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 dehyrogenase 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 pathenogens 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 Liu[1], Julie Sun[2], John Rose[1], David Hsiao[2], Wen-Rui Chang[1], Yong-Je Chung[2], Ingrid Kuo[3], John Hempel[3] Ronald Lindhal[4] and Bi-Cheng Wang[1], [1]Dept. of Biochemistry and Molecular Biology, Univ. of Georgia, Athens, GA 30602, U.S.A., Dept. of Crystallography, [2]Univ. of Pittsburgh, Pittsburgh, PA 15260, U.S.A., [3]Dept. of Molecular Genetics and Biochemistry, Univ. of Pittsburgh, Pittsburgh, PA 15219, U.S.A. and [4]Dept. 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.

Work supported by a grant AA06985 from the National Institute of Alcohol Abuse and Alcoholism, and resources from the Pittsburgh Supercomputing Center.

PS04.01.19 X-RAY STRUCTURE OF INOSINE MONOPHOSPHATE DEHYDROGENASE FROM THE PROTOZOAN PARASITE *TRITRICHOMONAS FOETUS*. Frank G. Whitbyt†¶, 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.

Initial low-resolution SIR and MIR electron density maps indicated a flat, tetrameric molecule, consistent with the expected crystal packing with one monomer per asymmetric unit. Solvent flattening was performed with the program PHASES. From the solvent flattened maps, about 280 alanine residues were fit in an alpha(8)-beta(8) barrel (TIM barrel). Phase combination with the MIR and refined partial model phases was performed. Heavy-atom difference maps indicate clearly 5 cysteine residues that reacted with either PCMBS or thimerosal, and 3 methionine residues that bound platinum chloride. One disulfide bond has been discovered and may be related to the observed increase in activity of this enzyme in the presence of reducing agent. In addition, the active site has been identified by the position of an active-site cysteine and the positions of electron density in difference maps calculated from data collected from ligand and inhibitor-soaked crystals. Presently the crystallographic R-factor is 21% to 2.0 angstroms resolution for data greater than 2 sigma, and R-free is 27%. The model will hopefully serve as a model for solving the structure of the human enzyme and in the design of inhibitors.

PS04.01.20 STRUCTURAL STUDIES OF RAT LIVER 3α-HYDROXYSTEROID/DIHYDRODIOL DEHYDROGENASE. Melanie J. Bennett*, Brian P. Schlegelt, Joseph M. Jez*, Trevor M. Penning†, Mitchell Lewis*, *The Johnson Research Foundation, Department of Biochemistry and Biophysics and †Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Rat liver 3α-hydroxysteroid dehydrogenase/dihydrodiol dehydrogenase (3α-HSD) inactivates circulating steroid hormones and is involved in polycyclic aromatic hydrocarbon (PAM) carcinogenesis. This enzyme is a member of the aldo-keto reductase (AKR) superfamily, and its structure is likely to provide a paradigm for other mammalian HSDs in the family. The previously determined apoenzyme structure[1] showed that the protein folds into an α/ß barrel. Now, the structure of 3α-HSD complexed with NADPH has been determined at 2.7 Å resolution. This binary complex model reveals the mode of cofactor binding and provides insight into some features of substrate binding and catalysis. The model supports a catalytic mechanism in which Tyr 55 is the general acid, and we present evidence that the structurally distinct short-chain alcohol dehydrogenase (SCAD) family may have convergently evolved a similar catalytic mechanism. We also make predictions about substrate binding to 3α-HSD based on (1) a fortuitous crystal packing contact that may mimic a portion of a bound steroid hormone or PAH *trans*-dihydrodiol, (2) an active site water molecule that may indicate the position of the carbonyl or hydroxyl oxygen in a substrate, and (3) site-directed mutagenesis data. We are currently working to obtain crystal structures with a variety of bound inhibitors that will allow us to test these predictions and provide direct information about how the enzyme recognizes and reacts with apolar substrates.

1. Hoog, S. S. et al. (1994), Proc. Natl. Acad. Sci., USA 91, 2517-2521.

Supported by the Cancer Research Fund of the Damon Runyon-Walter Winchell Foundation Fellowship, DRG-1298

PS04.01.21 PRELIMINARY CRYSTALLOGRAPHIC ANALYSIS OF THE L-PHENYLALANINE DEHYDROGENASE NAD+ COMPLEX FROM RHODOCOCCUS. J. L. Vanhooke[2], N.M.W. Brunhuber[1], J.S. Blanchard[3], and H. M. Holden[2],Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin 53705[2], Department of Chemistry, University of California, Santa Barbara, California 93106[1], Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461[3]

Phenylalanine dehydrogenase (EC 1.4.1.20) catalyzes the reversible NAD+dependent oxidative deamination of L-phenylalanine to form phenylpyruvate, ammonia, and NADH. The enzyme has been identified in a small number of Gram-positive bacteria and is currently being investigated for use in neonatal screening for phenylketonuria (PKU) and for bulk production of racemically pure L-phenylalanine for the artificial sweetener aspartame (NutraSweet®)[1].

Single crystals of recombinant Rhodococcus sp. M4 phenylalanine dehydrogenase[2] complexed with NAD+ have been grown at 4 °C by macroseeding into batch solutions containing 5 mg/ml enzyme, 5 mM NAD+, 9 % poly(ethylene glycol) 8000, 175 mM NaCl, 100 mM MES (pH 5.6), and 5 mM NaN_3. The crystals are diamond-shaped and belong to the space group C2, with unit cell dimensions of a= 105.6 Å, b= 66.9 Å, c= 281.6 Å, ,ß= 91.6 °. Crystal dimensions of 0.6 x 0.6 x 0.25 mm are typically obtained in two weeks. Native x-ray data sets have been collected to 3.2 Å from several crystals at 4 °C. Heavy atom derivative searches are currently in progress.

The utilization of an aromatic substrate makes phenylalanine dehydrogenase unique among the known amino acid dehydrogenases. Thus, in addition to enhancing our understanding of the mechanism of pyridine nucleotide-dependent oxidative deamination, the three dimensional structure of phenylalanine dehydrogenase will assist in elucidating the determinants of substrate selectivity in the amino acid dehydrogenase family of enzymes.

1) Brunhuber, N.M.W. and Blanchard, J.S. (1994) Crit. Rev. Biochem. Mol. Biol. 29, 415-467.
2) Brunhuber, N.M.W., Banerjee, A., Jacobs, W.R. Jr., and Blanchard, J.S. (1994) J. Biol. Chem. 269, 16203-16211.

PS04.01.22 BAD TO SAD: ALCOHOL DEHYDROGENASE AS A "CRYSTALLOGRAPHIC ASSAY" FOR NAD ANALOGS Barry M. Goldstein, Thomas D. Colby, Krzysztof Pankiewicz and Kyoichi Watanabe, Dept. of Biophysics, University of Rochester Medical Center, Rochester, NY 14642, and OncorPharm Corp., Gaithersburg, MD 20877.

We have used liver alcohol dehydrogenase (LADH) to determine the ability of analogs of the cofactor nicotinamide adenine dinucleotide (NAD) to structurally mimic normal cofactor binding. LADH undergoes a cofactor-induced conformational transformation which can be used to discriminate between different classes of analogs.

NAD binding to LADH induces a conformational transition from an open to closed form of the enzyme. This transition requires the formation of specific hydrogen bonds by the nicotinamide carboxamide group, and is very sensitive to perturbations at the nicotinamide end of the ligand. An analog that can adopt the conformation required by the cofactor site will induce the transition to the closed form. An analog subject to constraints incompatible with the binding site cannot stabilize the closed form. The complex then remains in the open conformation.