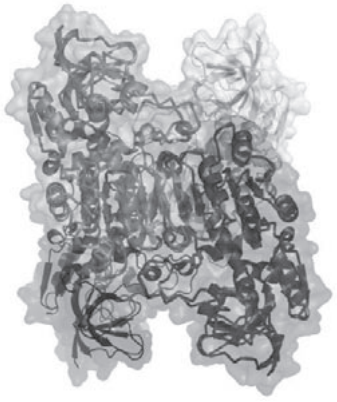
diet L-Threonine is an indispensable amino acid and under normal conditions is synthesised by microbes from oxaloacetate. Threonine degradation occurs by two major pathways: -either it is converted by TDH to 2-amino-3-ketobutyrate, which by the action of 2-amino-3-oxobutyrate CoA ligase produces glycine and acetyl CoA. -or alternatively L-serine/ threonine dehydratase converts threonine to NH_4^+ and 2-ketobutyrate and the latter is further metabolized by way of acetyl CoA.



Keywords: threonine dehydrogenase, enzyme, zinc

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Structural studies of acyl-CoA thioesterase 7 and its role in inflammation

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Acyl-CoA thioesterases (Acots) are important enzymes involved in the hydrolysis of acyl-CoA to free fatty acids and coenzyme A. The enzyme can be found in a large range of organisms from bacteria to humans and in all mammalian tissues and cells. The mammalian full length acyl-CoA thioesterase (Acot7) enzyme is comprised of two hotdog fold domains. The crystal structures of both the N- and C-terminal domains were determined separately at 1.8 and 2.5 Å resolution, respectively, and the structure of full length enzyme was inferred by a combination of chemical cross-linking, mass spectrometry, and molecular modeling. We have shown using functional and biochemical assays that both the domains are required for activity of enzyme and mutational studies have shown that out of the two possible actives sites, only one is functional. This is in contrast with the acyl-CoA thioesterases from the primitive organisms, in which the active form of the enzyme can be achieved from one single domain copy. Because, Acot7 is highly expressed in macrophages, up-regulated by LPS (lipoploysacchrides), has a strong affinity towards arachidonyl-CoA and its over expression in macrophages alters the D2 and E2 prostaglandin production, we propose that it plays an important role in inflammation. Collectively, our results link the molecular and cellular functions of Acot7 and identify it as a candidate drug target in inflammatory disease.

Keywords: X-ray crystallography, protein structure, acyl CoA thioesterase

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Structural proteomics of secreted proteases from the ovine footrot pathogen, *Dichelobacter nodosus*

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Dichelobacter nodosus is the primary cause of ovine footrot, a contagious disease that causes severe economic loss to the sheep industry. The severity of the infection can range from virulent to benign depending upon the causative *D. nodosus* isolate. Virulent footrot is characterised by the separation skin horn junction whilst benign isolates cause a mild inflammation of this tissue. Virulent isolates of *D. nodosus* secrete three subtilisin-like serine proteases, the acidic proteases AprV2 and AprV5 and the basic protease BprV. It is postulated that these proteases play an essential role in the pathogenesis of virulent footrot due to their ability to degrade components of the extracellular matrix at the site of infection. Benign isolates of *D. nodosus* secrete the closely related proteases AprB2 and BprB which differ from AprV2 and BprV by one amino acid (Y92R) and ten amino acids respectively. In this study, X-ray crystallography has been used to elucidate the structural differences between the proteases secreted by virulent and benign isolates. The crystal structures of AprV2, AprB2, BprV and BprB have been determined to 2 Å, 1.7 Å, 2 Å and 1.8 Å respectively. The structures revealed a conserved subtilisin domain with a unique disulphide tethered, solvent exposed loop that partially occluded the active site. The single amino acid difference between AprV2 and AprB2 is located at the tip of this loop. Amino acid variations between BprV and BprB are located throughout the structure including surface residues and residues in the substrate binding site and the active site occluding loop of the protease. This study provides an insight into the structure of the secreted proteases from *D.nodosus* and the role of these proteases play in the pathogenesis of ovine footrot.

Keywords: enzyme structure determination, proteases, infectious diseases

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Structure determination of *E. coli* isocitrate dehydrogenase kinase/phosphatase

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The study of bacterial phosphorylation systems was advanced by the discovery of a phosphorylating activity in *E. coli* which regulates isocitrate dehydrogenase (IDH). This was the first prokaryote phosphorylation system to be identified in bacteria, and is the only known serine/threonine (Ser/Thr) phosphorylation system/pathway in E. coli. This phosphorylation-dephosphorylation system modifies the Ser-113 residue on IDH. It is this modification that regulates the amount of isocitrate going through the glyoxylate bypass. IDH competes with isocitrate lyase in directing isocitrate through the Krebs' cycle or glyoxylate bypass, respectively. When the organism

is grown on acetate, IDH is in its inactive phosphorylated form, thus inhibiting Krebs' cycle. Alternatively, a change of carbon source to glucose or pyruvate results in the activation of IDH by dephosphorylation, and the initiation of Krebs' cycle. The function of AceK and its involvement in the regulation of Krebs' cycle and the glyoxylate bypass is well-characterized, but its structural and mechanistic qualities have remained relatively unknown. The determination of the crystal structure could provide confirmation of the function of AceK by identifying the various kinase, phosphatase and ATPase domains and insights into the coordination of the kinase and phosphatase activity of AceK. Of note, it is currently unknown how, or if at all, the active site changes conformation as it switches between kinase and phosphatase activity. Three Acek crystal forms were obtained and SAD datasets were collected at CHESS and BNL synchrotron source. The AceK structure is determined at 2.6 Å. The overall AceK structure displays a typical eukaryotic kinase folding.

Keywords: kinase, phosphatase, isocitrate dehydrogenase

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Structure of decameric PLP-dependent acid induced arginine decarboxylase from *Escherichia coli*

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In order to infect a human host, enteric *Escherichia coli* must pass through the stomach, which has a pH of around 2.0, and survive there for approximately two hours before the stomach is emptied. *E. coli* is capable of surviving in the highly acidic environment of the stomach since it possesses systems that make it acid resistant. Three such systems have been identified, one of which is the arginine-dependent acid resistance system (AR3). AR3 requires the presence of arginine and is dependent on the acid induced arginine decarboxylase (AdiA) and the arginine-agmatine antiporter (AdiC). AdiA converts one molecule of arginine into agmatine and AdiC transports the agmatine out of the cell in exchange for arginine. Through this enzymatic cycle, AR3 acts to protect the organism by preventing the accumulation of protons inside the cell. In order to investigate the enzymatic mechanism involved in AR3, we have determined the structure of the acid induced arginine decarboxylase, AdiA, by X-ray crystallography. The AdiA structure, solved by multiple isomorphous replacement (MIR), revealed a ca. 800 kDa decameric assembly with unusual five-fold non-crystallographic symmetry. Presented here is the structure of AdiA from E. coli refined to 2.4 Å resolution, its pyridoxal-5'-phosphate (PLP) containing active site and its large macromolecular assembly.

Keywords: arginine decarboxylase, macromolecular proteins, acid resistance

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The substrate recognition and the catalytic reaction mechanisms of D-3-hydroxybutyrate dehydrogenase

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D-3-Hydroxybutyrate dehydrogenase (HBDH) is an NAD$^+$-dependent enzyme for reversible conversion between D-3-hydroxybutyrate and acetoacetate. These ketone bodies are important energy sources, but their excess level causes ketoacidosis. HBDH can be used as a marker in the assay of diabetes mellitus, and/or ketoacidosis. To reveal the reaction mechanism, the crystal structures of *Alcaligenes faecalis* HBDH in the *apo* form and two different *holo* forms, one with a substrate analogue, acetate (HBDH-NAD-AC) and the other with the reaction product, acetoacetate (HBDH-NAD-AA), have been determined by X-ray crystallography. The physiologically active enzyme is a homo tetramer assembled according to the non-crystallographic 222 point symmetry. Each subunit has a principal domain with a typical Rossmann fold. The two helices, H6 and H7 of the small domain, move to the principal domain to trap NAD$^+$ in a cavity. In HBDH-NAD-AC complex, an acetate ion is directly bound to the residues Q94, H144, K152 and Q196 through hydrogen bonds. A water molecule is bound to S142 and Y155, mimicking the hydroxyl group of the substrate D-3-hydroxybutyrate. The position of the water oxygen atom is near the methyl group of the acetate. These structural features strongly suggest the binding scheme of the true substrate in the enzyme-NAD complex. In the HBDH-NAD-AA crystal, obtained by adding the substrate, the reaction product acetoacetate has been found in the active site. This structure is consistent to our proposed reaction mechanism [1]. The basic structural architecture of the enzyme is highly conserved as a member of the SDR family with the catalytic triad of S142, Y155 and K159.

[1] Hoque *et al*., *Acta Cryst* . D **64**, In press (2008).

Keywords: hydroxybutyrate dehydrogenase, ketone bodies, X-ray structure

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Using natural variations among shikimate dehydrogenases to study modes of substrate selectivity

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