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Type II dehydroquinase (EC 4.2.1.10) catalyzes the third step in the shikimate pathway by dehydrating 3-dehydroquinate to dehydroshikimate. *Y. pestis* dehydroquinase is formed by monomers of 150 amino acid residues that assemble as dodecamers. The enzyme was purified by size-exclusion and ion-exchange chromatography and crystallized using 16% PEG 3350. The crystals diffracted to 2.9Å resolution and belong to space group $I4_1$ (a=b= 132.4 Å, c = 187.2 Å) with six monomers in the crystallographic asymmetric unit. The dodecamer is built up by a crystallographic two-fold axis that operates on two- and three-fold non-crystallographic operators. The structure was determined by molecular replacement techniques and refined. The monomers display a typical flavodoxin-like fold with a central parallel β-sheet connected by helices. The active site is on the C-terminal end of the parallel β-sheet, at the trimer interface, and near the external surface of the dodecamer.

The structure of the active site is similar to that reported for the enzyme in other species. A peculiar feature of the enzyme is a loop, residues 18 to 29, that closes down on the active site when substrates and inhibitors are bound, and is often ordered only in such cases. Loop residue Tyr 28 (24 in *M. tuberculosis* numbering) is critical in the dehydration step according to the proposed mechanism. In the *Y. pestis* enzyme the loop is ordered in only one out of six subunits, and is in a completely open conformation with a displacement of main chain atoms of up to 11 Å, relative to the closed state.

Keywords: shikimate pathway, Type II dehydroquinase, *Yersinia pestis*

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Crystal Structure of HAV 3C Protease Complexed with a β-lactone Inhibitor: a New Crystal Form of HAV 3C and Its Application for Studying Enzyme-inhibitor Interaction

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We obtained a new crystal form of Hepatitis A virus (HAV) 3C protease in complex with a serine-derived β-lactone inhibitor (N-benzyloxycarbonyl-L-serine-β-lactone). The crystals, which diffract to the highest resolution known to date (1.4 Å) for HAV and related viral proteases, are of space group $P2_12_12_1$ and contain one molecule per asymmetric unit. Serendipitously, the inhibitor covalently binds to a histidine residue (His102) on the surface of the enzyme opposite to the active site and leaves the catalytic residue Cys172 unmodified. Moreover, this is the first time that a functional arrangement of the catalytic triad (Cys172, His44, Asp84) was observed in HAV 3C crystals, making them ideal for the study of enzyme-inhibitor interactions in atomic detail.

The new crystal form seems to result at least partly from the binding of the benzene moiety of the lactone inhibitor to a hydrophobic pocket of a neighboring molecule. Structural alignment with previously solved HAV 3C crystal structures indicates significant conformational changes occur beyond the site of chemical modification. The two anti-parallel β-strands (aa 139-158) between the N- and C-terminal domains of HAV 3C exhibit less flexibility than previously observed in other crystal forms, which may have an important role in the organization of a catalytically competent triad. In particular, the hydroxyl of Tyr143 forms a hydrogen bond with $O^{\delta 2}$ of Asp84, facilitating the latter to interact with $N^{\varepsilon 2}$ of His44, the general base in catalysis. We subsequently soaked HAV 3C-lactone crystals in solution containing another irreversible inhibitor N-iodoacetyl-Val-Phe-amide to explore the feasibility of using these new HAV 3C crystals for the study of interactions between the enzyme and active site-reacting inhibitors.

Keywords: hepatitis, protease, inhibitor

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Crystal Structure of a Protein Disulfide Oxidoreductase from *Aquifex aeolicus*

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Protein disulfide oxidoreductases (PDO*s*) are ubiquitous redox enzymes that catalyse dithiol-disulfide exchange reactions. These enzymes include the families of thioredoxin (TRX), glutaredoxin (GRX), protein disulfide isomerase (PDI), disulfide bond forming (Dsb) and their homologues. In 1995 a hyperthermostable PDO has been purified from the archaeon *Pyrococcus furiosus* (*Pf*PDO). This protein has an unusual molecular mass of 26 kDa, compared to the small sizes of most GRX*s* and TRX*s*, and its amino acid sequence shows no overall similarity to previously studied PDO*s*. Interestingly, it presents two active sites with the conserved CXXC sequence motif. The resolution of its three-dimensional structure revealed important conformational details suggesting that *Pf*PDO may be related to the PDI, known only in eukaryotes.

Comparison of the genomes from archaea and bacteria showed the existence of a group of redox proteins similar to *Pf*PDO. The unusual features of these enzymes suggest that they could constitute a new family of PDO*s*. We have recently focused our attention on a protein isolated from the thermophilic bacterium *Aquifex aeolicus* (*Aa*PDO) and belonging to this putative new enzyme family. In order to provide insights into the function, structural diversity and evolution of PDO*s*, a structural and functional study on this protein has been carried out.

Keywords: crystal structure, PDO, thermostable enzymes

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***Pseudomonas aeruginosa* PA3859: from Structure to Function**

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We have recently purified a carboxylesterase from *Pseudomonas aeruginosa* that corresponds to the open reading frame PA3859 [1]. The biochemical characterization of this enzyme showed that it hydrolyzes short chain fatty acid esters with a broad substrate specificity. However, its biochemical characterization did not provide insights into a clearly defined *in vivo* function.

Neither bioinformatics tools did provide hints on its physiological function: useful information could not be gained by data mining for homologs or orthologs.

The successful crystallization and 3D structure determination of PA3859 at 2.1Å resolution provided new insights into the structural basis for its *in vivo* substrate(s) specificity. The presence on the protein surface, next to the active site, of an hydrophobic cleft exposed to the solvent has been hypothesized as the possible protein *hot spot* binding site that may accommodate an alkylic chain. This hypothesis was also supported by the localization of genes involved in lipid metabolism in the vicinity of the PA3859 locus. Docking of a variety of phospholipids suggested lysophopsphatidylcholine as potential substrate. We have established, by an enzymatic bioassay, that PA3859 is indeed able to release free fatty acid from lysophosphatidylcholine.

[1] Pesaresi A., Devescovi G., Lamba D., Venturi V., Degrassi G., *Curr. Microbiol.*, 2005, **50**, 102.

Keywords: *Pseudomonas aeruginosa*, carboxylesterase, lysophospholipase