Catalytic Mechanism of Haloalkane Dehalogenase

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the signature motifs and the binding of substrates (ATP, tRNA) or the oligomeric state of the protein. It also showed that this family of enzymes is modular and can be built around a central active site for ATP, amino acid and CCA recognition, with the addition of extra domains for functions specific of each synthetase.
The crystal structure of the complex formed by yeast aspartyl-tRNA synthetase, ATP, aspartic acid and $tRNA^{Asp}$ will be presented, with a detailed description of the active site of the enzyme. The class II synthetases exhibit a novel ATP binding fold, where the ATP molecule is anchored by highly conserved motif II and motif III residues. The binding pocket of the amino acid which has been inferred by model building and sequence analysis will be described. This model has been tested and confirmed by a number of site-directed mutations. The mode of binding of the receptor stem of the tRNA which is also class specific, explains the correlation of the two classes of synthetases with the primary site of attachment of the amino acid to the terminal adenosine.

MS-03.05.04 CATALYTIC MECHANISM OF HALOALKANE DEHALOGENASE: CRYSTAL STRUCTURES OF ENZYME-SUBSTRATE, ALKYL-ENZYME AND ENZYME-PRODUCT COMPLEXES

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Haloalkane dehalogenase, an enzyme of molecular weight 36,000, catalyzes the conversion of 1,2-dichloroethane into chloroethanol and chloride:
$Cl\text{-}CH_2\text{-}CH_2\text{-}Cl + H_2O \rightarrow Cl\text{-}CH_2\text{-}CH_2\text{-}OH + H^+ + Cl^-$
Dehalogenase is a member of the α/β hydrolase-fold family[1], to which family also belong acetylcholinesterase, lipases, dienelactone hydrolase and serine carboxypeptidases. These other enzymes are all esterases or peptidases, whereas the dehalogenase hydrolyses carbon-halogen bonds. Moreover, the proposed nucleophilic residue in dehalogenase (Asp124)[2] is quite different from the nucleophile in the other enzymes (Ser or Cys). Therefore, we analysed the reaction mechanism in detail by soaking dehalogenase crystals in the substrate 1,2-dichloroethane at different temperatures and pH values.
1. Substrate could be bound in the active site of the dehalogenase at 0°C and pH 5.0 without appreciable conversion. One chlorine atom is bound between the sidechain nitrogen atoms of two tryptophan residues. For the other chlorine no clear density is available suggesting that part of the molecule is flexible and not bound very tightly.
2. Soaking a crystal at room temperature and pH5.0 produces a covalent intermediate, bound to the Asp124 side-chain. This shows that Asp124 is the nucleophile attacking the C1 atom of the substrate. A chloride ion is produced, the density of which is clearly visible between the sidechains of the two tryptophans in the active site. A hydrolytic water molecule is in attacking position from the Cγ atom of Asp124.
3. Soaking crystals at room temperature and pH6.0, produces a chloride ion. No density is visible anymore for the alkyl-enzyme, nor for the product chloroethanol. Also no density for the hydrolytic water molecule is present anymore, suggesting that one reaction cycle has nearly completed.

From the above we conclude: Asp124 is the nucleophile in the active site of dehalogenase. It attacks the C1 carbon atom of the substrate. The negative charge which develops on the chlorine atom is stabilized by the side-chains of two tryptophan residues. An intermediate, covalently bound to the Asp124 side-chain in an ester bond, is formed. This ester is subsequently hydrolysed by a water molecule, assisted by His289 as a general base.

1 Ollis, D. L. et al. Protein Engin. 5, 197-211 (1992)
2 Franken, S. M. et al. EMBO J. 10, 1297, 1302 (1991)

MS-03.05.05 ELUCIDATING THE MECHANISM OF β-LACTAMASE BY CRYSTALLOGRAPHIC STUDIES. Osnat Herzberg*, Celia C.H. Chen & John Moult. Center for Advanced Research in Biotechnology, Maryland Biotechnology Institute, University of Maryland, 9600 Gudelsky Dr., Rockville MD 20850, USA.

The hydrolysis of β-lactams by β-lactamases constitutes the major bacterial defence against this class of antibiotics, diminishing the effectiveness of some of the most powerful therapeutic compounds. Following the determination of the crystal structure of a class A β-lactamase from *Staphylococcus aureus* we have proposed a catalytic mechanism that shares common features with that of the serine proteases. However, unlike the latter group of enzymes, the assisting catalytic machinery for acylation of β-lactamase differs from that of deacylation (Herzberg and Moult, *Science*, (1987), 236, 694). Support for this proposal has been provided by solution and crystallographic studies, investigating substrate and inhibitor binding, as well as mutant structures. The structure determination of the mutant protein P54 revealed that its activity is impaired due to a disordered loop at the active site. This disorder includes a glutamic acid residue, Glu166, whose main role is in the deacylation step, but not in acylation. Stopped flow measurements substantiated the crystallographic results, showing that the deacylation step becomes the rate-limiting step. The crystal structure of a phosphonate-inhibited β-lactamase, an analog of the tetrahedral transition state/intermediate of β-lactam hydrolysis enabled the modeling of the tetrahedral transition state associated with the acylation step of the true substrate. Because of the conformational rigidity of the fused rings of the penicillin molecule, the orientation of the substrate is fixed. The model supports our previously proposed mechanism of hydrolysis and provides new information about the mode of binding. We have also succeeded in trapping the acyl-enzyme products of clavulanate, a naturally occurring inhibitor, using cryo-crystallographic techniques. The crystal structure has been interpreted as containing a mixture of two acyl-enzymes representing two different stages of inhibitor degradation. This helped in differentiating between various proposed mechanisms of inactivation.

