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Lectin are carbohydrate binding proteins which binds to sugars specifically and reversibly. Their binding to the surface of cells leads to cross-linking of glycoconjugate receptors, including glycoproteins and glycolipids, which, in many cases, is related to a variety of biological signal transduction and recognition processes including mitogenesis. We have observed that many naturally occurring cell surface oligosaccharides and synthetic analogs are multivalent and capable of binding and precipitating with specific lectins (cf. Bhattacharyya, L., Ceccarini, C., Lorenzini, R. and Brewer, C. F. (1987) *J. Biol. Chem.*, 262, 1288 – 1293). Our recent studies show that many of these lattices are crystalline, and that structural information can be obtained using electron microscopy, x-ray diffraction, and molecular modeling techniques. We have also demonstrated that similar highly organized cross-linked lattices form between glycoproteins and both plant and animal lectins including the 14 kDa β-galactoside specific lectin from calf spleen. Quantitative precipitation analyses of binary mixtures of a variety of glycoproteins in the presence of the mannose-specific lectin concanavalin A lectin provide evidence that each glycoprotein forms a unique homopolymeric cross-linked lattice with a specific lectin which excludes the lattice of other glycoproteins. Our recent quantitative precipitation studies of binary mixtures of different galactose specific plant and animal lectins including the 14 kDa β-galactoside specific lectin from calf spleen in the presence of the glycoprotein asialofetuin also demonstrates the formation of unique homogeneous cross-linked lattices between each lectin and asialofetuin. These findings thus represent a new source of binding specificity between lectins and glycoproteins; namely, the formation of homogeneous cross-linked complexes. These results are discussed in terms of the possible biological recognition properties of lectins and glycoproteins as receptors.

S.9 STRUCTURAL ANALYSIS OF GLYCOCONJUGATES: GLYCOPROTEINS

S9.1
Applications of Mass Spectrometry to Glycobiology

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High field magnetic sector double focusing Fast Atom Bombardment (FAB) mass spectrometers have played a major role in biopolymer analysis in the past 13 years and will continue to do so for the forseeable future. This lecture will review the wide range of structural problems that have been and continue to be solved using high field FAB-MS. Recently the sensitivity of this type of instrumentation has been significantly improved by array detector technology and a new mass spectrometer, the ZAB-2SE FPD, which incorporates a wide angle (1.5: 1) multichannel array focal plane detector for high sensitivity, low resolution survey analysis up to 15 kilodalton has now been installed in our laboratory. A second array (1.05: 1) permits narrow angle high resolution analysis. The performance of this instrument and its potential for solving the next generation of structural problems in glycobiology will be assessed.

A powerful complementary method for analysing intact macromolecules, Electrospray Mass Spectrometry (ES-MS), was first introduced several years ago and is now being successfully applied to biopolymer analysis in many laboratories. This talk will show how we are addressing structural problems in glycobiology using a combination of FAB-MS and ES-MS strategies which have been optimised for high sensitivity production of molecular and fragment ions (1,2). These afford information on degree of heterogeneity, sites of glycosylation, glycoform compositions, branching patterns, sequences, functional groups and, in certain cases, data on linkage positions and anomeric stereochemistry. Current achievements with these methodologies will be illustrated with data from three broad classes of structural problems: (i) glycoproteins including *O*-linked GlcNAc glycoproteins (collaborations with G. W. Hart, Johns Hopkins and R. Treisman, ICRF London), (ii) the glycocalyx of *Mycobacteria,* (collaboration with M. McNeil and P. Brennan, Fort Collins), and (iii) glycosaminoglycan oligosaccharides (collaborations with U. Lindahl, Uppsala and K. Sugahara, Kobe).

In our studies of a variety of glycoproteins and glycopeptides including the 16 K fragment of pro-opiomelanocortin (POMC) (collaboration with H. P. J. Bennett, Montreal), excretory/secretory glycoproteins from nematodes (collaborations with R. Maizels and M. Selkirk, Imperial College; W. Harnett, Glasgow; and J. Appleton (Cornell)), and several cathepsin enzymes (collaboration with M. Fusek, Prague and M. Baudys, Utah), we have employed a strategy based on ES-MS analysis of intact glycoproteins, glycopeptides and peptides, FAB-MS analysis of derivatised oligosaccharides and GC-MS linkage analysis. We have defined novel mono-sulphated *N*- and *O*-linked oligosaccharides in POMC including a biantennary structure which is capped on one antenna with sulphate and on the second with fucose (3). The major glycan present in the acidic *O*-glycan fraction is a tetrasaccharide containing a sulphated GalNAc residue. In a study of site occupancy in the cathepsins we have defined the glycoforms of bovine cathepsin D and shown that fucosylation of the core region of high mannose and truncated structures is confined to the second of the two glycosylation sites. The truncated *N*-glycans which are common in the cathepsins are also dominant structures in all the nematode glycoproteins that we have examined to date.

FAB-MS is a very powerful tool for characterising the complex carbohydrates present in the glycocalyx of *Mycobacteria* (4). We have developed strategies based on derivatisation together with hydrolysis and methanolysis which we are using to characterise glycopeptidolipids (GPL), lipooligosaccharides (LOS), lipoarabinomannans (LAM) and arabinogalactans (AG) derived from several species of *Mycobacteria.* Derivatisation also underpins our glycosaminoglycan studies