PS–03.11.07 CRYSTALLOGRAPHIC STUDY ON A SERIES OF NEUROTOXINS WITH DIFFERENT TOXICITIES FROM CHINESE SCORPIONS. By Lei Jin*, Hongming Li, Miao Wang, Zonghao Zeng and Dacheng Wang, Institute of Biophysics, Academia Sinica, Beijing 100101, P.R.China.

Scorpion neurotoxins form a homologous family of small-sized proteins. Their neurotoxic activities are attributed to their capacity to interact with ion channels on excitable membranes. Elucidating and comparing the three-dimensional structures of a series of scorpion neurotoxins with different toxicities is significant in understanding both their structure-function relationships and the nature of ligand-receptor interaction in excitable membranes. From the venom of scorpion *Buthus martensii Karsch* distributed in China, three mammalian neurotoxins(Bmk1, Bmk5 and Bmk8) have been purified to a high degree, showing single band on SDS- and IEF-PAGE and one peak on HPLC. They possess strong(Bmk1), medium(Bmk5) and weak(Bmk8) toxicities respectively so as to form an excellent series for exploration of the structure-function relationship. Furthermore, Bmk8 is an acidic protein(pI 5.3), differing distinctly from all the basic scorpion neurotoxins yet reported and making the study more informative.

The three neurotoxins have been crystallized in orthorhombic and monoclinic forms respectively. All the crystals obtained diffract to resolutions beyond 0.2nm, laying down a solid foundation for further study. High resolution diffraction data have been collected and crystal structure determinations are in progress. The details of crystallizations, crystallographic analysis, partial sequences and preliminary structure analyses for these three neurotoxins will be presented.

PS–03.11.08 STRUCTURAL COMPARISON OF TWO CRYSTAL FORMS OF TRICHOSANTHIN. By Fu Zhuji*, Zhou Kangjing, Lin Yujuan, Chen Minghuang and Pan Kezhen, Fujian Institute of Research on the Structure of Matter, Chinese Academy of Sciences, Fuzhou 350002, P.R.China.

Trichosanthin, being one kind of plant toxins, belongs to ribosome inactivating proteins with single chain, which have strong inhibition on the protein synthesis of the cell-free system, and produce immunotoxin if they are bound with monoclonical antibody.

Two crystal forms of trichosanthin(TCS) crystallized in monoclinic with space group (S.G.) C2, a=75.64, b=75.52, c=88.88Å, β=99.51°, z=8, which has 2 molecules in an asymmetric unit and othorombic with S.G. $P2_12_12_1$, a=38.31, b=76.22, c=79.21Å, z=4 at pH=8.6 and pH=5.4 respectively. Two crystal structures have been refined with data to 1.9Å and 1.88Å resolution respectively by using the restrained least squares; PROLSQ and the molecular dynamics refinement program, XPLOR. The refined structures have been used as the starting point for a comparison of molecular structure. Both structures are very similar although the crystals of two forms grow at different pH values. The structure homologies between two forms are calculated by the least squares of equivalent superposition according HOMOLOG program. The results are as follows: average error is 1.472Å for all 1914 nonhydrogen atoms; 1.211Å for main chain atoms; 1.163Å for Cα atoms. It follows that the variation of pH condition does not cause violent change in molecule conformation of TCS. α_5-helix of three molecules which come from these two crystal forms lying in the center of the molecule is an irregular helix. The helix begins to bend outward at Gln156, thus making Gln156 locate at the bottom of the concave and expose on the surface of the molecule, Meanwhile, Glu160 and Arg163 residues also locate on the surface of concave. The upper half and the lower half part of the α_7 from a turn of about 60° at the Ser191. This result compels the side chain of the Trp192 to parallel with the side chain of Arg163. This kind of α helix with angle turn is conspicuous in the molecular structure of TCS. There are some differences from each other in three molecular stuctures in two forms. The variances of polypeptide chain are mainly shown on the C terminal and extended polypeptide chain regions, particularly on the molecular surface. Owing to the packing environment of molecules in crystal, there are also some differences in the orientations of some side chains, the forms of hydrogen bonds and ion pairs as well. For instance, Arg5 in Mol.A of C2 S.G. forms an ion pair with Glu28 in Mol.B, but Arg5 in Mol.B, in the same way as Arg5 in $P2_12_12_1$, forms an ion pair with Asp57 in itself molecule.

PS–03.11.09 THREE DIMENSIONAL STRUCTURE AND FUNCTION OF TRICHOSANTHIN. By Pan Kezhen*, Lin Yujuan, Zhou Kangjing, Fu Zhuji and Chen Minghuang, Fujian Institute of Research on the Structure of Matter, Chinese Academy of Sciences, Fuzhou 350002, P.R.China.

It is known that higher plants contain ribosome inactivating proteins(RIPs), recognized as one kind of plant toxins, which have a strong inhibitory effect on protein synthesis of the eucaryotic intact cell and cell free system, particularly due to inactivation of ribosomes. Trichosanthin(TCS) belongs to the single-chain RIPs. The C2 crystals grow under basic conditions (pH≈8.6), while $P2_12_12_1$ crystals grow at an acidic pH(5.4). We have completed the refinements of the two crystal forms at 1.9Å and 1.88Å resolution, respectively. There are two molecules of TCS in the C2 asymmetric unit, their crystallographic environments are different, but their molecular structures are very similar except some differences in the orientation of some extended polypeptide chain regions and some side chain. Although the crystallization conditions of the two crystal forms have great differences(from basicity to acidity), their molecular structures are extremely similar. They suggest that it doesn't lead to any considerable changes in the molecular conformation. The TCS has a rather wide adapting range in playing its biological function in the cell. The active centers of the biological function of the two molecules in the asymmetric unit of C2 crystals both locate on its own molecular surface but not on the boundary of the two molecules, and have the same molecular structure as well as the same biological function.

There are many similarities in the crystal structures and the amino acid sequences between the single-chain RIPs(e.g. TCS) and the A-chain of double-chain RIPs(e.g. Ricin). It is very important to compare the amino acid sequences between the homologous proteins so as to find out which amino acid residues are important in the relationship of the structure and function in TCS. The amino acid sequences of RIPs from ten different kinds of plants already published in literature are listed in sequence and comprehensively compared. Among these plant toxins, it is essential to know which are the most conservative residues in the evolution of plants, which are certainly very important in the study on the relationship of structure and function of all these proteins. Based on the summary of the regularity of amino acid sequences of the ten kinds of RIPs, together with the crystal and molecular structures of TCS, seventeen most conservative polar amino acid residues are surveyed and analyzed. We found that five most conservative polar amino acid residues Arg122, Gln156, Glu160, Arg163 and Glu189 gather in the concave on the boundary of the large and the small domains, and locate on the molecular surface. They are supported by the actions of the two ion pairs, the hydrogen bonds and the Van der Waal's forces, thus form the possible active center of TCS. Meanwhile, Tyr14, Arg22, Tyr70, Tyr111 and Trp192 lie around this active center. Trp192 in helix α_7 lies at the bottom of the concave. Its side chain is parallel to that of Arg163, and its C atoms come in contact with this residue,thus having an important role in the relative stabilization of Arg163 in the active region. Some hydrophobic residues Phe17, Gly128, Leu132, Ala148, Ile167, Val232 and Leu241 take part in the formation of hydrophobic areas in the large and the small domains, support the folding of polypeptide chain of protein and form the above-mentioned active center structure.

