C-1.01.94 PRELIMINARY X-RAY STRUCTURE ANALYSIS OF ACC DEAMINASE
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1-Aminocyclopropane-1-carboxylic acid (ACC) is isolated from several plant tissues including pears and apples, and regarded as a key intermediate in the biosynthesis of ethylene, a plant hormone that affects diverse growing and developmental processes, including fruit ripening, leaf and flower senescence, and stress responses. ACC is the simplest compound in which amino and carboxyl groups bind to cyclopropane ring directly. The enzyme ACC deaminase catalyzes opening of this ring to give a-ketobutyrate.

ACC deaminase from a yeast Hansenula saturnus has been crystallized by a hanging-drop vapour diffusion method. The diffracted data from native crystal has been collected to 4.0 Å resolution with Rmerge=11.3% (82.1% of expected reflections) on a Weissenberg camera using synchrotron radiation at Photon Factory (KEK, Japan). The crystals belong to C2221 space group with two dimers of an estimated 69,000 per asymmetric unit. VN was calculated to be 3.0 Å3/Da and solvent volume fraction was 58%. Assuming three dimers per asymmetric unit, VN was calculated to be 2.0 Å3/Da and solvent volume fraction was 38%. Diffraction data set of PHMB derivatives was collected to 4.0 Å resolution with Rmerge=12.8% (89.0% of expected reflections). From difference Patterson and anomalous difference Patterson maps of PHMB derivative, we could find heavy atom peaks. Phasing and a further search for heavy-atom derivatives for multiple isomorphous replacement method are now in progress.

P804.01.95 2.2 Å STRUCTURE OF D84E MUTANT OF PORPHOBILINOGEN DEAMINASE, N. C. Picken1, R. Lambert2, S. Awan3, P. M. Jordan4, S. P. Wood5, Department of Crystallography, Birkbeck College, Malet St., London, UK, Institute of Biology, University of Southampton, Southampton Institute of Ophthalmology, University College London, UK

Porphobilinogen deaminase (PBGD) is the third enzyme in the biosynthetic pathway of tetrapyrroles. PBGD catalyses the stepwise polymerization of four molecules of the substrate porphobilinogen forming the highly unstable intermediate uroporphyrinogen.

The structure of native E. coli PBGD has been solved to 1.7 Å. However a flexible loop region of this structure remains invisible. Therefore it was decided to try freezing the crystal to gain more information about this missing loop.

Asp 84 is a catalytically important residue in the active site cleft. This residue hydrogen bonds to the pyrrole nitrogens of the cofactor and facilitates deamination and stabilizes the developing positive charge throughout the reaction. The D84E mutant of this protein retains 1% of its catalytic activity whereas other mutants, including fruit hormone that affects diverse growing and developmental processes such as fruit ripening, leaf and flower senescence, and stress responses.

Due to the shrinkage in the unit cell molecular replacement methods had to be used before any structural refinement could take place.

P804.01.96 STRUCTURAL STUDY OF SESQUITERPENE CYCLASES.

Cyclopentene terpenoids are found throughout nature and comprise a medicinally important class of compounds from plants. The biosynthesis of cyclic terpenes is determined by branch point enzymes referred to as terpene cyclases. The objective of our research program is to understand the structural, functional, and chemical features governing two distinct stereochemically controlled cyclizations of farnesyl diphosphate (FPP) catalyzed by two homologous plant sesquiterpene cyclases that result in two unique bicyclic products. Our current crystallographic and enzymatic studies will provide the foundation for our long range goal that focuses on a rationally and combinatorially based redesign of terpene cyclases for the enzymatically directed syntheses of pharmacologically important terpenoids. This structure/function analysis should also deepen our understanding of the biosynthesis of the larger class of essential terpenoids including cholesterol, steroid hormones, and lipid soluble vitamins.

Our experimental system encompasses two homologous sesquiterpene cyclases (77% amino acid identity), 5-epi-aristolochene synthase from N. tabacum (TEAS) and vetispiradiene synthase from H. muticus (HVS), which cyclize FPP into products which are structurally quite different. Chemical rationalization of the reaction mechanisms suggests several partial reactions common to both enzymes and at least one final step unique to each. We have grown crystals of TEAS and an active TEAS/HVS chimera in the space group P41212 (65% solvent). We have obtained a native chimera data set to 2.8 Å and four derivative data sets resulting in our initial 2.8 Å MIRAS map. Solvent flattening greatly improved the quality of the initial electron density map; solvent boundaries, connectivity, and secondary structural features are readily apparent. We have begun model building. Once the chimera structure is interpreted and refined, it should provide us with a search model for the structural determination of the wild type enzymes as well as other chemically interesting chimeras. By comparing the structures of these enzymes and their complexes with products and inhibitors we should gain insight into the particular active site residues and surfaces responsible for common and specific reactions among the cyclases.

P804.01.97 TOWARDS THE STRUCTURE OF HEMAGGLUTININ-NEURAMINIDASE FROM NEWCASTLE DISEASE VIRUS. S. J. Crennell, A. Porter, T. Takimoto1, W.G. Laver2, W. J. Wilmot and G.L. Taylor, Department of Crystallography, University of Bath, BA1 7RH, U.K., Saint Jude's Children Research Hospital, Memphis, TN, USA. 1Australian National University, Canberra, Australia.

Hemagglutinin-Neuraminidase, (HN), one of the two surface proteins of paramyxoviruses, mediates the attachment of the virus to host cells and as such a candidate for drug design not only against Newcastle disease (NDV), but also mumps and parainfluenza whose HN share 33 and 25% sequence identity respectively with NDV HN.

HN was crystallized by the hanging drop method from 0.1M acetate buffer pH 4.6, 0.2M (NH4)2SO4 and 25%PEG4K. Native X-ray data were collected both on the inhouse Siemens and on beamline X11 of the DESY synchrotron, Hamburg, to 2.7 Å resolution. No HN structure has been determined, although there is a predicted structural similarity to influenza neuraminidase which shares 17% sequence identity. Molecular replacement using the structures of influenza neuraminidase A or B, the known bacterial neuraminidases or models based on these has been unsuccessful.