05-Molecular Modelling and Design for Proteins and Drugs

binding (lectin) characteristics of this particular pentraxin. Significantly the structure reveals an arrangement of beta strands very similar to the subunit fold of the legume lectins Concanavalin A and pea lectin.

Possible clinical applications and rational drug design:

Amyloidosis is defined as a group of biochemically diverse conditions in which normally innocuous soluble proteins polymerize to form insoluble fibrils. This growing mass of amyloid fibrils is universally bound by SAP to form amyloid deposits which persist and invade the extracellular spaces of organs destroying normal tissue architecture and function. One of our aims is to try and disperse these deposits by designing drugs to remove bound SAP allowing normal in vivo clearance mechanisms to act upon the fibrils. Further possiblities are to use the specificity of SAP in designing magic bullets. This idea is well founded in the use of radiolabelled SAP as the only means of imaging amyloid in vivo using whole body scans.

DS–05.03.04 A STRUCTURAL COMPARISON OF BACTERIAL AND VIRAL NEURAMINIDASES G.L. Taylor*, S.J. Crennell, , E.F. Garman§, W.G. Laver† and E. Vimr‡, Department of Biochemistry, University of Bath, Claverton Down, Bath, BA2 7AY, U.K.

§Laboratory of Molecular Biophysics, University of Oxford, Oxford, OX1 3QU, U.K.

† The John Curtin School of Medical Research, P.O. Box 334, Canberra, Act 2601, Australia.

‡College of Veterinary Medicine, University of Illinois, Urbana, IL 61801, U.S.A.

We have determined the structure of *S. Typhimurium* LT2 neuraminidase/sialidase using MIR techniques to 1.6Å, and have obtained inhibitor complexes to 1.6Åand 2.2Å. This is the first bacterial sialidase whose structure has been determined, and despite only 16% sequence homology shares the same fold as the influenza virus enzyme. The viral enzyme requires Ca^{2+} for optimal activity; the bacterial enzyme does not. The viral enzyme possesses at least 8 conserved disulphides; the bacterial only one. The fold of six four-stranded antiparallel β-sheets is very similar, but the strand and loop lengths vary considerably. The active site shares features with the viral enzyme: an arginine triad, a hydrophobic pocket, and a tyrosine which most probably stabilizes a carbonium ion intermediate in the catalysis similar to β-galactosidase. There are differences, however, which explain the differential binding of various substituted sialic acids. The structure provides valuable information for those designing inhibitors targeted at this enzyme against various pathogens.

DS–05.03.05 STRUCTURAL, FUNCTIONAL AND EVOLUTIONARY IMPLICATIONS OF THE THREE-DIMENSIONAL CRYSTAL STRUCTURE OF MURINE INTERFERON-β. By Y.Mitsui* and T.Senda, Nagaoka University of Technology, Nagaoka, Niigata, 940-21, Japan

Interferons (IFN) are proteins showing antiviral, antitumor and immunomodulator activities with significant therapeutic value for Type C hepatitis and other diseases. The IFN's have been classified into two categories on the basis of their biological and physical properties. Type I IFN's include fibroblast interferon (IFN-β) and the leukocyte family of interferons (IFN-α) which is composed of at least 10 subspecies. Each member of Type I IFN's contain ~165 amino acid residues exhibiting considerable sequence homology to each other, and competes for the same receptors. In contrast, Type II IFN (IFN-γ) is produced in response to mitogens and antigenic stimuli, contains ~146 amino acid residues exhibiting no significant sequence homology to Type I IFN's and displays no measurable binding to Type I interferon receptors.

The first and still the only three-dimensional structure of Type I IFN, the crystal structure of recombinant murine interferon-β, was elucidated by T.Senda *et al.* (*Proc.Japan Acad.*, 1990, **66B**, 77 - 80; *The EMBO J.*, 1992,**11**, 3193 - 3201). It appears to represent the basic structural framework of all Type I IFN's including IFN-β and all subtypes of IFN-α of various mammalian origin. The huge accumulated data on the structure activity relationship of Type I IFN's using various chemical and genetical techniques have been systematically evaluated in terms of the three-dimensional structure. Several intriguing observations have also been made through 1) structural comparison with other cytokines, for which three-dimensional structure has been established, including interferon-γ (Ealick,S.E. *et al.*, *Science*, 1991, **252**, 698 - 702; Samudzi,C.T. *et al.*, *J. Biol. Chem.*, 1991, **266**, 21791 - 21797), and 2) considerations on evolution of cytokines and cytokine receptors (Mitsui,Y., Senda,T., Shimazu,T., Matsuda,S. and Utsumi,J., *Pharmacology and Therapeutics*, 1993, in press; also see the poster presented by T.Senda and Y.Mitsui). Some of the results follow. a) Basic structural framework of Type I IFN is a four-α-helix bundle with 2 overhand inter-helical connections as in the case of growth hormone, GM-CSF and (the revised structure of) IL-2. b) Type II IFN (IFN-γ) exhibits only a topological (if any) structural similarity with Type I IFN's (IFN-α and IFN-β). c) Functionary important sites on the Type I IFN molecules are located on the two separate polypeptide chain segments which, however, form an apparently one contiguous receptor-binding site. d) Comparisons between human and murine amino-acid sequences for various cytokines and their cognate receptor proteins show that 1) the speed of their evolutionary variation is generally much faster than in other proteins (*e.g.* enzymes) and 2) amino acid substitution rate of cytokines correlates with that of their cognate receptors. "Coupled evolution" shared by a cytokine and its cognate receptor protein(s) may well be an explanation for this phenomenon.

DS–05.03.06
STRUCTURAL IMMUNOLOGY OF MOUSE MHC CLASS I
Ian A. Wilson, David H. Fremont, Masazumi Matsumura, Enrico A. Stura and Per A. Peterson, Depts. of Molecular Biology and Immunology, The Scripps Research Institute, 10666 N. Torrey Pines Road, La Jolla, CA 92037 USA

Structures of murine MHC class I H-2K^b have been determined in complex with three different peptide antigens at 2.3 - 2.5Å resolution. The structures reveal, when compared with the corresponding human HLA structures of B27, Aw68.1 and A-2 from the Wiley laboratory, a general mechanism for peptide binding. The peptides are embedded deeply within the 25Å binding groove and specific hydrogen bonds to the peptide backbone as well as the amino carboxyl terminal provide specificity for peptides of 8 to 9 residues in length. This mode of binding explains the higher affinity but low sequence specificity of the MHC interaction. Comparison of the three H-2K^b peptide complexes has revealed small but significant changes in the MHC structure itself which may affect T-cell recognition. Such sequence changes are synergistic in as much as different peptide sequences can cause different changes in the MHC so that the information content of the peptide is enhanced. The question of whether the empty MHC class molecule has the same conformation as in the peptide complex is being addressed.

REFERENCES:

1. Fremont, D.H., Stura, E.A., Matsumura, M., Peterson, P.A. and Wilson, I.A. (1992) Crystal Structures of Two Viral Peptides in Complex with Murine MHC Class I H-2K^b. Science 257: 919-926.

2. Matsumura, M., Fremont, D.H., Peterson, P.A. and Wilson, I.A. (1992) Emerging Principles for the Recognition of Peptide Antigens by MHC Class I Molecules. Science 257: 927-934.

PS-05.03.07

THE MODEL OF SUBSTRATE INTERACTION WITH α - MOMORCHARIN BY COMPUTER GRAPHIC SIMULATION Yi-Cheng Dong[1], Ricky Ngok-Shun Wong[2], Pang-Chui Shaw[3], Hei-Wun Leung[4] and Hin- Wing Yeung[3]

[1] Institute of Biophysics. Academia Sinica. Beijing 100101. China

[2] Department of Biology and [4]Chemistry. Hong Kong Baptist College. Kowloon. Hong Kong

[3] Department of Biochemistry. Chinese University of Hong Kong. Shatin , N. T. . Hong Kong

The mechanism of N-glycosidase activity of ribosome- inactivating proteins (RIPs) was studied through the model of substrate interaction by using computer graphic simulation. The crystal structure of α -momorcharin(α -MMC) at 2 A resolution was used as a model of RIP. The natural substrate of α -momorcharin is the 28S rRNA. The target site is the N- glycosidic bond at A4324 of the 28S rRNA which is a highly conserved region near the 3'-end of the rRNA. A tetraribonucleotide GAGA representing the conserved sequence at the site of cleavage was chosen as the substrate for computer graphic simulation. Fitting the substrate GAGA into the putative active site of α -MMC revealed several interesting observations which helps to explain the possible mechanism of its N- glycosidase activity. Firstly, the second nucleotide (adenosine) , which is the target for RIP protrudes out from the rest of the molecule and this facilitates the enzyme- substrate interaction. Secondly, the adenine at the active site was found to adopt the *sync* onformation. In fact, the glycosidic torsion angle of adenine was best described as the "high anti" which was intermediate between the classic *syn* and *anti* position. The binding of adenine in this sterically unfavoured conformation would place strain on the glycosidic bond. Furthermore, the active site residues of α -MMC such as E160 and R163 are in close proximity to the bond of cleavage. According to the model, the NH1 of R163 is hydrogen bonded to N9 of the adenine ring. This interaction helps to withdraw electrons from the imidazole ring and further weakens the glycosidic bond. The C1 on the ribose ring would become slightly electropositive owing to the electron withdrawing effect of N9 as well as the ribose oxygen. The OE1 of E160 is available for nucleophilic attack on C1 leading to bond cleavage. Hydrolysis of the intermediate regenerates the active site. This model helps to explain the alteration in activity of some mutants in homologous RIPs such as trichosanthin and ricin A. Besides catalysis, substrate specificity can also be interpreted from the interaction between the RIP and the sugar-phosphate backbone of the substrate .

PS-05.03.08 THE STRUCTURE OF DES-F1-MEIZO-THROMBIN: THE NATURE AND LOCATION OF KRINGLE-THROMBIN AND KRINGLE-KRINGLE INTERACTIONS. By Philip D. Martin*, Michael G. Malkowski, Charles T. Esmon, and Brian F. P. Edwards, Wayne State University School of Medicine, Detroit, Michigan 48201 USA

We have solved the structure of bovine des-F1-meizothrombin inhibited with D-FPR-chloromethyl ketone at 2.8 Å resolution by molecular replacement and difference Fourier techniques. The space group is $P4_12_12$, a=186.5 Å, c=120.3Å, with a dimer in the asymmetric unit. A monomer consists of prothrombin residues 156 to 579 (prothrombin numbering system) with a covalent break at Arg-320 (15/16 in the chymotrypsin numbering system) separating the A and B chains of thrombin. The current model, which lacks the first 14 amino terminal residues of the kringle 2 domain and a 35 residue linker chain connecting kringle 2 to the thrombin A chain, has a standard crystallographic R-value of 0.28 with RMS deviations of 0.022 Å from ideal on bond lengths and 3.6° on ω angles. The active site of the thrombin B-chain is inhibited by D-FPR which is covalently bound in the active site to both His-363 (His-57) and Ser-525 (Ser-195). The dimer has crystallized in a manner that gives significant information on the interactions of the different domains of the structure. There are two distinct interactions of kringle 2 with thrombin. The first is a primary ionic interaction through a cluster of kringle glutamic and aspartic acids centered at Asp residue 225 which form ionic bonds with thrombin Arg residues 418 and 500 (but are not limited to these residues alone). A second interaction is seen in which the kringle domain related by local symmetry blocks the entrence to the thrombin active site *via* two stretches of chain (182 to 188 and 243 to 247). There is also a kringle-kringle interaction directly across the local two-fold which is characterized by imidazole ring stacking between residues His 187 from each kringle and hydrogen bonds from these residues to the carbonyl oxygens of Glu-241 across the dimer interface. Completely unique to this structure is the presence of density for the carbohydrate at the Asn-373 attachment site. Previously reported structures of both bovine and human thrombins, and these thrombins complexed with hirudin, have reported that there is no traceable density for the sugar at this position. This work is supported in part by grant GM33192 and the Molecular Biology Center at Wayne State University.

PS-05.03.09 DESIGN AND PROPERTIES OF INHIBITORS OF INFLUENZA VIRUS NEURAMINIDASE. P.M.Colman*[1] and J.N.Varghese[1], Mark von Itzstein[2], and C.R. Penn[3]. [1]Biomolecular Research Institute, 343 Royal Parade, Parkville, Victoria 3052, Australia, [2]Victorian College of Pharmacy Ltd., 381 Royal Parade, Parkville, Victoria 3052, Australia, [3]Glaxo Group Research Ltd, Department of Virology, Greenford Road, Greenford, Middlesex, BU6 OHE, UK.