PS-03.07.06 STRUCTURE COMPARISON BETWEEN TRICHO
SANTHIN AND MOMORCHARIN. By Gao Ben*, Wang Yingting, Chen Shixi, Wu Shen, Ma Xingyi and Dong Yichang, Institute of Biophysics, Academia Sinica, Beijing, 100101, PRC.

The similarities and differences between the two RPs (polysine inactivating protein) molecular structures were determined and analyzed on the basis of the refined structure models of Trichosanthin at 1.7Å resolution (Gao Ben, et al., Sciencia Sinica B, 1993, 4, in press) and α-Momorcharin at 2.0Å resolution provided by Ren Jinghua and Wang Yingting (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 segments, the superpositions of the pairs of different polypeptide fragments have been done in turn and the RMS deviations for the main-chain atoms of four fifth polypeptide fragments are smaller than 0.20Å. 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 indicate that the backbone of the two proteins have very similar three dimensional structures. There are three domains of the greatest deviation for the main-chain conformation of the two proteins, 38-45, 172-182 and 216-222, which are the flexible loops on the surface 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 water molecules which form hydrogen bonds to the main-chain atoms. The patterns of thirteen percent of the hydrogen bonds for the a-helix 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 areas of the water bound to the main-chain atoms are different, respectively.

Ten highly conserved residues among primary amino acid sequences of 12 RPs (Funatsu, C., et al., Biochimia, 1991, 73, 1157-1161; Gao Ben, et al., Sciencia Sinica B, 1993, 4, in press) were analyzed and those corresponding to Trichosanthin are 14Fy, 22Arg, 207Ser, 11Fy, 124Arg, 33Leu, 16Glu, 16Ala, 163Arg, and 197Phe. 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 0.80Å and that for the side-chain atoms is 0.18Å. The differences in distributions of hydrogen bonds and bound water 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 these residues except 122Arg are smaller than 0.15Å. Therefore, the three-dimensional structures of nine residues which are invariant among the known sequences of these RPs are highly conserved. These results have an important significance for researching the RPs structure-function relationship.


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 activity 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 26S 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 P21 (Xia et al., Chine J. Chem., 1991, 9, 563-564) and it has been refined to 2.7Å resolution (Xia et al., Abstracts of 6th PDB 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 P21(212) 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-00B area detector. The three-dimensional structure of the complex has been solved by molecular replacement method (program MERGET) using one molecule of the monoclinic trichosanthin structure as the search model. 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 3-2.0Å with the rms deviation of 0.02Å 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 Tyr70 and Tyr71. The adenine interacts with Arg161 which is absolutely conserved and located in the deep center of the cleft, Ser159 which is conserved in some of the RPs, and the main chain of the protein. The phosphate at the position O2' of the ribose of the adenosine interacts with several conserved residues in the cleft and is important for stabilizing the complex, as shown by the fluorescence spectra. The further refinement with NADPH present in the model is in progress.