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 913, 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 acid s 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.

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.

02.1–42 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.

Phosphoglucomutase (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 $P4_12_12$ 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. B293, 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

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 2.6Å 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.