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 immunosuppressors. 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 gene encoding it were determined to 2.8 Å 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 IC50 values in the 6-30µM range. The inhibitor 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'-dideoxynucleosine 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 ACETYLCOLINESTERASE 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 used under clinical consideration for the management of Alzheimer's disease. Kinetic and physicochemical analyses show that the active site of AChE comprises an ionic subsite, containing the catalytic machinery, and an 'amionic' subsite, assumed to contain several negative charges, which binds the quaternary group of the neurotransmitter ACh. Inspection of AChE 3D structure (Sasson, J.L., et al., Science 1993, 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 'amionic' 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 'amionic' attachment site for the quaternary group of two cholinergic drugs and of a bis-quaternary inhibitor is, in fact, W34. Furthermore, both crystalline tryptophan and photoaffinity labelling implicate an additional aromatic residue, F370, 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 positive 'peripheral' binding site of AChE.


Serum amyloid P component (SAP) is a glycoprotein composed of ten identical 28kDa 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 systemic amyloidosis.

Structure determination of SAP: SAP crystallises in the spacegroup P21 with cell dimensions a=86.9Å b=99.3Å c=96.7Å and β=95.9°. In vivo it is known to exist as two pentamers aggregated into a dimer. The self rotation function studies seemed to confirm this showing a 5 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 neutrons scattering studies on solutions of SAP showed the predominance of pentamers in the conditions of pH and high calcium ion concentration which makes up the crystallisation buffer. This work was performed using the MULTAN program to determine the structure of 2.6 Å 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 surmounted at the atomic level using different Fourier 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 topology and a core of hydrophobic side chains. On one side of the barrel there is a short helix which sits above the disulphide bridge Cys 36 - Cys 95, adjacent to this is the glycosylation site Asn 52. On the other side is the calcium binding site which uniquely shows two loops involved in co-coordinating two calcium ions which are 4 Å apart. SAP and CRP are characterised by a number of distinct calcium dependent 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 agomere. The latter two affinities are likely to be related to the calcium dependent sugar