05-Molecular Modelling and Design for Proteins and Drugs

The binding of over 50 inhibitors to T state GPb in the crystal have been studied to 2.3Å resolution and the structures refined to R values less than 0.20. One year ago, the best inhibitors were 1-α-amidoglucose and N-methyl-1-β-amidoglucose with K_i values of 0.37 and 0.16mM, respectively. Attempts to improve the inhibition by making further substitutions to either compounds have not led to a better inhibitor. In the case of the α-anomer this may be due to a conformational change to the ring geometry and subsequent loss of some hydrogen bonding to O2 and O3 of the sugar moiety. A very encouraging result has been in the recent study of N-acetyl-1-β-glucosylamine leading to a K_i of 0.032mM. It is postulated, that the reversal of the amide portion of the β C1 substitution has led 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.

PS-05.03.13 CRYSTAL STRUCTURE OF CHOLERA TOXIN. *By R.G. Zhang, M.L. Westbrook, S.L. Nance and E.M. Westbrook, Biological and Medical Research Division, Argonne National Laboratory, and D. Scott, Department of Molecular Biophysics and Biochemistry, Yale University, U.S.A.*

We have determined the crystal structure of the entire cholera toxin hexamer (A_1B_5) at 2.3 Å resolution, using a combined phasing approach of molecular replacement, multiple heavy-atom isomorphous replacement, and phase extention. The molecular replacement probe was the B_5 pentameric "choleragenoid" structure, determined earlier by isomorphous replacement, and 5-fold rotational averaging, in collaboration with Graham Shipley and his colleagues at Boston University. Two heavy atom derivatives were needed to improve phases sufficiently to initially fit the map. The structure was first determined at 2.6 Å resolution with a rotating-anode x-ray source and a Siemens/Xentronics multiwire detector. The structure has been re-refined against new data, to 2.3 Å resolution, collected on synchrotron beamline X8C of the NSLS, using a newly developed CCD area detector (17,381 data to 2.6 Å; 29,484 data to 2.3 Å). Currently the crystallographic R-factor is 21.3% with 0.023 Å rms bond distance deviations and 3.1° rms bond angle deviations. No solvent has yet been included in this refinement. Ganglioside GM1, its cell-surface receptor, has been fitted to putative B-subunit binding sites and we discuss functional implications of the molecular design.

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PS-05.03.14 THE BINDING STRUCTURES OF ANTITHROMBOSIS AND ANTIPANCREATITIS DRUGS WITH THROMBIN AND TRYPSIN. By C. Sasaki, H. Kubodera, C. Okumura, R. Kikumoto and T. Matsuzaki*, Mitsubishi Kasei Corporation, Yokohama, 227 Japan

MQPA is the first synthetic thrombin inhibitor which has been clinically used in Japan as an antithrombosis drug since 1990. We reported its unusual binding motif to trypsin (Matsuzaki,T. et al., 1989, J.Biochem., 105, 949-952). Despite of the similar chemical structure to that of BPTI, MQPA does not utilize the oxyanion hole for binding, instead, it forms antiparallel β 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 1.8-2.5 Å and R factors range 17-22 %. The results are; (1)The MQPA's unique binding motif is not due to its carboxyl group but to the molecular conformation, which places restrictions on the enzyme-inhibitor interaction. (2)The decrease of inhibitory activity of (2R,4S)MQPA 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 amidinonaphthalene group goes into the specificity pocket, while the more basic guanidinobenzene group lies near His57. These findings will be useful for the design of second generation drugs.

PS-05.03.15 STRUCTURAL PROPERTIES OF FUNCTIONAL GROUPS WHICH PRODUCE CLASS III ANTIARRHYTHMIC ACTION. By Xiaoling Sui and Penelope W. Codding*, Departments of Chemistry and of Pharmacology and Therapeutics, University of Calgary, Calgary, Alberta, Canada.

Arrhythmias 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 anesthetics, 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 sematilide and the acetylated derivative of a class I agent, N-acetylprocainamide (NAPA), which has weak class III activity. Morgan, et al[1] have reported a series of compounds which contain an imidazole ring in place of the methane sulfonamide present in sematilide 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[2] 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[3]), 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.

[1] T.K. Morgan, Jr., *et al.*, *J. Med. Chem.*, **1990**, *33*, 1091-1097.
[2] F.H. Allen, *et al.*, *Acta Cryst.*, **1979**, *B35*, 2331-2339
[3] F. Mohamadi, *et al.*, *J. Comp. Chem.*, **1990**, *11*, 440-467.

PS-05.03.16 CORRELATION OF INHIBITORY POWER WITH STRUCTURES OF SULFONAMIDE DRUGS COMPLEXED TO HUMAN CARBONIC ANHYDRASE I ENZYME By S.Chakravarty and K.K. Kannan, Solid State Physics Division, Bhabha Atomic Research Centre, Bombay 400085, India.

Sulfonamide drugs, being extremely potent inhibitors of Human Carbonic Anhydrase

isozymes, are widely used in the treatment of several types of physiological disorders. Individual data sets for three sulfonamides, namely, Acetazolamide, Methazolamide and Amsulf complexed to Human Carbonic Anhydrase I (HCAI) enzyme were collected using both conventional X-ray generators with photographic films and Photon factory synchrotron source 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 2Å 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 essential 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 loop region residues Leu198 and His200 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 sulfonamide-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 thiophene/benzene based sulfonamide drug molecules with known inhibition constants for HCAI were docked into the active site of HCAI using TOM/FRODO package on IRIS-4D/20 graphics workstation after anchoring the sulfamido and the aromatic groups approximately around the positions found from the three refined structures above. These starting models were energy minimised 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.

PS-05.03.17 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 glycolysis to the stage of pyruvate as its sole source of energy supply. Therefore, the trypanosomal glycolytic enzymes are attractive targets for designing selective inhibitors which should obviously exhibit minimal affinity for the equivalent enzymes of the human host. The design of selective active site inhibitors is difficult because the active site of an enzyme is usually well conserved in the course of evolution. In contrast, selective inhibition may be easier in case an enzyme makes use of a large cofactor. A substantial part of such a cofactor is not directly involved in the catalytic reaction, and as a consequence, its environment is less conserved. We wondered whether the adenosine part of NAD would be a good lead for trypanosomal glyceraldehyde phosphate dehydrogenase (GAPDH) and inhibitor design.

Careful comparison of the *Trypanosoma brucei (T.b.)* GAPDH structure, obtained from Laue diffraction study at 3.2 Å, and a 2.4 Å resolution human GAPDH structure reveals: (1) the presence of a small hydrophobic pocket next to the adenine C2-position in *T.b.* GAPDH versus the presence of Asn in human GAPDH; (2) the proximity of a hydrophobic canyon next to O2' of the adenosine ribose 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 C8-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 adenine substituent 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-thienyl substituent enhances inhibition 100-fold for parasite GAPDH and 20-fold for mammalian GAPDH. More recently, a meta-methoxy benzamide derivative of 2'-deoxy-2'-amino adenosine appeared to have a 170-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.

We would like to thank our colleagues for their numerous contributions to this work. Fred Opperdoes, Paul Michels, Mia Callens and colleagues in Brussels for providing protein material and carrying out kinetic studies; Fred Vellieux, now in Grenoble, and Randy Read, now in Edmonton, for their X-ray studies leading to the three-dimensional structures of GAPDH; and Piet Herdewijn and Arthur van Oirschot for the synthesis of the new inhibitors.

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

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Since 1980 nearly fifty pharmaceutical companies worldwide have established protein crystallography laboratories, most 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 but does not replace the need for traditional screening and SAR studies, (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 enzyme or receptor assay and ADME data is crucial, (4) It 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 greatly on the degree of integration and interaction between scientists performing the functions shown in the flowchart above.