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

(Rsym = 0.109) were collected on a Siemens multiwire X200-B area detector. The structure was solved by molecular replacement with Crowther's fast rotation and translation function, and the search molecule constructed from one subunit(L) of *C. atrox* venom PLA_2. The structure was refined using programs PROLSQ and X-PLOR and model building techniques. The current model gives a crystallographic R-factor of 0.19. The root-mean-square (rms) deviation from ideality for bond lengths is 0.012Å, for bond angles is 2.9°, and for planarity of the peptide bond is 23.8°. The structure of acidic PLA_2 shows that the main chain folding is similar to that of *C. atrox* venom PLA_2, with the exception of several flexible loops and the stretch 115-124 where the extra residue Ser121 is inserted. The enzymatic active center, the hydrophobic channel to accessing the catalytic center and the binding region of calcium ion resembles closely those of other PLA_2. The structure details will be described and the relationship between the structure and function including inhibiting platelet aggregation will be discussed.

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PS–03.07.29 THE CRYSTAL STRUCTURE OF THE TERNARY COMPLEX OF MUNG BEAN TRYPSIN INHIBITOR WITH TWO PORCINE TRYPSIN (PTRY-MBI-PTRY) AT 2.8 Å RESOLUTION. Jin Yi, Liu Shenping, Li Genpei, Tang Youchi, Institute of Physical Chemistry, Peking University, Beijing 100871, P.R. China; Chi Zhengwu, Shanghai Institute of Biochemistry Academia Sinica, Shanghai 200031, P.R. China

Mung bean trypsin inhibitor is a Bowman-Birk type serine proteinase inhibitor with a single peptide chain of 72 residues and possesses two active sites of antitrypsin. Both sites bind strongly to trypsin synchronously, but not to chymotrypsin. The crystallization of the complex was carried out by the combination of vapor diffusion and reseeding methods. The crystal was orthorhombic with space group I222 and unit cell dimensions of a=122.4, b=123.0, c=112.8Å.The 2.8Å resolution data set composed of 18409 unique reflections above significant level, corresponding to 88.7% expected unique reflections, was collected on a Simens X-200B area detector.The structure of the complex was determined by molecular replacement method and refined by stereochemically restrained least-squares and simulating annealing procedure to a current R value of 0.217 at 2.8Å resolution. The root-mean-square deviations from the ideal bond distances and angles for the model are 0.015Å and 3.4°,respectively. The model of MBI molecule is composed of two structural domains which are similar in conformation, but distinctly different in atomic details.The two domains relate to each other by an approximate dyad axis and their binding modes with trypsin are remarkably similar. A model of ternary PTRY-MBI-PTRY complex will be presented.

PS–03.07.30 THE CRYSTAL STRUCTURE OF THE COMPLEX OF PORCINE PANCREATIC ELASTASE WITH C/E-1 INHIBITOR, A MEMBER OF A NOVEL INHIBITOR FAMILY OF SERINE PROTEINASES. Kui Huang, Robert J. Peanasky and Michael N.G. James, Dept. of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7 and Dept. of Biological Chemistry, The University of South Dakota Medical School, Vermillion, South Dakota 57069, U.S.A.

A 63 amino acid protein isolated from the intestinal parasite *Ascaris suum* is a very strong inhibitor of chymotrypsin and elastase, with a Ka of 1.6×10^{10} M^{-1} for porcine pancreatic elastase. This inhibitor (C/E-1) shares little sequence homology with known protein inhibitors of proteolytic enzymes, thus representing a distinct family of protein inhibitors of serine proteinases, including several other small proteins isolated from *Ascaris suum*.

The C/E-1 inhibitor has been crystallized in complex with porcine pancreatic elastase. The crystals grow as thin plates from 12% PEG (6000), at pH 6.5, in 50 mM sodium citrate buffer. The space group was determined from precession photos, as $P2_12_12$, with $a = 74.51$ Å, $b = 114.33$ Å, $c = 70.45$ Å. There are two molecules of complex per asymmetric unit. X-ray diffraction data have been collected using synchrotron radiation to 2.5 Å resolution. The initial phases for the structure of the complex were determined by the molecular replacement method, using the refined crystal structure of porcine pancreatic elastase as a search model. The highest peak given by the Navaza fast rotation function was 9.2 sigma above the mean. The second highest peak was significantly lower (4.5 sigma), suggesting that the two complex molecules are in almost the same orientation. The Brute translation search carried out with two molecules in the same orientation gave two peaks, one for each of two molecules. The best orientation for each molecule was determined by a 6-dimensional fine search of one molecule, while the other one was fixed. The molecular replacement solution gave a reasonable crystal packing pattern, exhibiting abundant intermolecular interactions and no collision of main chain atoms in the interfaces of neighboring molecules. The relatively limited number of interactions along the b-axis explains the fact that the complex crystal is very thin in that direction (about 0.01 mm thickness).

The initial electron density map reveals parts of the polypeptide chain of the C/E-1 inhibitor in the vicinity of the reactive site. Progress on the detailed structure analysis of the C/E-1 inhibitor, the comparisons of the structure and inhibitory mechanism of C/E-1 inhibitor with those of inhibitors from other families, and prediction of common features of the novel *Ascaris suum* inhibitor family represented by C/E-1 will be reported.

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