and porcine fibrinogen. These were derivatized by attaching t-BOC-tyrosine to the β-glycosylamine of the reducing GlcNAc residue. The tyrosinated-oligosaccharides were resolved into single structures on RP-HPLC and characterized by high-field proton NMR. The non-reducing end residues of asialo-biantennary and triantennary oligosaccharides were removed sequentially with β-galactosidase and β-*N*-acetyl glucosaminidase. In addition, α2,6-sialyltransferase was used to replace terminal sialic acid residues on terminal galactose residues. These modifications provided an array of related triantennary and biantennary oligosaccharide structures differing in their non-reducing end sugars.

The pharmacokinetics and biodistribution of each tyrosinated-oligosaccharide was studied in mice. Radiolabeling was accomplished by iodination of tyrosine with ^{125}I. The oligosaccharides' half-lives and clearance rates were analyzed by dosing and sampling from the jugular vein. Each oligosaccharide's biodistribution was determined by cryosectioning mice and performing whole body autoradiography on sections from multiple depths to reveal the amount of radiolabel in each organ.

Comparison of the serum half-life and biodistribution of oligosaccharides differing in number of antenna and/or their non-reducing end residues indicates that certain *N*-linked oligosaccharides are taken-up into organs by receptor (lectin) mediated endocytosis. Oligosaccharides demonstrating narrow biodistribution may be suitable for further development as targeted drug delivery carriers. Furthermore, this approach holds promise to reveal the location and ligand specificity of endogenous mammalian lectins.

S8.14

Sequence and Post-Translational Processing of *Bowringia mildbraedii* Agglutinin

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Bowringia mildbraedii agglutinin (BMA) is a plant lectin which identifies isomers of kinetically early intermediates of oligosaccharide processing. Moderate affinity binding of hybrid and bi-antennary glycans to BMA shows many features in common with those required for high affinity binding to Con A. The aminoacid sequences of the subunits of the lectin BMA from seeds of *Bowringia mildbraedii* have been determined. The lectin consists of a precursor polypeptide of M_r ~29,000 that is cleaved almost completely into two fragments of M_r ~13,300 (α subunit) and M_r ~11,900 (β subunit) respectively. The β subunit represents the *N*-terminal half of precursor polypeptides and is disulphide linked in a ββ dimer in the nature $(\alpha\beta)_2$ protein. BMA shows extensive aminoacid sequence homologies with Con A and other legume lectins. The site of post-translational proteolysis of the putative precursor occurs as a position similar to that identified in lectins obtained from other Sophoreae plants such as *S. japonica* and in *Diocleae* lectins such as Concanavalin A, but different from that of two chain lectins obtained from other tribes of the *Papilionaceae*. BMA is a rare plant even in indigenous regions of West Africa. Seeds were germinated at Plant Science Ltd., Sheffield, U.K. and small quantities of stem and leaves were obtained. Genomic DNA was isolated from the leaves and DNA coding for BMA was amplified by polymerase chain reaction (PCR). A product of 0.6 Kb (⅔ of full length clone) was obtained, cloned into vector PMX 163 and sequenced by the dideoxy method. This product had an additional 75 nucleotides (25 aminoacids) between the β and α subunits sequences and supported the prediction of a post-translational processing in BMA biosynthesis. By changing the oligonucleotide primers, a full length product of 1 Kb was obtained and cloning is in progress.

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S8.15

Complementary DNA Sequence and Organization of the Gene of Conglutinin, a C-Type Mammalian Lectin Containing Collagen-Like Domain

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Conglutinin is a unique bovine plasma protein which mediates the agglutination of the sensitized erythrocyte-solid phase iC3b. It is a Ca^{2+}-dependent serum lectin specific for *N*-acetylglucosamine with overall structural similarity to other animal C-type lectins containing collagen-like domain (1,2). In the present study, we characterized the cDNA structure and organization of the gene of conglutinin to shed light upon its evolutional origin and biological function. With the polymerase chain reaction technology, the full-length conglutinin cDNA (1550 bp) structure was determined including a 5′-non-coding region (245 bp and 160 bp, generated by alternative splicing) and a 3′-non-coding region (192 bp). The coding region consisted of the signal sequence encoding a hydrophobic peptide of 20 amino acids and the sequence encoding the 351 amino acids of the mature protein, which were in good agreement with those determined by Lee, Y.-M. *et al.* (2) using the protein chemistry method, except for one amino acid residue. The coding region of conglutinin mRNA is encoded by seven exons. Exon I (5′-non-translating (3 bp) and 199 bp) encodes the signal peptide, a cysteine-rich NH_2-terminal region and six repeats of Gly-X-Y motif. The remaining long collagen-like domain (49 Gly-X-Y repeats) was encoded by four separate exons (Exon II~V) of almost equal lengths (117 bp and 108 bp). The neck region and the carbohydrate-recognition domain were encoded by exon VI (84 bp) and VII (380 bp and 3′-non-translating), respectively. The exon-intron boundaries of conglutinin are very similar to those of other C-type collagen-like domain-containing lectins, rat serum and liver, and human serum mannan-binding proteins, suggesting that these lectins are derived from a common ancestral gene.

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(2) Lee, Y.-M. *et al.* (1991) *J. Biol. Chem.*, **266**, 2715 – 2723.

S8.16

Studies on the Molecular Recognition of the Galβ(1,4)Glc(GlcNAc) Sequence by the Soluble 14-kDa Bovine Heart Lectin