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 3.0$Å^3$/Da and solvent volume fraction was 58 %. Assuming three dimers per asymmetric unit, Vm was calculated to be 2.0$Å^3$/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.

Heavy atom soaks under the crystallisation buffer conditions have not yielded an isomorphous derivative. To increase side chain reactivity the crystals were transferred to MES buffer at pH6 but soaking under these conditions in the presence or absence of $(NH_4)_2SO_4$ have been similarly unsuccessful. The current buffer, 30%PEG4K, 10mM Tris pH7, has at least induced reproducibility in cell dimensions between crystals. Good derivatives are still elusive, the most promising having a phasing power of 1.3 to 6Å resolution.

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PS04.01.98 CRYSTAL STRUCTURES OF CYCLOPHILIN A COMPLEXED WITH PROLINE-CONTAINING DIPEPTIDES AND TETRAPEPTIDE. Yingdong Zhao and Hengming Ke, Department of Biochemistry and Biophysics, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

Cyclophilins are the cellular binding proteins for the immunosuppressive drug cyclosporin A and enzymes which catalyze the peptidyl-prolyl cis-trans isomerization. Isomerization of a peptidyl-prolyl amide bond is considered to be a rate-limiting step in protein folding.

The crystal structures of human cyclophilin A complexed with proline-containing dipeptides and tetrapeptide have been determined and refined at high resolution, including cyclophilin A complexed with Ser-Pro at 1.9 Å, with His-Pro at 2.1 Å, with Gly-Pro at 2.1 Å, and with the standard tetrapeptide substrate succinyl-Ala-Ala-Pro-Phe-p-nitroanilide at 2.4 Å.

The following arguments are discussed on the basis of the crystal structures:
1. Unique cis conformation of the bound peptides suggests that cyclophilin A catalyzes the trans to cis isomerization of prolyl amide bond much faster than the reverse reaction.
2. The side chains of the Xaa amino acid in Xaa-Pro do not strongly interact with cyclophilin, implying their minor contribution to the cis-trans isomerization and thus accounting for the broad specificity of cyclophilin.
3. Substantial differences in the binding of dipeptides and tetrapeptide imply that either the dipeptides are not the substrates but inhibitors of cis-trans isomerase or that the dipeptides have a different catalytic mechanism from the tetrapeptide.

PS04.01.99 X-RAY STRUCTURE OF GELONIN AND GELONIN-AMP COMPLEX. M. V. Hosur, Bindu Nair, P. Satyamurthy, S. Misquith*, A. Surolia*, K. K. Kannan, Solid State Physics Division, B. A. R. C., Bombay-400085, *Molecular Biophysics Unit, I. I. Sc., Bangalore-560012 India

Ribosome Inactivating Proteins (RIPs) are applications in the treatment of cancer and AIDS. Detailed structure at the atomic level of RIP's and their substrate complexes are needed to understand the molecular mechanism of their immunotoxicity and N-glycosidase activity. Gelonin, is a type I RIP isolated from seeds of the plant Gelonium multiflorum. Single crystals of gelonin, grown using PEG4000, belong to the space group $P2_1$, with a=49.4 Å, b=44.9 Å, c=137.4 Å and beta=98.4 deg. There are two molecules of gelonin in the asymmetric unit, and these are related by a non-crystallographic two fold symmetry axis. X-ray diffraction data collected to 1.8A resolution limit has a Rm value of 7.3%. We have recently solved and refined this structure of Gelonin (J. Mol. Biol. (1995) 250, 368-380). We have soaked for 72 hours crystals of native gelonin into a solution containing 0.1M Tris buffer of pH 8.5, 23% PEG4000 and saturated amounts of AMP, which is the nucleotide base that is specifically cleaved during catalytic activity. The unit cell dimensions of these crystals are; a = 48.4 b = 44.5 c = 137.4A and beta = 94.5 deg., showing a significant change in the beta angle. Diffraction data collected on a RAXIS IIC system has a Rm value of 6.4% to 2.2A for the space group $P2_1$. We have solved the structure of the complex using MR, and the current R-factor is 21%. Difference electron density for the nucleotide is seen in the active site region. The refined structure of the complex, and a comparison with the native structure will be reported.

PS04.01.100 STRUCTURE AND INTERACTIONS OF A COMPLEX OF SNAKE VENOM TOXIN AND ACETYLCHOLINESTERASE. Michal Harel[1], Gerard J Kleywegt[2], Raimond BG Ravelli[3], Israel Silman[4], Joel L Sussman[1]. [1]Dept of Structural Biology, Weizmann Inst of Science, Rehovot, Israel; [2]Dept of Molecular Biology, Uppsala University, Uppsala, Sweden; [3]Dept of Crystal and Structural Chemistry, Bijvoet Center for Biomolecular Res, Utrecht, The Netherlands; [4]Dept of Neurobiology, Weizmann Inst of Science, Rehovot, Israel; [5]Dept of Biology, Brookhaven National Lab, Upton, NY, USA

The selective inhibition of acetylcholinesterase (AChE) by the snake toxin fasciculin-II was analysed using the 3D structure at 3.0 Å resolution of the 1:1 complex of the two proteins. The x-ray structure of the complex shows the 3-fingered toxin to be situated at the opening of the active-site gorge of the enzyme at the proposed peripheral anionic binding site. The toxin is interacting with AChE through an unusually large contact area and through a number of residues which are unique to fasciculin(FAS) or rare in other 3-fingered toxins. Two of the three tips of the toxin show multiple interactions with the enzyme with one tip blocking the entrance to the active-site gorge and the other interacting with the thin wall of the gorge. Both AChE and FAS maintain their 3D structures upon complexation. The interactions between FAS and AChE are mainly hydrophobic and the crucial role of aromatic residues in the activity of AChE is demonstrated again in the fact that cholinesterases lacking 2 aromatic residues in their peripheral anionic site show reduced affinity to FAS.

PS04.01.101 INSIGHTS INTO THE MECHANISM OF RUBISCO. Thomas C. Taylor, Inger Andersson. Swedish University of Agricultural Science, Uppsala, Sweden

So far little has been known about the precise mechanism by which Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) is able to catalyse the enolisation, carboxylation and hydrolysis of its substrate. We have collected data from several complexes of Rubisco in both the non-active and the active forms with the substrate; the inhibitors 2-carboxyarabitinol bisphosphate (2-CABP), 4-CABP, and xylulose bisphosphate (XuBP); with the product, 3-phosphoglycerate, and also the unliganded active enzyme. With this information it has been possible to identify residues in the active site capable of assisting the chemistry, to identify active site loop movements necessary for correct catalysis and to propose a catalytic mechanism for the carboxylation reaction.

Rubisco is activated by the post-translational carbamylation of a specific lysine residue. The carbamylate, along with an aspartate and a glutamate coordinates a magnesium ion. The magnesium ion and the carbamate appear to play a key role in the reaction. Activation does not seem to affect the conformation of the enzyme. In the unliganded form the large subunit N-terminus and C-terminus along with an active site loop (Loop 6) are disordered and the active site is open to bulk solvent. When liganded with 2-CABP, a reaction intermediate analogue, these disordered regions are locked over the active site thus shielding it from the solvent and protecting the ligand. This is important during the slow reaction cycle to prevent misprotonation of the reaction intermediates.