we have determined the crystal structure of YlqF from Thermotoga maritima in complex with GDP, GTP, and a non-hydrolyzable GTP analog. YlqF is a circularly permuted GTPase. It is composed of two domains: an N-terminal G domain and a C-terminal basic alpha-helical bundle domain. The structures of Tm YlqF bound with different ligands reveal a significant difference in the relative orientation of the two domains, when we compare the GDP-bound structure with either the GTP- or GNP-bound structure. Our study thus provides a glimpse of a possible conformational change of YlqF upon GTP hydrolysis.

Keywords: GTPase, circular permutation, YlqF

P04.02.79

Acta Cryst. (2008). A**64**, C255

Crystal structure of L-sorbose reductase from *Gluconobacter frateurii* at 2.4 Å resolution

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L-Sorbose reductase (SR) from *Gluconobacter frateurii* is an NADPH-dependent reductase that catalyzes the reduction of L-sorbose to D-sorbitol with a high substrate specificity. To understand the structural bases of substrate specificity and catalytic mechanism of SR, we have determined the first crystal structure of SR in complex with L-sorbose at 2.4-Å resolution. SR adopts the Rossmann fold and belongs to the short-chain dehydrogenase/reductase family. SR takes a tetrameric assembly in crystal and solution at optimal pH's, pH 6.2 (for the conversion of L-sorbose to D-sorbitol) and pH 9.0 (for the conversion of D-sorbitol to L-sorbose). Although the crystal structure does not contain the co-factor NADPH, the NADPH-binding site can be predicted by comparing the structures of the SDR family members. The bound L-sorbose was located in the active-site pocket near the putative NADPH-binding site and recognized by η1, α5 and α6 helices and loops.

Keywords: crystal structures, enzyme structure, enzyme ligand complexes

P04.02.80

Acta Cryst. (2008). A**64**, C255

Structural studies by X-ray on enzymes involved in propionate metabolism from membrane integrated protein leukotriene C4 synthase

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Propionate is the second most abundant organic compound in the soil. The metabolism of propionate has been investigated in *Salmonella enterica serovar typhimurium* and *Escherichia coli*. The first reaction in the anaerobic breakdown of L-threonine to propionate is catalyzed by the biodegradative threonine deaminase (TdcB), which catalyzes the deamination of L-threonine to α-ketobutyrate. The X-ray structure of TdcB from S. typhimurium was determined in two forms to resolutions of 1.7 Å and 2.2 Å, respectively. The dimeric protein displays an interesting variation in the quaternary association of subunits in one of the crystal forms. CMP binding alters the oligomeric state of TdcB from a dimeric to a tetramerc form. The structure of the TdcB-CMP determined at 2.5 Å resolution suggests that the changes induced at the dimer interface by ligand binding are essential for tetramerization. The structural alterations also appear to account for enzyme activation and increased affinity for L-threonine. Propionate kinase (TdcD) catalyzes the last step of non-oxidative degradation of thronine to propionate by enabling the conversion of propionyl phosphate and ADP to propionate and ATP. The crystal structures of unliganded TdcD from *Salmonella typhimurium* and its complexes with ADP and AMPPNP have been determined to resolutions of 2.6, 2.2 and 2.3 Å, respectively. Examination of active site pocket revealed the plausible structural rationale for the higher specificity of the enzyme towards propionate than acetate. The structure of TdcD in complex with ATP determined at 1.98 Å resolution revealed a novel reaction catalyzed by the enzyme, i.e., synthesis of 5', 5'''-P1, P4-tetraphosphate (Ap4A). This was further confirmed by determination of the structure of TdcD - Ap4A complex.

Keywords: propionate, threonine deaminase, propionate kinase

P04.02.81

Acta Cryst. (2008). A**64**, C255-256

Crystal structure of tetrameric malate dehydrogenase from *Antarctic psychrophile*

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The psychrophilic and thermolabile malate dehydrogenase was isolated from *Flavobacterium frigidimaris* KUC-1(FfMDH) living in Antarctic seawater. The enzyme is a homotetramer with a molecular weight of 123 kDa and consists of 311 amino acid residues per subunit. To understand structural features of psychrophilic enzymes adapting to cold environment, the structure of FfMDH has been solved by X-ray analysis. FfMDH was crystallized by a hanging-drop vapor diffusion method at 288 K. The crystal has space group $P3_221$ with unit cell dimensions of a=b=147.8 Å and c=165.1 Å, and contains four subunits in the asymmetric unit. Diffraction data were collected at 100 K to 1.8 Å resolution at Beamline BL-5A, PF, Tsukuba. The crystal structure was solved by molecular replacement as the search model of a hybrid MDH (PDB code 1GUZ) using the program MOLREP. It was refined for 1.8 Å diffraction data to R_{work}=0.146 and R_{free}=0.164 with the programs CNS and Refmac5. The structure of FfMDH is a dimer of dimmers and similar to those of MDHs from other bacteria. A structural comparison of four MDHs from the present psychrophilic, mesophilic, moderate thermophilic and thermophilic bacteria, however, reveals several differences in molecular architecture, especially intersubunit interaction. The number of ion pairs in the FfMDH tetramer is smallest among the MDH molecules compared. FfMDH has no intersubunit ion-pairs, although three other MDHs have two to six ion-pairs between every two subunits in each molecule. The lack of intersubunit ion-pair in the oligomeric molecule results in the loose contact among the subunits

in the molecule. This probably contributes to the activity expression of the present psychrophilic enzyme at low temperatures.

Keywords: biological macromolecules, dehydrogenases, cold adapted enzymes

P04.02.82

Acta Cryst. (2008). A**64**, C256

Crystallographic analysis of complexes of bovine trypsin and Schiff base metal chelate

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Studies on trypsin-specific compound are useful for the design of clinical useful compounds, since a variety of physiologically important enzymes (e.g. thrombin, kallikrein and urokinase) have trypsin-like specificity. Several benzamidine and phenylguanidine derivative have been reported to be potent inhibitor. In our previous papers, we have reported of the Schiff base copper(II) chelates carrying a guanidinium group. The chelates are strong inhibitor for trypsin (K_i =～10^{-5} M). To elucidate the structure-activity relationship in this novel series of inhibitors, the crystal structure of complexes between trypsin and guanidine-containing inhibitors were determined. The crystals (**1**,**2**) were obtained by equilibrating the droplet containing 1.25 mM trypsin, 0.1 M Tris-HCl buffer (pH 8.0), 22%(v/v) PEG 4000 and 0.2 M lithium sulfate. Guanidinium group of crystal **1** forms hydrogen bonds with Asp189O$^{\delta 2}$, Ser190O$^{\gamma}$, and Gly219O. The copper(II) ion of inhibitor is in close contact with His57 and Ser195. The imidazole nitrogen of His57 is directly coordinated with the copper(II) ion (2.33 Å). The copper(II) ion coordinated by the imine nitrogen, the phenolic oxygen and one carboxyl oxygen of the Schiff base ligand. In conjunction with detailed structural analysis, this approach will hopefully lead to the development of more potent inhibitor specifically targeting trypsin-like protease, as well as other physiologically important enzymes.

Keywords: X-ray crystallography of proteins, enzyme inhibitor design, medicinal chemistry

P04.02.83

Acta Cryst. (2008). A**64**, C256

Evolution of nylon-oligomer-degrading enzyme based on high resolution crystal structure analysis

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Carboxylesterase with b-lactamase folds on plasmid pOAD2 in *Arthrobacter* sp. KI72, was evolved to be a nylon-oligomer degrading enzyme through directed-evolution using error-prone PCR and DNA shuffling. The activity in a mutant (Hyb-S4M94) was enhanced 80-folds by substitutions of seven amino acid. Site-directed mutagenesis for parental carboxylesterase revealed that the enhancement of the activity is due to three mutations (R187S/F264C/D370Y). To understand the directed evolution of this enzyme based on the ultra-high resolution crystal structures, we have tried to grow the crystals in a good quality under the microgravity condition. X-ray crystal structure analyses of the various mutants and of S112A-activity-deficient-enzyme/Ald complexes were successfully carried out at high resolutions. In G181D mutant (20-folds activity) and in G181D/H266N double mutant (180-folds activity), substrate was stabilized in the active site by electrostatic interaction between amino group in Ald and D181-carboxyl. In contrast, in D370Y mutant (8-folds activity) and Hyb-S4M94, Ald was stabilized by hydrogen bonding and hydrophobic interaction between carboxyl group in Ald and Tyr370, located opposite side in the active site. In G181D/H266N/D370Y triple mutant, the kcat/Km value was increased 5-folds of the G181D/H266N double mutant, and the substrate was stabilized at both amino and carboxyl groups of Ald. Here we discuss the cumulative effects of amino acid substitutions for generating the catalytic centers responsible for nylon oligomer hydrolysis.

Keywords: nylon-oligomer-degrading enzyme, evolution of enzyme, high-reolution crystal structure analysis

P04.02.84

Acta Cryst. (2008). A**64**, C256

Structural biology study in biosynthesis of plant natural products

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Plants may be regarded as biofactories and synthesize over 200,000 natural products. Many of plant natural products can be used for the benefit of human and animal health. The biosynthesis of plant natural products is a very complex process including many different chemical reactions. We are working on three types of enzymes important for plant natural product biosynthesis, glycosyltransferases involved in glycosylation reactions, reductases involved in reductions, and cytochrome P450s involved in hydroxylation and dehydration. We determined crystal structures of several uridine diphosphate glycosyltransferases, NADPH-dependent reductases, and a cytochrome P450 enzyme. Theses structures provide essential insights into their structure-function relationships and catalytic mechanisms in the complex biosynthetic processes. Structure-based mutagenesis and the further functional study help to explore the roles of key residues for catalysis and specificity, and decipher the mechanisms. These studies may also provide us the basis for in vitro manipulation of enzyme activity and substrate specificity, and further rationally-based metabolic engineering and manipulation.

Keywords: enzyme structure function, crystal structures, biosynthesis