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PS–03.07.06 STRUCTURE COMPARISON BETWEEN TRICHOSANTHIN AND MOMORCHARIN. By Gao Ben*, Wang Yaoping, Chen Shizhi, Wu Shen, Ma Xingqi and Dong Yicheng, Institute of Biophysics, Academia Sinica, Beijing, 100101, PRC.

The similarities and differences between the two RIPs (ribosome inactivating proteins) molecular structures were determined and analysed on the basis of the refined structure models of Trichosanthin at 1.73Å resolution (Gao Ben, et al., Scientia Sinica B, 1993, 3, in press) and α-Momorcharin at 2Å resolution provided by Ren Jingshan and Wang Yaoping (Private communication). The superposition of the two models was performed using the main-chain atoms and the RMS deviation for all the main-chain atoms of the 246 residues is 0.70Å. Dividing the two models into many pairs of different polypeptide fragments, the superpositions of the pairs of all polypeptide fragments have done in turn and the RMS deviations for the main-chain atoms of four fifth polypeptide fragments are smaller than 0.30Å. The RMS deviation for the side-chain atoms of more than half of all residues are smaller than 0.50Å and the great majority of these residues form six hydrophobic cores in the interior of the two proteins. These results indicates that the backbones of the two proteins have very similar three-dimensional arrange. There are three domains of the greatest deviation for the main-chain conformations of the two proteins, 38-45, 172-182, and 216-222, which are the flexible loops on the surfaces of the two proteins and corresponding to the sequence fragments with the greatest residue differences. Therefore, this result indicates that the residue differences have evidently brought about the three-dimensional conformational differences.

There are the residue differences corresponding to primary amino acid sequences for one third residues of the two models. As the result of these residue differences, the differences were found to exist not only in the main-chain conformations but also evidently in the secondary structures and in distributions of the other hydrogen bonds relative to the main-chain atoms and bound waters which form hydrogen bonds to the main-chain atoms. The patterns of thirteen percent of the hydrogen bonds for the α helics of the two models, that of seventeen percent of the hydrogen bonds for the β sheets, and that of thirty eight percent of the hydrogen bonds for the turns are different from each other, respectively. The patterns of thirty percent of the other hydrogen bonds relative to the main-chain atoms and thirty seven of the waters bound to the main-chain atoms are different, respectively.

Ten highly conserved residues among primary amino acid sequences of 12 RIPs (Funatsu, G., et al., Biochimie, 1991, 73, 1157-1161. Gao Ben, et al., Scientia Sinica B, 1993, 3, in press) were analysed and those corresponding to Trichosanthin are 14Tyr, 22Arg, 70Tyr, 111Tyr, 122Arg, 132Leu, 160Glu, 161Ala, 163Arg, and 192Trp. Superposition of ten residues of Momorcharin on those corresponding to Trichosanthin was done together using all the 40 atoms of the main-chains. The RMS deviation for the main-chain atoms is 0.30Å and that for the side-chain atoms is 0.18Å. The differences in distributions of hydrogen bonds and bound waters relative to the corresponding ten residues in the two models are a little. A summary of the analysis statistics by superimposing, in turn, ten pairs of residues of the two models indicates that the RMS deviations for the main-chain atoms are all smaller than 0.10Å and that for the side-chain atoms of those residues except 122Arg are smaller than 0.15Å. Therefore, the three-dimentional structures of nine residues which are invariant among the known sequences of those RIPs are highly conserved. These results have an important significance for researching of the RIPs structure-function relationship.

PS–03.07.07 THREE-DIMENSIONAL STRUCTURE OF THE COMPLEX OF TRICHOSANTHIN WITH NADPH AT 1.7Å RESOLUTION. By J.-P. Xiong*, L. Zhang, Z.-X. Xia, and Y. Wang. Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China.

Trichosanthin is a toxic protein (Mr 27,000) used as a traditional Chinese drug for inducing abortion and recently found to be an anti-human immunodeficiency virus agent. Trichosanthin is a type-I ribosome-inactivating protein (RIP) with the acitivity of RNA N-glycosidase, and it was reported that ricin, a type-II RIP, catalyzes the cleavage of the N-glycosidic bond of a specific adenine within 28s rRNA, resulting in the inhibition of protein synthesis (Endo et al., J. Biol. Chem., 1987, **262**, 8128-8130). We have determined at 3Å resolution the three-dimensional structure of trichosanthin crystallizing in monoclinic space group C_2 (Xia et al., chinese J. Chem., 1991, **9**, 563-564) and it has been refined at 2.7Å resolution (Xia et al., Abstracts of 6th FAOB Congress, 1992, **16-21**, 99). The molecule shows a cleft near the interface of the two domains and the cleft is likely to be the active site region in which several absolutely conserved residues are located.

The complex of trichosanthin with nicotinamide adenine dinucleotide phosphate (NADPH), a substrate analogue, was prepared and crystallized in space group $P2_12_12_1$ with unit cell dimensions a=38.39Å, b=76.81Å and c=79.93Å, similar to orthorhombic native crystals. The diffraction data up to 1.7Å resolution were collected on an X-200B area detector. The three-dimensional structure of the complex has been solved by molecular replacement method (program MERLOT) using one molecule of the monoclinic trichosanthin structure as the search molecule. The complex structure was refined at 1.7Å resolution, using program PROFFT, in which 170 bonded water molecules were included but NADPH was absent in the model, giving an R-factor of 18.9% in the resolution range 5.0-2.0Å with the rms deviation of 0.025Å from ideal bond lengths. The resulting (2Fo-Fc) map shows excellent electron density for the protein and an additional piece of continuous electron density. The NADPH has been fitted into it with the adenine ring in the strong and flat electron density which is located between the aromatic rings of Try70 and Try111. The adenine interacts with Arg163 which is absolutely conserved and located in the deep center of the cleft, Ser159 which is conserved in some of the RIPs', and the main chain of the protein. The phosphate at the position O_2' of the ribose of the adenosine interacts with serveral conserved residues in the cleft and is important for stabillizing the complex, as shown by the fluorescence spectra. The further refinement with NADPH present in the model is in progress.

PS–03.07.08 X-RAY STUDIES ON THE TRYPSIN INHIBITOR I-2 FROM WHEAT GERM AND ITS COMPLEX WITH TRYPSIN. By A. Suzuki[1], T. Kurasawa[1], C. Tashiro[1], T. Yamane[1]*, T. Ashida[1], and S. Odani[2], [1]Department of Biotechnology, School of Engineering, Nagoya University, Nagoya 464-01, Japan, [2]Department of Biology, Faculty of Science, Niigata University, Niigata 951, Japan.

