

reaction of dCTP - hereby directly producing dUMP from dCTP. In this way the organism avoids to accumulate the toxic intermediate dUTP. dCTP deaminase:dUTPase was first discovered in the archaea *Methanocaldococcus jannaschii* and the *M. tuberculosis* enzyme is the second bifunctional dCTP deaminase:dUTPase to be characterized. Steady state kinetics has shown that the *M. tuberculosis* enzyme has a similar affinity for the two substrates dCTP and dUTP, while dTTP is shown to bind as an inhibitor. Structural comparison between the crystal structures of the enzyme complexed with dTTP and the enzyme alone made us able to demonstrate how the bifunctional enzyme may bind dTTP without hydrolyzing this regulatory molecule [1]. Further studies on selected mutant enzymes have currently been initiated and will be presented.

[1] Helt S.S., Thymark M., Harris P., Aagaard C., Dietrich J., Larsen S., Willemoes M., *J. Mol. Biol.*, 2008, **376**, 554-569.

Keywords: deoxy-ribonucleotide metabolism, deamination, enzyme regulation

P04.02.167

Acta Cryst. (2008). A64, C283

Expansion of substrate specificity and the structural basis of AzoR from *Escherichia coli*

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AzoR is an oxidoreductase isolated from *E. coli* as a protein responsible for the degradation of azo compounds. AzoR exists as a homodimer composed of 23 kDa subunits. The reaction follows a ping-pong mechanism requiring 2 mol of NADH to reduce 1 mol of methyl red, a typical azo dye, into 2-aminobenzoic acid and N,N'-dimethyl-p-phenylenediamine. To clarify the reduction mechanism of azo compounds and to expand the substrate specificity for the development of biodegradation technologies, we have carried out the X-ray crystallographic and enzymatic analyses of AzoR. The overall structures revealed that AzoR has a flavodoxin-like structure in spite of the explicit amino acid sequence homology, and act as a homodimeric FMN-containing enzyme. Superposition of the structures of oxidized AzoR from different crystal forms revealed the regions that participate in the conformational change of the active site, which would be a mechanism to accommodate substrates of different size. The structure of AzoR in the reduced form revealed a twisted butterfly bend of the FMN cofactor's isoalloxazine ring and a rearrangement of solvent molecules. The structure of the enzyme in complex with an inhibitor dicoumarol and the enzymological analysis, combined with the structures of oxidized AzoR, indicate that the formation of a hydrophobic part around the isoalloxazine ring is important for substrate binding, and an electrostatic interaction between Arg-59 and the carboxyl group of the azo compound determines a substrate preference. Based on these results, we succeeded in expanding the substrate specificity by the substitution of Arg-59 with Ala, and we built an authentic model of the AzoR-methyl red complex. This is the first structure of FMN-dependent NADH-azoreductase.

Keywords: azoreductase, substrate specificity, oxidoreductase

P04.02.168

Acta Cryst. (2008). A64, C283

The complex between a branched pentasaccharide and *Thermobacillus xylanilyticus* arabinofuranosidase

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The crystal structure of the family GH-51 α-L-arabinofuranosidase from *T. xylanilyticus* has been solved as a seleno-methionyl derivative and as an inactive mutant Glu176Gln in complex with a branched pentasaccharide. The overall structure shows the two characteristic GH-51 domains: a catalytic domain that is folded into a (α/β)8-barrel and a C-terminal domain that displays jelly-roll architecture. The pentasaccharide is bound in a groove on the surface of the enzyme, with the mono arabinosyl-branch entering a tight pocket harbouring the catalytic dyad. Detailed analyses of both structures and comparisons with the two previously determined structures from *G. stearothermophilus* and *C. thermocellum* reveal important details unique to the *T. xylanilyticus* enzyme. In the absence of substrate, the enzyme adopts an open conformation. In the substrate-bound form, the loop connecting β-strand 2 to α-helix 2 closes the active site and interacts with the substrate through residues His98 and Trp99. The results of kinetic and fluorescence titration studies using mutants underline the importance of this loop, and support the notion of an interaction between Trp99 and the bound substrate. We suggest that the changes in loop conformation are an integral part of the *T. xylanilyticus* α-L-arabinofuranosidase reaction mechanism, and ensure efficient binding and release of substrate.

Keywords: arabinofuranosidase, enzyme, crystal structure

P04.02.169

Acta Cryst. (2008). A64, C283-284

Structure of glycerol-3-phosphate dehydrogenase, an essential monotopic membrane enzyme

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Sn-glycerol-3-phosphate dehydrogenase (GlpD) is monotopic membrane enzyme, functioning at the central junction of respiration, glycolysis, and phospholipid biosynthesis. Its critical role is indicated by multi-tiered regulatory mechanisms. A key flavin-linked primary dehydrogenases of the respiratory electron transport chain, the activity of the enzyme is regulated through its interaction with the inner membrane in *E. coli*. We have determined several new structures of the fully active GlpD, up to 1.75 Å resolution. In addition to elucidating the structure of the native enzyme, we have determined the structures of GlpD complexed with various substrate analogues and product. These structural results reveal conformational states of the enzyme, delineating the residues involved in substrate binding and catalysis at the glycerol-3-phosphate site. Two probable mechanisms for catalyzing the dehydrogenation of glycerol-3-phosphate are envisioned, based on the conformational states of the complexes. To further correlate catalytic dehydrogenation to respiration, we have additionally determined the structures of GlpD bound with ubiquinone analogues, identifying a hydrophobic plateau that is likely to be the ubiquinone binding site. These structural results

illuminate probable mechanisms of catalysis and suggest how GlpD shuttles electrons into the respiratory pathway. Homologs of GlpD are found in practically all organisms, from prokaryotes to humans, with over 45% consensus protein sequences, signifying that these structural results on the prokaryotic enzyme may readily be applied to the eukaryotic GlpD enzymes.

Keywords: membrane, proteins, mechanism

P04.02.170

Acta Cryst. (2008). A64, C284

The crystal structure of capsule synthesis protein CapD, a major virulence factor in *B. anthracis*

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The capBCADE genes are responsible for synthesis and transport of the poly- γ -D-glutamic acid capsule known to protect *Bacillus anthracis* from phagocytic killing during infection. Here, we present the crystal structures of CapD with and without the dipeptide α -L-Glu-L-Glu in the active site of the enzyme. CapD shares the structure of N-terminal nucleotide hydrolases and in particular *Escherichia coli* and *Helicobacter pylori* γ -glutamyltranspeptidases. Unlike bacterial γ -glutamyltranspeptidases, CapD displays transpeptidation activity and its structure reveals a wide open active site for poly- γ -glutamate binding and processing. Based on sequence and structure comparison, we propose that Pro427, Gly428, Gly429 contribute to CapD activity by activating Thr352 and stabilizing an oxyanion hole via classical main chain amides hydrogen bonds. Structural observations are corroborated by mutagenesis and functional studies. Modeling of a di- γ -D-Glu substrate in the active site suggests that Thr352 could form an acyl-enzyme intermediate with γ -carboxylate of poly- γ -glutamate chains. Resolution of this intermediate by a nucleophile would result in transpeptidation activity.

Keywords: CapD, PDGA, Ntn-hydrolase family

P04.02.171

Acta Cryst. (2008). A64, C284

Crystal structure of adenosine kinase from *M. tuberculosis* in complex with nucleoside analogs

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Adenosine kinase (AK) is a key enzyme in the purine salvage pathway of *Mycobacterium tuberculosis*, an intracellular pathogen that causes tuberculosis (TB). Mtb AK, a unique bacterial adenosine kinase catalyzes the phosphorylation of adenosine to adenosine monophosphate and is involved in the bioactivation of some nucleoside analogs that have demonstrated selective activity against *M. tuberculosis*. The mechanism of action of these adenosine analogs is likely to be different from those of current TB treatments; therefore, specific activation of nucleoside analogs by Mtb AK may prove to be a novel therapeutic intervention for TB, particularly for multi-

drug resistant TB. Specific inhibition of this key enzyme in the purine salvage pathway may also be exploited for therapeutic development. The crystal structures of the enzyme in complex with adenosine or one of three selected nucleoside analogs have been determined at 1.9 Å resolution with *R* factor of 0.19 and *R*_{free} of 0.25. The structure reveals a tightly associated homo-dimer, which is different from the known human and *T. gondii* AK, but rather resembles the structure of ribokinases. The monomer consists of a small domain that is responsible for the dimer formation and a large catalytic domain. The active sites reveal the protein-ligand interaction and significant structural differences between the human and Mtb AK. The structural information provides the structural basis for the specific activation of nucleoside analogs by Mtb AK and should aid in the design of more potent and selective antimycobacterial agents. We thank SBC-CAT and NE-CAT at APS for access to beam lines 19-BM and 8-BM. This research is supported in part by NIH grants, AI55344 to RL.

Keywords: nucleoside analogs, tuberculosis, drug design

P04.02.172

Acta Cryst. (2008). A64, C284

Structure and mechanism of rhamnogalacturonan lyases

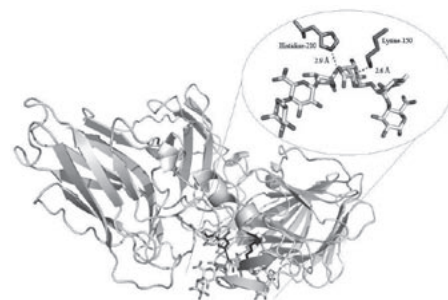
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We present the first experimental evidence of rhamnogalacturonan lyase (RGL), belonging to family PL4 according to CAZY classification (www.CAZy.org.[1]), with bound substrate in the active site. *Aspergillus aculeatus* RGL (AARGL), which native structure is published as 1NKG in the PDB [2], is involved in the degradation of rhamnogalacturonan-I, an important plant cell wall polysaccharides. Through enzyme variants H210A and K150A, characterized kinetically and structurally, we have shown that His210 and Lys150 are key active site residues. X-ray diffraction data collected at MaxLab, Lund, Sweden, after soaking the K150A variant with a rhamnogalacturonan digest shows evidence of bound substrate at the -3/+3 subsites (Fig.1). Considering our results and structural and sequence comparisons to PL family enzymes, we propose a beta-elimination mechanism on [2]- α -L-Rhamno-(1,4)- α -D-Galacturonic acid (1,). The lack of calcium in the mechanism significantly differs from other characterized lyases, also supporting a different mechanism.

[1] Coutinho et al. in Recent Advances in Carbohydrate Bioengineering, 1999, Cambridge: RSC.

[2] McDonough, M. A. et al., FEBS Lett, 2004. 565 (1-3): p. 188-94.



Keywords: biological macromolecular crystallography, carbohydrate degradation, pectate lyases substrate binding