03-Crystallography of Biological Macromolecules

03.11 – Proteins of Medical Interest

PS-03.11.01 THE CRYSTAL STRUCTURE OF PORCINE PANCREATIC SPASMOLYTIC POLYPEPTIDE: A MEMBER OF A NOVEL FAMILY OF GROWTH FACTORS, by Anitha Dev, M. A. Guzman, D. Brown*, M. E. Sanderson, M. Carr* and M. Lane*, and P. S. Freemont, Protein Structure Laboratory, Imperial Cancer Research Fund, The Randall Institute, King's College, National Institute of Medical Research, London, U.K.

Porcine pancreatic spasmolytic polypeptide (PSP) belongs to a family of novel growth factor-like polypeptides which have been isolated from a variety of sources and includes spasmolyin (from Hoffmann, M., J. Biol. Chem., 1986, 261, 7684-7689) AFGF (from Machle, M. et al., FRG Lett., 1990, 260, 415-418), hSP (from Tommietto, C. et al., EMBJ J., 1990, 9, 407-417) and pSP (from Rio, M. et al., Science, 1988, 691, 705-708). PSP is produced in large amounts in the pancreas and has been shown to inhibit both intestinal motility and gastric acid secretion and to suppress involvement in the excruciating function of the pancreas, Jaegeren, F. D. et al., Regul. Peptides, 1982, 2, 231-241. Recent studies of the human analogue hSP suggest a role in the healing of damaged endothelial tissue, such as in gastro-oesophageal ulcers (Weight, N. A. et al., Nature, 1992, 360, 567-570). PSP (106 amino acids, 11.700 daltons) is a hormone and comprises seven disulfide bonds and two highly homologous domains, residues 8-47 and 58-96, which are approximately 50% identical (Thim, L. et al., Biochem. Biophys. Acta, 1985, 827, 410-418). Each domain has three disulfide bonds which give rise to the proposed three looped "triplet-like" motifs which is distinct from any other characterised growth factors (Thim, L. et al., FEBS Lett., 1989, 250, 85-90). These repeated domains are also seen in human hSP and a single domain is found in IGF (intestinal trefoil factor) from cat (81 residues) and human pSP (60 residues). Understanding the role of pSP in particular in the absence of 50% of heparin-dependent human breast tumour secreted pSP (Brown, A. et al., J. Biol. Chem., 1990, 265, 13807-13812) whereas in normal breast tissue these are not significant. The role of pSP in human breast cancer is still unknown.

In order to understand the molecular function of PSP, hSP and more importantly pSP, we have grown crystals of PSP suitable for high resolution structural analysis (Gozman, M. A. et al., Proc. Natl. Acad. Sci., 1992, 90, 11830-11834) and have now solved the structure by the single isomorphous replacement method combined with solvent modification. The preliminary model and protein fold was interpreted with the help of the recently determined NMR structure of one domain of PSP (Carr, M. D., Biochemistry, 1990, 29, 5198-5204) which proved vital in assigning unambiguous parts of the structure. The structure consists of two domains, as suggested by the sequence, which are related by an approximate two-fold axis. Each domain consists of 7 beta-strands followed by a short anti-parallel /-strand which gives rise to three expanded loop regions between secondary structure elements. The topology of PSP is novel and has not been observed in any other protein to date. The crystal structure of PSP will be presented and compared to the NMR solution structure and bioassays will be discussed in terms of the function of PSP as a growth factor.

PS-03.11.02 PRELIMINARY CRYSTALLOGRAPHIC STUDIES OF MOUSE LIVER GLUTATHIONE S-TRANSFERASE YbI. By G. Zhao, I. Haynes, R. Wolf and H. D. Dressen, Imperial Cancer Research Fund, Unit of Structural Molecular Biology, Synthecell, London WC1 7TH, UK

The glutathione S-transferases (GST) are a family of enzymes which play a critical role in cellular detoxification processes of mutagens, carcinogens, and other noxious chemical substances and are implicated in the development of drug resistance to electrophilic anticancer drugs, pesticides and herbicides. The enzymes catalyze the nucleophilic addition of the thiol group (SH) to a large number of structurally diverse electrophiles making them more polar. A second function for GST is that they serve as intracellular protein carriers, binding with high affinity a number of hydrophobic compounds such as drugs, dioxin and bile salts.

In order to understand the mechanism by which the enzymes bind GST and to establish structural relationships, we have crystallized mouse liver GST isozyme Yb1 (1a class) for three-dimensional structure analysis using single crystal X-ray diffraction methods. The enzyme has been crystallized by the vapour diffusion method in the presence of octyl-glutathione and diethyl-glyoxylisulfide using polyethylene glycol 6000. The crystals are monoclinic, space group P21, cell dimensions a=71.52, b=74.81, c=79.86 Å, α=β=90°, γ=90.0°, β=90.0° and diffract to at least 2.5 Å resolution on a rotating anode source. Two dimers in the asymmetric unit (Vmol=227) intersect around Z4 screw axes in the pseudo-orthorhombic space group P212121, while the intra- dimer two-fold axes are at an angle of 17.5° and 17.5° from the Z-axis.

An initial attempt to solve the structure of mouse Yb1 with the technique of molecular replacement using the 1a class (Rainwater, U., L. Landensteim, Schiffer, Galway and Huc, EMBL Journal, 1992) has failed. The sequence identity is only 32.9% in this case. The structure of mouse Yb1 has now been solved to 3.0 Å with molecular replacement using the coordinates of one human class of the rat Yb1 (1a class) structure (A. Zhang, Armstrong and Gilchrist, Biochemistry, 1992, 31, 1060-1064). Rat and mouse Yb1 have a sequence identity of 93.1%. Currently the mouse Yb1 structure has been refined to a resolution of 2.3 Å. Initial results show that all 4 GST molecules have one O3-5 binding is the G site (GST and its derivatives binding position). The O3-5 is well defined electron density and the O3-5 atom will be used to refine the crystal structure. The GST binding site is adjacent to the catalytic triad and the catalytic serine is conserved in all 4 GST molecules. The binding is M. A. Guzman, D. Brown, M. E. Sanderson, M. Carr, M. Lane, and P. S. Freemont, Protein Structure Laboratory, Imperial Cancer Research Fund, The Randall Institute, King's College, National Institute of Medical Research, London, U.K.

Detailed analysis has not yet been possible, as the refinement has not yet been completed. However, there appears to be a novel site, where the dimer has different interactions in the mouse and human GST. The dimer may be an interesting feature. The substrate binding sites in the sequence and the dimer site

PS-03.11.03 THE CRYSTAL STRUCTURE OF CHOLESTEROL OXIDASE COMPLEXED WITH A DEHYDROISOANDROSTERONE SUBSTRATE AT 1.9A RESOLUTION. By Jieqo Li*, Peter Brick & David Blow Imperial College of Science, Technology and Medicine, London, UK

The crystal structure of Cholesterol Oxidase from Brevibacterium Sterolicum complexed with a dehydroisoandrosterone substrate was determined by difference Fourier techniques at 1.9A resolution. The Kim measurements and the solubility was no more than 50%. The model refined up to R=14.5% by PROLSQ contains 3877 nonhydrogen protein atoms plus a PAP molecule, a sodium molecule and 435 waters. The structure of this complex with the active enzyme structure (Vandis, A., et al. 1991, J. Mol. Biol., 219, 533-544) shows some interesting features. The substrate binding site which is moved buried inside the protein molecule and was filled with an ordered lattice of 13 waters was now replaced by density for the
PS-03.11.04 X-RAY STRUCTURE ANALYSIS OF AKRIN-A FROM THE SLETS OF ABRUS PRECATORIUS. By Fahid H. Tahirev*, Tim- che-Liu, Department of Physics, National Tsing Hua University, Hsin-chu, Taiwan 300, China; Yen-Chyon Lin, Institute of Molecular Biology, Academia Sinica, Nankang Taipeh, Taiwan 11529, China and Jung-Tzu Lin, Institute of Biochemistry, College of Medicine, National Taiwan University, Taipei 10018, Taiwan, China.

Akrin-a is one of the four isomers isolated from the seeds of Abrus precatorius (Lin, J. Am. Chem. Soc. 1961, 53, 41-51). The x-ray diffraction analysis of the four isomers were studied, and we found that akrin-a showed the highest inhibitory effects on the protein biosynthesis of Sarcoma 180 tumor cells and on the growth of Sarcoma 180 tumor cells in mice. Toxin-like and antimicrobial activities are present in the x-ray structure of akrin-a. The x-ray structure of akrin-a in the form of a single crystal obtained by vapor diffusion method and belong to monoclinic space group P21, a = 84.5, b = 73.0, c = 48.2 Å, β = 96.2°. There is one molecule in an asymmetric unit. Unit cell dimensions and intensity data for native crystal and three heavy-atom derivatives were collected on the SDM-5 twin area detector system. The x-ray diffraction data were collected on a Rigaku D-Max-1000 diffractometer running at 50 kV and 80 mA. A total of 113,198 reflections were measured from native crystal at 2.7 Å, of which 27,140 were independent, giving a merging R factor of 0.063. A three-dimensional structure determination simultaneously by the MAD method and by the molecular replacement method using model of ricin (Baker et al., Bioc. 1987, 287, 5398-5403) homology 42% for A-chain and 59% for B-chain are in progress.

PS-03.11.05 MOLECULAR BASIS OF CELL ADHESION: CRYSTAL STRUCTURES OF A FIBRONECTIN FRAGMENT AND A MOLECULAR MIMIC FOR FIBRINOGEN. By K. R. Elby*, C. Dejksonin, B. Veerasapathan, C.-Z. Ni, and R. Kodandapanj. La Jolla Cancer Research Foundation, La Jolla, CA 92037 USA.

Cell surface receptors (integrins) mediate diverse cell-adhesion phenomena through recognition of an RGD sequence present in proteins such as fibronectin and fibrinogen. This recognition important in cell attachment and metastasis (fibronectin) and platelet aggregation and thrombosis (fibrinogen). Efforts to date to use NMR analyses to examine the RGD structure have been unsuccessful due to marked flexibility of a loop containing the tripeptide. We have studied two proteins to study the conformation of the RGD site. Both of these structures have been solved by molecular replacement methods: 1) the 10th Type I1 module (RGD) of fibronectin (space group P21, a = 30.7, b = 36.1, c = 37.9 Å, β = 107°, and 2) the OP-52 Fab fragment that contains an RYD sequence and functions as a molecular mimic for fibrinogen (space group P212121 with a = 93.1, b = 83.8 and c = 53.7 Å). The high-resolution structures of these proteins will be presented and the atomic configuration of the RGD (RYD) sequences will be compared.

PS-03.11.06 A NEW STRUCTURE TYPE OF INSULIN HEXAMER, T3R, BY Da-Cheng Wang*, Zong-Hao Zeng and Yang-Lin Hsu, Institute of Biophysics, Academia Sinica, Beijing 100080, China.

Some recent finding from X-ray structural analysis revealed that the hexamer of insulin displays the properties of an allosteric protein. So far, three conformational states designated as T3, T3R2, and K8 have been found in the crystal structures of 2 Zn insulin, 4 Zn insulin and phenol insulin respectively. Here we report a new structure type of insulin hexamer which was observed in the crystal structure of a protein-engineered mutant, A21-Ser human insulin, at 1.8 Å resolution and designated as T3R1. Compared with the R2 conformational state discovered in T3R2 and R2 the R1 structure is the new structural species possesses the following characteristics: (1) the first cycle of B-chain helix appeared in R1 is lost in T3; (2) the coordination pattern of zinc ion on the 3-fold axis adopts an octahedral array composed of six ligands (like a T type) including three Asn and three His: B10, other than the tetrahedral array composed of four ligands including one chloride ion or water molecule and three His: B10 in R structure; (3) the effector inducing the conformational change to K8 is a neutral organic molecule, I,4-dioxane, which binds to a pocket on the hexamer surface to trigger the conformational transition, other than the phenol or chloride ion.

The T3R1 seems to represent a new particularly stable intermediate in the T3 to R2 conformational transition and may provide a new insight into the insulin property as an allosteric protein. The detail characters of T3R1 structure and its significance will be presented and discussed.