05-Molecular Modelling and Design for Proteins and Drugs

05.03 – Proteins of Medical Interest and their Interactions with Drugs

DS–05.03.01 THE STRUCTURE OF HUMAN PURINE NUCLEOSIDE PHOSPHORYLASE AND ITS USE IN INHIBITOR DESIGN.
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Purine nucleoside phosphorylase (PNP) catalyzes the reversible phosphorolysis of purine-ribo- and 2'-deoxyribonucleosides to the base and ribose or 2'-deoxyribose-1-phosphate. Since the enzyme is required for T-cell immunity, but not for B-cell functions, inhibitors of PNP have potential as T-cell specific immunosuppressives. In addition, inhibitors might be used to prevent the degradation of chemotherapeutic nucleoside analogs that serve as substrates for the enzyme. The structure of the native enzyme and the guanine complex were refined to 2.7Å resolution. Approximately 30 complexes of PNP with substrates, substrate-analogs and inhibitors were examined by difference Fourier methods. The analysis was facilitated by the high solvent content (approximately 80%) of the crystals, which are enzymatically active. Inhibitors were developed by an iterative process that involved crystallographic analysis of lead compounds bound to the enzyme, molecular modeling studies, organic synthesis, and measurement of inhibition constants. This approach led to the development of several different types of potent inhibitors with IC_{50} values in the 6-30nM range. The inhibition is competitive with respect to inosine. Selected inhibitors have been shown to effectively inhibit PNP in whole cells and in animals, and to reduce the catabolic cleavage of 2', 3'-dideoxyinosine and other purine nucleosides. Clinical trials are in progress to test the usefulness of these inhibitors for treatment of T-cell mediated diseases.

DS–05.03.02
QUATERNARY LIGAND BINDING SITES OF ACETYLCHOLINESTERASE AS REVEALED BY X-RAY CRYSTALLOGRAPHY
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The primary role of acetylcholinesterase (AChE) is the termination of impulse transmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter acetylcholine (ACh). Symptomatic treatment of diseases whose etiology involves depletion of ACh levels can be achieved by controlled inhibition of AChE. Anticholinesterase agents are thus of therapeutic importance in countering the effects of acute glaucoma and myasthenia gravis and are under active consideration for the management of Alzheimers' disease. Kinetic and physicochemical analyses show that the active site of AChE comprises an esteratic subsite, containing the catalytic machinery, and an 'anionic' subsite, assumed to contain several negative charges, which binds the quaternary group of the neurotransmitter ACh. Inspection of AChE 3-D structure (Sussman, J.L. et al., Science 1991, 252, 872-879) shows that it contains a catalytic triad, similar to that of other serine hydrolases; this triad is located at the bottom of a deep and narrow cavity, which was named the 'aromatic gorge' since 40% of its lining is composed of the rings of 14 highly conserved aromatic amino acids. Three X-ray structures of AChE-ligand complexes, refined at 2.8Å, show that the primary 'anionic' attachment site for the quaternary group of two cholinergic drugs and of a bis-quaternary inhibitor is, in fact, W84. Furthermore, both crystallography and photoaffinity labelling implicate an additional aromatic residue, F330, in the AChE binding site and suggest a distal binding site, W279, at the entrance gorge, which binds the second quaternary group of the bis-quaternary inhibitor, as the putative 'peripheral' binding site of AChE.

DS–05.03.03 THE THREE DIMENSIONAL STRUCTURE OF HUMAN SERUM AMYLOID P-COMPONENT DEFINED AT 2.0Å REVEALS A LECTIN LIKE FOLD AND CALCIUM MEDIATED LIGAND BINDING.
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Serum amyloid P component (SAP) is a glycoprotein composed of ten identical 25kDa subunits arranged as two cyclic pentamers stacked face to face. It is a member of the small family of plasma proteins called the pentraxins. This group includes C-reactive protein (CRP) and even has a homologue in the Horseshoe crab, indicating stable conservation throughout evolution and an important role in physiology. Human SAP is the precursor of amyloid P, the only non-fibrillar component of amyloid deposits in vivo. This is a direct relationship whereby SAP leaves the circulation and decorates amyloid fibrils. Such deposits are known to be associated with conditions such as Alzheimer's disease, Gerstmann-Strassler syndrome and systematic amyloidosis.

Structure determination of SAP: SAP crystallises in the spacegroup $P2_1$ with cell dimensions a=68.9Å b=99.3Å c=96.7Å and β=95.9°. In vivo it is known to exist as two pentamers aggregated into a decamer. The self rotation function studies seemed to confirm this showing a five fold axis direction with a two fold perpendicular to it. However a decamer in the asymmetric unit would have required an unusually low crystal solvent content. Furthermore neutron scattering studies on solutions of SAP showed the predominance of pentamers in the conditions of low pH and high calcium ion concentration that makes up the crystallisation buffer. The initial model of SAP was based on structure determination at 2.8Å resolution using the multiple isomorphous replacement (MIR) technique followed by solvent flattening and five fold molecular averaging. The two key derivatives were prepared from novel heavy atom compounds thorium (IV) nitrate and cerium (IV) sulphate. Close clustering of sites in these derivatives proved to be a problem which was only solved at the atomic level using difference Fouriers when phasing to sufficient resolution became available from other derivatives.

The SAP structure: The tertiary fold of the subunit is dominated by antiparallel beta sheets which form a flattened β-barrel with a jellyroll topolgy and a core of hydrophobic side chains. On one side of the jellyroll there is a short helix which sits above the disulphide bridge Cys 36 - Cys 95, adjacent to this is the glcosylation site Asn 32. On the other side is the calcium binding site which uniquely shows two loops involved in co-ordinating two calcium ions which are 4Å apart. SAP and CRP are characterised by a number of distinct calcium dependant binding reactions including the closely related ligands of phospho-ethanolamine and phospho-choline respectively. SAP also displays a number of extra reactivities which includes binding specifically to DNA, amyloid fibrils and agarose. The latter two affinities are likely to be related by the calcium dependant sugar

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.

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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
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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.