

MS04.11.02 X-RAY CRYSTAL STRUCTURES OF GALECTINS AND THEIR CARBOHYDRATE COMPLEXES.

James M. Rini¹, Amit I. Kanigsberg¹, J. Seetharaman¹, Catherine S. Kayden¹, Hakon Leffler^{2,3}, and Samuel H. Barondes². Departments of Molecular and Medical Genetics and Biochemistry¹, University of Toronto, Toronto, Ontario, M5S 1A8, Canada, and the Department of Psychiatry and Langley Porter Psychiatric Institute² and Department of Pharmaceutical Chemistry³, University of California, San Francisco, CA 94143.

The Galectins are a large family of β -galactoside binding animal lectins characterized by a conserved ~15 kDa carbohydrate recognition domain (CRD). Both intracellular and extracellular functions have been proposed for these lectins, including roles in T cell apoptosis, pre-mRNA splicing and the modulation of cell-cell and cell-matrix interactions. Although the smallest members of the family are composed of a single canonical CRD (which may or may not dimerize), others contain an additional non-carbohydrate binding domain or two CRDs linked in tandem. In addition to differences in structural organization, members of the family show differences in their oligosaccharide binding affinity and specificity. We have now solved the x-ray crystal structures of rat Gal-1, human Gal-2, the CRD of human Gal-3 and the N-terminal CRD of rat Gal-4, as well as their lactose or N-Acetyllactosamine complexes. In addition, we have solved the structure of human Gal-3 in the presence of Lacto-N-neotetraose. The structures have allowed us to not only determine the basis for some of their carbohydrate binding specificity differences, but to shed light on the structural organization of the Gal-3 and Gal-4 CRD, relative to that seen in the 2-fold symmetric dimers (Gal-1 and Gal-2).

MS04.11.03 RECEPTOR RECOGNITION, PROTEIN-CARBOHYDRATE INTERACTIONS AND A SEARCH FOR RECEPTOR ANTAGONISTS OF THE CHOLERA TOXIN FAMILY.

Wim G.J. Hol, Ethan Merritt, Focco van den Akker, Ingeborg Feil, Steve Sarfaty, Wendy Minke, and Christophe Verlinde, Howard Hughes Medical Institute, Biomolecular Structure Center, Departments of Biological Structure and Biochemistry, University of Washington, Box 357742, Seattle, Washington 98195-7742.

Cholera toxin and the closely related heat-labile enterotoxin of *E. coli* are the prime virulence factors secreted by *Vibrio cholerae* and enterotoxigenic *E. coli*. These pathogens are responsible for significant mortality during epidemics as well as occurring endemically in third world countries. The toxins recognize as receptor the pentasaccharide head group of the glycolipid ganglioside GM1 on the outer surface of epithelial cells. In collaboration with three other groups, this recognition process has been studied crystallographically by determining the structures of:

1. The cholera toxin B-pentamer complexed with GM1 pentasaccharide;
2. The heat labile enterotoxin in complex with galactose, lactose, and galactose- β 1,3-N-acetylgalactosamine (the Thomsen-Friedenreich 'T-antigen' disaccharide used in cancer diagnosis);
3. The crystal structures of mutants of the two toxins which have impaired receptor recognition properties. In particular position Gly33 is intriguing since this residue does not interact directly with the receptor and yet some, but not all, amino acid substitutions at this position affect receptor binding.

In three of the mutant structures it was discovered that the imidazole ring of a histidine of a neighboring B-pentamer is positioned above the indole ring of Trp88. This indole ring is the prime hydrophobic interaction in the GM1:cholera toxin complex. So we have two starting points for our attempts to arrive at molecules which might interfere with receptor binding: X-ray structures with sugars bound, and the mutant structures with imidazole rings in the sugar binding site.

MS04.11.04 SUBSTRATE RECOGNITION BY ENZYMES THAT RELEASE OLIGOSACCHARIDES FROM GLYCOPROTEINS.

Patrick Van Roey, Wadsworth Center, New York State Dept. of Health, Albany, NY 12201-0509, USA

Flavobacterium meningosepticum secretes four oligosaccharide releasing enzymes: three glycohydrolases, endo- β -N-acetylglucosaminidase (Endo) F₁, F₂ and F₃, and the amidohydrolase, peptide-N-(N-acetyl- β -D-glucosaminy)asparagine amidase (PNGase) F. The enzymes remove asparagine-linked oligosaccharides and are used as biochemical tools for the analysis of glycoproteins. All four enzymes have unique substrate specificities. PNGase F removes the intact oligosaccharide chain and converts the asparagine to an aspartic acid. The minimum substrate for PNGase F consists of the asparagine residue with both the carboxyl and amino groups in peptide linkage and the chitobiose core of the oligosaccharide. Endo F₁, F₂ and F₃, as well as the related Endo H, cleave the β (1-4)-glycosidic bond between the two N-acetylglucosamines of the chitobiose core. They differ strongly in their respective specificities for different oligosaccharide structures: F₁ (and H), high-mannose; F₂, biantennary; and, F₃, triantennary. Crystallographic studies of the enzymes, mutants and complexes are aimed at the analysis of the mechanisms of action and the basis for the substrate specificities of the enzymes. PNGase F is composed of two 8-stranded β -sandwich domains that are positioned side-by-side. Loops that connect the β -strands form a cleft at the interface between the two domains. Site directed mutagenesis studies combined with crystallographic analysis of the chitobiose complex have shown that this cleft contains the active site residues and the oligosaccharide binding site. The endoglycosidases are (α/β)₈-barrels. The structures of Endo F₁ and H reveal distinct features associated with the recognition of the branched high-mannose chain, the difference in tolerance for an α (1-3)-fucose on the asparagine-proximal N-acetylglucosamine and the interaction with the protein component of the glycoprotein substrate.

MS04.11.05 STRUCTURAL STUDIES ON CELLULASE-OLIGOSACCHARIDE COMPLEXES

Gideon Davies¹, Gerlind Sulzenbacher¹, Bernard Henrissat², Hugues Driguez² and Martin Schülein³. 1. Department of Chemistry, University of York, Heslington, York, YO1 5AT, Great Britain. 2. CERMAV, CNRS, BP-53, F38041, Grenoble, France and 3. Novo-Nordisk a/s, Novo allé, 2880-Bagsvaerd, Denmark.

Since the seminal structure determination of hen-egg white lysozyme over 25 years ago, the number of glycosyl hydrolase structures has blossomed. There are now over 54 sequenced-based families of glycosyl hydrolases with structural representatives for approximately 25 of these. Oligosaccharide-bound structures are somewhat more scarce. We have been studying the cellulases, enzymes which hydrolyse the β -1,4 linkages of cellulose. In particular, the endoglucanase V from *Humicola insolens*, which performs catalysis with inversion of the anomeric configuration and the endoglucanase I's from *H. insolens* and *Fusarium oxysporum* which both act with a net retention of configuration. We have obtained saccharide-bound complexes of these inverting and retaining endoglucanases by techniques including:

- mechanism-based inhibitors and suicide substrates
- active -enzyme with reaction products
- inactive-mutants plus substrates
- non-hydrolysable substrate analogues

Analysis of these structures reveals the methods utilised by these enzymes to facilitate catalysis, such as substrate distortion and favoured binding of the elongated glycosidic bond found in the transition state of the glycosyl hydrolysis. The non-hydrolysable