

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 3 α -HYDROXYSTEROID/DIHYDRODIOL DEHYDROGENASE. Melanie J. Bennett*, Brian P. Schlegel†, 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 3 α -hydroxysteroid dehydrogenase/dihydrodiol dehydrogenase (3 α -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 structure¹ showed that the protein folds into an α/β barrel. Now, the structure of 3 α -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 3 α -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.

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PS04.01.21 PRELIMINARY CRYSTALLOGRAPHIC ANALYSIS OF THE L-PHENYLALANINE DEHYDROGENASE NAD⁺ COMPLEX FROM RHODOCOCCLUS. J. L. Vanhooke², N.M.W. Brunhuber¹, J.S. Blanchard³, and H. M. Holden², Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin 53705², Department of Chemistry, University of California, Santa Barbara, California 93106¹, Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461³

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 dehydrogenase² 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 NaN₃. 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, β = 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.