02.1-03 THE RELAXIN STRUCTURE, by Maxine McCall*, Guy Dodson**, Elias Eliopoulos*, Neil Isaacs† and Hugh Niall††, *Astbury Department of Biophysics, University of Leeds, Leeds, U.K. ** Department of Chemistry, University of York, York, U.K. †St Vincent's School of Medical Research, Melbourne, Victoria, Australia, ††Howard Florey Inst. of Experimental Physiology and Medicine, University of Melbourne, Victoria, Australia.

Analyses of relaxin molecules from a variety of species show that they have a chain structure comparable to insulin's and may have a similar 3-dimensional conformation (Isaacs et al (1978) Nature, 271, 278-281; Bedarker et al (1977) Nature, 270, 449-451).

We have used a computer graphics system to fold various relaxin chains into the structures of 2Zn insulin, molecule II (Blundell et al (1971) Nature, 231, 506-511) and 4Zn insulin, molecule I (Bentley et al (1976) Nature, 261, 166-168). The results demonstrate that a common conformation may indeed exist for insulin and relaxin, with possible variations for a few residues at both ends of the B chain. Secondary structure prediction studies support these observations.

There is a striking surface feature common to all the predicted structures: of the 6 polar, essentially-invariant residues, 5 are charged and form a contiguous surface on the relaxin molecule. This nicely explains the antigenic overlap that is observed for rat and pig relaxin which otherwise have completely different surface residues (John et al (1981) Endocrinology, in press). It also sustains the principle that the folding of relaxin is akin to insulin.

02.1-04 X-RAY AND COMPUTER GRAPHICS STUDIES OF POLYPEPTIDE HORMONES AND GROWTH FACTORS AND THEIR PRECURSORS. By S. Bedarkar, T. L. Blundell, J. Gunning, J. E. Pitts, G. L. Taylor, I. J. Tickle and S. P. Wood, Department of Crystallography, Birkbeck College, Malet Street, London WC1E 7HX, United Kingdom.

Although smaller peptide hormones such as glucagon have no well-defined conformers in dilute aqueous solutions, larger polypeptides such as proinsulin, relaxin, insulin-like growth factor, nerve growth factor and neurophysin probably have well-defined globular structures. Crystals of proinsulin ($P4_12_12$, a = 51.16, c = 150.79Å), nerve growth factor ($P6_122$, a = 56.1, c = 181.4Å) (in collaboration with A. Wlodawer, K. Hodgson, E. Shooter and J. Goodfellow) and neurophysin ($P2_12_12$, a = 152.78, b = 69.08, c = 36.30Å) have been obtained and structure analyses are in progress using the method of multiple isomorphous replacement and, in the case of proinsulin, molecular replacement. The well-defined structure of 2Zn-insulin provides the base for the latter study.

Relaxin and insulin-like growth factor have not been crystallised, but their structures have been predicted using computer graphics model building. Amino acid sequence homology with insulin in structurally important areas has allowed models of these factors to be built with an insulin-like fold. The model structures are globular, with core volumes the same as insulin. The nature and surface distribution of side-chains for insulin, IGF and relaxin is consistent with their respective distinct biological properties.

Model building NGF and proinsulin has also been attempted. It is not possible to build a satisfactory model for NGF based on the insulin fold but several plausible placements of the C-peptide of proinsulin on the insulin surface have been investigated. The structure analyses of these proteins will test the conclusions of model building.

02.1-05 MODELLING STRUCTURE AND FUNCTION IN HOMOLOGOUS PROTEINS (MEMBRANE-ACTIVE PLANT TOXINS). By M.M. Teeter and M. Whitlow, Dept. of Chemistry, Boston University, Boston, MA 02215.

The crystal structure of the hydrophobic plant protein crambin which has 46 amino acids has been recently determined (Hendrickson and Teeter, Nature, in press). Comparison of the completed sequence of crambin (Teeter, Mazer and L'Italien, submitted to Biochemistry) to that of other proteins revealed that it was homologous to a class of membrane-active plant toxins which are hemolytic, cause skeletal and cardiac muscle contraction and kill diverse types of phytopathogenic bacteria. To date, no toxicity has been demonstrated for crambin. Although different in function, circular dichroism studies in our laboratory indicate crambin and the toxins are similar in secondary structure in solution. We have chosen to use these small (45-46 amino acids), disulfide crosslinked proteins to establish the principles of a structure prediction scheme which could be applied to other series of homologous proteins such as snake venom neurotoxins or immunoglobulins. The residues conserved between crambin and the plant toxins may show which features are necessary to preserve the secondary and tertiary structure. The aspects different from crambin but common to all the toxins might define the functional site for these proteins.

Three steps were involved in our procedure. First, we built the amino acid sequence of two representative plant toxins onto the backbone of crambin using an interactive graphics system in the laboratory of Dr. Greg Petsko at MIT. Second, we subjected the molecules to potential energy minimization using the AMBER molecular modelling programs of Paul Weiner at U. Cal. at San Francisco. Finally, we evaluated similarities and differences in the surface features of crambin and the toxins.

Residues conserved for all the proteins lie primarily in the inner bend of the Γ-shaped molecules. The interaction of residue Arg 10 with residues 46 and 2 would be present throughout. The toxins are very basic (pI $\simeq$ 11) but crambin is neutral. The basic residues for the toxins fall into two systems, one parallel and one perpendicular to the helix axes. These residues may define a toxic surface to interact with lipid or membrane protein.