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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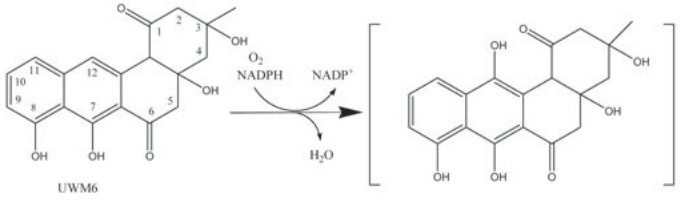
Aromatic hydroxylases in polyketide antibiotic biosynthesis

Hanna Koskiniemi[1], Mikko Metsa-Ketela[1,2], Pauli Kallio[2], Doreen Dobritzsch[1], Liu Zhanliang[2], Pekka Mantsala[2], Jarmo Niemi[2], Gunter Schneider[1]
[1]Karolinska Institutet, Department of Medical Biochemistry and Biophysics, Tomtebodavägen 6, 4. tr., Stockholm, Stockholm, 17177, Sweden, [2]University of Turku, Vatselankatu 2, 20014 University of Turku, Finland, E-mail：hanna.koskiniemi@ki.se

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

Nobuhiko Watanabe[1], Maia M Cherney[1], Marco J van Belkum[2], Sandra L Marcus[2], Mitchel D Flegel[2], Matthew D Clay[2], Michael K Deyholos[3], John C Vederas[2], Michael N. G. James[1]
[1]University of Alberta, Biochemistry, 429 Medical Sciences Buildings, Department of Biochemistry, Edmonton, Alberta, T6G 2H7, Canada, [2]University of Alberta, Department of Chemistry, Edmonton, Alberta, T6G 2G2, Canada, [3]University of Alberta, Department of Biological Sciences, Edmonton, Alberta, T6G 2E9, Canada, E-mail：nobuhiko@ualberta.ca

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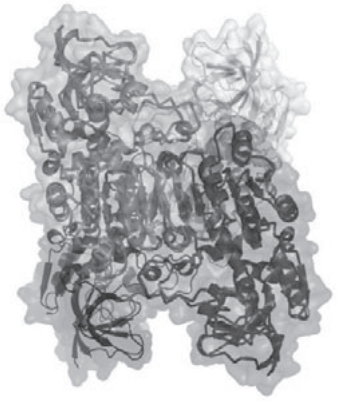
L-Threonine dehydrogenase (TDH) from *T. kodakaraensis*, an enzyme involved in amino acid metabolism

Alexandra Bowyer, Jon B Cooper, Muhammad Akhtar
University of Southampton, School of Biological Sciences, Biochemistry, Bassett Crescent East, Southampton, Hampshire, SO16 7PX, UK, E-mail：alexr@soton.ac.uk

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

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

Anil S Thakur[1], Jade K Forwood[1,2], Gregor Guncar[1,2], Mary Marfori[1], Dmitri Mouradov[1], Weining Meng[1], Jodie Robinson[1], Thomas Huber[1], Stuart Kellie[1,2,3], Jennifer L Martin[1,2,4], David A Hume[1,2,3,4], Bostjan Kobe[1,2,4]

[1]University of Queensland, School of Molecular and Microbial Sciences, Bld 76, Coopers Road, St. Lucia, Brisbane, Queensland, 4072, Australia, [2]Institute for Molecular Bioscience, [3]Cooperative Research Centre for Chronic Inflammatory Diseases, [4]Australian Research Council Special Research Centre for Functional and Applied Genomics, University of Queensland, Brisbane, Queensland 4072, Australia, E-mail：s4095060@student.uq.edu.au

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*

Wilson Wong[1,3], Corrine J Porter[1,3], Ruth M Kennan[2,3], Dane Parker[2,3], Julian I Rood[2,3], James C Whisstock[1,3]

[1]Monash University, Biochemistry and Molecular Biology, 940 Heatherton Rd, Springvale South, VIC 3172, Australia, Melbourne, VIctoria, 3172, Australia, [2]Monash University, Department of Microbiology, Melbourne, VIC, 3800, Australia, [3]Monash University, ARC Centre of Excellence in Structural and Functional Microbial Genomices, Melbourne, VIC, 3800, Australia, E-mail：wilson.wong@med.monash.edu.au

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

Jimin Zheng, Zongchao Jia

Queen's University, Biochemistry, 623 Botterell Hall,18 Stuart Street, Kingston, Ontario, K7L 3N6, Canada, E-mail：1jz9@queensu.ca

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