enzymes. Papain, the archetype member of this family has industrial and pharmaceutical applications. The papain-family cysteine proteases share a typical overall fold, comprising two domains, with the active site located in a cleft at their interface. Their enzymatic activity is related to a catalytic dyad formed by a Cys(-) and His(+). Despite the similarities, variations in properties like substrate specificity, activity and thermal stability have been observed in some of these proteases. Three such proteases, Ervatamins A, B and C, have been isolated from the latex of a tropical plant *Ervatamia coronaria* and characterized. Structural and biochemical studies on these proteases have helped us to identify a few amino acid residues which may be thought to be responsible for substantial changes in their functional properties. This structure-based knowledge is being utilized to design proteases for improved industrial applications.

Keywords: proteases, structure-based protein engineering, industrial applications

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Aromatic hydroxylases in polyketide antibiotic biosynthesis

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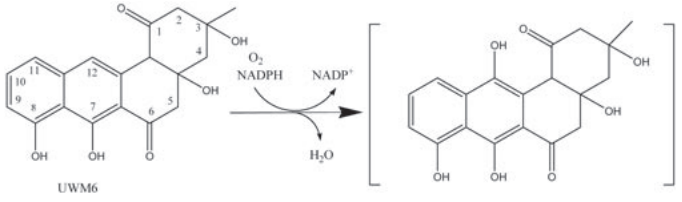
Aromatic polyketides are a major class of natural products with great medical significance: many representatives are used as e.g. anticancer and antimicrobial agents. However, their use is limited by harmful side-effects and drug resistance, hence new therapeutic agents are needed. Polyketides are produced in complicated enzymatic pathways by certain bacteria, plants and fungi. Engineering of the biosynthesis routes is a promising means of producing novel polyketide drugs, but requires detailed structural and mechanistic information of the biosynthetic enzymes. PgaE and CabE are homologous aromatic hydroxylases from the biosynthesis route of angucycline class of polyketides in *Streptomyces* sp. PGA64 and S. sp. H021. They catalyze the hydroxylation of the C12 of the substrate, UWM6. Their structures have been determined by X-ray crystallography to 1.8Å and 2.7Å. CabE and PgaE belong to the p-hydroxybenzoate hydroxylase (pHBH)-family of flavin adenine dinucleotide (FAD)-dependent aromatic hydroxylases. The ordered reaction mechanism includes dynamic rearrangements of the protein and the bound FAD. Unlike pHBH, PgaE and CabE do not appear to activate their substrate via deprotonation.



Keywords: flavin, polyketide antibiotic, aromatic hydroxylase

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Mechanism of stereospecific substrate recognition by LL-diaminopimelate aminotransferase

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The lysine biosynthetic pathway is an attractive target for the development of new antibiotics or herbicides because it is absent in humans. LL-diaminopimelate aminotransferase (LL-DAP-AT) is a newly discovered enzyme in the novel lysine biosynthetic pathway in Chlamydia and plants. Previously, three different lysine biosynthetic pathways have been characterized in bacteria. However, none of the previous bacterial lysine biosynthetic pathways were found in Chlamydia or in plants. Recently, LL-DAP-AT was discovered to be the missing piece in Chlamydial and plant lysine biosynthetic pathways, and this enzyme bypasses three enzymatic pathways in the previously described bacterial lysine biosynthetic pathway. In order to understand the mechanism of this enzyme and to assist in the design of inhibitors, we have determined the three-dimensional structures of LL-DAP-AT from A. thaliana in native and with two substrate-analogues (LL-DAP-PLP, Glu-PLP) bound. LL-DAP-AT is a pyridoxal-5'-phosphate (PLP) dependent enzyme and belongs to the type I fold family of PLP-dependent enzymes. Comparison of the active site residues of LL-DAP-AT and aspartate aminotransferases revealed that the PLP binding residues in LL-DAP-AT are well conserved in both enzymes. However, Tyr37, Tyr152, Glu97 and Asn309 are unique to LL-DAP-AT. Tyr37 and Tyr152 are positioned to recognize distal carboxylate groups of both LL-DAP and glutamate. Glu97, Asn309 and water molecules form an array of hydrogen-bonds to stereospecifically recognize LL-DAP in the active site. Our studies revealed the unique stereospecific recognition mechanism used by this newly discovered LL-DAP-AT.

Keywords: drug targets, aminotransferases, enzymatic mechanisms

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L-Threonine dehydrogenase (TDH) from *T. kodakaraensis*, an enzyme involved in amino acid metabolism

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We have determined the structure of threonine dehydrogenase (TDH) from the hyperthermophillic archaeon *Thermococcus kodakaraensis*. It exists as a homotetramer with 1 structural zinc ion per monomer, but is unclear whether a second zinc is required for catalytic activity at the active site, as in many alcohol dehydrogenases. Data was collected to 2.3Å and molecular replacement was used to solve the structure. Amino acids are essential for cellular growth, repair, and maintenance, although organisms are unable to synthesise all the ones they need themselves. Whilst they are able to synthesise some from chemicals and amino acids, others must be absorbed through the