P.04.02.115

Acta Cryst. (2005). A**61**, C207

Crystal Structure of a Glycyl Radical Enzyme from *Archaeoglobus fulgidus*

Lari Lehtiö[a], Gunter Grossmann[b], Bashkim Kokona[c], Robert Fairman[c], Adrian Goldman[a], [a]*Institute of Biotechnology, University of Helsinki, PO Box 65, FIN-00014, Helsinki, Finland.* [b]*CLRC Daresbury Laboratory, Daresbury, Warrington, WA4 4AD, United Kingdom.* [c]*Department of Biology, Haverford College, 370 Lancaster Ave, Haverford, PA 19041,USA*. E-mail: lari.lehtio@helsinki.fi

We have solved the crystal structure of a PFL2 from Archaeglobus fulgidus at 2.9 Å resolution. Of the two previously solved enzyme structures of PFL-family, pyruvate formate lyase and glycerol dehydratase , the latter is clearly more similar to our model. In our structure we have a glycerol molecule bound to the active site, which, however, is bound in a different orientation than in glycerol dehydratase, due to the changes in the residues forming the binding site. However,the active site of PFL2 is so similar to that of glycerol dehydratase, that it is likely to catalyze, not the same, but perhaps a very similar reaction.

Crystal packing, small angle scattering and ultaracentrifugation experiments show that PFL2 is a tetrameric protein in contrast to our earlier result indicating trimeric arrangement [1]. This higher oligomeric state is one of the explanations for the thermostability of the enzyme.

[1] Lehtio L., Goldman A., *Protein Eng Des Sel*, 2004, **17**, 545.

Keywords: pyruvate formate lyase, glycerol dehydratase, glycyl radical

P.04.02.116

Acta Cryst. (2005). A**61**, C207

Crystal Structure of Dihydropteroate Synthase from *Streptococcus pneumoniae*

Colin Levy, Jeremy Derrick, *Faculty of Life Sciences, The University of Manchester, Manchester, U.K.* E-mail: c.levy@manchester.ac.uk

Dihydropteroate synthase (DHPS) catalyses a key step in the biosynthesis of folic acid, joining *para*-aminobenzoic acid to hydroxymethylpterin pyrophosphate. It is also the target for the sulphonamide family of antimicrobial drugs [1], and hence a well-established target for anti-infectives. We have determined the crystal structure of DHPS from the respiratory pathogen *Streptococcus pneumoniae* [2], as a prelude to a study of the recognition of sulphonamides and mechanisms of drug resistance by the enzyme. Data was collected from DHPS crystals which diffracted to 1.90Å resolution: attempts to solve the structure by molecular replacement using DHPS structures from other organisms failed, however. A second crystal form was obtained from a DHPS preparation with selenomethionine incorporation. Data were collected to 2.32Å resolution (97% complete), and the structure was subsequently solved using a single wavelength SAD data set. This model was subsequently used to solve the structure of the first crystal form. *S. pneumoniae* DHPS has a TIM-barrel fold and the active site is surrounded by several extensive loop regions which appear to play an important part in substrate recognition and the compulsory order ternary complex mechanism of the enzyme [2].

[1] Bermingham A., Derrick J.P., *Bioessays*, 2002, **24**, 637. [2] Vinnicombe H.G., Derrick J.P., *Biochem Biophys Res Commun*, 1999, **258**, 752.

Keywords: folate dependent enzymes, biosynthesis, antifolates

P.04.02.117

Acta Cryst. (2005). A**61**, C207

Crystal Structure of NH_3-dependent NAD+ Synthetase from *Helicobacter pylori*

Mun-kyoung Kim, Soo Hyun Eom, *Department of Life Science, Gwangju Institute of Science and Technlogy*. E-mail: eom@gist.ac.kr

Nicotinamide adenine dinucleotides (NAD+ and NADP+) play a central role in all living systems. They are essential and ubiquitous coenzymes, and are involved in biochemical processes ranging from redox reactions to DNA repair, DNA recombination, and protein-ADP ribosylation.

The prokaryotic and eukaryotic forms of NAD+ synthetase differ in terms of their substrate requirements. Prokaryotic NAD+ synthetase uses ammonia as a nitrogen source, whereas eukaryotic NAD+ synthetase requires glutamine. However, eukaryotic NAD+ synthetase belongs to the amidotransferase family, and has an additional domain that enables the enzyme to use glutamine as a nitrogen source. The amidotransferases are composed of a glutamine amide transfer (GAT) domain and a synthetase domain, and can be divided into F- and G-type amidotransferase families based on their GAT domains.

The need to identify new targets for antibacterial agents is growing due to increasing drug resistance. NAD+ synthetase from bacterial pathogens like *Helicobacter pylori, Mycobacterium tuberculosis, and Peudomonas aeruginosa*, is an attractive target for the development of new antibacterial drugs. We determined the crystal structures of *H. pylori* NAD+ synthetase in apo- and complex forms with NaAD & ATP to a resolution of 2.3 and 1.7 Å, respectively.

[1] Ozment C. e*t al.*, *J Struct Biol*, 1999, **127**, 279. [2] Devedjiev Y. *et al.*, *Acta Crystallogr D*, 2001, **57**, 806. [3] Gil Bu K. *et al.*, *Proteins*, 2005, **58(4)**, 985.

Keywords: NH_3-dependent NAD+ synthetase, drug targets, enzimatic proteins

P.04.02.118

Acta Cryst. (2005). A**61**, C207

Structural Insights into a Glysoside Hydrolase Family 26 lichenase

Victoria A. Money[a], Edward J. Taylor[a], Carlos M. G. A. Fontes[b], Harry J. Gilbert[c], Gideon J. Davies[a], [a]*York Structural Biology Laboratory, Department of Chemistry, University of York, York, YO10 5YW UK.* [b]*CIISA-Faculdade de Medicina Veterinária, Rua Prof. Cid dos Santos, 1300 477 Lisbon, Portugal.* [c]*Biological and Nutritional Sciences, Univerisity of Newcastle upon Tyne, Newcastle upon Tyne, NE2 4HH, UK*. E-mail: money@ysbl.york.ac.uk

Hydrolysis of the glycosidic bond is one of the most critical processes in nature and has considerable technical importance. Glycoside hydrolases have been shown to be extremely proficient at the acceleration of this reaction increasing rates by a factor of 10^{17}, this makes them among the most effective of enzymes. This effectiveness is reflected in the tight binding of the oxocarbenium transition state. Determination of the conformation of the substrate whilst in this transition state is of importance not only for improved understanding of the action of these enzymes but also for the design of specific and powerful enzyme inhibitors. We present here the results of a crystallographic analysis of a member of the glycoside hydrolase family 26. The structure of the native enzyme and the successful structural determination of the enzyme complexed with an inhibitor is described. The enzyme inhibitor interactions revealed as a result of this study are discussed.

Keywords: protein ligand complex, glycoside hydrolase, protein structure

P.04.02.119

Acta Cryst. (2005). A**61**, C207-C208

Crystal Structure of Methylthioribose-1-phosphate Isomerase from *Bacillus subtilis*

Haruka Tamura[a], Hiroyoshi Matsumura[a], Tsuyoshi Inoue[a,c], Hiroki Ashida[b], Yohtaro Saito[b], Akiho Yokota[b], Yasushi Kai[a], [a]*Department of Materials Chemistry, Graduate School of Engineering, Osaka University, Japan.* [b]*Department of Molecular Biology, Graduate School of Biological Science, Nara Institute of Science and Technology (NAIST), Japan.* [c]*Structure and Function of Biomolecules Group, PRESTO, Japan Science and Technology Agency.* E-mail: haruka@chem.eng.osaka-u.ac.jp

Methionine salvage pathway plays an important role to recycle organic sulphur from a sulphahydryl derivative of the nucleoside. Recently, five enzymes in the pathway have been characterized from *Bacillus subtilis* [1]. One of them was characterized as a methylthioribose-1-phosphate isomerase (MtnA), which catalyzes the isomerization of methylthioribose-1-phosphate to methylthioribulose-