05-Molecular Modelling and Design for Proteins and Drugs

PS-05.01.10 Tampering with Success: Using Structure to Develop Second Generation Engineered Subtilisins" By. R. Bott, J. Dauberman, L. Wilson, B. Schmidt, G. Ganshaw, J. Sanford, D. Estell, H. Sagar, and T. Graycar, Genencor International, So. San Francisco CA 94080 USA

We have determined the three-dimensional structure of a variant of *Bacillus lentus* having four site-specific substitutions: K27R/V104Y/N123S/T274A which has the benefit of providing twice the commercially important performance as the native enzyme. This structure has been used to identify additional sites which can confer increased chelant and thermal stability, as well as, sites which can dramatically alter the kinetic parameters of this variant enzyme. Substituting S128G results in an enzyme with substantially increased activity on a synthetic substrate and reduced performance of proteinaceous substrates while substitutions at other sites produce the reverse effects. We are in the process of determining how the structure function relationships deduced from these structures can be incorporated to produce a second generation engineered enzyme.

PS-05.01.11 MODELS FOR CATALYTIC ANTIBODIES: A TOOL FOR UNDERSTANDING THEIR MECHANISM OF ACTION. By M. Eisenstein[1], D.S. Schindler[2], R. Zemel[2] B.S. Green[3] and Z. Eshhar[2], Departments of Structural Biology[1] and Chemical Immunology[2], The Weizmann Institute of Science, Rehovot, Israel, and Pharmaceutical Chemistry[3], The Hebrew University, Jerusalem, Israel.

Immunization with transition state analogs of a chemical reaction can give antibodies which catalyze the reaction. Such catalytic antibodies have the potential to provide us with proteins that have enzymatic activities with novel specificities. In order to engineer these antibodies and understand their mechanism of action we are studying catalytic antibodies with esterolytic activity on p-nitrophenyl esters.
Antibodies were raised in mice by immunization with p-nitrophenyl phosphonate as the hapten and six monoclonal antibodies with catalytic activity were found by extensive screening. Biochemical experiments divide these antibodies into two groups according to their substrate specificity and inactivation, sensitivity to chemical modification and affinity to transition state analogs.
Sequencing of these antibodies showed that the two groups have different light chains. Models of the antibodies were built on the basis of their sequence and the known three-dimensional structures of antibodies. A deep L-shaped groove is present in one group of the antibodies and it is proposed that this is where the transition state analog binds. This groove is wider and shallower in the other group of antibodies explaining the difference in binding affinity. The phosphonate moiety of the transition state analog binds to a Tyr residue which is conserved in all six antibodies and is located at the bend of the groove. It also binds to a Gln / Tyr from the heavy chain. Additional interactions between the antibodies and the p-nitrophenyl and the aliphatic chain of the transition state analog stabilize the binding.
An evident structural difference in the binding grooves of the two groups of antibodies is that a Tyr residue in one group, which is bound to the phosphonate-binding Tyr, is replaced by an Arg in the other group. This change can explain the differences in substrate inactivation and chemical modification. It also hints at possible dissimilarities in the ester hydrolysis mechanism between the two groups of antibodies.

PS-05.01.12 MODELLING STUDY OF A NEUTRAL PHOSPHOLIPASE A_2 FROM THE VENOM OF AGKISTRODON HALYS PALLAS. By X.Q. Wang*, Z.J. Lin, National Laboratory of Biomacromolecules, Institute of Biophysics, Academia Sinica, Beijing, China.

The neutral phospholipase A_2 (PLA_2), isolated from *Agkistrodon halys* pallus venom, has strong presynaptic neurotoxin activity and designated as agkistrodotoxin (ATX). The sequence of ATX (Kondo,K. et al.,(1989),J.Biochem.105,196-203) is highly homologous to that of the toxic basic subunit of crotoxin. ATX has a tendency to associate with identical molecules to form dimer or higher aggregates from crystallization and M.W. determination (Jin,L.,et al.,(1991),Chinese J. Biochem.Biophys.23,269-276). Sequences alignment between ATX and non-toxic *Crotalus atrox* venom PLA_2 shows the identity of 50% amino acids and presence of almost same residues involving subunit interaction. In order to study the possibility of dimerization from a structure point of view,three dimensional models of both monomeric and dimeric ATX have been graphically built using the X-ray structure of *C. atrox* venom PLA_2 and optimized by energy minimization technique with programs QUANTA and CHARMm. The result shows that the structure seems essentially similar to that of *C. atrox* venom PLA_2(the r.m.s. deviation of corresponding C_α atoms is 1.46Å except the fragment 85-89 in a loop region. The dimer-stabilizations of ATX dimeric model are very similar to those of *C. atrox* venom PLA_2 except that the interaction between His34 and Glu6 is replaced by that between Glu34 and Asn6. The energy calculation shows that the dimer's total energy and hydrogen bond energy are 241 and 41 kcal/mol respectively lower than twice of monomer's energies. These suggest that the C.atrox-like dimer is a more stable form than monomer. We plan to complete the modeling using molecular dynamics simulation method in next step. The definitive determination of the structures will be done by X-ray crystallography, which is currently underway.

PS-05.01.13 DISTANCE GEOMETRY AND MOLECULAR DYNAMICS CONFORMATIONAL SEARCH OF A CYCLIC PEPTIDE FOR PROTEIN DE NOVO DESIGN. Zhaowen Luo*, Luhua Lai, Xiaojie Xu, Department of Chemistry, Peking University, Beijing 100871, CHINA

A cyclic peptide, designed to act as topological template of the four–helixes bundle, was investigated by distance geometry and molecular dynamics conformational search. The sequence of the cyclic peptide is (Phe–Lys–Pro–Gly–Lys–Gly)2. Firstly, 100 conformations were generated by distance geometry with constraints for ring closure. Seven conformation clusters were classified according to their mutual rms. A representative conformation from the cluster with highest density was selected to

