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 severe 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 (Pi), 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

P04.02.158


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

Marc-Michael Blum1,2,3, Marat Mustyakimov4, Heinz Rueterjans2, Benno P. Schoenborn4, Paul Langan1, Julian C.H. Chen1
1Blum - Scientific Services, Ledererstrasse 23, Munich, Bavaria, 80331, Germany, 2J.W. Goethe University, Institute of Biophysical Chemistry, Max-von-Laue-Strasse 9, 60438 Frankfurt, Germany, 3Bundeswehr Institute for Pharmacology and Toxicology, Neuherbergstrasse 1, 80937 Munich, Germany, 4Los Alamos National Laboratory, Bioscience Division, 87545 Los Alamos, NM, USA, E-mail : mmb@blum-scientific.de

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: 1PIX) 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 highly symmetrical β-propeller structure of DFPase. Also we were able to determine the positions and orientations 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 ordered 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 diffraction, 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 experimental methods for investigating internal water dynamics like NMR-spectroscopy.

Keywords: neutron crystallography, molecular dynamics simulations, protein dynamics

P04.02.159


Structural study of putative aminotransferase from Thermus thermophilus HB8

Ikuko Miyahara1,2, Mitsuyoshi Matsumura1,2, Masaru Goto1,2, Rie Omi1,2, Ken Hirotsu1,2, Hiroyuki Mizuguchi3, Hideyuki Hayashi1
1Osaka City University Graduate School of Science, 3-3-138, sugimoto, sumiyoshi-ku, Osaka, Osaka, 558-8585, Japan, 2RIKEN/Harima Inst., sayo-gun, Hyogo 679-5148, Japan, 3Dept. of Biochem., Osaka Medical Coll., Takatsuki, Osaka 569-8666, Japan, E-mail : miyahara@sci.osaka-cu.ac.jp

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

Keywords: plants, enzyme mechanics, ATP dependent reactions