03-Crystallography of Biological Macromolecules

MS–03.06.03 CRYSTAL STRUCTURE OF PEANUT LECTIN, A PROTEIN WITH AN OPEN QUATERNARY ARRANGEMENT. By Rahul Banerjee, Sekhar C. Mande, V. Ganesh, Kalyan Das, V. Dhanaraj, Sanjeev K. Mahanta, K. Suguna, A. Surolia 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 2.95Å 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 in four of the six known structures of legume lectins involves the formation of a 12-stranded ß-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 lead to 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.COLI.
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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 Gsα, causing this G protein to remain in a permanently activated state, stimulating adenylate cyclase, thereby increasing cAMP levels in the cell. This leads to dehydration which, if untreated, has often fatal consequences. Healthy adults can succumb to an attack of *Vibrio cholerae* within 6 to 8 hours.

The structure elucidations 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 GM_1, 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 know 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 crystallized as well as a mutant where an essential residue in the active site has been mutated rendering the A subunit inactive. Progress on these studies will be reported.

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§ T. K. Sixma, S. E. Pronk, K. H. Kalk, E. S. Wartna, B. A. M. van Zanten, B. Witholt and W. G. J. Hol 1991 Crystal structure of a cholera toxin-related heat-labile enterotoxin from *E. coli*. Nature 351: 371-377.
† T. K. Sixma, S. E. Pronk, K. H. Kalk, B. A. M. van Zanten, A. M. Berghuis and W. G. J. Hol 1992 Lactose binding to heat-labile enterotoxin revealed by x-ra crystallography. Nature 355: 561-564.
‡ Ethan Merritt, Steve Sarfaty, Focco van den Akker, Stewart Turley, Cécile l'Hoir, Joseph Martial and Wim G.J. Hol, submitted.

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CALCIUM-DEPENDENT CARBOHYDRATE RECOGNITION BY C-TYPE ANIMAL LECTINS. By William I. Weis,[1*] Kurt Drickamer[1,2] and Wayne A. Hendrickson[1], Department of Biochemistry and Molecular Biophysics[1] and Howard Hughes Medical Institute[2], 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-dependence and homologous domains that are responsible for carbohydrate-binding. C-type lectins include the asialoglycoprotein receptors, which are involved in clearing desialated 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 mode of binding is unlike that in other sugar-protein interactions, including other calcium-containing lectins. In the case of MBP, the mannose residues coordinate directly to one of the calcium ions, displacing a water ligand with the 3- and 4-hydroxyl oxygen atoms of the sugar. Each of these hydroxyl oxygens also donates a hydrogen bond to a carboxyl oxygen atom of a glutamate residue and receives one from the amido group of an asparagine residue. Thus, the sugar group is fixed specifically to the protein by elegant tetrahedral interactions, as if perched on two three-legged stools. The mode of binding seen here helps to rationalize the specificities observed for various C-type lectins.

