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.