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.

NAD-dependent dehydrogenases are involved in numerous metabolic processes, and have become attractive targets for drug design. A number of laboratories have been involved in the design and synthesis of neutral isosteric NAD analogs. These compounds differ in the heterocycle used to replace the nicotinamide group, as well as in the particular functional groups modified to improve both specificity and transport properties.

LADH complexes with several classes of C-glycosyl analogs have been examined. The thiazole and selenazole dinucleotides have constrained rotation about their C-glycosyl bonds. These complexes crystallize in the open conformation. (Li et al., Biochemistry, 1994, v33, 23). The pyridine dinucleotides are unconstrained, closely mimic NAD binding, and stabilize the closed conformation (Li et al., Biochemistry, 1994, v33, 11734).

We have recently examined complexes with the new dinucleotide inhibitor benzamide adenine dinucleotide (BAD), the antitumor agent selenazole-4-carboxamide adenine dinucleotide (SAD), and analogs of these compounds with additional modifications. SAD is constrained, binding in the open conformation. BAD is unconstrained, and is accommodated by the cofactor site in the closed conformation. The enzyme may also distinguish between more subtle changes in the ligand, such as the replacement of the phosphate ester oxygen with a methylene bridge.

PS04.01.23 TWO MUTATIONS IN THE ACTIVE SITE OF ALCOHOL DEHYDROGENASE PERTURB THE CATALYTIC GEOMETRY Thomas D. Colby, Brian J Bahnson, Jodie K. Chin, Judith P. Klinman, Barry M. Goldstein, Dept. of Biophysics, University of Rochester Medical Center, Rochester, NY 14642, Dept. of Chemistry, University of California, Berkeley, CA 94720.

Two structures of ternary complexes of active site mutants of horse liver alcohol dehydrogenase with NAD and substrate analog have been solved, revealing perturbations that change the relative orientation of the substrate and the cofactor ring. The two mutations are F93->W (in the substrate site) and V203->A (at the nicotinamide end of the cofactor site). Both mutants were designed in order to study the kinetic contribution of quantum mechanical tunneling to the hydride transfer step of alcohol oxidation. Structural results are consistent with kinetic measurements.

Both mutants were crystallized in the presence of NAD and the substrate inhibitor trifluoroethanol.The V203A structure is unusual, having four unique monomers in the asymmetric unit. Complexes were solved by molecular replacement, and refined to 2.0Å (F93W) and to 2.5Å (V203A). Both complexes adopt the catalytically competent "closed form" of the enzyme, characterized by a narrowing of the inter-domain active-site cleft . In both mutants, hydrogen bonds between the carboxamide group of NAD and mainchain atoms from two domains maintain the closed conformation. However, significant differences in cofactor-substrate geometries are observed between the two structures.

In the F93W mutant, the substrate is positioned very close to the nicotinamide ring of NAD, with ~3.1Å between hydride donor and acceptor carbons.This geometry is stabilized by the bulky Trp 93 substitution. In the second mutant, Val is replaced by the reduced bulk of Ala at position 203. The neighboring nicotinamide ring of NAD rotates toward the resulting pocket, away from the substrate. In each of the four monomers, the cofactor -substrate distance is increased to ~3.5Å, compromising the catalytic geometry.

The F93W mutant was designed to increase the off-rate of bulky alcohol substrates in order to make the hydride-transfer step rate-limiting. Kinetic isotope effects for this mutant suggest an enhanced tunneling contribution. The V->A mutation reduces the kcat/km of the enzyme dramatically, and displayes no increase in tunneling.Structural perturbations in the active site geomeries are consistent with these observations.

PS04.01.24 NATIVE AND COMPLEX CRYSTAL STRUCTURES OF THE FLAVIN ENZYME DIHYDROOROTATE DEHYDROGENASE. Paul Rowland, Finn S. Nielsen, Kaj Frank Jensen & Sine Larsen. Centre for Crystallographic Studies and Center for Enzyme Research, University of Copenhagen, Denmark

High resolution crystal structures of *Lactococcus lactis* dihydroorotate dehydrogenase "A" have been determined in the native form and also as a complex with the product of the enzyme reaction, orotate. Dihydroorotate dehydrogenase (DOD) is an FMN containing enzyme catalysing the oxidation of L-dihydroorotate to orotate. *Lactococcus lactis* is the only organism known to contain two functional dihydroorotate dehydrogenases (the "A" and "B" forms). Both consist of polypeptides of 311 residues, though they share only 30% sequence identity. The A form is active as a dimer, whereas the catalytic function of the B form depends strongly on the presence of an iron-sulphur containing protein of 262 amino acids. Well diffracting crystals have been obtained of the A form, which belong to space group $P2_1$ and have a dimer in the asymmetric unit. The native DODA structure was solved by isomorphous replacement using multiple datasets of a single gold cyanide derivative. Data to 2.0Å has been refined to an R-factor of 16.8% (free R=21.2%). The DODA/orotate complex structure was obtained by soaking a native crystal with the enzyme substrate and has a final R of 16.1% to 2.0Å (free R=18.7%). This first example of a dihydroorotate dehydrogenase structure folds into a classical α/β barrel. The flavin binding site is between the top of the barrel and a small β-strand subdomain formed by two barrel inserts. A small cavity above the flavin ring system is completely enclosed by the surrounding protein. In the complex this space is taken up by the orotate without any changes in the protein structure. Many of the conserved residues among this class of enzymes are associated with interactions between the protein residues and the flavin and substrate groups. There are also some important differences between the A and B enzyme forms in nonconserved binding residues.

The DODB/Fe-S protein complex crystallises in space group R32 with one protein complex per asymmetric unit. The results from the investigations of this enzyme will also be presented.

PS04.01.25 CRYSTALLOGRAPHIC ENZYMOLOGY OF ω-AMINO ACID PYRUVATE AMINOTRANSFERASE. S. Ikemizu, K.Sasaki[1], N.Watanabe[2], K. Yonaha[3], N. Sakabe & K.Sakabe[4]. Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki, 305 Japan; [1]College of Medical Technology, Nagoya Univ., Higashi, Nagoya, 461 Japan; [2]PF, KEK, Tsukuba, Ibaraki, 305 Japan; [3]Dept. of Agricultural Chem, Univ. of the Ryukyus, Nishihara, Okinawa, 903-01 Japan; [4]Dept. of Chemistry, Nagoya Univ. Chikusa, Nagoya, 464 Japan

Many enzyme reaction mechanisms has been established with the crystal structures of enzyme and enzyme-inhibitor complexes. However synchrotron radiation brought the progress of time-resolved protein crystallography. Thus we have to think about that the structure determination of enzyme is the starting point of the time resolved crystallography. Namely the crystal structure of substrate-enzyme complex should be determined in addition to those of inhibitor-complex because former complex can transfer to next reaction state but the latter complex can not do. On the other hand, in many reactions the solvent has also very important role of the reaction. Therefore high resolution structure determination is also necessary to study a time resolved protein crystallography. We determined crystal structure of ω-aminoacid pyravate aminotransferase containing PLP and PMP as a co-factor, substrate-enzyme complexes with ß-alanine, L-alanine, γ-aminobutyric acid, 6-aminohexonate, isoamylamine and pyruvate at better than 1.8Å resolution and we could obtain atomic parameters including solvent molecules. The reaction mechanism on the bases of these structures as been proposed. The application to time resolved Laue method has been testing.