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

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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

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Crystal Structure of Dihydropteroate Synthase from *Streptococcus pneumoniae*

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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

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

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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

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Structural Insights into a Glysoside Hydrolase Family 26 lichenase

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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

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

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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-

1-phosphate.

In this work, we report the three-dimensional structure of MtnA from *B. subtilis* at 2.4 Å resolution, which is the first structure of the well-characterized MtnA. The crystal structure reveals the homodimeric architecture, which corresponds to the result observed in the analysis of dynamic light scattering. A search of protein coordinates in the Protein Data Bank with the program DALI [2] shows that probable MtnA from *Thermotoga maritima*, regulatory subunit of aIF2B from *Pyrococcus horikoshii* and yeast Ypr118w are structurally the most similar to that of MtnA from *B. subtilis*. Although insertion/deletion occur frequently in the sequence alignment, *B. subtilis* MtnA has a high degree of similarity with the secondary structures and the active site structures of these proteins. These observations probably suggest that these functionally unknown or putative proteins have the same function as that of *Bacillus* MtnA. For the purpose of investigation into the detailed catalytic mechanism of MtnA, the crystallization of MtnA complexed with its substrate is currently in progress.

[1] Ashida H., Saito Y., Kojima C., Kobayashi K., Ogasawara N., Yokota A., *Science*, 2003, **302**, 286. [2] Holm L., Sander C., *J. Mol. Biol.*, 1993, **233**, 123.

Keywords: crystal structure determination, metabolism enzyme, isomerase

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Structural and Functional Studies of Carbohydrate Esterase Family 7 Enzymes

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The Carbohydrate Esterase family 7 (CE-7, CAZy, April 2005) includes 26 bacterial oligomeric α/β hydrolases with multifunctional deacetylase activity. Their primary role is the deacetylation of the decorated xylooligosaccharides that are transported into the bacterium cytoplasm. Therefore, the enzymes could be considered accessory ones in the plant cell wall biodegradation. Despite the crystal structure of two family members was determined, it is still unclear how the substrate reaches the catalytic site, and how the product is released from the oligomeric enzymatic assembly. To further characterize the CE-7 family, *Bacillus pumilus* acetyl xylan esterase (AXE) was expressed, purified and crystallized - alone and in complex with the reaction products xylose and acetate. The 3D structures were determined by X-ray analysis at 1.9Å and 2.6Å respectively, each one showing two doughnut-like hexamers with local 32 symmetry in the asymmetric unit. Snapshots of the enzymatic process were obtained. The identified xylose binding sites let us hypothesize a route connecting the active site to the exterior of the self-compartmentalizing enzymatic assembly. The CE-7 family representative, *Thermoanaerobacterium sp*. AXE1, was also characterized, and its crystal structure determined at 1.9Å. As a result, new insights into the CE-7 family mechanism of action are suggested, and structural basis for their different sensitivity to the commonly used serine-modifying reagent, PMSF, are provided.

Keywords: macromolecular X-ray crystallography, esterases, MR

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Differential Maturation of SUMO Precursors by SUMO-specific Protease, SENP1

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Small ubiquitin-related modifier (SUMO) is a member of the ubiquitin-like protein family that regulates cellular function of a variety of target proteins. SUMO proteins are expressed as their precursor forms. Cleavage of the residues after the "G-G" region of these precursors by SUMO specific proteases in maturation is a prerequisite for subsequent sumoylation. To further understand this proteolytic processing, we expressed and purified SENP1, one of the SUMO specific proteases, using an *E. coli* expression system. We show that SENP1 is able to process all SUMO-1, -2 and -3 *in vitro*, however the proteolytic efficiency of SUMO-1 is the highest followed by SUMO-2 and SUMO-3. We further demonstrate the catalytic domain of SENP1 (SENP1C) alone can determine the substrate specificity towards SUMO-1, -2 and -3. Using mutagenesis analysis, two residues immediately after the "G-G" region are mapped to be essential for the differential maturation. At present, crystals of inactive SENP1C and SUMO-1 have been obtained. Future structural analysis will provide insight into the molecular basis of the differential maturation process.

Keywords: small ubiquitin-related modifier (SUMO), sentrin-specific protease 1 (SENP1) proteases, sumoylation

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High Resolution Structures of Formate Dehydrogenase Mutants from *Candida boidinii*

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Formate dehydrogenase from *Candida Boidinii (Cbfdh)* is an intensively studied cofactor regenerating biocatalyst. To improve its thermal stability and minimize the oxidative deactivation occurring under the conditions of the industrial process, modifications were designed based on the available X-ray structure from *Pseudomonas sp*. [1]. Structural information on *Cbfdh* is required for engineering coenzyme specificity changes in order to generate regeneration systems for additional applications [2]. Since *Cbfdh* remained reluctant to crystallisation we applied rational site-directed mutagenesis of surface patches based on the results obtained by Derewenda and coworkers [3] and using the FoldIndex prediction software. Dramatic improvement resulting in crystals diffracting to 1.6 Å resolution could be achieved. Structural analysis is ongoing and the results will be presented on the poster. Rational site-directed mutagenesis of selected surface amino acids could become a routine application to decrease the entropy on the protein surface and therefore improve the crystallisation process.

[1] Slusarczyk H. *et al*., *Eur. J. Biochem*., 2000 **267**, 1280-1289. [2] Tishkov V.I., *FEBS Letters*, 1996, **390**, 104-108. [3] Derewenda Z.S., *Structure*, 2004, **12**,529-535.**Keywords: X-ray protein protein, engineering mutagenesis crystallography, protein disorder**

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Novel Mechanisms of pH Sensitivity in Tuna Hemoglobin

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The crystal structure of fish hemoglobin (Hb) has been known for several years, yet various features of the molecule remain unexplained or controversial. Fish Hbs are well known for their widely varying interactions with heterotropic effector molecules and pH sensitivity. Some fish Hbs are almost completely insensitive to pH, whereas others show extremely low oxygen affinity under acid conditions, a phenomenon called the Root effect. We have solved the crystal structure of tuna Hb in the deoxy form at low and moderate pH and in the presence of carbon monoxide at high pH. In the T state a novel salt salt bridge is formed between His69β and Asp72β. This salt bridge is broken in the R state structure, releasing a proton. Additional proton binding to the T state occurs through a pair of carboxyl groups, Asp96α1 and Asp101β2. The dramatic change between the two T state models is found at His60 of one α subunit. At low pH, this residue swings out the of the heme pocket. Removal of His60α from the heme pocket will significantly reduce the α subunit affinity for oxygen.

Keywords: crystallography macromolecular, hemoglobins, structure and function