160 05-Molecular Modelling and Design for Proteins and Drugs

The influenza virus neuraminidase is a surface antigen of the virus. Its best characterised role in the life cycle of the virus is facilitating the release of progeny virions from the surface of infected cells. Inhibitors of neuraminidase do not prevent infection or single cycle replication of virus in tissue culture. However they do prevent multi-cycle replication and could therefore be expected to have an effect on the course of the infection in animals.

Based upon the three-dimensional structures of neuraminidases from different strains of human and animal influenza viruses, and of their complexes with substrate (sialic acid) and putative transition state analogues, a number of tightly binding inhibitors of the enzyme have been designed and synthesised. The structures of these compounds complexed to the enzyme have been determined, and show that the designed molecules generally bind as predicted by the design process. The compounds show antiviral activity in an animal model of influenza.

PS–05.03.10 THE DESIGN OF POTENTIAL DRUGS FOR THE TREATMENT OF DIABETES: A QSAR STUDY. K.A. WOODS*, L.N. JOHNSON, *Laboratory of Molecular Biophysics, University of Oxford, Rex Richards Building, South Parks Road, Oxford OX1 3QU, England;* G. CRUCIANI, *Laboratorio di Chemiometria, Dipartimento di Chimica, Universite di Perugia, Via Eece di Sotto 10, Perugia, Italy.*

The primary goal in any drug design project is to predict the activity of new compounds. Design methods have evolved to study the comparative properties of ligands.

The 3-dimensional structure of the receptor is often not known and information regarding ligand-receptor interactions is therefore unavailable. In such cases a method for finding relationships between the ligands is known as Principal Component Analysis (PCA) (Wold, S., Esbensen, K., Geladi, P., Chemometrics and Intelligent Laboratory Systems, 1987, 2, 37-52). PCA is used to build a model that describes how certain selected physical or chemical properties are interrelated. This results in a clustering of similar and different ligands which may be useful in the search for new compounds by indicating regions of structure that have not been previously explored.

When the activity coefficients for a series of ligands are known, a useful drug-design method involves the determination of Quantitative Structure/Activity Relationships (QSAR) (Martin, Y., *Quantitative Drug Design*, 1978, Marcel Dekker, New York). A new computer-aided QSAR methodology employs Generating Optimal Linear PLS (Partial Least-Squares) Estimations (GOLPE) (Baroni, M., Costantino, G., Cruciani, G., accepted to Quantitative Structure-Activity Relationships, 1992), an advanced procedure aimed at obtaining optimal PLS regression models with a very high predictive ability.

We have used a PCA and QSAR/GOLPE study to explore and predict new compounds in a search for an inhibitor of glycogen phosphorylase. We report the results of such a study based on a selected database of inhibitors of glycogen phosphorylase, where both the 3-dimensional structure of the ligand bound complexes and the kinetic coefficients of binding are known.

PS–05.03.11 THE STRUCTURES AND BIOLOGICAL PROPERTIES OF THREE INSULIN MUTANTS

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Diabetics may benefit in the near future from the mutant insulins prepared by genetic engineering, which have long and rapid-acting effects. The site-specific mutagenesis of individual amino acids has an important role in altering molecular assembly, increasing their biological activity and improving the therapeutic properties. The human insulin mutants (1) HB10D/PB28D, (2) TA8H/SB9D/TB27E and (3) HB10D were designed for the above purpose. Their associative states deduced from osmometry are 1.6, 1.1 and 2.2 respectively at 1mM of concentration, and (1) and (3) have double the biological potence of human insulin. Their crystal structures have been determined by X-ray analysis, the solutions have been obtained using the molecular replacement methods. The crystallographic parameters are listed below:

mutant	space group	cell parameters	Reso(Å)	R-f
(1)	C2	a=66.0 b=46.5 c=45.0 β=128.79°	2.1	0.19
(2)	$P2_12_12_1$	a=b=51.9 c=89.7	2.0	0.20
(3)	$P2_13$	a=b=c=113.2	3.0	0.21

There are two molecules in the asymmetric unit of structure (1) which are organised in the crystal as dimers disposed about the two fold crystal axis. The roles of B10D and B28D in the dissociation of insulin and in the interactions of insulin with its receptor will be discussed.

Surprisingly, structure (2) which was designed as a monomeric insulin, crystallised as a dimer with two dimers in the asymmetric unit. This unexpected aggregation state may be explained by the high concentration of (2) used to grow crystals. The details of the affects of the mutated residues on the molecular stability and the receptor binding sites will be given.

Structure (3) has an unusual aggregation of six dimers forming a dodecamer through zinc coordination with B5 His imidazoles around the three fold crystallographic axis. The implication of producing a more stable aggregation state opens a new possibility for combining traditional pharmaceutical formulation and protein engineering to alter insulin's physico-chemical properties.

PS–05.03.12 GLUCOSE ANALOGUE INHIBITORS OF GLYCOGEN PHOSPHORYLASE: THE DESIGN OF POTENTIAL DRUGS FOR DIABETES. K.A. WOODS*, E.P. MITCHELL, L.N. JOHNSON, *Laboratory of Molecular Biophysics, Rex Richards Building, University of Oxford, South Parks Road, Oxford, England;* G.W.J. FLEET, M. ORCHARD, J.C. SON, C. BICHARD, *Dyson Perrins Laboratory, University of Oxford, South Parks Road, Oxford, England;* N. OIKONOMAKOS, D. LEONIDAS and A. PAPAGEORGIU, *The National Hellenic Foundation, 48, Vas. Constantinou Avenue, Athens, Greece.*

Glucose is known to be a weak inhibitor of glycogen phosphorylase (GP) and helps to control blood glucose levels by binding to phosphorylase at the catalytic site, resulting in a conformational change which stabilises the inactive T state of the enzyme, and promotes glycogen synthesis. It has therefore been suggested that inhibition of GP by glucose-analogues may help shift the balance, between glycogen synthesis and glycogen degradation, in favour of glycogen synthesis in both the muscle and liver. By exploiting the knowledge of the crystal structures of T state rabbit muscle GP*b* and the glucose-enzyme complex it is hoped to design an inhibitor that will be more effective than glucose.

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