P.04.02.33

Acta Cryst. (2005). A**61**, C188

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

P.04.02.34

Acta Cryst. (2005). A**61**, C188

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

P.04.02.35

Acta Cryst. (2005). A**61**, C188

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

P.04.02.36

Acta Cryst. (2005). A**61**, C188

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

P.04.02.37

Acta Cryst. (2005). A**61**, C188-C189

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

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