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

