03-Crystallography of Biological Macromolecules

PS-03.11.10

PRELIMINARY CRYSTALLOGRAPHIC STUDIES OF GROTM II. By Chen Minghuang, Zhou Xiangji, 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 100104.

Two new toxins, cotrin I and cotrin II have been isolated and purified from the seeds of Euphorbiae Costonigum, which is a Chinese medicinal herb named Ba Dou. The molecular weight (M.W.) of cotrin I and cotrin 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 (Stempf, F. et al., 1976, Biochem. J. 136, 1-6) and depurinate rat liver ribosomes (Barbieri, L. et al., 1992, Biochem. J. 286, 1-41). They belong to so-called ribosome inactivating proteins (RIPs). The experiments show that cotrin II has much higher inhibitory activity than cotrin I. Cotrin II has a neutral pl and a lower M.W., it is different from single chain RIPs e.g. Thromostatin, which have a basic pl and a higher 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 cotrin II is important in the relationship of structure and function in single chain RIPs.

The crystallization was performed by using the hanging-drop method. The crystals of cotrin II with high quality grew at room temperature in a 2:1 ratio buffer solution with KCl 0.1 M as the precipitant. The crystal grows to a size of 0.7 mm x 0.3 mm x 0.3 mm within ten days. Precipitation photographs of the crystals mounted in the thin-wall siliconized glass capillary tubes were taken by using a H-filtered CuKα radiation (40 KV, 100mA). The cell parameters were determined to be a = b = 94.62 A, c = 28.43 A, α = β = γ = 90°, y = 120°. The extinction rules and intensity distribution of the reflections show that the crystal belongs to space group P61 22 22. Assuming one molecule in an asymmetric unit, the Vm value of 2.46 Å3/Å2 and 44% solvent contains are calculated (Matthews, B.M., 1986, J. Mol. Biol., 188, 411). X-ray diffraction data for native crystals were collected on area detector (Siemens X-2000). Each oscillation frame covered 0.26° and was measured for 120s. Total 720 frames were collected. The data were reduced by using the XENGEN program. Final merged diffraction data have 1,822 unique reflections within the 1.82Å resolution. Rmerge = 0.219.

PS-03.11.11

STRUCTURE OF ORTHORHOMBIC CRYSTAL OF TRICHOSANTHIN AT 1.88Å RESOLUTION. By Zhou Xiangji, Fu Zhuli, Chen Minghuang, Li. Yu-jie and Pan Kezhen, 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 Trichosanthes kirilowii, Moschin Cucurbitaceae. It consists of 247 amino acids with Mr = 27,112.3. The orthorhombic crystal of trichosanthin have been obtained by using hanging drop method under the condition of pH 5-6. The crystal belongs to the space group P212121 with a = 38.365, b = 76.221, c = 70.231 Å. The X-ray intensity data of 15466 reflections were collected on a Siemens X-2000 area detector. The structure was solved by molecular replacement methods, using the model of trichosanthin molecule of mononuclear crystal as the known structural model. The initial model was refined using the programs of XPLOR and PROLSQ to an R-factor of 0.191 for the reflections between 6.1-1.88Å. The r.m.s. deviations of bond length and bond angle are 0.013Å and 0.055Å, respectively. Trichosanthe molecule can be divided into two structural domains with different size. The molecule contains 6 α helices and 13 β strands, the characteristic of which is that almost all α helices were in the inner of the molecule, whereas all β strands were near the surface. The active site of the molecule consisted of 5 conservative residues is located on the concave region between the two domains. In the active site Arg122 and Gin189, Arg163 and Gln160 form two ion pairs, Gin189 and Gin156 are hydrogen bonded to each other. A total of 229 solvent molecules are included in the final refined model. Comparing with the structure of mononuclear crystal of trichosanthin grown under the condition 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 2.8Å 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 has recently been shown to play an important role in sexual behavior and response, as well as in 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 61 M in the neurosecretory granules of the posterior pituitary complex 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. 221, 43). The crystals diffract to at least 3Å resolution, belong to Laut group 4mm and exhibit systematic absences consistent with either space group P412121 or P41222. 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 2.9 Å3/Å2, the solvent content is calculated to be 58%. The structure of the hormone-receptor 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-45828 and a grant from the Pittsburgh Supercomputer Center.

PS-03.11.13

THE REFINED CRYSTAL STRUCTURE OF A NEUROPHYSIN-DIPEPTIDE COMPLEX AT 2.5Å RESOLUTION. Chia-Kuei Wu, John P. Rose and Bi-Cheng Wang, Department 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 anterior pituitary hormones oxytocin and vasopressin. The crystal structure of a complex of NP-II (a vasopressin-associated NP) with N-Phe-Tyr-NH2 which binds at the hormone-binding site was determined by using single wavelength anomalous scattering data (Chen, et al. (1991) Proc. Natl. Acad. Sci. USA, 88, 4246). 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 P21212 and diffracts to 2.5 Å. In this structure, which is