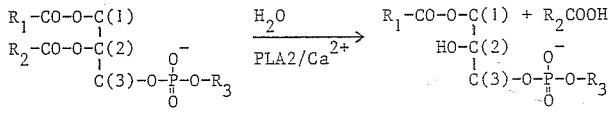
02.1–28 STRUCTURAL DIFFERENCES IN CLOSELY RELATED PANCREATIC PHOSPHOLIPASES A_2. By R.Renetseder, B.W.Dijkstra, J.Drenth, Laboratory of Chemical Physics, State University of Groningen, Nijenborgh 16, Groningen, The Netherlands

Phospholipases A_2 are lipid degrading enzymes which catalyze the reaction



with Ca^{2+} as an essential cofactor bound to the enzyme. The postulated reaction mechanism is similar to that of the serine proteinases, with an Asp-His couple as proton donor and the nucleophilic serine-OH replaced by a water molecule bound to the essential Ca^{2+}-ion in PLA2 (Dijkstra et al.,Nature 289,604). The extracellular enzymes of this type (e.g. pancreatic and venom PLA2's) have molecular weights of about 15000 and their amino acid sequences are highly homologous. Their specific activity with aggregated substrates is much higher than with monomeric substrates. The structures of bovine and porcine pancreatic PLA2 were determined at 1.7 Å and 2.6 Å resolution, respectively (Dijkstra et al., J.Mol.Biol. 147,97 and Dijkstra et al., J.Mol.Biol. 168,163). There are a total of 21 amino acid differences, which hardly affect the secondary structure. However, the single change of a Val in the bovine into a Phe in the porcine enzyme at position 63 completely alters the conformation of the chain between residues 58 and 70, resulting in the disappearance of a small α-helix. Such a change would not have been predicted by any of the current secondary structure predicting procedures. The concomittant loss of rigidity in this part of the structure may be compensated for by a second, low affinity Ca^{2+}-binding site (Glu71+Glu92) in porcine PLA2 which is not present in the bovine enzyme (Asn71). These structural differences can, at least in part, account for the observation that porcine PLA2 binds to micelles better than bovine PLA2, while their affinities for monomeric substrates are similar, which can be attributed to the almost identical active sites.

02.1–29 THE CRYSTALLOGRAPHIC STRUCTURE OF FRAGMENT 1 FROM BOVINE PROTHROMBIN TO 2.7 A RESOLUTION. By Lennart Sjölin, Leif Andersen, Oliver Lindqvist and Gustaf Olsson, Dept. of Inorganic Chemistry, Chalmers University of Technology, S-412 96 Göteborg, Sweden and Staffan Magnusson, Dept of Molecular Biology, University of Aarhus, DK-8000, Aarhus C, Denmark.

When blood clotting is activated through factor XA, prothrombin is split into a pro-fragment (A+S) and thrombin. In the thrombin-catalyzed inactivation of excess prothrombin, prethrombin 1 and fragment 1 may be formed. The fragment 1 from bovine prothrombin contains 10 substituted gamma carboxy glutamic acids, which are almost certainly involved in the binding of Ca^{2+} ions. The fragment 1 thus seems to be responsible for the binding of prothrombin to the phospho-lipid layers. The molecular weight of fragment 1 from bovine prothrombin is appr. 25500. About 20% of the molecular weight is due to carbohydrates substituted at ASN 77 and ASN 101 on the amino acid chain. Single crystals of fragment 1 have been grown and the cell constants are A=39 A, B=54 A and C=129 A. Three-dimensional data to 2.7 a resolution have been collected from native crystals. In addition, data have been collected from two different derivatives and a low resolution model (4 A resolution) has been published (Olsson,Andersen,Lindqvist,Sjölin,Magnusson, Petersen and Sottrup-Jensen, Febs Letters, 145, 317, 1982). We have now traced 140 amino acid residues out of 156 and subsequent model building and refinement are under way. We aim to present a model containing the "Kringle" fold, a fold which has until now been found in prothrombin plasminogen, eurokinase and tissue plasminogen activator (TPI).

02.1–30 X-RAY STRUCTURAL STUDIES OF THE ASPARTIC PROTEINASES. By T. L. Blundell, L. H. Pearl, S. P. Wood, S. I. Foundling and F. E. Watson, Laboratory of Molecular Biology, Department of Crystallography, Birkbeck College, University of London, London WC1E 7HX, UK

The X-ray structure of endothiapepsin has been refined at 2.1Å resolution using restrained least-squares (RESTRAIN) to an R-value of 0.16. Fragments of known sequence (V. Pedersen, private communication) have been aligned in the electron density to give an almost complete primary structure. The two catalytically important aspartyl groups (Asp 32 and Asp 215) are hydrogen-bonded together and lie on a pseudo dyad with equivalent contacts to a bound water molecule lying on this dyad. The arrangement of the catalytically active groups appears to be inconsistent with a mechanism based on carboxylates of different pKa.

A 4.3Å electron density map of mucor chymosin allows tentative identification of the molecular boundary and work is in hand to confirm this by molecular replacement and computer graphics techniques. Native data have been collected to 2.6Å and further heavy atom data are being collected to obtain an improved MIR (multiple isomorphous replacement) map.

Work on inhibitors and substrates is in progress. This is being carried out making use of kinetic studies at Cardiff University (J. Kay) and using model compounds prepared at the Royal Postgraduate School of Medicine (M. Szelke). The inhibitors (H256 and H142) which have reduced peptide bonds have been soaked into crystals of endothiapepsin and are being studied by difference Fourier techniques. Similar work is being carried out on soluble derivatives of pepstatin complexed to mucor chymosin.