

PS04.01.16 PURIFICATION AND CRYSTALLIZATION OF RECOMBINANT LACTATE DEHYDROGENASE OF PLASMODIUM FALCIPARUM. Debasish Chattopadhyay, Dwight Moore, Patrick Campbell, David Bzik*, Barabara A. Fox*, Lawrence J. DeLucas & Sthanam V. L. Narayana. Center for Macromolecular Crystallography, University of Alabama at Birmingham, 1918 University Blvd. Birmingham, AL 35294; *Department of Microbiology, Dartmouth-Hitchcock Medical Center, Hanover, NH 03755.

Infection with the malaria parasites is one of the major infectious causes of mortality in worldwide. Management of the infection is increasingly compromised by the incessant spread of drug resistance. It is necessary to identify new exploitable therapeutic targets and discover potential inhibitors for these targets. Lactate dehydrogenase enzyme of malaria parasite plays an important role in regulating glycolysis. The enzyme possesses distinctive features in its physicochemical and biochemical properties as compared to the host enzyme. We have purified a recombinant lactate dehydrogenase (LDH) of *Plasmodium falciparum* to explore the possibility of using this enzyme as a target for structure based drug design. Protein is purified from the soluble extract of *Escherichia coli* using anion exchange chromatography on Q-Sepharose followed by chromatography on Blue Sepharose and HPLC gel filtration. Purified protein is active in an NADH dependent LDH assay. The protein is crystallized using hanging drop vapor diffusion technique with PEG 20,000 as precipitant at pH 6.3 -6.7 and 10% (v/v) glycerol as an additive.

PS04.01.17 CRYSTALLOGRAPHIC STUDIES OF HOMOSERINE DEHYDROGENASE FOR THE DESIGN OF NOVEL ANTIFUNGAL AGENTS. DeLaBarre, Byron; Wright, Gerald D.; Berghuis, Albert. M. McMaster University, Hamilton, ON, CANADA

We are attempting to apply protein crystallography towards the design of antifungal agents. Fungal pathogens have become a serious problem because of their acquired resistance to existing antifungal agents (1). Agriculture, where fungi can cause crop losses both before and after the harvest, would benefit greatly from an improved fungicide. The worldwide sales of antifungal agents is estimated to be close to \$5 billion US (2). Fungal pathogens are also an important problem for people with compromised immune systems such as AIDS victims, burn patients, and chemotherapy subjects.

The approach we are using is known as structure based drug design (3); it provides the researcher with the *a priori* knowledge necessary for a rational search for drug compounds. Structure based drug design requires a thorough understanding of the underlying biochemistry of the pathology. An important contribution to this understanding is the determination of the three dimensional structure of the biological molecule with which the drug molecule interacts; such information is obtained by the technique of X-ray crystallography.

An excellent target for structure based drug design is the altered amino acid metabolism of fungi. The pathway shown generates essential amino acids for the fungus, but has no corresponding pathway in animals (animals obtain the required amino acids through their diet). We have selected the homoserine dehydrogenase (HSD) enzyme as a subject for structural determination. HSD was chosen for two reasons:

- (1) The protein can be obtained in large quantities - an overexpression vector and a purification scheme have already been established.
- (2) It is known that 2-amino-4-oxo-5-hydroxypentanoic acid inhibits fungal growth by interacting with HSD (4).

We have crystallized the HSD protein as plates which diffract to 2.5 Angstrom™ resolution. The enzyme has been cocrystallized with the NAD+ cofactor and also with an NAD+ analogue and the substrate L-homoserine. Data collection for these crystals is currently underway.

- Holloman, D.W. (1993) *Biochem. Soc. Trans.* 21, 1047-1055.
- Clough and Godfrey (1995) *Chemistry in Britain*, 466.
- Whittle, P.J. and Blundell, T. L. (1994) *Annu. Rev. Biophys. Biomol. Struct.* 23, 349-375.
- Yamaguchi, M. Uamaki, H., Shinoda, T. Tago, Y., Suzuki, T. & Yamaguchi, H. (1990) *J. Antibiot.* 43, 411-418.

PS04.01.18 THE CRYSTAL STRUCTURE OF CLASS 3 ALDEHYDE DEHYDROGENASE: IMPLICATIONS TO THE CLASS 1 AND 2 ENZYMES. Zhi-Jie Liu¹, Julie Sun², John Rose¹, David Hsiao², Wen-Rui Chang¹, Yong-Je Chung², Ingrid Kuo³, John Hempel³, Ronald Lindhal⁴ and Bi-Cheng Wang¹, ¹Dept. of Biochemistry and Molecular Biology, Univ. of Georgia, Athens, GA 30602, U.S.A., ²Univ. of Pittsburgh, Pittsburgh, PA 15260, U.S.A., ³Dept. of Molecular Genetics and Biochemistry, Univ. of Pittsburgh, Pittsburgh, PA 15219, U.S.A. and ⁴Dept. of Biochemistry and Molecular Biology, Univ. of South Dakota, Vermillion, SD 57069, U.S.A.

The first structure of an aldehyde dehydrogenase (class 3, 452 residues) from rat liver has been determined at 2.6Å resolution using SIRAS data and solvent flattening. There are two molecules in the crystallographic asymmetric unit which self-associate to form a homodimer. The structure shows two open α/β domains. The NAD binding domain (residues 1-200) shows a variant Rossmann fold with the glycine-rich segment at the end of β -strand 4 instead of at the end of β -strand 1 found in comparable enzymes. The transition to the catalytic domain is punctuated by a highly conserved Gly-Gly segment, residues 211-212. The catalytic domain (residues 201-400) bears an intriguing resemblance to the catalytic domain of dihydrofolate reductase. The apparent aldehyde binding site contains the strictly conserved catalytic Cys243 and the highly conserved Glu209. Another interesting feature of the structure is a 55 residue segment at C-terminus which extends back from the catalytic domain over the co-enzyme binding domain with the final 30 residues completing of the catalytic domain of the related ALDH molecule in the homodimer. Details of the structure and its implications to the Class 1 and 2 structures will be presented.

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PS04.01.19 X-RAY STRUCTURE OF INOSINE MONOPHOSPHATE DEHYDROGENASE FROM THE PROTOZOAN PARASITE TRITRICHOMONAS FOETUS. Frank G. Whitby[†]¶, Hartmut Luecke[‡], John Somoza[†], Hiro Tsaruta[‡], Jorge Huete-Perez[†], Christopher P. Hill[¶], Robert J. Fletterick[†], Ching Chung Wang[†], [†]Department of Pharmaceutical Chemistry, University of California, San Francisco, California [‡]Stanford Synchrotron Radiation Laboratory, Stanford University, Palo Alto, California [¶]Department of Biochemistry, University of Utah Medical Center, Salt Lake City, Utah 84132

Inosine monophosphate dehydrogenase (IMPDH) catalyzes the NAD-dependent oxidation of inosine monophosphate (IMP) to xanthine monophosphate (XMP). This is the rate-limiting step in purine biosynthesis. Inhibitors of this enzyme have been shown to have anti-tumor, immunosuppressive, and antiparasitic effects.

The enzyme is a tetramer of 230 kD total weight. Crystals of the recombinant enzyme were grown in 2.2 M ammonium sulfate and crystallized in the cubic space group P432. Data were collected at beamlines 1-5 and 7-1 at SSRL, and at beamline F1 at CHESS.