

**01.4-04** X-RAY CRYSTALLOGRAPHIC ANALYSIS OF HORSE SPLEEN APOFERRITIN. By P.E. Bourne, G.A. Clegg, P.M. Harrison, J.M.A. Smith and R.F.D. Stansfield, Department of Biochemistry, The University, Sheffield S10 2TN, U.K.

Horse spleen apoferritin, the protein component of the iron-storage protein ferritin, crystallizes in space group F432, with  $a = 184\text{\AA}$  and  $Z = 4$ . Electron density maps calculated at  $2.8\text{\AA}$  resolution with phases obtained from two isomorphous derivatives showed each of the 24 equivalent subunits to contain a bundle of four long helices, together with two short helices and stretches of irregular conformation (Banyard et al. Nature 271, 282 (1978)). Detailed investigation showed two possible tracings of the polypeptide chain (Clegg et al. Nature 288, 298 (1980)).

The amino acid sequence (Heusterspreute et al. 16th Congr. Int. Soc. Hematology, Montreal (1980)) has now been compared with the electron density by use of an interactive graphics facility. Approximately 60% of the side chains could be defined at  $2.8\text{\AA}$  resolution and atomic co-ordinates were obtained for these and for the backbone atoms (174 amino acid residues). The fitting of the electron density, the positions of regions of secondary structure predicted from the amino acid sequence and an examination of the distribution of side chains with respect to the three-dimensional structure allows a choice between the alternative subunit conformations to be made, that of Banyard et al. (loc. cit.) now being preferred. The subunit shows internal structural homology and some sequence homology can be seen.

We will describe attempts to improve the structure by means of restrained least-squares refinement (Hendrickson and Konnert in Biomolecular Structure, Conformation, Function and Evolution ed. Srinivasan, Vol. I, 43 (1980)) and interpretation of combined maps phased by both MIR and partial structure information (Bricogne, Acta Cryst. A32, 832 (1976)).

**01.4-05** REFINEMENT OF AVIAN PANCREATIC POLYPEPTIDE AT  $0.98\text{\AA}$  RESOLUTION. By I. J. Tickle, I. D. Glover, J. E. Pitts, S. P. Wood and T. L. Blundell, Department of Crystallography, Birkbeck College, University of London, Malet Street, London WC1E 7HX, United Kingdom

The crystal structure of avian pancreatic polypeptide (APP), a 36-residue polypeptide chain with some hormonal properties, has been determined using single isomorphous replacement and anomalous scattering phases at  $2.1\text{\AA}$  resolution, extended to  $1.4\text{\AA}$  resolution by a tangent formula technique. A model consisting of all 302 atoms in the polypeptide and 40 water molecules with individual isotropic thermal parameters has been refined by structure factor least-squares with bond length and interbond angle restraints to a residual  $R = 0.21$  for 6283 reflections at  $1.4\text{\AA}$  resolution. Refinement is continuing at  $0.98\text{\AA}$  resolution (17058 reflections) with a view to comparison of various refinement techniques, including fast Fourier least-squares alternating with regularisations, restrained least-squares and block diagonal least-squares with individual anisotropic thermal parameters.

The structure comprises a stretch of polyproline-like helix folded on to an  $\alpha$ -helix to give a small but stable globular core. The molecule forms dimers and zinc-coordinated oligomers.

**01.4-06** FAST FOURIER REFINEMENT OF THE STRUCTURE OF HAGFISH INSULIN AT  $1.9\text{\AA}$  RESOLUTION.

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Insulin from the Atlantic hagfish, *Myxine glutinosa*, is the most primitive insulin for which the sequence and structure are known. There are 19 changes in its amino acid sequence relative to that of pig insulin and an additional residue (Met) at the C terminus of the B chain. High resolution X-ray studies on hagfish insulin were undertaken on tetragonal crystals, space group  $P4_12_12$  with  $a = b = 38.4\text{\AA}$  and  $c = 85.3\text{\AA}$ , containing one molecule per asymmetric unit but organised as a symmetric dimer lying on a 2-fold axis. The hagfish insulin structure has been refined at  $1.9\text{\AA}$  resolution using the Agarwal fast Fourier least squares method. Starting coordinates were measured from the  $3.1\text{\AA}$  isomorphously phased electron density map. Individual atomic parameters were refined with periodic regularisation of the structure. After initial refinement with data to  $3.1\text{\AA}$  resolution, the R factor fell from 52.7% to 40.4%. Seven rounds of refinement with data to  $1.9\text{\AA}$  resolution reduced R from 44.5% to 23.1%.  $|F_o|$  maps,  $|F_o| - |F_c|$  maps and systematic (1/8th removed) difference maps were examined at various stages of the refinement, the  $|F_o|$  maps proved particularly useful for locating water molecules. A total of 26 water positions have so far been determined, all of which make chemically reasonable contacts with the insulin molecule. Within each of the 2Zn and 4Zn pig insulin dimers, one molecule has essentially the same structure which closely resembles the conformation of the hagfish insulin monomer.

**01.4-07** REFINEMENT OF CONCANAVALIN A WITH THE FAST FOURIER LEAST-SQUARES METHOD. Karl D. Hardman, Ramesh C. Agarwal and Marvin Freiser, IBM Thomas J. Watson Research Center, Yorktown Heights, NY. 10598.

Concanavalin A, a lectin which specifically binds saccharides containing mannosyl and glucosyl residues, has been refined with the fast Fourier least-squares method of Agarwal, Acta Cryst. A34, 791 (1978). The starting atomic coordinates were read directly from a skeletal model that was built into an MIR map and had no further modification or refinement other than geometric regularization. Two significant improvements to the refinement procedure were added: 1/, a method for computing all derivatives of  $x, y, z$  and  $B$  in a single Fourier transform (previously 4 were required), and 2/, a space group general method for computing the diagonal terms of the normal matrix (which is also faster). After 15 cycles of refinement, with geometric regularization about every 5 cycles, the R factor was reduced from 0.48 for  $2.0\text{\AA}$  data, to 0.22 before and 0.25 after regularization, respectively, for  $1.75\text{\AA}$  data. The most dramatic changes in the model at this stage are the improvements in the symmetries of the Mn and Ca ions. Both are now significantly more octahedral with average Mn-O and Ca-O distances of  $2.43 + 0.086$  and  $2.41 + 0.055\text{\AA}$ , respectively. The 'cis' peptide between Ala 207 and Asp 208 still remains. This was also checked by removal of all atoms in this region, refining several cycles and calculating an Fo-Fc electron density map. The densities for the deleted atoms reappeared in very good agreement with their refined positions. Work in progress is the removal of large errors in atomic positions (those over  $1.5\text{\AA}$ ) by the same general procedure as mentioned for checking the 'cis' peptide, and the addition of solvent molecules.