PS04.01.16 PURIFICATION AND CRYSTALLIZATION OF RECOMBINANT LACTATE DEHYDROGENASE OF PLASMODIUM FALCIPARUM. Debasish Chattopadhyay, Dwight Moore, Patrick Campbell, David Bezik*, Barbara A. Fox**, Lawrence J. DeLucas & Sihaman 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 6.5 - 6.7 and 10% (v/v) glycerol as an additive.

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

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 are using this knowledge as a framework to design novel antifungal agents. We are using structure based drug design to design new antifungal agents that will be effective against fungal pathogens in a variety of environments.

PS04.01.18 THE CRYSTAL STRUCTURE OF CLASS 3 ALDEHYDE DEHYDROGENASE: IMPLICATIONS TO THE CLASS 1 AND 2 ENZYMES. Zhi-Jie Liu*, Julie Sun*, John Rose1, David Hsiao2, Wen-Rui Chang1, Yong-Jo Chung2, Ingrid Ku02, John Hemptel1 Ronald Lindahl4 and Bi-Cheng Wang1. *Dept. of Biochemistry and Molecular Biology, Univ. of Georgia, Athens, GA 30602, U.S.A., 2Dept. of Crystallography, 3Univ. of Pittsburgh, Pittsburgh, PA 15260, U.S.A., 4Dept. of Molecular Genetics and Biochemistry, Univ. of Pittsburgh, Pittsburgh, PA 15219, U.S.A. and 5Dept. 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 2.6A resolution using SIRAS data and solvent flattening. There are two molecules in the crystallographic asymmetric unit which self-assemble to form a homodimer. The structure shows two open a/b domains. The NAD binding domain (residues 1-200) shows a variant Rossman fold with the glycine-rich segment at the end of a-strand 4 instead of at the end of b-strand 1 found in comparable enzymes. The transition to the catalytic domain is punctuated by a highly conserved Gly-Gly residue, 211-212. The catalytic domain (residues 201-400) bears an intriguing resemblance to the catalytic domain of dihydrololate 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 PROTOZOA PARASITE TRITRICHOMONAS FOETUS. Frank G. Whitby1, Hartmut Luecck2, John Somozza3, Hiro Tsarural, Jorge Huete-Perez1, Christopher P. Hill1, Robert J. Fletterick1. Ching Chung Wang1. 1Department of Pharmaceutical Chemistry, University of California, San Francisco, California 2Stanford Synchrotron Radiation Laboratory, Stanford University, Palo Alto, California 3Department of Biochemistry, University of Utah Medical Center, Salt Lake City, Utah 84152

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 antiapoptotic 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 SIRL, and at beamline F1 at CHESS.