03-Crystallography of Biological Macromolecules

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

Two plant toxins, crotin I and crotin II have been isolated and purified from the seeds of Euphorbiacea *Croton tiglium*, which is a Chinese medicinal herb named Ba Dou. The molecular weight (M.W.) of crotin I and crotin 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 (Stirpes, F. et al, 1976, Biochem. J. 156, 1-6) and depurinated 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 crotin II has much higher inhibitory activity than crotin I. Crotin II has a neutral pI and a lower M.W., it is different from single chain RIPs(e.g. Trichosanthin), which have a basic pI and a heiger 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 crotin 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 crotin II with high quality were grown at room temperature in a citrate buffer solution with KCl as the precipitant. The crystals grow to a size of 0.7 mm x 0.3 mm x 0.3 mm within ten days. Precession photographs of the crystals mounted in the thin-wall siliconized glass capillary tubes were taken by using a Ni-filtered CuKα radiation(40 KV, 100mA). The cell parameters were determined to be a = b = 94.62Å, c = 28.43Å, $\alpha = \beta = 90°$, $\gamma = 120°$. The extinction rules and intensity distribution of the reflections show that the crystal belongs to space group $P6_1$ or $P6_5$. Assuming one molecule in an asymmetric unit, the V_m value of 2.46Å/Da and 44% solvent contains were calculated (Matthews, B.M., 1968, J.Mol. Biol., 33, 491).

X-ray diffraction data for native crystals were collected on area detector (Siemens X-200B). Each oscillation frame covered 0.25° and was measured for 120s. Total 720 frames were collected. The data were reduced by using the XENGEN program. Final merged diffraction data have 11,822 unique reflections within the 1.82Å resolution. R_{merge} =0.0819.

PS-03.11.11 STRUCTURE OF ORTHORHOMBIC CRYSTAL OF TRICHOSANTHIN AT 1.88Å RESOLUTION. By Zhou Kang-jing*, Fu Zhu-ji, Chen Ming-huang, Lin Yu-juan 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 *Trichosanthes, Kirilowii, Maxim* Cucurbitaceae. It consists of 247 amino acids with Mr=27,132D. The orthorhombic crystals of trichosanthin have been obtained by using hanging drop method under the condition of pH=5.6. The crystal belongs to the space group $P2_12_12_1$ with a=38.305, b=76.221, c=79.213Å. The X-ray intensity data of 15466 reflections were collected on a Siemens X-200B area detector. The structure was solved by molecule replacement methods using the model of trichosanthin molecule of monoclinic 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 6Å-1.88Å. The r.m.s. deviations of bond length and bond angle are 0.013Å and 0.055Å, 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 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 Glu189, Arg163 and Glu160 form two ion pairs, Glu189 and Gln156 are hydrogen bonded to each other. A total of 219 solvent molecules are included in the final refined model. Comparing with the structure of monoclinic crystal of trichosanthin grown under the condition of pH=8.4, 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, Departments 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 bonding between parent and offspring. Vasopressin plays an important role in influencing kidney function, blood pressure and body fluids. Both hormones are nonapeptides and are found in concentrations as high as 0.1 M in the neurosecretory granules of the posterior pituitary complexed in a 1:1 ratio with a class of small (11 kD) disufide-rich proteins called neurophysins.

Single crystals of a bovine neurophysin II - oxytocin complex have been obtained using $(NH_4)_2SO_4$ as the precipitating agent (Rose *et al.* (1991) *J. Mol. Biol.***221**, 43). The crystals diffract to at least 3 Å resolution, belong to Laue group 4/mmm and exhibit systematic absences consistent with either space group $P4_12_12$ or $P4_32_12$. 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 V_m of 2.9 $Å^3$/dalton, the solvent content is calculated to be 58%. The structure of the protein-hormone complex has been determined by molecular replacement using the structure of a bovine neurophysin II Phe-Tyr-NH_2 complex (Chen, *et al.*(1991) *Proc. Natl. Acad. Sci. USA*, **88**, 4240) 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.

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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, 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 I-Phe-Tyr-NH_2 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**, 4240). It is an intermediate step in solving the structure of the native neurophysin - dipeptide complex.

The native NPII-Phe-Tyr-NH_2 complex was crystallized in space group $P2_12_12_1$ and diffracts to 2.5 Å. In this structure, which is

isomorphous with the I-Phe-Tyr-NH_2 complex, the four crystallographically distinct neurophysin molecules were found to associate into an elongated tetramer consisting of two similar neurophysin dimers. However, in contrast to the structure of the I-Phe-Tyr-NH_2 complex, in which the neurophysin tetramer was found to contain five dipeptide molecules, the native neurophysin tetramer contains only four bound dipeptide molecules. A full crystallographic refinement using XPLOR is underway. The current R factor is 20.1%. Details of the structure determination as well as analysis of the structure will be presented.

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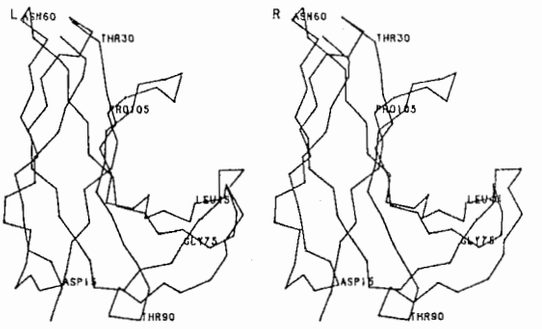

PS-03.11.14 REFINEMENT AND INTERPRETATION OF THE X-RAY STRUCTURE OF APO-NEOCARZINOSTATIN AT 1.8Å RESOLUTION. by M.Ramanadham*, Solid State Physics Division, Bhabha Atomic Research Centre, Trombay, Bombay 400085, India, and Larry C. Sieker, Department of Biological Structure, University of Washington, Seattle, WA 98195, USA

Apo-neocarzinostatin (apo-NCS), the 113 amino-acid long polypeptide component of the antitumour, antibiotic protein neocarzinostatin (NCS), has been refined at 1.8Å resolution by the method of stereochemically restrained least-squares. (Sp.gr. $P2_12_12_1$, a=27.36, b=33.89, c=101.9Å, Z=4). Prior to this, the structure was refined at 2.25Å resolution to an R-value of 0.37 for a model consisting of 751 out of 778 protein atoms. Numerous difficulties encountered in extending this refinement further had taken us back to the MIR map. A thorough re-examination of this map resulted in a model, which is consistent with not only the electron density distribution, but also the accepted rules of conformation and inter-atomic interactions. Further model improvements were affected by the use of Fourier maps phased by the atomic positions obtained during the previous step. Refinement and periodic model editing at 2.25Å resolution, commenced only after the conclusion of these steps, had resulted in an R-value of 0.27. The data were then extended to 1.8Å resolution (7,559 observations in the d-spacing range 10-1.79Å), and refinement was continued, eventually leading to a model consisting of all the 778 protein atoms and 248 solvent atoms with an R-value of 0.155. The final model is completely unambiguous in terms of the Fourier map interpretation, and is quite satisfactory from the point of view of structural and stereochemical considerations. A detailed analysis of the structure, conformation, and the chromophore binding site has been carried out. This model is currently in use for interpreting the binding of ethidium bromide to NCS at 2.5Å resolution. Efforts are also underway to model the chromophore binding to NCS at 2Å resolution.

PS-03.11.15 CRYSTALLISATION AND X-RAY DIFFRACTION STUDIES OF RECOMBINANT HUMAN PLATELET-DERIVED ENDOTHELIAL CELL GROWTH FACTOR

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Thymidine phosphorylase (TP) is one of two pyrimidine phosphorylases in the base and nucleoside salvage pathway. A search of the NBRF-PIR sequence database has revealed a striking homology (40% identity over 438 aligned positions) between TP derived from *E. coli* and human platelet-derived endothelial cell growth factor (PD-ECGF) (Barton *et al.* , 1992). This strongly suggests that human PD-ECGF is identical to human TP. PD-ECGF is known to stimulate the growth and chemotaxis of endothelial cells *in vitro* and possesses angiogenic activity *in vivo*. It is likely that endothelial cells respond specifically to a modulation in intracellular DNA precursor pool brought about by thymidine phosphorolysis.

Five different crystal forms of recombinant human PD-ECGF from yeast were produced from initial screening of crystal growth parameters using the "magic fifty" set of conditions (Jancarik & Kim, 1991) and the hanging drop vapour diffusion technique (Hampel *et al.*, 1968) One of the forms proved suitable for X-ray analysis. These crystals belong to the space group $P2_12_12_1$ with unit cell dimensions a=63.7 b=70.4 c=219.6 Å $\alpha = \beta = \gamma = 90°$. Assuming a 47% solvent content the crystals contain a single dimer in the asymmetric unit . A set of diffraction data has been obtained using station X11 of the Hamburg synchrotron source which is 84% complete at the 1σ level to 3.5Å Bragg spacings.

PS-03.11.16 CRYSTAL STRUCTURE ANALYSIS OF HUMAN DIHYDROFOLATE REDUCTASE INHIBITOR COMPLEXES. Vivian Cody*, Joseph R. Luft, Erik Jensen, Walter Pangborn, Andrzej Wojtczak, Nikolai Galitsky, J.H. Freisheim[!] and R. Blakley[+], Medical Foundation of Buffalo, Buffalo, NY 14203, [!]Medical College of Ohio, Toledo, OH 43699, [+]St. Jude Children's Hospital, Memphis, TN 38101.

Data from a methotrexate (MTX)-resistant human cancer cell line reveal a natural F31S mutation in the enzyme dihydrofolate reductase (DHFR). Kinetic data for F31 mutants shows greater effects on binding for folates than antifolates. To understand the structural basis for selectivity and specificity for binding to DHFR, and to determine the fundamental role of F31 in binding and catalysis, we have co-crystallized a series of antifolate inhibitors with both human wild type and F31 mutant DHFR as binary and ternary complexes with the cofactor NADPH. We report structural data for isomorphous R3 crystal complexes with anti-