The binding of over 50 inhibitors to T state 02g in the crystal has been studied to 2.3 Å resolution and the structures refined to R values less than 0.20. One year ago, however, we were unable to obtain crystals of the 02g type, which are needed for a conformational change to the 02g state and subsequent loss of some hydrogen bonding to O2 and O3 of the sugar moiety. A very encouraging result has been the recent success of N-acetyl-β-glucosamine leading to K_i of 0.023 M. It is postulated, that the presence of the amide portion of the β-Cl substitution has led in to more favourable electrostatic interactions between the ligand and the protein. Further modelling studies and syntheses are in progress with this compound as our new lead.

We are starting to use data base analysis to identify particular probe sites for favourable binding and to search for compounds of known structure that have the required conformation.

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MOPA is the first synthetic thrombin inhibitor which has been clinically used in Japan as an antithrombotic drug since 1990. We reported its unusual binding motif to trypsin (Matsuoka, T. et al., 1992, J. Biochem., 110: 969-952). Despite of the similar chemical structure to that of PTI, MOPA does not utilize the oxyanion hole for binding, instead, it forms antiparallel A type hydrogen bonds with Gly216 of trypsin. The binding structures of 19 enzyme-inhibitor complexes including a new monoclinic crystal form of h-α-thrombin, have been determined to elucidate the details of the binding mechanisms. The resolution is 1.8-1.5 Å and R factors range 17-22 %. The results are: (1) The MOPA's unique binding motif is due to its carbonyl group but to the molecular conformation, which places restrictions on the enzyme-inhibitor interaction. (2) The decrease of inhibitory activity of (2S,4S)MOPA isomer is not due to a change in the binding structure but to the lack of surface complementarity. (3) An antipancreatitis drug, Nafamostat, also assumes an unexpected binding structure, where the less basic amidonaphthalene group goes into the specificity pocket, while the more basic guanidinozinc group lies near His97. These findings will be useful for the design of second generation drugs.

Arhythmias are a major cause of sudden cardiac death. Several types of drugs, which modulate the function of the various ion channels involved in heart muscle contraction, have been studied as potential treatments for arrhythmias. While Class I drugs, based on local anaesthetics, have some beneficial effects through blocking sodium channels, they have recently been shown to be proarrhythmic. In contrast, Class III drugs, which prolong action potential duration by blocking potassium ion channels are more promising, including anilide and the acetylated derivative of a class I agent, N-acetylprocainamide (NAPA), which has weak class III activity. Morgan, et al 4 have reported a series of compounds which contain an imidazole ring in place of the amide sulfonamide present in anilide or the amide group present in NAPA. To compare the Class III antiarrhythmics, we sought to explain how these three groups could replace one another at a common binding site. Using the Cambridge Crystallographic Database and results from our crystal structure determinations of NAPA and three imidazole derivatives we have determined a common interaction pattern for the three moieties. Using molecular modeling calculations (MACROMODEL), we can explain the reduced activity of NAPA and the inactivity of imidazole derivatives with bulky substituents on the amine N atom. Taken together these studies provide a beginning model for the potassium channel recognition site for Class III antiarrhythmics.

Sulfonamide drugs, being extremely potent inhibitors of Human Carbonic Anhydrase
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isoenzymes, are widely used in the treatment of several types of physiological disorders. For example, clotting factors for valeric safinamides, namely, acetazolamide, methazolamide and clauslul complexed to human carbonic anhydrase I (HCAI) enzyme were collected using both conventional X-ray generators with photographic film and Photon Factory synchrotron sources with image plates. Starting from the refined phases of the native HCAI model without the solvent molecules, the structures of the three complexes were refined at 2A resolution to R-factors of 0.179, 0.186 and 0.192 respectively using molecular dynamics / simulated annealing method of XPLOR. These refined structures show differences in the orientations of the sulfamido group of the drug molecules bound to the active zinc ion in the active site of the enzyme. The aromatic ring of these molecules is always found to be sandwiched between the active site ligand residues Leu198 and His202 resulting thereby in large shifts of the imidazole ring and indicating thereby the importance of this loop in the catalytic activity / inhibition of this enzyme. Since differences in the orientation of the sulfamido group indicate corresponding differences in the inhibitory power of these drugs, correlation of inhibition constants with the structures was investigated on the basis of computed energetics of sulfamido-HCAI interactions.

From the force fields used for refinement above, the various energy terms were estimated for the refined structures of the drug complexes. A series of twenty different chlorophene/benzene based sulfonamide drug molecules with known inhibition constant for HCAI were docked into the active site of HCAI using TOM FRDDO package on IRIS 4D/20 graphics workstation after anchoring the sulfamido and the aromatic groups approximately around the position found from the three refined structures above. These starting models were energy minimized using different trial force fields in XPLOR. The set of parameters which resulted in models of the drug molecules having minimum differences with the common parts of the refined structures was accepted as the correct one for the corresponding model complex. Various energy terms were computed again from these reparametrized force fields for each of the model complexes and variations of these terms plotted against the nature of the substituents on the aromatic rings. These were then correlated with the known inhibition constants of the corresponding drug molecules, details of which will be discussed.

Successful Rational Selective Inhibition of a Trypanosomal Enzyme

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The bloodstream form of the trypanosome, the causative agent of sleeping sickness, relies upon glycogenolysis to the stage of pyruvate as its sole source of energy supply. Therefore, the trypanosomal glyceraldehyde phosphate dehydrogenase (GAPDH) and inhibitor design.

Careful comparison of the Trypanosoma cruzi (T.B.) GAPDH structure, obtained from Laue diffraction study at 3.2 A, and a 2.4 A resolution human GAPDH structure reveals: (1) the presence of a small hydrophilic pocket next to the adenine C2-position in T.B. GAPDH versus the absence of Arg in human GAPDH; (2) the presence of a hydrophobic region next to O2 of the adenine base in T.B. GAPDH versus this region being occupied by protein atoms in human GAPDH due to a different loop conformation; (3) the presence of a hydrophobic patch near the adenine C9-position in both T.B. and human GAPDH.

After one design-synthesis-testing cycle starting from adenosine the following preliminary results were obtained: (1) a 2-methyl adenosine salt improves inhibition 43-fold in T.B. and 16-fold in human GAPDH; (2) 2'-benzamide-2'-deoxy-adenosine selectively inhibits parasite GAPDH and shows a 16-fold better inhibition; (3) an 8-thionyl substituted benzene inhibits 100-fold for parasite GAPDH and 20-fold for mammalian GAPDH. More recently, a meta-nitrobenzamide derivative of 2'-deoxy-2'-amino adenine appeared to have a 70-fold higher affinity for the parasite enzyme and a 3-fold lower affinity for the human enzyme, compared to adenosine. In one step selectivity was hence improved over 500-fold.

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PS-05.03.18 STRUCTURE-BASED DRUG DESIGN IN THE PHARMACEUTICAL INDUSTRY: SOME LESSONS LEARNED

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Since 1990 nearly fifty pharmaceutical companies worldwide have established protein crystallography laboratories, many with an emphasis on structure-based drug design. This iterative process uses experimentally determined or modeled structures of macromolecules and their complexes with potential drug molecules as a guide to the design of enzyme inhibitors and receptor agonists and antagonists. The goal is to develop tighter binding, more selective drugs with improved pharmacokinetics. Several companies are now entering clinical trials with compounds derived from this process. Experience in several laboratories indicates that: (1) Structure-based drug design reduces substantially the number of compounds tested. (2) Improving the properties of lead compounds obtained from screening is generally more efficient than de novo drug design. (3) Rapid and early access to enzymatic or receptor assay and ADME data is crucial. (4) 1) is more productive to start with leads which already have acceptable pharmacokinetics and then improve their binding and selectivity. (5) Success in structure-based drug design and the speed with which a drug candidate can be developed depend heavily on the degree of integration and interaction between scientists performing the functions shown in the flowchart above.