemical mechanism exhibited by the Triad-type amidotransferases which serve as mimics for the Michaelis complex and the tetrahedral intermediates, respectively. In conjunction with the previously solved glutamyl-thioester intermediate complex, the stereochemical course of glutamine hydrolysis in CPS has been outlined. Specifically, attack by the thiolate of Cys 269 occurs at the Si face of the carboxamide group of the glutamine substrate leading to a tetrahedral intermediate with an S-configuration. Both the backbone amide groups of Gly 241 and Leu 270, and Og of Ser 47 play key roles in stabilizing the developing oxyanion. Collapse of the tetrahedral intermediate leads to formation of the glutamyl-thioester intermediate which is subsequently attacked at the Si face by an activated water molecule positioned near His 353. These results serve as a paradigm for other members of the Triad class of amidotranferases.