05-Molecular Modelling and Design for Proteins and Drugs

PS-05.01.17 STUDIES OF THE STRUCTURE-ACTIVITY RELATIONSHIP OF TTI INHIBITOR by Liu Shenping*, Huang Qichen, Tang Youqi and Qi Zhenwu[2]. Institute of Physical Chemistry, Peking University, Beijing 100871, China, [2]Shanghai Institute of Biochemistry, Academia Sinica, Shanghai 200031, China.

The serine proteinase inhibitor from Trichosanthes Kirilowii, Cucurbitaceae (TTI) belongs to Squash family and is the smallest protein proteinase inhibitor (27 a.a), so this protein is a suitable target for structure-function relationship studies.
We build the model of TTI and its complexes with enzyme based upon structure of complex formed by porcine β-trypsin with inhibitor from bitter gourd determined in our laboratory. It is apparent that the hydrophobic amino acid within TTI's primary binding segment make contribution to the inhibition. In TTI P1' mutant ILE/SER, the P1' side chain forms a hydrogen bond with and prevents the orientation of 57HIS side chain of the enzyme toward 195SER and prevents the proton transfer from 195SER to 57HIS which is essential to serine proteinase's mechanism. It explained the fact that mutant TTI was observed to exhibit much higher inhibition than the native inhibitor.

PS-05.01.18 MODELLING OF HYDROGEN BONDING IN X-RAY PROTEIN STRUCTURES USING NEUTRON DATA FROM AMINO ACIDS AND SMALL PEPTIDES. by M.Ramanadham, V.S.Jakkal and R.Chidambaram*, Solid State Physics Division and Applied Chemistry Division, Bhabha Atomic Research Centre, Trombay, Bombay 400085, India

Modelling of the hydrogen-bond interaction in x-ray protein structures can be carried out using data from high precision neutron studies on small biomolecules. However, in the case of a carboxyl group, it is necessary to know whether it is neutral, and, if so, which is the hydroxyl position, before assigning the hydrogen atom to it. We have analysed 42 carboxyl groups in neutron studies of amino acids and small peptides in terms of hydrogen bond populations and bond length, bond valence correlations, and come up with criteria that can help in making an unambiguous distinction between $-COO^-$ and -COOH, and in identifying the -OH position in -COOH groups, in x-ray protein structures. These criteria have been successfully applied to the carboxyl groups, GLU-35, ASP-52 (involved in function), GLU-7 and ASP-101 (involved in protein-protein contact) in the x-ray structure of triclinic HEW lysozyme (Ramanadham et al, 1990).

Ramanadham, M., Sieker, L.C. and Jensen, L.H. (1990) Acta Cryst. B46, 63-69

PS-05.01.19 IDENTIFICATION OF KEY RESIDUES FOR CARBONIC ANHYDRASE ISOENZYMES: USE OF SEQUENCE AND CRYSTALLOGRAPHIC DATA. By Vinay Kumar* and K.K. Kannan, Solid State Physics Division, Bhabha Atomic Research Centre, Bombay- 400085, India.

Seven forms of Carbonic anhydrase (CA) enzymes (CAI-CAVII) with an overall sequence identity of about 17% are known in vertebrates and catalyze the reversible hydration of CO_2 to varying efficiencies. They also differ in their stability towards various denaturing agents. The catalytic tuning and stabilty of the folded state of various forms of CA isoenzymes is encoded by the amino acid substitutions. However, the three known structures HCAI, HCAII, BCAIII and BCAII (Kannan et al., *Ann. N. Y. Acad. Sci.* 1984, 429,49-60; Eriksson, A. E. & Liljas, A. *Ph.D. Thesis of Eriksson,* 1988, Uppsala University, Uppsala, Sweden; Vinay Kumar et al., *Current Science,* 1989, 58, 344-348.) with 60% sequence identity, are observed to be highly homologous, the pairwise rms displacement being about 1Å. The analysis of the structure, obtained from strictly conserved residues, could be useful in determining the key residues in catalysis and/or folding. The conserved structure for CA enzyme was built by preserving only sequence wise and structurally identical residues with BCAII structure (Vinay Kumar et al., *ibid*) as a template. The conserved structure reveals interesting H-bonding networks. The analysis of strictly invariant residues reveal that the network involving Tyr194, His107 and Glu117 residues may restrict the side chain conformation of Glu117 which in turn that of the peptide segment 105-107 by its H-bonding to the main chain NH and thus the position of the catalytically important Glu106 in the active site (Vinay Kumar, *Ph.D. Thesis,* 1992, University of Bombay, India). The side chain of ionized Glu106 accepts H-bonds from Thr199, peptide NH of Arg246 and a water molecule. The polypeptide chain 245-250 runs in the vicinity of conserved type VIb turn which arises due to Cis-Pro30. It may be inferred from modelling studies that substitution of Cis peptide at Pro30 with a trans peptide may affect the peptide segment at 245-250 residues and thereby the orientation of essential Glu106 side chain which in turn may affect the catalytic activity. This is consistent with the recent experimental observations on HCAII. Similarly a few residues responsible for the CA folding may be identified. Gln222, situated on a Alpha-helix (221-226) forms two H-bonds with the main chain atoms of Ala154, which is preceeding a long helix (155-162; 163-166). Another conserved residue Leu164 from the long helix points into a hydrophobic pocket. These residues may be important for packing of the corresponding helices. Site directed mutagenesis may confirm the importance of His107, Glu117, Leu164 and Gln222 amino acid residues. The stability of HCAI, HCAII and BCAII isoenzymes towards various denaturing agents can be correlated with of sulphur-aromatic and aromatic-aromatic interacting pairs. On the basis of observed correlation and amino acid sequence of BCAII isoenzyme it seems that BCAIII may be less stable than the other two isoenzymes. The "conserved sructure of CA" and its implications to function and folding will be discussed.

