decreased flexibility provides a basis to explain the low affinity of ONC towards nucleotides and, more generally, its lower catalytic activity. The results also suggest the basis of the unusually high thermal stability of the enzyme.[1]

[1] Merlino A., *et al.*, *J. Biol. Chem.*, *accepted for publication*.

Keywords: mutations, dynamic properties, crystallography

P.04.02.25

Acta Cryst. (2005). A**61**, C186

Structural Basis for Tumor Pyruvate Kinase M2 Allosteric Regulation and Catalysis

Jill D. Dombrauckas, Bernard D. Santarsiero, Andrew D. Mesecar, *Center for Pharmaceutical Biotechnology and Department of Medicinal Chemistry, University of Illinois at Chicago, Chicago, Illinois*. E-mail: jdombr5@uic.edu

Pyruvate Kinase plays catalyzes the last step of the glycolytic cycle, turning over the substrate phosphoenolpyruvate, PEP, into pyruvate, producing one molecule of ATP per reaction. Four isozymes of this enzyme exist in humans: R, L, M_1, and M_2. The R and L isoforms are present in the erythrocytes and liver cells, respectively. Both M_1 and M_2 are encoded by the M gene. The M_1 isoform is found in skeletal muscle and brain tissue. The M_2 isoform is predominately present in fetal tissue and is progressively replaced by the other isoforms after birth. However, the M_2 isoform is again reexpressed in numerous tumor cells.

The overexpression of the M_2 isoform in tumor cells invokes many mechanistic questions regarding the role of $hPKM_2$ in tumorgenesis, as well as offers an intriguing anti-cancer target. Therefore, our structure may be useful as a template for the discovery of novel compounds that may serve as possible anti-cancer drug leads. We cloned, overexpressed, and purified $hPKM_2$ from inclusion bodies in *E.coli* through a unique refolding protocol. The enzyme was crystallized and x-ray data were collected at the APS (Argonne National Labs). The human PKM_2 crystal structure was determined to 2.8 Å resolution. Structural analysis and comparison of structural differences among isozymes is presented here.

Keywords: pyruvate kinase, allosteric, conformational change

P.04.02.26

Acta Cryst. (2005). A**61**, C186

Structural Studies of the Sucrose Isomerase MutB from *Pseudomonas mesoacidophila*

Stéphanie Ravaud[a], Hildegard Watzlawick[b], Richard Haser[a], Ralf Mattes[b], Nushin Aghajari[a], *[a]LBC, IBCP, Lyon, France. [b] Institut für Industrielle Genetik, Universität Stuttgart, Germany*. E-mail: s.ravaud@ibcp.fr

The sucrose isomerase, MutB, from *Pseudomonas mesoacidophila* belongs to glycoside hydrolase family 13, and catalyzes the isomerization of sucrose into isomaltulose and trehalulose [1]. The 64 kDa enzyme has been crystallized [2] and the three dimensional structure of MutB has been solved to 1.6 Å resolution by the molecular replacement method using the isomaltulose synthase, PalI, from *Klebsiella* sp. LX3 as a search model [3]. The overall structure of MutB is made up of three domains: an N-terminal and catalytic $(\beta/\alpha)_8$ domain, a subdomain and a C-terminal domain made up of seven β-strands [4].

The structures of various complexes with inhibitors and/or substrate analogues have been obtained and are currently under refinement. Once the detailed analyses of these structures have been completed, a better understanding of the molecular basis of sucrose decomposition, isomerization as well as the selectivity of this enzyme leading to the formation of different products should be gained.

[1] Nagai Y., Sugitani T., Tsuyuki K., *Biosci. Biotechnol. Biochem.*, 1994, **58**, 1789. [2] Ravaud S., Watzlawick H., Haser R., Mattes R., Aghajari N., *Acta Cryst. F.*, 2005, **61**, 100. [3] Zhang D., Li N., Lok S.M., Zhang L.H., Swaminathan K., *J. Biol. Chem.*, 2003, **278**, 35428. [4] Ravaud S., Watzlawick H., Haser R., Mattes R., Aghajari N., *submitted.*

Keywords: X-ray crystal structure determination, enzymatic structure-activity relationships, sucrose

P.04.02.27

Acta Cryst. (2005). A**61**, C186

Recovery of Argininosuccinate Lyase Activity in Duck δ₁Crystallin

May Y. W Tsai[1,2], Jason Koo[1,2], Liliana M. Sampaleanu[1], Caroline Greene[1], Louise Creagh[3], Charles Haynes[3], P. Lynne Howell[1,2], *[1]Hospital for Sick Children. [2]University of Toronto, Canada. [3]Univerisity of British Columbia, Canada.* E-mail: tsai_may@yahoo.com

δ-Crystallin is directly related to argininosuccinate lyase (ASL). Two isoforms exist in ducks, δ2 and δ1, which are 94% identical. δ2 is the duck orthologue of ASL, while δ1 is enzymatically inactive. Chimeras of the two isoforms have shown that domain 1 of δ2 is sufficient to recover activity in δ1. Structural comparisons of various δ-crystallin proteins revealed that conformational differences between δ1 and δ2 are localized to residues 23-32 and 74-89 (20's and 70's loops). As the putative catalytic residues are conserved in δ1, the amino acid substitutions in these loops are thought to prevent substrate binding in δ1. However, a δ1 double loop mutant (DLM), with all residues in the 20's and 70's loops replaced with those of δ2, was found to be inactive and binding of the substrate to the DLM could not be detected by ITC. To further investigate this result, crystal structures of the DLM with and without sulfate bound have been determined to 2.2 and 2.5Å resolution, respectively. The conformations of the 20's and 70's loops in the DLM and δ2 are very similar, suggesting the remaining five amino acid differences in domain 1 of the DLM relative to δ2 are important for ASL activity. Mutagenesis experiments reveal that ASL activity can be recovered in the DLM by mutating Met-9 to Trp. Truncation mutants of δ2 demonstrate that although the N-terminal arm is conformationally flexible, this region of the protein is critical for ASL activity. The N-terminal segment is likely involved in stabilizing regions of δ2 involved in substrate binding and catalysis.

Keywords: δ-crystallin, argininosuccinate lyase, enzyme mechanism

P.04.02.28

Acta Cryst. (2005). A**61**, C186-C187

Crystal Structure of a Native Chitinase from the Fungal Pathogen *Aspergillus fumigatus* YJ-407 (afCHI)

Kailin Yang[a], Ganggang Wang[a], Haitao Yang[a], Mark Bartlam[a], Chen Jin[b], Zihe Rao[a,c], *[a]Laboratoy of Structural Biology, Department of Biological Sciences and Biotechnology, Tsinghua Universite. [b]State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences. [c]National Laboratory of Macromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, P. R. China.* E-mail: yangkl@xtal.tsinghua.edu.cn

Chitinase hydrolyzes chitin, which is a structural component of cell walls and coating of many organisms. In fungi, chitinase is thought to contribute to a number of morphogenetic processes in filamentous fungi. Therefore, fungal chitinase is thought to be a putative virulence factor and a promising anti-fungi target molecule. As one of the most ubiquitous of the airborne saprophytic fungi, Aspergillus fumigatus has been shown to be an opportunistic pathogen causing pneumonia and other fatal invasive inflection. Except for endo- and exo-hydrolytic activities, a transglycosyl activity was observed in the extracellular chitinase (afCHI) from Aspergillus fumigatus YJ-407.

This native chitinase from the fungal pathogen Aspergillus fumigatus YJ-407 (afCHI) has been crystallized and the X-ray structure has been solved to 2.1Å resolution by molecular replacement. Like other members of the class 18 hydrolase family, this fungal enzyme is of an eight_stranded b/a-barrel. And a GlcNAc was observed in the glycosylation site (Asn257-Asp258-Thr259). Structural comparisons revealed that structural features such as substrate binding site, residues in active site and catalytic acid are conserved. Furthermore, the physiological role of saccharide and the structural basis of transglycosyl activity were discussed.

[1] Xia G., Jin C., Zhou J., Yang S., Zhang S., *Eur. J. Biochem*, 2001, **268**, 4079. [2] Fusetti F., von Moeller H., Houston D., Rozeboom H.J., Dijkstra

B.W., Boot R.G., Aerts J.M., van Aalten D.M., *J. Biol Chem* 2002, **277**, 25537.
Keywords: chitinase, crystal structure, glycoprotein

P.04.02.29
Acta Cryst. (2005). A**61**, C187
Crystal Structures of Two *Bacillus subtilis* Protein YjcG and YflL
Dan Li, Lan-Fen Li, Yu-He Liang, Xiao-Dong Su, *Department of Biochemistry and Molecular Biology, College of Life Sciences, Peking University, Beijing, China.* E-mail: li-dan@pku.edu.cn

YjcG is a putative protein from *Bacillus Subtilis* with 171 amino acids, it was cloned into the vector pET21-DEST by Gateway system. YjcG was expressed in *E. coli* strain BL21(DE3), purified and crystallized by hanging-drop vapor diffusion method. Since there is no homologous structures, MAD (multi-wavelength anomalous diffraction) method was chosen to solve the YjcG structure. The crystal belongs to spacegroup C2 with unit-cell parameters a=99.30 Å, b=73.77 Å, c=61.58 Å, β=113.541°. The structure is refined to R_{cryst} 0.237 and R_{free} 0.267 at 2.3 Å. Dali search showed that YjcG structure is similar to 2'-5' RNA ligase with Z=14.7, RMSD 2.5 Å with 138 residues.

YflL is a function uncharacterized *B. subtilis* protein with 39.5% identity to acylphosphatase from horse muscle. Crystals suitable for structural studies were obtained from 50 mM potassium phosophate, pH 6.5. The structure was determined by MR (molecular replacement). The crystal diffracted to 1.3 Å and belongs to space group $P2_12_12_1$ with unit-cell parameters of a=26.86 Å, b=48.27 Å, c=59.57 Å. The structure is refined to R_{cryst} 0.181 and R_{free} 0.234. We have identified in vitro that the YflL is not only an acylphosphatase but also an apyrase.
Keywords: *Bacillus subtilis*, MAD method, MR

P.04.02.30
Acta Cryst. (2005). A**61**, C187
Crystal Structures of DAH7PS Synthase from *Pyrococcus furiosus*
Bryan Frederick Anderson, G.E. Norris, L.R. Schofield, M.L. Patchett, G.B. Jameson, E.J. Parker, *College of Sciences, Massey University, Palmerston North, New Zealand.* E-mail: B.F.Anderson@massey.ac.nz

The enzyme 3 deoxy-D-arabino-heptulosate 7-phosphate synthase (DAH7PS) catalyses the condensation reaction between phosphoenol pyruvate (PEP) and the 4 carbon saccharide D-erythrose-4-phosphate (E4P). This reaction is the first step in the shikimate pathway used in plants and microorganisms to synthesize aromatic amino acids and many secondary aromatic metabolites.

DAH7PS from *P furiosus* requires a metal ion for activity and, unlike DAH7PS from other species, is not subject to regulation by aromatic amino acids, It is also able to utilize both 4-carbon and 5-carbon phosphorylated monosaccharides with similar K_{cat} but increased K_m values. This broad substrate specificity and lack of regulation indicates it may be the closest protein to the ancestral enzyme of the type 1 DAH7PS enzymes.

Two crystal structures will be presented. The first – the apo form of the molecule, crystallizes in space group I222 with *a*=87.23Å, *b*=110.02Å, *c*=144.35Å. There are two molecules in the asymmetric unit (the biological unit) which associate with a 2-fold related pair to form distinct tetramers. The structure was solved by molecular replacement using a hybrid model formed from elements of two related structures with low sequence identity (26% and 30%). Maps show the presence of PEP at low occupancy.

The second form, the Cd loaded derivative crystallizes in space group $P2_1$, *a*=48.94Å, *b*=84.33Å, *c*=139.16Å, β=92.60° with two intact tetramers in the unit cell. Current maps show a greatly expanded metal binding site, the presence of PEP at low occupancy, and the presence of some ribose 5-phosphate.
Keywords: enzyme, condensation, synthase

P.04.02.31
Acta Cryst. (2005). A**61**, C187
Crystal Structure of the *Bacillus subtilis* YwlE Protein
Erik Brostromer[a], Huimin Xu[b], Yuhe Liang[a], Changwen Jin[b], Xiao-Dong Su[a], [a]*Department of Biochemistry and Molecular Biology, College of Life Sciences, Peking University, Beijing China.* [b]*Beijing NMR center, Peking University, Beijing China.* E-mail: su-xd@pku.edu.cn

YwlE is a 150 residue protein encoded by the ywlE gene in *Bacillus subtilis*. It is a putative low-molecular-weight protein tyrosine phosphatase (LMW PTP) by sequence comparison.

The full length YwlE was cloned into pET28a and expressed in good amount in soluble form, it was purified by two-step conventional chromatography on a FPLC system. Crystallization trials of the purified protein were preformed by hanging-drop vapor diffusion method, usable crystals appeared in some of the screen drops after a few days. One set of native diffraction data was collected at the MAD beamline of Beijing Synchrotron Radiation Facilities (BSRF), using the oscillation method, and processed to 1.8 Å. The crystal belongs to space group C2 and there is 1 molecule per asymmetric unit. The sequence homology to bovine LMW PTP (Blast score 50.2 bits, 29 %) enabled the structure to be determined through molecular replacement using the CNS program. This gave a model with an R_{free} of 0.48. The program ARP/wARP was used for automated model building and further refinement. A much improved model was obtained after this procedure with R_{free} of 0.27.

The YwlE structure shows significant structural homology to protein-tyrosine-phosphatases, especially in the active site (CX_5R motif). It's hoped that this structure together with enzymology studies will deepen our understanding of protein phosphorylation and signal transduction in general.
Keywords: YwlE protein, tyrosine-phosphotase, bacterial

P.04.02.32
Acta Cryst. (2005). A**61**, C187
Structure of Aldehyde Reductase Complex: Implications for Inhibitor Specificity
Vincenzo Carbone[a], Roland P.-T. Chung[a], Alberto Podjarny[b], Ossama El-Kabbani[a], [a]*Department of Medicinal Chemistry, Monash University.* [b]*UPR de Biologie Structurale, IGBMC, CNRS INSERM ULP, Illkirch France.* E-mail: Vincenzo.Carbone@vcp.monash.edu.au

Aldehyde and aldose reductases are members of the aldo-keto reductase superfamily of enzymes that catalyze the NADPH-dependent reduction of a number of different aldehydes to their corresponding alcohols. Both enzymes share significant sequence homology and form a classic α/β TIM-barrel structure with the least conserved residues lining the active site located at the C-terminal loop. Aldose reductase activity has been implicated in glucose over utilization and the aetiology of diabetic complications. Given the abilities of both enzymes to bind common inhibitors, the X-ray structure of porcine aldehyde reductase holoenzyme in complex with NADPH and the potent aldose reductase inhibitor Fidarestat (SNK-860) was determined at an atomic resolution of 1.85Å to elucidate the mechanism of inhibition. The hydrogen bonds between the active site residues Tyr50, His113 and Trp114 are conserved in aldose and aldehyde reductases. In the case of aldehyde reductase residues from the C-terminal loop do not form hydrogen bonds with the inhibitor Fidarestat. Leu300, a residue previously identified as essential in the determination of inhibitor potency for aldose reductase is a Pro in aldehyde reductase and can not form a hydrogen bond. Furthermore, molecular modelling calculations suggest that the conserved Trp220 may play a role in the disparity in IC_{50} values for the two enzymes. These results could provide a structural basis in the design of potent and specific inhibitors for aldose reductase.
Keywords: aldehyde reductase, inhibitor interactions, diabetes