03-Crystallography of Biological Macromolecules

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

Two plant toxins, crotonin I and crotonin II have been isolated and purified from the seeds of Euphorbiaceae Cistus cignus, which is a Chinese medicinal herb named Da Dou. The molecular weight (M.W.) of crotonin I and crotonin II measured by SDS-PAGE are about 46,000 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 (Stripes, F. et al., 1976, Biochem. J. 156, 1-6) and depurinate rat liver ribosomes (Barbieri, L. et al., 1992, Biochem. J. 286, 1-14). They belong to so-called ribosome inactivating proteins(RIPs). The experiments show that crotonin II has much higher inhibitory activity than crotonin I. Crotonin II has a neutral pH and a lower M.W., it is different from single chain RIPs.e.g. Trichosanthin, which have a basic pl and a hager 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 grew 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. Percussion photographs of the crystals mounted in the thin-wall siliconized glass capillary tubes were taken by using a N-filtered CuKα radiation (40 KV, 100 mA). The cell parameters were determined to be a = 94.62 Å, b = 28.43 Å, c = 90°, 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 Vₐ value of 2.46 Å³/D and 44% solvent contents were calculated (Matthews, B.M., 1986, J. Mol. Biol., 185, 53-91).

X-ray diffraction data for native crystals were collected on an 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 1.82Å unit reflections within the 1.82Å resolution. R(free) = 0.2619.

PS-03.11.11
STRUCTURE OF ORTHOCHRISTIAN OF TRICHOSANTHIN AT 1.8Å RESOLUTION. By Zhou Kang-jiing, Fu Zhi-ji, Chen Ming-huang, Lu Ya-jian 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 Trichosanthis, Kirilowii, Monac Cucurbitaceae. It consists of 247 amino acids with Mr = 27,137.2. The orthochristian 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 = 38.37 Å, b = 76.22 Å, c = 79.21 Å. The X-ray intensity data of 15466 reflections were collected on a Siemens X-200B area detector. The structure was solved by molecular replacement methods, using the model of trichosanthin molecule of monochromatic 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 Å-1.8Å. 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 site. 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 consisted of 5 conservative residues is located on the concave region between the two domains, in the active site Arg122 and Gin189, Arg163 and Gin160 forms two ion pairs, Gin189 and Gin165 are hydrogen bonded to each other. A total of 2/7 solvent molecules are included in the final refined model. Comparing with the structure of monochromatic crystal of trichosanthin grown under the condition of pH 5-6, it is shown that there are little differences between the two structures.

PS-03.11.12
THE REFINED CRYSTAL STRUCTURE OF THE NEUROPHYSIN-PYRINOXIDIN 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 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 complex, in a 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 3 Å resolution, belong to Laue group 4mm and exhibit systematic absences consistent with either space group P4₁2₁2 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 s PVA of 2.9 Å, the solvent content is calculated to be 58%. The structure of the hormone-binding complexes 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.

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PS-03.11.13
THE REFINED CRYSTAL STRUCTURE OF A NEUROPHYSIN-DIPEPTIDE COMPLEX AT 2.5Å RESOLUTION. Chia-Koei 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 posterior pituitary hormones oxytocin and vasopressin. The crystal structure of a complex of NP-II (a vasopressin-associated NP) with 1-Phe-Tyr-NH₂ which binds at the hormone-binding site has been derived from 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 P2₁2₁2 and diffractions to 2.5 Å. In this structure, which is
isomorphous with the 1-Phe-Tyr-NH₂ 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-NH₂ complex, in which the neurophysin center 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 of analysis of the structure will be presented.

Work supported by NH grant GM-46828 and a grant from the Pittsburgh Supercomputing Center.

PS-03.11.14 REFINEMENT AND INTERPRETATION OF THE X-RAY STRUCTURE OF APC-NEOCARZINOSTATIN AT 1.8Å RESOLUTION. by M. Ramamadhan¹, 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-NC), the 113 amino-acid long polyepitope 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. P2₁2₁2₁, a=27.36, b=31.89, c=101.9A, Z=4). Prior to this, the structure was refined at 2.25 Å resolution to an R-value of 0.37 for a model consisting of 751 out of 776 protein atoms. Numerous difficulties encountered in extending this refinement further had taken us back to the NCS 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 interatomic 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 2.25 Å resolution, commenced only after the conclusion of these steps, had resulted in an R-value of 0.27. The data were then extended to 1.8 Å resolution (7,569 observations in the d-spacing range 10-1.79 Å), and refinement was continued, eventually leading to a model consisting of all the 776 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 2.2 Å resolution. Efforts are also underway to model the chromophore binding to NCS at 2 Å resolution.

PS-03.11.15 CRYSTALLISATION AND X-RAY DIFFRACTION STUDIES OF RECOMBINANT HUMAN PLATELET-DERIVED ENDOTHELIAL CB11 GROWTH FACTOR. by Spraggan G.,1 Poulton, C. F.,1 Jones E. Y.,1 Barton, G. J.,1 Stuart D. I.,1 Finus C. and 1Sleep D.1

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Thymidine phosphorylase (TP) is one of two purine nucleoside phosphorylases in the base and nucleoside salvage pathway. A search of the NBRF-PFIR 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-EGF) (Barton et al., 1992). This strongly suggests that human PD-EGF is identical to human TP. PD-EGF is known to stimulate the growth and chemotaxis of endothelial cells in vivo and possesses angiogenic activity in vitro. It is likely that endothelial cells respond specifically to a modulation in intracellular DNA precursor pool brought about by thymidylate phosphorylase.

Five different crystal forms of recombinant human PD-EGF from yeast were produced from initial screening of crystal growth parameters using the 'magic fifty' set of conditions (Jancarik and Kim, 1991) and the hanging drop vapour diffusion technique (Hampel et al., 1988). One of the forms proved suitable for X-ray analysis. These crystals belong to the space group P2₁2₁2₁ with unit cell dimensions a=37.7 b=70.4 c=93.6 Å β=α=γ=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 XII of the Hamburg synchrotron source which is 84% complete at the 1σ level to 3.5 Å data spacings.

PS-03.11.16 CRYSTAL STRUCTURE ANALYSIS OF HUMAN DIPPYRROLE REDUCTASE INHIBITOR COMPLEXES. by Vivian Cady¹, Joseph R. Luft, Erik Jensen, Walter Pangborn, Andzej Wojtczak, Nikolai Galitsky, J. H. Freisheim¹ and K. Pakesley¹, Medical College of Ohio, Toledo, OH 43619, Medical College of Ohio, Toledo, OH 43669, 6 St. Jude Children's Research Hospital, Memphis, TN 38101.

Data from a methotrexate (Mtx)-resistant human cancer cell line reveal a natural F535 mutation in the enzyme dihydropyrimidine reductase (DHFR). Kinetic data for F51 mutants show greater effects on binding for folates than anticlastoles. To understand the structural basis for selectivity and specificity for binding to DHFR, and to determine the fundamental role of F51 in binding and catalysis, we have co-crystallized a series of anticlastoles with both human wild type and F51 mutant DHFR as binary and ternary complexes with the cofactor NADPH. We report structural data for isomorphous P3 crystal complexes with anti-