03-Crystallography of Biological Macromolecules

PS-03.11.10
PRELIMINARY CRYSTALLOGRAPHIC STUDIES OF GROTIN II. By Chen Minghuang, Zhou Kangiing, Fu Zhuli and Pan Kezhen*, Fujian Institute of Research on the Structure of Matter, Chinese Academy of Sciences, Fuzhou 350002, and National Lab. of Biomacromolecules, Beijing 100101.

Two plant toxins, crotonin and crotonin II have been isolated and purified from the seeds of Euphorbiae Crotoxylon sagittatum, which is a Chinese medicinal herb named Ba Dou. The molecular weight (M.W.) of crotonin I and crotonin II measured by SDS-PAGE are about 40,000 and 15,000 Da, respectively. (Chen M.H. & Pan K.Z., Chinese Biochemical J., in press). It was observed that these two proteins inhibit protein synthesis in a cell-free system (Stolpes, F. et al. 1976, Biochem. J. 156: 1-6) and deproteinat rat liver ribosomes (Barbieri, L. et al., 1992, Biochem. J. 286: 1-4). They belong to so-called ribosome inactivating proteins(RIPs). The experiments show that crotonin I has much higher inhibitory activity than crotonin II. Crotonin II has a neutral pl and a lower M.W., it is different from single chain RIPs like Trichosanthin, which have a basic pl and a heger M.W.(e.g 27,000 Da). However, they have a similar function to inhibit protein synthesis. So the study of the three-dimensional structure of crotonin II is important in the relationship of structure and function in single chain RIPs.

The crystalization was performed by using the hanging-drop method. The crystals of crotonin II with high quality were grown at room temperature in a tris-buffer solution with KCl as the precipitant. The crystal grows to a size of 0.7 mm x 0.3 mm x 0.3 mm within ten days. Precission photographs of the crystals mounted in the thin-wall siliconized glass capillary tubes were taken by using a N-filtered CuKα radiation (40 KV, 100mA). The cell parameters were determined to be a = b = 18.4 Å, c = 28.4 Å, α = β = 90°, γ = 120°. The extinction rules and intensity distribution of the reflections show that the crystal belongs to space group P61 or P65. Assuming one molecule in an asymmetric unit, the Vd value of 2.4 Å3/Å3 and 44% solvent contents were calculated (Matthews, B.W., 1968, J. Mol. Biol. 41, 451).

X-ray data collection for native crystals were collected on a detector (Siemens X-200B). Each oscillation frame covered 0.2° and was measured for 120°. Total 720 frames were collected. The data were reduced by using the XENGEN program. Final merged data collection have 17,822 unique reflections within the 1.82 Å resolution. R(F) = 0.2619.

PS-03.11.11
STRUCTURE OF ORTHORHOMBIC CRYSTAL OF TRICHOSANTHIN AT 1.8Å RESOLUTION. By Zhou Kangiing*, Fu Zhuli, Chen Minghuang, He Yu-Jing and Pan Ke-Zhen, Fujian Institute of Research on the Structure of Matter, Chinese Academy of Sciences, Fuzhou, 350002, Fujian, China.

Trichosanthin is one of the ribosome inactivating proteins (RIPs) extracted from a Chinese herb medicine, the root tuber of Trichosanthus, Kiriunki, Morin Cucumbaraceae. It consists of 247 amino acids with Mr = 27,137.2. The orthorhombic crystals of trichosanthin have been obtained by using hanging drop method under the condition of pH5-6. The crystal belongs to the space group P212121, with a = 38.4 Å, b = 76.2 Å, c = 79.3 Å. The X-ray intensities were collected on a Siemens X-200B area detector. The structure was solved by molecular replacement methods, using the model of trichosanthin molecule of monocrystalline crystal as the known structure model. The initial model was refined using the programs of XPLOR and PROLSQ to an R-factor of 0.191 for the reflections between 6Å-1.8Å. The r.m.s. deviations of bond lengths and bond angles are 0.013 Å and 0.055 Å, respectively. Trichosanthin molecule can be divided into two structural domains with different size. The molecule contains 8 α helices and 13 β strands, the characteristic of which is that almost all α helices are in the inner of the molecule, whereas all β strands were near the surface. The active site of the molecule is located on the concave region between the two domains. In the active site Arg122 and Gin185, Arg123 and Gin160 form two ion pairs, Gin189 and Gin156 are hydrogen bonded to each other. A total of 279 solvent molecules are included in the final refined model. Comparing with the structure of monocrystalline crystal of trichosanthin grown under the conditions of pH5-6, it is shown that there are little differences between the two structures.

PS-03.11.12
THE REFINED CRYSTAL STRUCTURE OF THE NEUROPHYSIN-OXYTOCIN COMPLEX AT 2Å RESOLUTION. John P. Rose* and Bi-Cheng Wang, Department of Crystallography and Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260 USA.

The posterior pituitary produces two important regulatory hormones, oxytocin and vasopressin. Oxytocin is known to mediate uterine contraction and milk ejection and the recently been shown to play an important role in sexual behavior and response, as well as bonding between parent and offspring. Vasopressin plays an important role in influencing kidney function, blood pressure and body fluids. Both hormones are neuropeptides and are found in concentrations as high as 6.1 M in the neurosecretory granules of the posterior pituitary complexed in a 1:1 ratio with a class of small (11 Kd) disulfide-rich proteins called neurophysins.

Single crystals of a bovine neurophysin II - oxytocin complex have been obtained using (NH4)2SO4 as the precipitating agent (Rose et al., 1991 J. Mol. Biol. 223:43). The crystals diffract to at least 3Å resolution, belong to the space group P4mm and exhibit systematic absences consistent with either space group P412121 or P41212. The cell dimensions are a = b = 69.07 Å and c = 113.26 Å. The crystals contain one neurophysin-oxytocin dimer per asymmetric unit. Based on a Vm of 2.1 Å3/Å3, the solvent content is calculated to be 58%. The structure of the hormone-peptide complex has been determined by molecular replacement using the structure of a bovine neurophysin II Phe-Tyr-NH2 complex (Chen, et al., 1991 Proc. Natl. Acad. Sci. USA 88, 4246) as the search model. A full crystallographic refinement of the neurophysin-oxytocin complex is underway. Details of the structure and crystallographic analysis will be presented. Work supported by NIH grant GM-46828 and a grant from the Pittsburgh Supercomputing Center.

PS-03.11.13
THE REFINED CRYSTAL STRUCTURE OF A NEUROPHYSIN-DIPEPTIDE COMPLEX AT 2.5Å RESOLUTION. Chui-Koei Wu*, John P. Rose and Bi-Cheng Wang, Departments of Crystallography and Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260 USA.

Neurophysins are small disulfide rich proteins. They are found in concentrations as high as 0.1 M in the neurosecretory granules of the posterior pituitary where they are involved in the binding and storage of the posterior pituitary hormones oxytocin and vasopressin.

The crystal structure of a complex of NP-II (a vasopressin-associated NP) with L-Phe-Tyr-NH2 which binds at the hormone-binding site was determined using single wavelength anomalous scattering data (Chen, et al., 1991 Proc. Natl. Acad. Sci. USA, 88, 4240). It is an intermediate step in solving the structure of the native neurophysin-dipeptide complex.

The native NP-II Phe-Tyr-NH2 complex was crystallized in space group P212121 and diffracts to 2.5 Å. In this structure, which is