05-Molecular Modelling and Design for Proteins and Drugs

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THE MODEL OF SUBSTRATE INTERACTION WITH α - MOMORCHARIN BY COMPUTER GRAPHIC SIMULATION Yi-Cheng Dong[1], Ricky Ngok-Shun Wong[2], Pang-Chui Shaw[3], Hei-Wun Leung[4] and Hin- Wing Yeung[3]

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The mechanism of N-glycosidase activity of ribosome- inactivating proteins (RIPs) was studied through the model of substrate interaction by using computer graphic simulation. The crystal structure of α -momorcharin(α -MMC) at 2 A resolution was used as a model of RIP. The natural substrate of α -momorcharin is the 28S rRNA. The target site is the N- glycosidic bond at A4324 of the 28S rRNA which is a highly conserved region near the 3'-end of the rRNA. A tetraribonucleotide GAGA representing the conserved sequence at the site of cleavage was chosen as the substrate for computer graphic simulation. Fitting the substrate GAGA into the putative active site of α -MMC revealed several interesting observations which helps to explain the possible mechanism of its N- glycosidase activity. Firstly, the second nucleotide (adenosine) , which is the target for RIP protrudes out from the rest of the molecule and this facilitates the enzyme- substrate interaction. Secondly, the adenine at the active site was found to adopt the *sync* onformation. In fact, the glycosidic torsion angle of adenine was best described as the "high anti" which was intermediate between the classic *syn* and *anti* position. The binding of adenine in this sterically unfavoured conformation would place strain on the glycosidic bond. Furthermore, the active site residues of α -MMC such as E160 and R163 are in close proximity to the bond of cleavage. According to the model, the NH1 of R163 is hydrogen bonded to N9 of the adenine ring. This interaction helps to withdraw electrons from the imidazole ring and further weakens the glycosidic bond. The Cl on the ribose ring would become slightly electropositive owing to the electron withdrawing effect of N9 as well as the ribose oxygen. The OE1 of E160 is available for nucleophilic attack on C1 leading to bond cleavage. Hydrolysis of the intermediate regenerates the active site. This model helps to explain the alteration in activity of some mutants in homologous RIPs such as trichosanthin and ricin A. Besides catalysis, substrate specificity can also be interpreted from the interaction between the RIP and the sugar-phosphate backbone of the substrate .

PS-05.03.08 THE STRUCTURE OF DES-F1-MEIZO-THROMBIN: THE NATURE AND LOCATION OF KRINGLE-THROMBIN AND KRINGLE-KRINGLE INTERACTIONS. By Philip D. Martin*, Michael G. Malkowski, Charles T. Esmon, and Brian F. P. Edwards, Wayne State University School of Medicine, Detroit, Michigan 48201 USA

We have solved the structure of bovine des-F1-meizothrombin inhibited with D-FPR-chloromethyl ketone at 2.8 Å resolution by molecular replacement and difference Fourier techniques. The space group is $P4_12_12$, a=186.5 Å, c=120.3Å, with a dimer in the asymmetric unit. A monomer consists of prothrombin residues 156 to 579 (prothrombin numbering system) with a covalent break at Arg-320 (15/16 in the chymotrypsin numbering system) separating the A and B chains of thrombin. The current model, which lacks the first 14 amino terminal residues of the kringle 2 domain and a 35 residue linker chain connecting kringle 2 to the thrombin A chain, has a standard crystallographic R-value of 0.28 with RMS deviations of 0.022 Å from ideal on bond lengths and 3.6° on ω angles. The active site of the thrombin B-chain is inhibited by D-FPR which is covalently bound in the active site to both His-363 (His-57) and Ser-525 (Ser-195). The dimer has crystallized in a manner that gives significant information on the interactions of the different domains of the structure. There are two distinct interactions of kringle 2 with thrombin. The first is a primary ionic interaction through a cluster of kringle glutamic and aspartic acids centered at Asp residue 225 which form ionic bonds with thrombin Arg residues 418 and 500 (but are not limited to these residues alone). A second interaction is seen in which the kringle domain related by local symmetry blocks the entrence to the thrombin active site *via* two stretches of chain (182 to 188 and 243 to 247). There is also a kringle-kringle interaction directly across the local two-fold which is characterized by imidazole ring stacking between residues His 187 from each kringle and hydrogen bonds from these residues to the carbonyl oxygens of Glu-241 across the dimer interface. Completely unique to this structure is the presence of density for the carbohydrate at the Asn-373 attachment site. Previously reported structures of both bovine and human thrombins, and these thrombins complexed with hirudin, have reported that there is no traceable density for the sugar at this position. This work is supported in part by grant GM33192 and the Molecular Biology Center at Wayne State University.

PS-05.03.09 DESIGN AND PROPERTIES OF INHIBITORS OF INFLUENZA VIRUS NEURAMINIDASE. P.M.Colman*[1] and J.N.Varghese[1], Mark von Itzstein[2], and C.R. Penn[3]. [1]Biomolecular Research Institute, 343 Royal Parade, Parkville, Victoria 3052, Australia, [2]Victorian College of Pharmacy Ltd., 381 Royal Parade, Parkville, Victoria 3052, Australia, [3]Glaxo Group Research Ltd, Department of Virology, Greenford Road, Greenford, Middlesex, BU6 OHE, UK.

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.