

## 05-Molecular Modelling and Design for Proteins and Drugs

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### 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. C.E. Bugg<sup>\*1</sup>, S.E. Ealick<sup>2</sup>, J.A. Montgomery<sup>3</sup>, J.A. Secrist, III<sup>3</sup>, Y.S. Babu<sup>4</sup>, M.D. Erion<sup>5</sup>, and W.C. Guida<sup>5</sup>. <sup>1</sup>Center for Macromolecular Crystallography, University of Alabama at Birmingham, Birmingham, AL 35294-0005; <sup>2</sup>Dept. of Biochemistry, Cornell University, Ithaca, NY 14853; <sup>3</sup>Southern Research Institute, Birmingham, AL 35255; <sup>4</sup>BioCryst Pharmaceuticals, Inc., 2190 Parkway Lake Dr., Birmingham, AL 35244; <sup>5</sup>Ciba-Geigy Corporation, Summit, NJ 37901.

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<sub>50</sub> 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

M. Harel<sup>1</sup>, I. Silman<sup>2</sup> and J.L. Sussman<sup>1</sup>, Department of Structural Biology<sup>1</sup>, Department of Neurobiology<sup>2</sup>, The Weizmann Institute of Science, Rehovot 76100, Israel.

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. By J. Emsley\*, H. E. White, G. O. Oliva, B. P. O'Hara, S. P. Wood, I. J. Tickle, M. B. Pepys and T.L. Blundell, Department of Crystallography, Birkbeck College, London, Great Britain.

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<sub>1</sub> 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 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 jellyroll there is a short helix which sits above the disulphide bridge Cys 36 - Cys 95, adjacent to this is the glycosylation 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