There are an estimated 300-500 million cases of malaria and up to 3 million people die from this disease annually. Plasmodium falciparum is the causative agent of the most lethal and sever form of human malaria. Chemotherapy of malaria is available, but is complicated by both adverse effects and widespread resistance to most of the currently available anti-malaria drugs. The malaria parasite depends on de novo synthesis of pyrimidine nucleotides, whereas the human host has the ability to synthesize them by both de novo and salvage pathways. The de novo pathway contains six reaction steps. In the final two steps, uridine 5'-monophosphate (UMP) requires the addition of a ribose phosphate moiety from 5-phosphoribosyl-1-pyrophosphate to orotate by orotate phosphoribosyltransferase (OPRT) to form orotidine 5'-monophosphate (OMP) and pyrophosphate (PPi), and the subsequently decarboxylation of OMP to form UMP, by OMP decarboxylase (OMPDC). Here, we report the X-ray analysis of OMP or UMP-complex forms of OMPDC from Plasmodium falciparum (PfOMPDC) at 2.65 Å resolution. The structural analysis provides the substrate recognition mechanism with dynamic structural changes. And anti-malaria drugs design by using the structure of OMPDC is in progress.

Keywords: malaria, X-ray analysis, structural analysis

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Structural study of enzyme inhibitor complexes of eukaryotic glutamine synthetase from *Zea mays*

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Plants provide nourishment for animals and other heterotrophs as the sole primary producer in the food chain. Glutamine synthetase (GS), one of the essential enzymes for plant autotrophy catalyzes the incorporation of ammonia into glutamate to produce glutamine with concomitant hydrolysis of ATP, and plays a crucial role in the assimilation and re-assimilation of ammonia derived from a wide variety of metabolic processes during plant growth and development. We have determined the crystal structures of maize glutamine synthetase in complexes with three kinds of substrate analogues (J. Biol. Chem., 281, 29287-29296(2006)). From these structures we found a unique decameric structure of the enzyme which is significantly different from the bacterial glutamine synthetase and proposed a phosphate transfer reaction mechanism of ATP. In this study, we aims at gaining insights how the enzyme recognizes the substrate glutamic acid by the methods of mutagenesis and X-ray crystal structure analysis and we prepared several mutant enzymes. We determined new two crystal structures. One structure (WT/PPT/AMPPNP) is a wild type (WT) enzyme in complex with phosphinothricin (PPT) and AMPPNP (ATP analog). The other (H249A/MetSox-P/ADP) is an H249A mutant enzyme in complex with methionine sulfoximine phosphate (MetSox-P) and ADP. Crystal structures of the two complexes were determined at 3.06 and 2.97Å resolutions, respectively. In comparison to the previously reported structures, PPT in the WT/PPT/AMPPNP complex is closer to the γ-phosphate group than MetSox in the WT/MetSox/AMPPNP complex. It is expected that the phosphotransfer energy to PPT is smaller than that of MetSox. It was confirmed that the H249A/MetSox-P/ADP complex contains three manganese ions.

Keywords: plants, enzyme mechanics, ATP dependent reactions

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H/D-exchange and water structure in diisopropyl -fluorophosphatase as reveald by neutron diffraction

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The calcium-dependent phosphotriesterase Diisopropylfluorophosphatase (DFPase) from the squid Loligo vulgaris is an enzyme capable to detoxify a range of highly toxic organophosphorus compounds including DFP ad the nerve agents Tabun (GA), Sarin (GB), Soman (GD) and Cyclosarin (GF). In addition to an already existing atomic-resolution X-ray structure (0.85 Å, PDB: 1PJX) neutron diffraction was employed to reveal the protonation states and indentity of a catalytically important water molecule in the DFPase active site. Additional information was gained on the extend and distribution of H/D-exchange in the protein leading a detailed picture of structural rigidity in the hightly symetrical β-propeller structure of DFPase. Also we were able to determine the positions and oritentations of water molecules in the central tunnel of DFPase with high accuracy. Based on these finding we employed Molecular Dynamics (MD) simulation to investigate the dynamics of these internal water molecules that form an extended network connecting both metall ions in the protein. The results of several simulation runs of 30 ns each obtained using the OPLS all-atom force field and both TIP4P and SPC water models suggest a highly odered and correlated movement of water molecules in the tunnel. As DFPase is currently the protein structure with the largest extended network of internal water molecules characterized by neutron difraction, it might serve as a valuable model for other water filled narrow tunnel and channel like structural moieties. We show that information obtained from neutron diffraction and computational simulations is complementary to other currently employed exterimental methods for investigating internal water dynamics like NMR-spectroscopy.

Keywords: neutron crystallography, molecular dynamics simulations, protein dynamics

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Structural study of putative aminotransferase from *Thermus thermophilus* HB8

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Amiontransferase (AT) is one of pyridoxal 5'-phosphate (PLP) -dependent enzyme and plays an important role in amino acid

metabolism. AT reverbly catalyzes the transamination reaction in which the alpha-amino acid of amino acid 1 is transferred to the 2-oxo acid of amino acid 2 to produce the 2-oxo-acid of amino acid 1 and amino acid 2. Thus, AT recognizes two different kind of amino acid 1 and 2 (Dual substrate recognition). psi-BLAST search showed that TTHA0173 is a dual functional AT which works at serine and alanine metabolic pathway. TTHA0173 is serine:pyruvate AT and at the same time, alanine:glyoxylate AT. Three-dimensional structure of unliganded TTH0173 was determined by MAD method using Se-Met tSPAT at 1.45 Å resolution. TTH0173 is a homodimer and the polypeptide chain is folded into the small and large domains. The active-site pocket is formed at the subunit interface and the domain interface with the coenzyme PLP forming a Schiff base with the catalytic lysine residue. Program DALI was used to search PDB for proteins possessing structures similar to that of TTHA0173, therefore the highest Z-score was calculated to be 47.4 with sequence identity of 33% for human serine pyruvate-alanine:glyoxylate AT. The kcat and Kd values of tSPAT for various amino acid and their 2-oxo acid measured. Unexpectedly, glutamate and its 2-oxo acid which have bulky side chain, were good substrate. In order to elucidate the substrate recognition mechanism, we have determined the crystal structure of complex with 2-methylalanine and 2-methyl glutamine at 1.55 and 1.50 Å resolution, respectively.

Keywords: substrate binding, transaminases, vitamin B6

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Crystal structure of mouse sulfotransferase 2A4 (SULT2A4)

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Cytosolic sulfotransferase (SULT) sulfates specific substrates such as hormones, neurotransmitters, xenobiotics, drugs by transferring sulfate group from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to hydroxyl group of substrate. It is known that SULT regulates concentration of endogenous compounds or detoxifies xenobiotics for increasing hydrophilicity of them and excreting them out of body through blood or urine. The mouse SULT2A4 has unique substrate specificity for cholic acid. To gain insight into a molecular basis for the substrate specificity, we solved the crystal structure of SULT2A4 complexed with 3'-phosphoadenosine 5'-phosphate (PAP) and cholic acid. The overall structure is similar to those of SULT enzymes and the PAP binding site is conserved, however, significant differences exist in the positions of loops Pro14-Ser20, Glu79-Ile82 and Tyr234-Gln244 in the colic acid binding pocket. Moreover, completely conserved His, which is proposed catalytic base is not conserved in SULT2A3. However the functionary identical His residues exist in PSB (phosphosulfate binding motif) loop. The difference should need to sulfuryl transfer to 7'-OH of steroid ring position not in 3'-OH.

Keywords: crystal structure analysis, enzyme catalytic reaction mechanism, sulfotransferase

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Unusual comformational pathways of mismatched dNTP incorporation by DNA Polß

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Understanding how DNA polymerases control fidelity requires elucidation of the mechanisms of matched and mismatched dNTP incorporations. Little is known about the latter because mismatched complexes do not crystallize readily. In this report, we employed small-angle X-ray scattering (SAXS), X-ray crystallography and structural modeling to probe the conformations of different intermediate states of mammalian DNA polymerase ß (Pol ß) in its wild-type and an error-prone variant, I260Q. Our structural results indicate that the mismatched ternary complex lies in-between the open and the closed forms, but more closely resembles the open form for WT and the closed form for I260Q. On the basis of molecular modeling, this over-stabilization of mismatched ternary complex of I260Q is likely caused by formation of a hydrogen bonding network between the side chains of Gln260, Tyr296, Glu295 and Arg258, freeing up Asp192 to coordinate MgdNTP. These results argue against recent reports suggesting that mismatched dNTP incorporations follow a conformational path distinctly different from that of matched dNTP incorporation, or that its conformational closing is a major contributor to fidelity.

Keywords: crystal structure, DNA polymerase & error-prone variant I260Q, small-angle X-ray scattering (SAXS)

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Crystal structure of Delta1-tetrahydrocannabinolic acid synthase from *Cannabis sativa*

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Delta1-tetrahydrocannabinolic acid (THCA) synthase is the enzyme that catalyzes oxidative cyclization of cannabigerolic acid into THCA, the precursor of Delta1-tetrahydrocannabinol. In order to investigate the structure-function relationship of THCA synthase, this enzyme was overproduced in insect cells, purified and finally crystallized in 0.1 M HEPES buffer pH 7.5 containing 1.4 M sodium citrate. A single crystal suitable for X-ray diffraction measurement was obtained in 0.09 M HEPES buffer pH 7.5 containing 1.26 M sodium citrate. The crystal diffracted to 2.8 Å resolution at beamline BL41XU, SPring-8. The crystal belonged to the primitive cubic space group *P*432, with unit-cell parameters $a = b = c = 178.2$ Å. *R* value of the structure model was 19.6%. Structure of THCA synthase was divided into two domains, and there was FAD of a coenzyme