02.1-39 CRYSTAL STRUCTURE OF A BOVINE NEUROPHYSIN II DIPEPTIDE AMIDE COMPLEX AT 3 Å RESOLUTION. John P. Rose[1], Daniel S. C. Yang[1], William Furey Jr.[1,3], +Chung Soo Yoo[1,3], Martin Sax[1,3], Esther Breslow[4] and Bi-Cheng Wang[1,2], Departments of [1]Crystallography and [2]Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, [3]Biocrystallography Laboratory, VA Medical Center, Pittsburgh, PA 15240 and [4]Department of Biochemistry, Cornell University Medical College, New York, NY 10021, USA.

The neurophysins are a family of closely related proteins with molecular weights of approximately 10,000 daltons. In general, they are in equilibrium with their corresponding dimers or higher oligomers. Neurophysins are carrier proteins for the posterior pituitary hormones, oxytocin and vasopressin, during axonal transport. They also provide stability to the hormones within the neurosecretory granules. In vivo solid materials have been observed in neurosecretory granules and in hedgehog neurohypophyseal neurosecretory granules, for example crystals which are similar to those of the neurophysins have been observed. The crystal structure of bovine neurophysin II Phe-Tyr-NH_2 has been phased with the ISIR method to 3.0 Å resolution. The dipeptide in the complex is known to bind the neurophysin at its hormone binding site. There are 4 monomers in an asymmetric unit. An electron density map is currently being interpreted. Crystal data are: space group $P2_12_12_1$, a = 120.17, b = 69.03, and c = 62.50 Å. Area detector data collected at the University of Virginia and at the Genex Corporation were used in this study.

+Deceased August 31, 1983.

02.1-40 THE CRYSTAL STRUCTURE AND IMMUNOLOGICAL CROSS-REACTIVITY OF MONELLIN AND THAUMATIN, TWO INTENSELY SWEET PROTEINS. By C.-H. Kang, A. de Vos, C. Ogata, M. Hatada, G. Tomlinson, W.-C. Shin and S.-H. Kim, Department of Chemistry, University of California, Berkeley, California 94720.

Thaumatin and monellin are the sweetest compounds known to mankind. They are about 100,000 times sweeter on a molar basis and several times sweeter on a weight basis than sugar. The former is a single chain protein of 207 amino acid residues, and the latter is composed of two peptides, one 45 residues and the other 50 residues long. Both proteins have no modified amino acids or carbohydrates. Although there is no sequence homology between the two proteins, they are immunologically cross-reacting. The 3-dimensional structures of both proteins have been determined at 3Å resolution, and sites for cross-reacting antibody binding have been identified. The crystal structures of both proteins will be discussed in relation to the known biochemical and immunological properties.

02.1-41 STRUCTURAL STUDIES ON SERUM TRANSFERRIN: THE CENTRAL PROTEIN OF IRON METABOLISM. By S.Bailey, H.Jhoti, R.Garratt, B.Gorinsky, P.Lindley and R.Sarra, Department of Crystallography, Birkbeck College, University of London, Malet St., London WC1E 7HX, R.Evans, UMDS Division of Biochemistry, Guy's Hospital, London SE1 9RT, and S.Hasnain, SERC Daresbury Laboratory, Daresbury, Warrington WA4 4AD, United Kingdom.

The transferrins are a class of homologous two-sited iron-binding proteins, molecular weight ≈80kD, comprising serum transferrin (from blood), lactoferrin (from milk and other secretory fluids), ovotransferrin (from egg white) and melanotransferrin (a cell surface protein on human melanoma cell), [for reviews see E.H.Morgan, Mol. Aspects of Med., (1981), 4, 1-123 and J.H.Brock, in 'Metalloproteins. Part 2', Ed. P.M.Harrison, (1985), 183-261, MacMillan, London]. Although significant amino acid sequence similarities exist between the various transferrins, there are thought to be important differences in their biological functions; serum transferrin is the only member of the class whose primary function is to transport iron. Extensive biochemical and spectroscopic studies have shed comparatively little light on either the nature of the iron binding sites or the uptake, release and transport mechanisms. Structural studies should provide an insight into this important metabolic activity.

Recently, the three-dimensional structure of human lactoferrin at 3.2 Å resolution has been reported, (B.F.Anderson, H.M.Baker, E.J.Dodson, G.E.Norris, S.V.Rumball, J.M.Waters and E.N.Baker, (1987), Proc.Natl.Acad.Sci.(USA), in press). We report here the single crystal X-ray structure analysis of diferric rabbit serum transferrin at a resolution of 3.3 Å. Assuming that the key amino acid residues are conserved, the protein has a similar iron binding site geometry and overall tertiary structure to lactoferrin. The structure is bilobal with one iron binding-site per lobe, and has a pseudo two-fold axis relating the two lobes of the molecule. A similar folding pattern is observed in each lobe, consistent with a gene duplication event. The super-secondary structure of each lobe exhibits two domains of βαβ units with the helices pointing away from the iron site, which is at the inter-domain interface. Each iron atom is coordinated by four protein ligands, two tyrosine, a histidine and an aspartic acid residue. An arginine residue is also in the vicinity of each iron site and this residue and the iron may be bridged by the (bi)-carbonate ion required for iron binding.

However, EXAFS studies, (R.C.Garratt, R.W.Evans, S.S.Hasnain and P.F.Lindley, Biochem.J., (1986), 479-484), suggest that the iron is at least six-coordinated and comparative spectra on freeze-dried and solution protein samples further suggest that the sixth ligand is a water molecule, (S.S.Hasnain, R.W.Evans, R.C.Garratt and P.F.Lindley, Biochem.J., (1987), in press). This is consistent with the electron density distribution around the iron sites which clearly shows that the four protein ligands are clustered on the opposite side to the inter-domain cleft. The EXAFS data is currently being used to refine the geometry at the iron binding sites.

In the vicinity of the C-terminal lobe there is also electron density which may correspond to a single branched-chain carbohydrate moiety.

Although the overall topology of rabbit serum transferrin is similar to human lactoferrin the presence of extra disulphide bridges results in structural changes; these may account for some of the significant differences in their physiological roles.