**03-Crystallography of Biological Macromolecules**

**MS-03.06.03** CRYSTAL STRUCTURE OF PEANUT LECTIN, A PROTEIN WITH AN OPEN QUATERNARY ARRANGEMENT. By Rahul Sanjeeva, Sekhar C. Mande, V. Ganesh, Kalpan Das, V. Dhanraj, Sanjeev K. Mahanta, K. Sugana, A. Saroja and M. Vijayan, Molecular Biophysics Unit, Indian Institute of Science, Bangalore - 560012, India.

The crystal structure of the tetrameric anti-T lectin from peanut, Mr 1,10,000, has been determined by the multiple isomorphous replacement method and refined at 2.95 Å resolution. The crystal asymmetric unit contains the whole tetramer. The tertiary fold in each subunit is similar to that observed in other legume lectins. The association of monomers into dimers is four of the six known structures of legume lectins involves the formation of a 12-stranded β-sheet, six strands from each monomer. Interactions involving covalently linked sugar have been suggested to be responsible for the different modes of association found in the other two lectins. Dimerisation in peanut lectin does not involve the formation of the 12-stranded sheet, although the molecule does not contain covalently linked sugar. Thus, the structure demonstrates that differences in subunit arrangement in legume lectins could be caused by factors intrinsic to the protein molecule. The most interesting feature of the peanut lectin molecule is its quaternary structure. Unlike other well-characterised tetrameric proteins with identical subunits, the molecule has neither 222 nor fourfold symmetry. Two halves of the molecule, each half probably corresponding to the natural dimer, are related by a non-crystallographic twofold axis. The two monomers in each half are related by a local twofold axis. The mutual disposition of the axes is such that they do not meet at a closed point group.

**MS-03.06.04** THREE-DIMENSIONAL STRUCTURE OF CHOLERATOXIN'S B PENTAMER IN COMPLEX WITH ITS PENTASACCHARIDE RECEPTOR AND COMPARISON WITH THE STRUCTURE OF THE RELATED ENTEROTOXIN FROM E.COLL.


Choleratoxin (CT) and heat-labile enterotoxin of E.coli (LT) are two closely related major virulence factors of two bacterial species responsible for the death of hundreds of thousands of people annually, in particular, young children in third world countries. Both toxins are heterohexamers with a B pentamer responsible for binding to the carbohydrate moieties of the gut epithelial cells. The single enzymatic A subunit carries out an ADP-ribosylation of Gαs, causing this G protein to remain in a permanently activated state, stimulating adenylate cyclase, thereby increasing AMP levels in the cell. This leads to dehydration which, if untreated, has often fatal consequences. Healthy adults succumb to an attack of Vibrio cholerae within 6 to 8 hours.

The structure elucidation of LT showed a remarkable architecture with the B pentamer forming a ring in the shape of a crown, and the A subunit extending its terminus through the central pore of the B pentamer forming a unique association of these two components of the toxin. Lactose binding studies to LT revealed the position of the terminal galactose of the receptor GM1, a ganglioside. Recently, we have succeeded in elucidating the structure of the B-pentamer of choleratoxin complexed with the full pentasaccharide of its receptor. This confirmed the binding site of the terminal galactose and revealed that of neuraminic acid as well, plus, in one of the subunits a well-resolved density for the entire pentasaccharide. The carbohydrate binding sites are at the "convoluted" side of the B pentamer. This makes it most intriguing how the A subunit, which after binding of the toxin to the cell is pointing away from the cell surface, can be translocated across the membrane.

Choleratoxin and heat-labile enterotoxin are also some of the most potent mucosal immune stimulating proteins known to date. We are involved in the engineering and structural studies of fusion proteins where the toxin is used as a framework molecule to which epitopes from "pathogenic" proteins are attached. One such fusion protein has been crystallised as well as a mutant where an essential residue in the active site has been mutated rendering the A subunit inactive. We are planning to report on this study at the meeting.

**MS-03.06.05** CALCIUM-DEPENDENT CARBOHYDRATE RECOGNITION BY C-TYPE ANIMAL LECTINS. By William J. Weg, Kurt Eriksson, and Wayne A. Hendrickson, Department of Biochemistry and Molecular Biophysics and Edward Hughes Medical Institute, Columbia University, New York, NY 10032 USA. (Present address: Department of Cell Biology, Stanford University School of Medicine, Stanford, CA 94305).

Proteins that specifically recognize saccharide moieties on glycoproteins and cell surfaces are involved in a number of important biological processes. A remarkably diverse set of such carbohydrate-recognition proteins are the C-type lectins, which have in common a calcium-dependent and homologous domains that are responsible for carbohydrate-binding. C-type lectins include the antiileocytic proteins, which are involved in clearing desialylated proteins by endocytosis; the selectins, which target leukocytes to sites of inflammation; and mannose-binding proteins, which are involved in an antibody-independent immune defense. We have determined structures for the carbohydrate-recognition domain (CRD) of a rat mannose-binding protein (MBP). The structure has been solved both with and without a high mannose ligand. The asymmetric unit of the complex crystal contains two CRD units that bind to distinctive terminal mannose residues from the oligosaccharide. The binding of CRD showed that the C-terminal carbohydrate recognition sites are present in the CRD and that the binding of carbohydrate to the CRD involves interactions with both the sugar and the protein. One of the mannose residues is bound by one of the calcium ions, displacing a water ligand with the 3 and 4-hydroxyl oxygen atoms of the sugar. Each of these proximal oxygens also donates a hydrogen bond to a carbonyl oxygen atom of a glutamine residue and receives one from the amide group of an asparagine residue. Thus, the sugar is bound specifically to the protein by evolutionarily conserved interactions, as if lingering on two three-legged stools. The binding of the lectin here helps to rationalize the specificities observed for various C-type lectins.