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The Refinement of the Yeast Phosphofructokinase-1 Atomic Model
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6-Phosphofructo-1-kinase (Pfk), a key enzyme in glycolysis, is a heterooctamer ($\alpha_4\beta_4$)of about 800 kDa (21S). The crystal structure of the limited proteolysis product (600 kDa, 12S Pfk) was determined to 2.9 Å resolution. The total number of atoms of the Pfk model exceeds 44,000 and subsequently the number of parameters to be refined is four times as many. Owing to the low data to parameter ratio at this resolution (172,763 unique reflections have been obtained) the refinement has been carried out under tight restraints and with careful monitoring of the R/R_{free} ratio. The bulk of the molecule has clear electron density.

Fructose-6-phosphate was present in the crystallization medium. The electron density clearly shows the mode of binding of the ligand in the active site and in the binding site of the allosteric effector: fructose-2,6-bisphosphate, unique to eukariotic Pfk. The Pfk molecule appears to be in the allosteric R-state.

Keywords: allostery, metabolism regulation, reaction mechanisms of enzymes

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The Crystal Structure of *Francisella tularensis* AcpA
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Francisilla tularensis is a category A pathogen found predominantly in the Northern Hemisphere. It has been utilized as a biological warfare agent and is considered a likely weapon of a bioterrorist attack. Delineating the mechanisms of survival and previous investigations suggest that *F. tularensis* acid phosphatase, AcpA, suppresses the respiratory burst and may be important for intracellular survival and multiplication within the host's professional phagocytes. To better understand the molecular basis of virulence, we initiated crystal structure determination studies of AcpA. The gene has been cloned and expressed to high levels in E. coli. The crystal structure has been solved to 1.75Å.

Keywords: *Francisella tularensis*, acid phosphatase, enzime

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Comparison between Crystal Structures of cMDH in Apo and NAD/NADH Binding Form
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Cytosolic malate dehydrogenase (cMDH) is generally known as a key enzyme in several metabolic pathways. An additional biological function associated with nucleic acid-conducting channel has been identified very recently [1]. Furthermore, the finding that anticancer natural products inhibit cMDH has raised the possibility of this multi-functional protein as a druggable target [2]. Since human cMDH alone is structurally unknown, we investigated the crystal structure of the apo form to be compared with the NAD/NADH complex.

We crystallized three types of cMDH (NAD-binding, NADH-binding and the apo forms), and collected the diffraction data at high resolution by Pharmaceutical Industry Beamline (BL32B2) in the SPring-8. As a result of structural determination, significant structural differences were observed in the NAD/NADH-binding site, especially in the entrance region including a long loop. NAD and NADH interacted with the loop to be stabilized although the loop in the apo form was included in the packing interaction of the crystal. These findings suggest that the loop moves flexibly to capture the coenzymes. On the other hand, the flexibility of the inner region seemed to be low. Finally, the possibility to use the structure information on drug design will be discussed.

[1] Basil H., *et. al*., *Proc. Natl. Acad. Sci.*, 2002, **99**, 1707. [2] Knockaert M., *et. al*., *J. Biol. Chem*., 2002, **277**, 25493.

Keywords: dehydrogenase, crystal structures, drug design

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Deamination and Dephosphorylation of dCTP – Two Reactions Catalysed by a Family of Enzymes
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dCTP deaminase is a homotrimeric enzyme found in Gram-negative bacteria catalysing the deamination of dCTP forming dUTP. Dephosphorylation of dUTP by the indispensable enzyme dUTPase provides the substrate for thymidylate synthase in the pyrimidine nucleotide biosynthesis. dCTP deaminase and trimeric dUTPases are structurally related [1] and are therefore interesting in an evolutionary perspective. Another member of this family of homotrimeric enzymes is found in the archaeon *Methanocaldococcus jannaschii*, which produces a bifunctional enzyme with both dCTP deaminase and dUTPase activities in one polypeptide chain [2].

dCTP deaminase and the bifunctional dCTP deaminase-dUTPase are unique among nucleoside and nucleotide deaminases as they function without a catalytic metal ion that deprotonates a water molecule for nucleophilic attack on the substrate. Based on structures of substrate and product complexes of the *E. coli* dCTP deaminase a detailed catalytic mechanism could be proposed for the deamination reaction [1]. The regulation of this enzyme and how the different catalytic machineries are tied to the same trimeric protein scaffold will be presented.

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Keywords: nucleotide metabolism, enzyme mechanism, enzyme specificity

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Structural and Dynamic Studies of Onconase Mutants
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Onconase (ONC), a member of the RNase A superfamily extracted from oocytes of Rana pipiens is used in treatment of various forms of cancer. ONC antitumor properties depend on its ribonucleolytic activity that is three-five order of magnitude lower than RNase A. The structural reasons for this very low ribonucleolityc activity are not yet clearly understood. The most damaging side effect from ONC treatment is renal toxicity, probably linked to the enzyme stability, which is unusually high for a protein isolated from a mesophilic source. In this context, we have prepared and determined the crystal structures of two ONC mutants (M23L and C87S,des103-104), and performed molecular dynamics simulations of ONC and C87S,des103-104 with the aim of explaining on structural grounds the modifications of the activity and thermal stability of these mutants. Despite the strict similarity in the β-sheet architecture, ONC does not possess the β-sheet breathing motion characteristic of other RNase-like molecules and considered to be functionally important. The

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

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Structural Basis for Tumor Pyruvate Kinase M2 Allosteric Regulation and Catalysis

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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

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

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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.

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

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Recovery of Argininosuccinate Lyase Activity in Duck δ₁Crystallin

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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

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

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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.

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