

PS04.01.102 FLEXIBLE ASSEMBLY OF PYRUVATE DE-CARBOXYLASE TETRAMERS EXAMINED BY COMPARISON OF MULTIPLE CRYSTAL FORMS. P. Arjunan, L. Chen, S. Swaminathan, W. Furey & M. Sax, Biocrystallography Laboratory, VA Medical Center, University Drive C, PO Box 12055, Pittsburgh, PA 15240 & Department of Crystallography, University of Pittsburgh, Pittsburgh, PA 15260 and Y. Gao, D. Zhang & F. Jordan, Department of Chemistry, Rutgers University, Newark, NJ 07102

Pyruvate decarboxylase (PDC) is a thiamin diphosphate (ThDP) dependent enzyme that catalyzes the non-oxidative conversion of pyruvate to acetaldehyde and carbon-dioxide. PDC is active as a tetramer of MW 250,000 kDa with four cofactor molecules (ThDP and Mg²⁺) tightly bound, and is hysteretically regulated by the substrate. It was shown by mutational analysis that Cys221 is required for the allosteric substrate activation, and low angle scattering studies indicate a change in tetramer assembly takes place upon activation. Kinetic studies also indicate that unlike the normal substrate pyruvate which results in reversible activation, the alternate substrate ketomalonate converts PDC to an activated form which remains "locked on" and does not undergo unimolecular inactivation.

Using wild type PDC from the yeast *Saccharomyces uvarum* and recombinant PDC from the yeast *Saccharomyces cerevisiae*, we have now determined the enzyme structure in five different crystal forms, two of which were obtained in the presence of the allosteric activator ketomalonate. The five crystal forms show three distinct variations of tetramer assembly, with the largest difference in one of the structures obtained in the presence of the irreversible activator. In all cases the major differences involve shifting of one dimer within the tetramer relative to the other, with the individual dimers remaining essentially intact. The central domain however, which is associated with regulation, shows significantly greater variation than either of the cofactor binding domains. Structural details and comparisons between the different crystal forms of PDC will be presented.

PS04.01.103 CRYSTALLOGRAPHIC STUDIES ON SERRALYSINS. Ulrich Baumann, Institut f. Organische Chemie und Biochemie, Albertstr. 21, D-79104 Freiburg, Germany

The serralysins represent a family of 50 kDa metallo-endoproteases which are secreted into the medium by Gram-negative bacteria. Examples for members of this family are the proteases B and C from *Erwinia chrysanthemi* (PrtB and PrtC) the major metallo protease from *Serratia marcescens* (SMP) and the alkaline protease (AP) from *Pseudomonas aeruginosa*. The 3D structures of several serralysins, in an unliganded state or in complex with various inhibitors, have been solved and refined to high resolution. Comparison of these structures reveal an induced-fit mechanism in which Tyr216 acts not only a switch to trigger this conformational rearrangement but most likely also as electrophile which stabilizes the transition state.

PS04.01.104 STRUCTURE OF AN ANTIBIOTIC DEACTIVATING ENZYME. Lars C. Pedersen & Hazel M. Holden, University of Wisconsin, Madison, WI 53705

Kanamycin nucleotidyltransferase (KNTase) is a plasmid-coded enzyme which deactivates various antibiotics by transferring a nucleoside monophosphate group from ATP to the 4' hydroxyl group of the drug. Recently, we have solved the structure of KNTase in the presence of both kanamycin and AMPCPP. Crystals were grown from polyethylene glycol solutions and belonged to the space group P2₁2₁2₁ with unit cell dimensions of $a=57.3$ Å, $b=102.2$ Å, and $c=101.8$ Å and one dimer in the asymmetric unit.

Least-squares refinement of the model at 2.5 Å resolution reduced the crystallographic R-factor to 16.8%. The binding pockets for both the nucleotide and the antibiotic are composed of residues from each subunit. There are few specific interactions between the protein and the adenine ring of the nucleotide. The majority of the nucleotide:protein interactions involve the phosphoryl oxygens and various side chain moieties. Kanamycin binds with the 4' hydroxyl group at 5 Å from the α -phosphorus of the nucleotide and is in the proper orientation for a single in-line displacement attack at the phosphorus. Based on the structure of the KNTase/AMPCPP/antibiotic complex, a possible mechanism for the enzyme has been proposed with Glu 145 serving as the active site base. This residue is in the proper position to extract the proton from the 4'-hydroxyl group thereby activating the kanamycin for subsequent attack at the α -phosphorus of the nucleotide. The Mg²⁺/pyrophosphate moiety would serve as an excellent leaving group. In addition, the close proximity of Lys 149 to one of the α -phosphoryl oxygens may increase the electrophilic character of the phosphorus center thus making it more susceptible to nucleophilic attack.

The overall goal of this investigation is to provide a structural framework by which new drugs may be designed. In addition, the elucidation of the mechanism of nucleotide phosphate transfer by KNTase could possibly serve as a model for the reactions catalyzed by DNA polymerases. Currently, we are in the process of testing our proposed mechanism by creating site-directed mutants of Glu 145 and Lys 149. Crystals of KNTase in the presence of both tobramycin and amikacin have also been obtained. Crystallographic analysis of KNTase in the presence of multiple antibiotics will help to address important protein:drug interactions.

PS04.01.105 CRYSTAL STRUCTURE OF L-2-HALOACID DEHALOGENASE FROM *Pseudomonas* sp. YL AT 2.5 Å RESOLUTION. Y. Hata, T. Hisano, T. Fujii, J.-Q. Liu, T. Kurihara, N. Esaki, K. Soda, Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan

The crystal structure of thermostable L-2-haloacid dehalogenase from *Pseudomonas* sp. YL has been solved at 2.5 Å resolution by multiple isomorphous replacement. This is the first report on the structure of 2-haloacid dehalogenase.

The enzyme from *Pseudomonas* sp. YL is composed of two identical subunits consisting of 232 amino acid residues each, and catalyzes the hydrolysis of carbon-halogen bond of L-2-haloalkanoic acids to produce D-2-hydroxyalkanoic acids. It has some remarkable properties. It can act not only on short carbon-chain substrates in aqueous solution but also on long chain substrates such as bromohexadecanoic acids in organic solvent. It is highly thermostable with optimum temperature of 65°C.

Crystals were obtained at 4°C by vapor diffusion against 50 mM potassium dihydrogenphosphate solution (pH 4.5) containing 15% (w/v) polyethylene glycol 8,000 and 1% (v/v) *n*-propanol. A seeding technique was applied for enlargement of the sizes of crystals. They belong to the space group C2 with unit cell dimensions $a = 92.21$ Å, $b = 62.78$ Å, $c = 50.84$ Å and $\beta = 122.4^\circ$. An asymmetric unit contains one subunit of the enzyme. Diffraction data were collected on an R-AXIS IIC imaging plate detector system. Au- and U-derivatives were prepared by soaking experiments. MIRAS phases were calculated using the program PHASES. An atomic model was built on an MIRAS map using the program TURBO-FRODO, and refined at 2.5 Å resolution with the program X-PLOR to the crystallographic R-value of 0.195 for 7,848 reflections ($I \geq 2\sigma(I)$).

The present model of the subunit includes residues 4-222 and 19 water molecules. The structure consists of two domains: the α/β -type core-domain and the α -type sub-domain. There is a cleft between the domains. It contains residues important for catalysis.