PS-03.07.25 X-RAY CRYSTAL STRUCTURE OF A BIFUNCTIONAL INHIBITOR OF TRYPSIN AND α - AMYLASE AT 2.78 A RESOLUTION. By B.Padmanabhan, Srinivasan Alagiri and T.P.Singh, Department of Biophysics, All India Institute of Medical Sciences, New Delhi - 110 029, India

A novel class of inhibitors with two highly specific functions is known as bifunctional inhibitors. We have isolated a bifunctional inhibitor of trypsin and α - amylase from the seeds of ragi (Indian finger millet). The inhibitor is very stable in various denaturing conditions. However, it shows reduction in its activity at low concentrations of NaCl. The protein of 122 amino acids has a molecular weight of 13,300 Da. It crystallizes from 1.7M ammonium sulfate. The crystals are stable in the X-ray beam for two weeks. The space group of the orthorhombic crystals was determined to be $P2_12_12_1$ with unit cell parameters a = 30.49A, b = 56.30A, c = 73.65A. There are four molecules in the unit cell with 48% solvent content. The structure of the inhibitor has been determined by molecular replacement method at 2.78 A resolution. The molecule folds into a non-helical conformation. Further refinement of the model is in progress. The detailed refined structure of the inhibitor will be presented.

PS-03.07.26 STRUCTURE OF A TERNARY COMPLEX OF PROTEINASE K, HG AND A SUBSTRATE ANALOG HEXAPEPTIDE N-AC-PRO-ALA-PRO-PHE-PRO-ALA-NH_2 By A.K. Saxena and T.P. Singh, Department of Biophysics, All India Institute of Medical Sciences, New Delhi - 110 029, India, Ch. Betzel and M. Visanji, EMBL c/o DESY, Notkestrasse 85, 2000 Hamburg, Germany, K. Peters and S. Fittkau, Physiologisches-Chemisces Institut der Martin-Luther-Universitaet, Wittenberg 4020 Halle, Germany

The crystal structure of a ternary analog complex of Proteinase K Hg and a substrate hexapeptide N-Ac-Pro-Ala-Pro-Phe-Pro-Ala-NH_2 has been determined by X-ray diffraction method at a resolution of 2.19A. The serine protease Proteinase K belongs to subtilisin family. It contains 5 cysteines. Four of them form disulphide bridges while one, Cys 73, is in the reduced form. The Cys 73 is located near the active centre residues. It has been known that this enzyme is inhibited by inorganic mercury (II). A ternary stable crystalline complex between Proteinase K, Hg and a substrate analog hexa peptideamide was prepared by soaking the crystals of Proteinase K in $HgCl_2$ and in peptide solution. The intensity data were collected upto 2.19A with 13198 unique reflections. The crystals were isomorphous to the native Proteinase K. The coordinates of the native protein atoms were used as a starting model. The mercury atom is located in the active site region at two different positions with partial occupancies. Both are covalently linked to Cys 73 S while one of them is bound to Met 225 as well. Both have compact 5-fold coordination geometries and are tightly held with coordinations from His 69 and Asp 39. The mercury shows a stabilizing effect on the Proteinase K structure.The structure of the complex clearly shows that the Hg interaction with the protein presents the enzymatic activity of Proteinase K.the substrate analog hexapeptide amide is held well in the recognition site and gives rise to a stable complex. The electron density of peptide is well defined in the recognition site and extends to the prime sites. The refinement is in progress and the current R factor is 0.180 for 13198 observed reflections upto 2.19A resolution.

PS-03.07.27 X-RAY STRUCTURE DETERMINATION OF SUBTILISIN E AT 2Å RESOLUTION. By N.-M. Chu , K. Shi, L. Zhou, R.-C. Bi*, L. Deng@ and B. Li@, Institute of Biophysics, Academia Sinica, Beijing 100101; @Shanghai Institute of Biochemistry, Academia Sinica, Shanghai 200031, China.

Subtilisin E from *B. subtilis* was produced by genetic engineering in Department of Biology, University of Science and Technology, Hefei, China. It has been crystallized in space group $P2_12_12_1$ with cell dimensions *a*=74.35Å, *b*=80.98Å, and *c*=88.5 9Å. These crystals contain two molecules of subtilisin E in an asymmetric unit ,and this is a rare case among the subtilisin crystal structures studied so far. The orientation and translation parameters of the two molecules were determined by molecular replacement methods. Simulated annealing followed by EREF refinement with X-PLOR has resulted in the crystallographic R-value of 0.19 for 5.0-2.0Å data with r.m.s. deviation from ideal bond lengths of 0.02Å and r.m.s. deviation from ideal bond angles of 3.6°.The unusual packing of the crystal structure is related to the residue substitution, and several intermolecular hydrogen-bonds are formed by the substituted residues. The r.m.s. displacement for the main-chain superposition of the two independent molecules is 0.402Å. Besides typical features of subtilisin, there are larger conformational alterations for some amino acid residues in comparison with known subtilisin structures. The inhibition by PMSF leads to noticeable change of the catalytic triad geometry in the electron density map. A further analysis of the structure is still under way.

PS-03.07.28 CRYSTAL STRUCTURE OF ACIDIC PHOSPHOLIPASE A_2 FROM THE VENOM OF AGKISTRODON HALYS PALLAS. By X.Q.Wang[1], J.Yang[1], L.L.Gui[1], Z.J.Lin[1]*, N.Q.Lin[2] and Y.C.Zhou[2], [1] National Laboratory of Biomacromolecules, Institute of Biophysics, Academia Sinica, Beijing, China, [2]Shanghai Institute of Biochemistry, Academia Sinica, Shanghai, China.

The venom of *Agkistrodon halys* pallas contains three highly homologous phospholipase A_2 (PLA_2), which are quite different from each other in enzymatic activity, toxicity and pharmacological activity(Chen,Y.C.et al.,(1987),Toxicon,25,401). The structural analysis and comparison of these three PLA_2 will be beneficial to understanding of the relationship between the structure and function. The acidic PLA_2 shows weak toxicity (LD_{50}=300mg/kg) and has a function of inhibiting platelet aggregation. The molecule is a single polypeptide chain of 124 residues cross-linked by 7 disulphide bridges. There is 80% sequence homology between this PLA_2 and *Crotalus atrox* venom PLA_2. Single crystals were grown from 12 μl droplets containing 10mg/ml protein and 10% 2,5-hexanediol in $Na_2(CH_3)_2AsO_2$-HCl buffer(pH 5.94), equilibrated by vapour diffusion against 65% 2,5-hexanediol. The space group was determined to be either $P6_1$ or $P6_5$ (Final solution of the structure identified as $P6_1$), with a=b=83.57Å and c=32.72Å, and one molecule per asymmetric unit. Diffraction data to 2.0Å

