02. STRUCTURAL MOLECULAR BIOLOGY

02.1-41 CRYSTALLOGRAPHIC STRUCTURE OF PIG PANCREAS α-AMYLASE (2.9 Å RESOLUTION). By G. Ghibaudo, E. Dale, D.R.P. Ceriotti, E.J. L. Sutcliffe, C. Grenoble Codex, France. H. Fager, E. Payoux, C. Richer, C. Grenoble Codex, France. The structure of Pig Pancreas α-amy lase (PNA) (M = 53,000) was solved at 2.9 Å resolution. The electron-density map obtained from multilane isomorphous replacement was improved by a density-modification procedure, recalculating phases from a modified electron-density map where all solvent regions were given a constant electron density. The resulting map was interpretable in terms of amino acid sequence. PNA contains two domains: a larger C-terminal domain (residues 1-410) with a 9-stranded singly wound parallel β-barrel (successive strands being often connected by parallel helical regions) and a smaller C-terminal domain (residues 410-496) with essentially β-sheets. The main chain arrangement in PNA is roughly similar to that in Taka-α-amylase A.

The activator CI ion is located at the center of the β-barrel (as shown by Br substitution). The essential Ca2+ ion that is essential for activity of the polypeptide chain in the vicinity of the β-barrel, which would explain why it is "essential" for the integrity of the 3D structure of PNA. The re-interpretation of previous substrate analogues binding experiments (Acta Cryst. 1980 B36, 416-421) indicates which amino acids are involved in substrate binding. The active site cleft is only part of the N-terminal domain and is delineated by residues, constant in all α-amylases sequenced so far. The secondary binding site is formed by residues belonging to the two domains.

02.1-43 STRUCTURE OF THE IRON STORAGE PROTEIN FERRITIN USING SYNCHROTRON DATA. By G.C. Ford, P.M. Harrison, D.W. Rice, J.M.A. Smith, J.L. White, Dept. of Biochemistry, The University, Sheffield S10 2TN U.K.

Ferritin is a hollow spherical molecule which has evolved to sequester iron and protect cells from the toxic effects of an excess of this essential metal and provide a reserve in times of need. The ferritin molecule consists of 24 protein subunits packing with 432 symmetry and containing a core of up to 4500 iron atoms in the form of an inorganic hydrous ferric oxide-phosphate complex (the rare mineral ferrihydrite). Horse spleen apoferritin (the protein shell without the iron core) forms large octahedral crystals from aqueous solutions of 40M Cryst. Its structure has been determined and refined to 2.6 Å resolution. The subunit consists of a bundle of four helices and a β-strand with a fifth short helix at an angle to the bundle. Each facet of the rhombohedral molecule contains a 2-fold generated dimer arranged with the two strands forming a stretch of antiparallel β-sheet. Dimer formation buries many hydrophobic side chains and each dimer, provides a deep groove, lined with potential iron ligands, to the wall of the inner cavity. This may be the site of Fe2+ oxidation to Fe3+ or initiation of iron core formation. The two short helices at the dimer tips are perpendicular and the association of their hydrophobic faces drives ferritin formation and ensures the 432 symmetry of the assembled molecule. Narrow channels through the protein shell at the 3- and 4-fold symmetry axes may provide access for iron atoms to the interior. 12 leucines, 3 from each of the 4 subunits surrounding the 4-fold channel, make this a very hydrophobic route. By contrast the 3-fold channel, lined with Ser, Asp and Glu side chains, is very hydrophilic. Functional implications of ferritin crystals grown in metals known to inhibit iron uptake will be discussed.