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.