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 Amitabha De, M. A. Gorman, D. Brown*, M. R. Sanderson*, M. Carr+, A. N. 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 spasmolysin (frog; Hoffmann, W., J. Biol. Chem., 1988, 263, 7686-7690). APEG (frog; Gmachl, M. *et al.*, FEBS Lett., 1990, 260, 145-148), hSP (man; Tomasetto, C. *et al.*, EMBO J., 1990, 9, 407-414) and pS2 (man; Rio, M. C. *et al.*, Science, 1988, 241, 705-708). PSP is produced in large amounts in the pancreas and has been shown to inhibit both intestinal motility and gastric acid secretion suggesting involvement in the exocrine function of the pancreas, Jorgensen, K. D. *et al.*, Regul. Peptides, 1982, 3, 231-243). Recent studies of the human analogue hSP suggests a role in the healing of damaged endodermally-derived tissue, such as in gastrointestinal ulcers (Wright, N. A. *et al.*, Nature 1990, 343, 82-85). PSP (106 amino acids, 11,700 daltons) is a monomer and comprises seven disulphides and two highly homologous domains, residues 8-47 and 58-96, which are approximately 50% identical (Thim, L. *et al.*, Biochim. Biophys. Acta, 1985, 827, 410-418). Each domain has three disulphide bonds which gives rise to the proposed three looped "trefoil-like" motif which is distinct from any other characterised growth factors (Thim,, L., FEBS Lett., 1989, 250, 85-90). These repeated domains are also seen in human hSP and a single domain is found in ITF (intestinal trefoil factor) from rat (81 residues) and human pS2 (60 residues). Understanding the role of pS2 is of particular importance since approximately 50% of oestrogen dependent human breast tumours secrete pS2 (Brown, A. M. C. *et al.*, P.N.A.S. 1984, 81, 6344-6348; Foekens, J. A. *et al.*, Cancer Res., 1990, 50, 3832-3837) whereas in normal breast tissue there is no significant expression of pS2 (Rio, M. C. *et al.*, Science, 1988, 241, 705-708). However, as yet the molecular role of pS2 is still unknown.

In order to understand the molecular function of PSP, hSP and more importantly pS2, we have grown crystals of PSP suitable for high resolution structural analysis (Gorman, M. A. *et al.*, J. Mol. Biol., 1992, 228, 991-994) 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, 1992, 31, 1998-2004) which proved vital in assigning unambiguous parts of the structure. The structure comprises two domains, as suggested by the sequence, which are related by an approximate two-fold axis. Each domain consists of a 7 residue α-helix followed by a short anti-parallel β-strand which gives rise to three exposed 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 both structures 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 Yb1. By Q. Zhao*, J. Hayes, R. Wolf and H.Driessen, Imperial Cancer Research Fund, Unit of Structural Molecular Biology, Birkbeck College, London WC1E 7HX, 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 catalyse the nucleophilic addition of the thiol group (-SH) of reduced glutathione (GSH) to a large number of structurally diverse electrophiles making them more polar. A second function for GST's is that they serve as intracellular carrier proteins, binding with high affinity a number of hydrophobic compounds such as heme, bilirubin and bile salts.

In order to understand the mechanism by which the enzymes bind GSH and to establish structure/function relationships, with an eye on selective inhibition of GSTs in anticancer chemotherapy, we have crystallised mouse liver GST isoenzyme Yb1 (μ 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 octyl-glucopyranoside using polyethylene glycol 6000. The crystals are monoclinic, space group $P2_1$, cell dimensions a=79.52 Å, b=74.81 Å, c=78.86 Å, $\alpha = \gamma = 90.0°$, ß=90.07° and diffract to at least 2.5 Å resolution on a rotating anode source. Two dimers in the asymmetric unit (V_m=2.27) interact around 2_1 screw axes in the pseudo-orthorhombic space group $P2_12_12_1$, while the intra-dimer two-fold axes are at an angle of 12.5° and 77.5° from the b-axis in the b-c plane.

An initial attempt to solve the structure of mouse Yb1 with the technique of molecular replacement using the pig π coordinates (Reinemer, Dirr, Ladenstein, Schäffer, Gallay and Huber, EMBO Journal, 10, 1997-2005, 1991) has failed. The sequence identity is only 32.9% in this case. The structure of mouse Yb1 has now been solved to 3.0 Åwith molecular replacement using the coordinates of one homo-dimer of the rat Yb1 (μ class) structure (Ji, Zhang, Armstrong and Gilliland, Biochemistry 31, 10169-10184, 1992). Rat and mouse Yb1 have a sequence identity of 93.1 %. Currently the mouse YB1 structure has been refined to a residual of 21.3% at 2.8Å. Initial results show that all 4 GST molecules have octyl-GSH bound in the G-site (GSH and its derivatives binding position). The octyl-tails have well-defined electron density and have very similar conformations. The octyl-tail bends away from Tyr (100)6, and from the putative H-site (Hydrophobic compounds binding site), and stretches across the cleft to within a distance of 4 Å from side chains on helix _4. The direction of the bending is completely different from that of the hexyl-tail in human .

Detailed analysis has not yet been possible, as the refinement has not been completed. However, there appear to several areas, where the dimer pairs have different interactions on the surface. These areas do not appear to correlate with the residues where rat and mouse differ and there are clear differences between the positions of the octyl-tail in GSH. Further work is under way to study in more details the substrate binding sites of the enzyme.

PS–03.11.03 THE CRYSTAL STRUCTURE OF CHOLESTEROL OXIDASE COMPLEXED WITH A DEHYDROISOANDROSTERONE SUBSTRATE AT 1.9A RESOLUTION by Jiayao 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 Km measurements and the solubility was no more than 50%. The model refined up to R=14.5% by PROLSQ contains 3877 nonhydrogen protein atom plus a FAD molecule, a steroid molecule and 435 waters.
Comparison of this structure with the native enzyme structure (Vrielink, A., et.al. 1991, J. Mol. Biol. 219, 533–554) shows some interesting features. The substrate binding site which is deeply buried inside the protein molecule and was filled with an ordered lattice of 13 waters is now replaced by density for the

steroid. The density for four rings and two methyl groups of the substrate is clear in the difference map. The steroid is surrounded by hydrophobic protein residues on all sides and makes no contact with external solvent. There is only one water molecule left in the active site. It bridges N5 of the FAD(3.04A), NE2 of His447(3.14A), OD1 of Asn485(2.88A), OE1 of Glu361(2.99–3.29A, because of flexibility) and O1 of the substrate(3.05A) with strong hydrogen bonding interactions to FAD, the protein and the substrate. A loop formed by residues from 72 to 86 constitutes of one wall of the active site, and is the only region to show a major protein conformational change during the substrate binding. It suggests that this is the entrance for the substrate to the enclosed binding site.
some hints have been received regarding the oxidation reaction, the reducability of the FAD, the activation and reoxidation of the enzyme from this substrate structure and related experiments, all of which lead to this protein crystallographic results. With other substrate binding structures and product complex structures which are in progress, combined with other information about related structures and biological research, would reveal that how does this enzyme work.

PS–03.11.04 X-RAY STRUCTURE ANALYSIS OF ABRIN-A FROM THE SEEDS OF ABRUS PRECATORIUS. By Tahir H. Tahirov*, Tian-Huey Lu, Department of Physics, National Tsing Hua University, Hsinchu, Taiwan 300, China; Yen-Chywan Liaw, Institute of Molecular Biology, Academia Sinica, Nankand Taipei, Taiwan 11529, China and Jung-Yaw Lin, Institute of Biochemistry, College of Medicine, National Taiwan University, Taipei 10018, Taiwan, China.

Abrin-a is one of the four isoabrins isolated from the seeds of Abrus precatorius (Lin, Lee, Hu & Tung, Toxicon 1981, 19, 41-51). The antitumor activity of the four isoabrins were studied, and was found that abrin-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 experimented animals (Lin, Lee & Tung, Cancer Res., 1982, 42, 276-279). The toxic protein consists of two subunits, the A and B chains containing 250 and 267 amino acids each, are linked by a disulfide bond. The crystals of abrin-a in the form of rhombic prisms were obtained by vapor diffusion method and belong to monoclinic system, space group $P2_1$, a=84.58, b=73.07, c=48.23 Å, β=96.20°. There is one molecule in an asymmetric unit. Unit cell dimensions and intensity data from native crystal and three heavy-atom derivatives [$(CH_3COO)_2Hg$, $HgCl_2$ and $DyCl_3 \cdot 6H_2O$] with appoximately equal dimensions 0.35x0.40 x0.25 mm were collected on the SDMS twin area detector system (Hamlin, Methods Enzymol., 1985, 114, 452). The X-rays were from a Rigaku RU-300 generator running at 50 kV and 80 mA. A total of 113,198 reflections were measured from native crystal at 2.2 Å, of which 27,470 were independent, giving the merging R factor 0.063. A three dimensional structure determination simultaneously by the MIR method and by the molecular replacement method using model of ricin (Monfort et al., J. Biol. Chem., 1987, 262, 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. Ely*, C. Dickinson, B. Veerapandian, C.-Z. Ni, and R. Kodandapani, 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 is 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 crystallized 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 III module (RGD) of fibronectin (space group $P2_1$ with a = 30.7, b = 35.1, c = 37.7 Å, β = 107°; and 2) the OP-G2 Fab fragment that contains an RYD sequence and functions as a molecular mimic for fibrinogen (space group $P2_12_12$ 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, $T_3R_3^t$ By Da-Cheng Wang*, Zong-Hao Zeng and Yong-Lin Hu, Institute of Biophysics, Academia Sinica, Beijing 100101, China.

Some recent finding from X-ray structural analysis revealed that the hexameric zinc insulin displays the properties of an allosteric protein. So for three conformational states designated as T_6, T_3R_3, and R_6 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 $T_3R_3^t$. Compared with the R_3 conformational state discovered in T_3R_3 and R_6, the R_3^t structure in the new structural species possesses the following characteristics: (1) the first cycle of B-chain helix appeared in R_3^t is lost to a β-turn-like conformation; (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-B3 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 changes to R_3^t is a neutral organic molecule, 1,4-dioxane, which binds to a pocket on the hexamer surface to trigger the conformational transition, other than the phenol or chloride ion.

The $T_3R_3^t$ seems to represent a new particularly stable intermediate in the T_6 to R_6 conformational transition and may provide a new insight into the insulin property as an allosteric protein. The detail characteristics of $T_3R_3^t$ structure and its significance will be presented and discussed.