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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.

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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.

[1] Johansson E., Fanø M., Bynck J.H., Neuhard J., Larsen S., Sigurskjold B.W., Christensen U., Willemoës M., *J. Biol. Chem*., 2005, **280**, 3050. [2] Johansson E., Björnberg O., Nyman P.O., Larsen S., *J. Biol. Chem.*, 2003, **278**, 27916.

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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