05-Molecular Modelling and Design for Proteins and Drugs

REFERENCES:


PS-05.03.07

THE MODEL OF SUBSTRATE INTERACTION WITH 3-MONOURACIL BY COMPUTER GRAPHIC SIMULATION YI-Cheng Song1, RICKY WONG2, WEI-CHUN Sheu2, HEI-WAN Leng1 and MIN-KING Yeung3

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The mechanism of 3'-glycosidase activity of ribosome-inactivating protein (RIP) was studied through the model of substrate interaction by using computer graphic simulation. The crystal structure of α-mannoribosyl-3'-RIP at 2.6 Å resolution was used as a model of RIP. The nature substrate of α-mannoribosyl-3'-RIP is the 3'-terminal ribose of the RNA, the 5'-terminal site is the N-glycosidic bond at 4324 of the 25S rRNA which is a highly conserved region near the 3'-end of the rRNA. A tetra ribonucleotide 3-OAPA representing the conserved sequence at the site of cleavage was chosen as the substrate for computer graphic simulation. Fitting the substrate 3-OAPA into the active site of α-mannoribosyl-3'-RIP revealed several interesting observations which help to explain the possible mechanism of its 3'-glycosidase activity. Firstly, the nucleotide adenine, which is in the motif for RIP cleavage of UTP, protrudes out from the rest of the molecule and this facilitates the attack of the substrate interaction. Secondly, the adenine at the active site was found to adopt the specific conformation. In fact, the glycosidic torsion angle of adenine is best described as the "high angle" which is intermediate between the "planar" and "ring" position. The binding of adenine in this sterically unfavoured conformation would place strain on the glycosidic bond. Furthermore, the active site residues of α-mannoribosyl-3'-RIP are in close proximity to the bond cleavage. According to the model, the NH1 of E163 is hydrogen bonded to the adenine ring. This interaction helps to withdraw electrons from the leucine ring and further weaken the glycosidic bond. The Cl on the ribose ring would become slightly electropositive owing to the electron withdrawing effect of NS as well as the ribose oxygen. The O3 of E160 is available for nucleophilic attack on Cl leading to bond cleavage. Hydrolysis of the intermediate regenerates the active site. This model helps to explain the alterations in activity and specificities of some mutants in homologous RIPs such as ricin A and ricin B.

PS-05.03.08

THE STRUCTURE OF DES-FI-MEIZOTHROMBIN: THE NATURE AND LOCATION OF KRINGLE-TIHIOMBIN AND KRINGLE-KRINGLE INTERACTIONS. By Philip D. Martin1, Michael G. Malkowski, Charles T. Rannan, and Brian E. P. Edwards, Wayne State University School of Medicine, Detroit, Michigan 48201 USA

We have solved the structure of bovine des-FI-meizothrombin inhibited with D-FPR-chloroethyl ketone at 2.8 Å resolution by molecular replacement and difference Fourier techniques. The space group is P412121, a=186.5 Å, c=120.3 Å, 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 (15/15 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 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 0.22 Å from ideal on bond lengths and 3.6° on α 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-523 (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 Itonic interaction through a cluster of kringle glutamic and aspartic acid residues centered at Asp residue 225 which form ion 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 to local symmetry blocks the entrance to the thrombin active site via two stretches of chain (182 to 188 and 243 to 247). There is also a kringle-kringle interaction across the local two-fold which is characterized by imidazole ring stacking between residues His 137 from each kringle and hydrogen bonds from these residues to the carboxyl 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 grants GM33519 and the Molecular Biology Center at Wayne State University.

PS-05.03.09

DESIGN AND PROPERTIES OF INHIBITORS OF INFLUENZA VIRUS NEURAMINIDASE. By S M Coleman1, and J N Varughese1. Mark von Itzstein2, and C R Penn1

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