

02.1-41 CRYSTALLOGRAPHIC STRUCTURE OF PIG PANCREAS α AMYLASE (2.9 Å RESOLUTION). By G. Buisson, E. Duée, D.R.F., CEN-G, C.E.A., 85 X, 38041 Grenoble Cedex, France. R. Haser, F. Payan, C.R.M.C.2., Campus de Luminy, Case 915, 13288 Marseille Cedex 9, France.

The structure of Pig Pancreas α Amylase (PPA) (MW=53,000) was solved at 2.9 Å resolution. The electron-density map obtained from multiple 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. P.P.A. contains two domains: a larger N-terminal domain (residues 1-410) with a 8-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 P.P.A. is roughly similar to that in Taka-amylase A.

The activator Cl^- ion is located at the center of the β -barrel (as shown by Br^- substitution). The essential Ca^{2+} ion maintains one stretch 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 P.P.A. 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.

02.1-42 PROGRESS IN THE STRUCTURE DETERMINATION OF RABBIT MUSCLE PHOSPHOGLUCOMUTASE. By Celerino Abad-Zapatero, Zheng-jiong Lin, Michiko Konno, William J. Ray, Jr. and Michael G. Rossmann, Department of Biological Sciences, Purdue University, West Lafayette, Indiana. 47907. USA.

Phosphoglucosmutase (PGM) is a widely distributed enzyme that interconverts glucose-1-P and glucose-6-P in both glycolysis and gluconeogenesis; it is an unusually large monomeric enzyme (Mr. 61,600).

The enzyme from rabbit muscle crystallizes in space group $P4_22_1$ with cell dimensions $a = b = 174.6$, $c = 101.1$ Å with two polypeptide chains in the asymmetric unit. A preliminary rotation function study indicated the presence and orientation of a non-crystallographic twofold axis of symmetry (Wierenga et al., Phil. Trans. Roy. Soc. Lond. B295, 205-208, 1981). Data sets were collected to 3.5 Å resolution for both native and an ethylmercury phosphate derivative by oscillation photography. The difference Patterson and subsequent difference Fourier maps revealed the presence of 12 heavy atom sites within the crystallographic asymmetric unit. These sites were related in pairs by a non-crystallographic diad coincident with the one obtained by the rotation function results.

Single isomorphous replacement phases aided by the anomalous dispersion for the derivative data were computed to 3.5 Å resolution for 13,025 reflections with an overall figure of merit of 0.672. Molecular replacement phase refinement in real space was performed using refined values for the position and orientation of the parameters of non-crystallographic symmetry until convergence was reached (7 cycles).

The final electron density map, skewed and averaged down the non-crystallographic diad, was of good quality and revealed at least three domains and many secondary structure elements. The amino-terminal domain appears

to have an α/β structure; the other two domains appear to consist mainly of β -sheet structure. Approximately 220 residues separated into three different sections have been built in this map. A 60-residue section is consistent with the amino acid sequence (Ray et al., J. Biol. Chem. 258, 9166-9174, 1983).

The data has now been extended to 2.7 Å resolution for both the native and the heavy atom derivative. Single isomorphous phases have been computed from 3.5 to 2.7 Å resolution and phase refinement by electron density averaging in real space is now underway. A new map to the resolution limit of the native data (2.5 Å) and with refined phases should be available shortly.

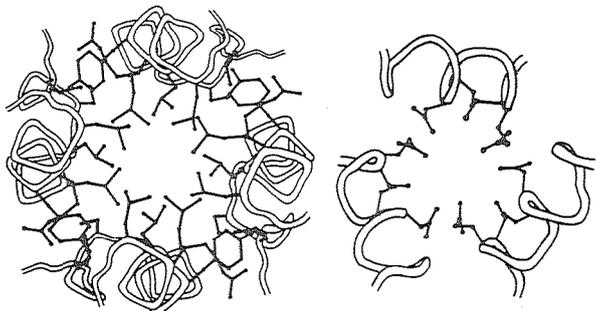
02.1-43 STRUCTURE OF THE IRON STORAGE PROTEIN FERRITIN USING SYNCHROTRON DATA.

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 so 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).

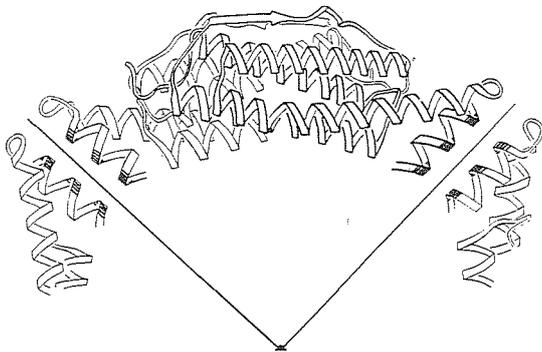
Horse spleen apoferritin (the protein shell without the iron core) forms large octahedral crystals from aqueous solutions of 40mM CdSO_4 . Its structure has been determined and refined to 2.6 Å resolution. The subunit consists of a bundle of four helices and a β -strand with a fifth short helix at an angle to the bundle. Each facet of the rhombic dodecahedral 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 Fe^{2+} oxidation to Fe^{3+} 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.



12 Leucines from 4 helices around the 4-fold axis make a hydrophobic channel.

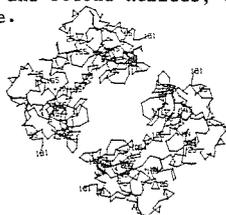
The 3-fold channel is very hydrophilic. Each subunit donates a SER, ASP and GLU.



Ribbon diagram of the apoferritin dimer and parts of two other subunits. The hydrophobic face of the short helix facing the 4-fold channel is shaded.

02.1-44 MANGANESE AND IRON SUPEROXIDE DISMUTASES ARE STRUCTURAL HOMOLOGS. W. Stallings, K.A. Patridge, and M.L. Ludwig, Biophysics Research Division and Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109.

The crystal structure of a tetrameric manganese superoxide dismutase from a thermophilic bacterium, *Thermus thermophilus* HB8, has been determined at 4.4 Å resolution by local averaging of electron density maps calculated by isomorphous replacement. The enzyme crystallizes from ammonium sulfate at pH 5.7 and pH 7.0 in space group $P4_12_12$ with $a = 146.6$ and $c = 55.6$ Å. The spatial arrangement of the principal secondary structural features of iron superoxide dismutase is repeated in manganese dismutase, as demonstrated by superposition of the polypeptide chains of Fe and Mn dismutases. Density peaks corresponding to bound Mn^{+3} occur at locations equivalent to the Fe positions in iron dismutase, indicating one metal binding site per chain, or four sites per tetramer. The Mn dismutase tetramers have molecular 222 symmetry with one of the twofold axes coincident with a crystallographic diad. The tetramer is approximately rectangular in shape and appears to be constructed with only two unique interfaces. One set of interchain contacts closely resembles the dimer interface of Fe dismutase, but the other interface utilizes a polypeptide segment, inserted between the first and second helices, that has no equivalent in Fe dismutase.



02.1-45 THE STRUCTURE ANALYSIS OF DIPHTHERIA TOXIN by B. McKeever and R. Sarma, Biochemistry Department, State Univ. of New York, Stony Brook, N.Y. 11794, U.S.A.

The Diphtheria Toxin, produced by *Corynebacterium diphtheriae* is responsible for the observed lesions associated with that disease. The Protein is a single polypeptide chain of molecular weight 60,000. It is made of two domains, the C terminal domain recognizes and binds to receptors on susceptible cell surface and internalizes the N terminal domain, which upon entering the cytoplasm catalyzes the hydrolysis of NAD and the ADP ribosylation of a unique diphthamide residue on the eukaryotic elongation Factor-2, resulting in the termination of Protein synthesis.

The purified Protein can be isolated into sixteen fractions with different identifiable properties; monomer-dimer; nucleotide bound or free and so on. The bound dimer fraction yields diffraction quality crystals belong to the space group $P3_12$ or its enantiomorph to the unit cell dimensions $a=b=97.9$ Å; $c=100.3$ Å. The diffraction data is being collected using oscillation photographs and the structure is being determined using multiple isomorphous replacement method. The results of the electron density map will be presented.

02.1-46 AN X-RAY DIFFRACTION STUDY OF A PEPTIDE HORMONE DEAMINO-OXYTOCIN. By T. L. Blundell, S. Cooper, J.-Y. Li, J. E. Pitts, I. J. Tickle, A. C. Treharne and S. P. Wood, Laboratory of Molecular Biology, Department of Crystallography, Birkbeck College, University of London, London WC1E 7HX, UK; V. J. Hruby, Department of Chemistry, University of Arizona, Tucson, Arizona 85721, USA; and H. R. Wyssbrod, Department of Physiology and Biophysics, Mount Sinai Medical Centre, New York 10029, USA.

Oxytocin, a neuropeptide hormone composed of a twenty-membered ring and acyclic tail, has hormonal activities eliciting smooth muscle contraction in mammary glands and uteri. The highly potent 6-sulphur deamino oxytocin has been crystallised in the dry form with space group C_2 and cell dimensions $a = 27.08$, $b = 9.06$, $c = 22.98$ Å, $\beta = 102.1^\circ$ and data collected to 1.20 Å resolution (after Low; Science 151 1552 (1966)). The 6-seleno deamino oxytocin analogue crystallised isomorphously with cell dimensions $a = 27.01$, $b = 9.14$, $c = 22.98$ Å, $\beta = 102.2^\circ$ and data have been collected to 1.92 Å resolution. The phases were calculated using anomalous and isomorphous differences and the models refined with restraints using SHELX and RESTRAIN (Moss, Morffew, Haneef, Stanford and Borkakoti). There are type II β -turns between residues 2 to 5 of the rings and type I β -turns involving residues 6 to 9. There is conformational disorder at the disulphide bridge. The crystal structure will be compared to conformations from NMR and other spectroscopic studies in water and DMSO and in terms of binding to neurophysin and receptors.