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Crystal Structure of the Catalytic Fragment of 2',3'-Cyclic nucleotide 3'-Phosphodiesterase from Human Brain

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2',3'-Cyclic-nucleotide 3'-phosphodiesterase (CNP), a member of the 2H phosphoesterase superfamily, is firmly bound to brain white matter and found mainly in the central nervous system of vertebrates, and it catalyzes the hydrolysis of 2',3'-cyclic nucleotide to produce 2'-nucleotide. Here we report crystal structure of the catalytic fragment (CF) of human CNP (hCNP-CF) at 1.8Å resolution [1]. On the basis of the present crystal structure of the hCNP-CF/phosphate complex, the available structure of the CPDase/cyclic nucleotide analogue complex, and the recent functional studies of rat CNP-CF, we propose a possible substrate-binding mode and catalytic mechanism of CNP. The proposed mechanism is basically equivalent to the second step of the well-accepted reaction mechanism of RNase A. Since the overall structure of hCNP-CF differs considerably from that of RNase A, it is likely that the similar active sites with two catalytic histidine residues in these enzymes arose through convergent evolution.

[1] Sakamoto Y., Tanaka N., Ichimiya T., Kurihara T., Nakamura, K. T., *J. Mol. Biol.*, 2005, **346**, 789.

Keywords: CNPase, myelin, phosphodiesterase

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Crystal Structure and Catalytic Mechanism of Proline Racemase

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Amino acid racemases catalyze an otherwise extremely unfavorable reaction: the stereoinversion of the chiral α-carbon. Amino acid racemization allows cells to produce the D-enantiomers that participate in biological processes such as bacterial cell wall construction or neuro/endocrine signaling in mammals.

Proline racemase has been extensively studied as a model of pyridoxal-phosphate-independent amino acid racemases. We report the crystal structure of the proline racemase from *Trypanosoma cruzi*, which is also known to be a powerful B-lymphocyte mitogen [1].

The enzyme is a homo-dimer, with each monomer folded in two α/β domains separated by a deep crevice. In contrast with the accepted model of one symmetric reaction center per dimer [2], the crystal complex with a transition-state analog (pyrrole-2-carboxylic acid) reveals one competent catalytic site per monomer, buried in the inter-subunit crevices. Two cysteine residues are optimally located to perform acid/base catalysis through a carbanion stabilization mechanism. Crystallographic and calorimetric evidence prove that proline racemase undergoes a substrate-triggered closure of the inter-domain crevice, which might regulate the protein's mitogenic activity.

[1] Reina-San-Martin B., Degrave W., Rougeot C., Cosson A., Chamond N., Cordeiro-da-Silva A., Arala-Chaves M., Minoprio P., *Nature Med.*, 2000, **6**, 890. [2] Rudnick, G., Abeles, R.H., *Biochemistry*, 1975, **14**, 4515.

Keywords: enzyme catalytic reaction mechanism, single anomalous diffraction, carbanion transition state species

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Crystal Structure of an Enzyme Involved in the Biosynthesis of Isoprenoids: 4-diphosphocytidyl-2C-methyl-D-erythritol Kinase from *E. coli*, a Potential Drug Target

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Isoprenoids are a diverse family of compounds consisting of isoprene units (five-carbons units) and are involved in many biological functions such as electron transport, hormone based signaling, apoptosis, also they provide structural components of cell membranes. In contrast to mammals, some pathogenic agents such as those responsible for serious human disease including leprosy, malaria, bacterial meningitis, tuberculosis and certain types of pneumonia use the non-mevalonate pathway to synthesis those compounds. If we could disrupt this pathway, it might provide the first step in the development of a broad-spectrum antimicrobial agent. With this in mind, we solved the structure of the 4-diphosphocytidyl-2C-methyl-D-erythritol kinase (CDP-ME kinase). The resulting model reveals information as to the specificity and the catalytic mechanism of the enzyme.

[1] Rohdich F., Hecht S., Bacher A., Eisenreich W., *Pure Appl. Chem.*, 2003, **75**, 393.

Keywords: structure, drug target, mechanism

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Crystal Structure of the Biotin Protein Ligase from *Pyrococcus horikoshii* OT3: Insights into the Mechanism of Biotin Activation

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Biotin protein ligase (BPL) catalyses synthesis of an activated form of biotin, biotinyl-5'-AMP, from substrates biotin and ATP, and followed biotinylation of the biotin carboxyl carrier protein subunit of acetyl-CoA carboxylase. The crystal structures of BPL from *Pyrococcus horikoshii* OT3 (*Ph*BPL) and its complexes with biotin, ATP, ADP and biotinyl-5'-AMP have been determined at 1.6, 2.0, 1.6 and 1.45Å resolution, respectively. Analysis of location of the activated intermediate and conformational rearrangements in the *Ph*BPL complexes allows us to propose structural guidelines for the biotin activation.

The structures reveal a dimer as the functional unit and each subunit contains two domains, a larger N-terminal catalytic and a smaller C-terminal domains. Dimer configuration of *Ph*BPL (enzyme) is different that of from BPL from *E.coli*, *Ec*BirA (enzyme-repressor): in *Ph*BPL, the tight dimer through N-termini shows no change upon ligand binding; in *Ec*BirA, the dimerization through the central regions of catalytic domain is controlled by the ligand binding. In crystals cocrystallized with biotin and ATP, electron density corresponding to a biotinyl-5'-AMP was observed due to the self-catalysis between substrates. An induced-fit ordering of the active site loop in the complexes makes the catalytic field suitable for the first step of BPL reaction. In *Ph*BPL, both biotin and ATP are fixed in spatially adjacent active site pockets in orientation allowing the reaction. In the bottom of the pockets, there are conserved residues like Gly45, Gly47, Gly127, Gly129 and Trp53 providing required space and orientation for substrates, as well as the conserved positively charged residues Arg48, Arg51, Arg233 and Lys111 located near to the reaction ends of substrates, which may facilitate the reaction.

Keywords: proteins structure, enzyme active site, biotinylation mechanism

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Structure and Mechanism of 2-C-methyl-D-erythritol 2,4-cyclodiphosphate Synthase

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Precursors for isoprenoid synthesis are essential in all organisms. These compounds are synthesized by one of two known routes: the well characterized mevalonate pathway [1] or a recently discovered non-mevalonate route which is used in many bacteria and human pathogens [2]. Since the second pathway is both vital and unlike any found in humans, enzymes catalysing reactions along this synthetic route are possible drug targets. The structure of one such enzyme from the thermophilic bacterium *Thermus thermophilus* has been solved to high resolution in the presence of substrate and with a substrate analogue. Enzyme co-crystallized with substrate shows only one product, cytosine monophosphate (CMP), in the active site. At the high resolution of the refinement (1.6 Å) the positions and coordination of the magnesium ions in the active site are clearly seen.

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Keywords: isoprenoid synthesis, non-mevalonate route, drug targets

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Crystal Structure of the β-subunit of Acetyl-CoA Carboxylase in *C. glutamicum*

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Acetyl-CoA carboxylases (ACCs) catalyze the first committed step of fatty acid biosynthesis. Although ACC is an essential enzyme (complex) in every organism, the structure-function relationship of ACC remains to be unclear. As the first step for elucidating the structure-function relationship of ACC, we started the crystallographic analysis of DtsR1. DtsR1 is the β-subunit of ACC multisubunit complex in *Corynebacterium glutamicum*, which catalyzes the transcarboxylation between biotin and acetyl-CoA.

DtsR1 was over-expressed in *E. coli*, purified, and crystallized by the sitting-drop vapor diffusion method using PEG 6000 as a precipitant. The approximate dimensions of the obtained crystals were 0.07x0.07x0.03mm^3. Diffraction data of the crystals were collected at NW12 of the Photon Factory (Tsukuba), revealing that the crystals belong to the space group *R*32. The crystal structure of DtsR1 was solved at 3.2Å resolution by the molecular replacement method using single-subunit coordinates of the 12S transcarboxylase (PDB ID: 1ON3) as a search model. The obtained structure suggests that the biological unit of DtsR1 is a ring-shaped hexamer with the *32*-point group symmetry. Crystallographic refinement of DtsR1 is in progress at 2.7Å resolution.

Keywords: carboxylases, fatty acid biosynthesis, structure-function enzymes

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Structural Studies of FlaA1, a UDP-GlcNAc 4,6-dehydratase

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FlaA1 is a UDP-GlcNAc 4,6-dehydratase believed to be involved in the protein glycosylation process of *Helicobacter pylori*. The crystal structures of FlaA1 in five different ternary complexes with various substrates were determined at resolutions between 1.9 and 2.8 Å. This represents the first structure of a 4,6-dehydratase that can catalyze a UDP-saccharide. Among 4,6-dehydratases, FlaA1 possesses several unique structural features including a novel C-terminal fold and a hexameric oligomerization state in the crystal. The catalytically productive conformation observed in the FlaA1•NADPH•UDP-GlcNAc ternary complex suggests that FlaA1 employs a different mechanism for the water elimination step from that proposed for other 4,6-dehydratases. Normally, an Asp and Glu residues are the two catalytic residues that effect dehydratase activity through a concerted mechanism. In FlaA1, the corresponding residues are Asp-132 and Lys-133, precluding an analogous mechanism. Computational analysis suggests that for the water elimination step in FlaA1, Lys-133 sequentially functions as catalytic acid and base while Asp-132 closely interacts with the leaving water group.

Keywords: dehydratase, catalytic mechanism, pKa calculation

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The Crystal Structure of Murine 11β-hydroxysteroid Dehydrogenase 1: an Important Therapeutic Target for Diabetes

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11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) catalyzes the conversion of 11-dehydrocorticosterone to its active form corticosterone in rodents (or cortisone to cortisol in humans). An excess of active glucocorticoids has been shown to play a key role in metabolic disorders such as diabetes and obesity. Therefore, 11β-HSD1 represents an important therapeutic target for the treatment of these diseases. To facilitate the iterative design of inhibitors, we have crystallized and determined the three-dimensional structures of a binary complex of murine 11β-HSD1 with NADP(H) to a resolution of 2.3 Å, and a ternary complex with corticosterone and NADP(H) to a resolution of 3.0 Å by X-ray crystallography. The enzyme forms a homodimer in the crystal. The structure shows a novel folding feature at the C-terminus of the enzyme. The C-terminal helix insertions provide additional dimer contacts, exert an influence on the conformations of the substrate binding loops, and present hydrophobic regions for potential membrane attachment. The structure also reveals how the 11β-HSD1 achieves its selectivity for its substrate.

Keywords: 11β-HSD1, SDR, corticosterone

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Crystal Structure of P-protein of the Glycine Cleavage System

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The glycine cleavage system (GCS) is a multienzyme complex composed of four different components (P-, H-, T- and L-proteins). In almost all organisms, the GCS plays a crucial role in the degradation of glycine, and it has been studied extensively. Three-dimensional structures of H-, T- and L-proteins from many species have been published, but only the structure of the P-protein has not yet been reported. We have determined the crystal structure of the P-protein from *Thermus thermophilus* HB8, which reveals that P-proteins do not involve the α_2-type active dimer universally observed in the evolutionarily related pyridoxal 5'-phosphate (PLP)-dependent enzymes. Instead, novel αβ-type dimers associate to form an $\alpha_2\beta_2$ tetramer, where the α- and β-subunits are structurally similar and appear to have arisen by gene duplication and subsequent divergence with a loss of one active site. The binding of PLP to the apoenzyme induces large open–closed conformational changes. The structure of the complex formed by the holoenzyme bound to an inhibitor, (aminooxy)acetate, suggests residues that may be responsible for substrate recognition. The molecular surface around the lipoamide-