B1. Carboxypeptidase

A peptide substrate binds at the active site of the carboxypeptidase enzyme. X-ray structures of the enzyme with and without a competitive inhibitor show a large conformational change at the active site when inhibitor or substrate is bound. Without inhibitor, several waters occupy the active site. When inhibitor and presumably substrate are bound, the water leaves (which is entropically favored), and Tyr 248 swings around from near the surface of the protein in the absence of a molecule in the active site to interact with the carboxyl group of the bound molecule, a distance of motion equal to about 1/4 the diameter of the protein. This effectively closes off the active site and expels the water. A \(\text{Zn}^{2+}\) ion is present at the active site. It is bound by His 69, His 196, Glu 72, and finally a water molecule as the fourth ligand. A hydrophobic pocket which interacts with the phenolic group of the substrate accounts for the specificity of the protein.

In the catalytic mechanism, \(\text{Zn}^{2+}\) helps polarize the labile amide bond, while Glu 270, acting as a general base, which along with \(\text{Zn}^{2+}\) helps promote dissociation of a proton from the bound water, making it a better nucleophile. Water attacks the electrophilic carbon of the sessile bond, with Glu 270 acting as a general base catalyst. The tetrahedral intermediate then collapses, expelling the leaving amine group, which picks up a proton from Glu 270, which now acts as a general acid catalyst. People used to believe that Tyr 248 acted as a general acid, but mutagenesis showed that Tyr 248 can be replaced with Phe 248 without significant effect on the rate of the reaction.

Contributors

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