Crystal Structure of P-protein of the Glycine Cleavage System

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The glycine cleavage system (GCS) is a multienzyme complex composed of four different components (P-, H-, T- and L-proteins). In almost all organisms, the GCS plays a crucial role in the degradation of glycine, and it has been studied extensively. Three-dimensional structures of H-, T- and L-proteins from many species have been published, but only the structure of the P-protein has not yet been reported. We have determined the crystal structure of the P-protein from *Thermus thermophilus* HB8, which reveals that P-proteins do not involve the α_2-type active dimer universally observed in the evolutionarily related pyridoxal 5'-phosphate (PLP)-dependent enzymes. Instead, novel αβ-type dimers associate to form an $\alpha_2\beta_2$ tetramer, where the α- and β-subunits are structurally similar and appear to have arisen by gene duplication and subsequent divergence with a loss of one active site. The binding of PLP to the apoenzyme induces large open–closed conformational changes. The structure of the complex formed by the holoenzyme bound to an inhibitor, (aminooxy)acetate, suggests residues that may be responsible for substrate recognition. The molecular surface around the lipoamide-binding channel shows conservation of positively charged residues, which are possibly involved in complex formation with the H-protein. These results provide insights into the molecular basis of nonketotic hyperglycinemia.

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