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.

