PS04.01.94 PRELIMINARY X-RAY STRUCTURE ANALYSIS OF ACC DEAMINASE Atsushi Horiuchi, Min Yao, Atsushi Nakagawa, Isao Tanaka and Mamoru Honma*, Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060, Japan, *Department of Bioscience and Chemistry, Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan

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 catalizes 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 diffraction 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 $C222_1$ space group with cell dimensions of a=276.7, b=66.1 and c=187.1 Å. Assuming two dimers of an estimated molecular weight of 69,000 per asymmetric unit, Vm was calculated to be 3.0Å^3/Da and solvent volume fraction was 58 %. Assuming three dimers per asymmetric unit, Vm was calculated to be 2.0Å^3/Da and solvent volume fraction was 38%. Diffraction data set of PHMBS derivatives was collected to 4.0Å resolution with Rmerge=12.8% (89.0% of expected reflections). From difference Patterson and anomalous difference Patterson maps of PHMBS derivative, we could find heavy atom peaks. Phasing and a searching for further heavy-atom derivatives for multiple isomorphous replacement method are now in progress.

PS04.01.95 2.2Å STRUCTURE OF D84E MUTANT OF PORPHOBILINOGEN DEAMINASE. N. C. Picken[1], R. Lambert[2], S. Awan[3], P. M. Jordan[2], S. P. Wood[2], [1]Department of Crystallography, Birkbeck College, Malet St., London, [2]Department of Biochemistry, University of Southampton, Southampton [3]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 preuroporphyrinogen.

The structure of native *E.coli* PBGD has been solved to 1.7Å. However a flexible loop region of this structure remains invisible. Therefore it was decided to try freezing crystals in order to try 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, such as D84A and D84N, are catalytically inactive. This makes this mutant an interesting mutant for structural studies.

Crystals of the D84E mutant have been grown and successfully frozen for data collection. A 2.2Å data set was collected and processed. The protein was found to have crystallized in space group $P2_12_12$, which is the same space group as the original structure, however the unit cell of a=84.97 b=75.09 c=48.29 $\alpha=\beta=\gamma=90°$ is significantly smaller than that of the native crystal. This shrinkage in the unit cell was assumed to be a result of freezing the crystal.

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

PS04.01.96 STRUCTURAL STUDY OF SESQUITERPENE CYCLASES. Starks, C.*, Back, K.†, Chappell, J.†, Noel, J.*, *Structural Biology Laboratory, Salk Institute, La Jolla, CA 92037, †Dept. of Agronomy, University of Kentucky, Lexington, KY 40546

Cyclic 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 pharmaceutically 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 $P4_12_12$ (65% solvent). We have obtained a native chimera data set to 2.8Å and four derivative data sets resulting in our initial 2.8Å 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.

PS04.01.97 TOWARDS THE STRUCTURE OF HEMAGGLUTININ-NEURAMINIDASE FROM NEWCASTLE DISEASE VIRUS. S.J. Crennell, A. Portner,† T. Takimoto,† W.G. Laver‡ and G.L. Taylor, School of Biology and Biochemistry, University of Bath, BA1 7RH, U.K., †St Judes Childrens Research Hospital, Memphis, TN, USA. ‡Australian 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 is a candidate for drug design not only against Newcastle disease (NDV), but also mumps and parainfluenza[1] whose HN share 33 and 25% sequence identity respectively with NDV HN.

HN was crystallised by the hanging drop method from 0.1M acetate buffer pH4.6, 0.2M$(NH_4)_2SO_4$ and 25%PEG4K. Native X-ray data were collected both on the inhouse Siemens and on beamline X11 of the DESY synchrotron, Hamburg, to 2.7Å resolution. No HN structure has been determined, although there is a predicted[2] 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.