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Reports of meetings

9th Annual Symposium on Biotechnology: The New Biology of Carbohydrates. University College London Medical School, 16–17 December 1993

This meeting was organized by Ten Feizi for the Institute of Biology and Biotechnology. The first session on Biosynthesis was chaired by Winifred Watkins and Ulf Lindahl. Harry Schachter opened the session with a report on the *N*-acetylglucosaminyltransferases involved in *N*-glycan branching. The gene encoding human *N*-acetylglucosaminyltransferase II (MGAT2) has been cloned and expressed in the baculovirus/Sf9 insect cell system (J. Tan, G. A. F. D'Agostaro, B. K. Bendiak, J. Squire and H. Schachter). The mouse *N*-acetylglucosaminyltransferase I gene (*Mgat-1*) has been inactivated in the transgenic mouse model (M. Metzler, A. Gertz, M. Sarkar, H. Schachter, J. W. Schrader and J. D. Marth). The homozygous *Mgat-1*$^-$/*Mgat-1*$^-$ genotype embryo did not survive past 10.5 days of development providing strong evidence that complex *N*-glycans are essential for normal embryogenesis. The ratio of high-mannose to complex *N*-glycans in the *Mgat-1*$^-$/*Mgat-1*$^-$ embryos was increased and the embryos were smaller and had various structural abnormalities.

Dirk van den Eijnden discussed several substrate-level controls operative in two biosynthetic pathways involved in the synthesis of *N*-glycan antennae and which he has named the *N*-acetyllactosamine (LacNAc) and diacetyllactosamine (LacdiNAc) pathways. LacNAc and LacdiNAc antennae are initiated by Galβ1-4GlcNAc and GalNAcβ1-4GlcNAc respectively. A GalNAc-transferase capable of synthesizing the GalNAcβ1-4GlcNAc moiety has been described in a schistosome and a snail (*Lymnaea stagnalis*). Preliminary attempts to clone the gene for the snail GalNAc-transferase were described.

John Lowe gave a progress report on the cloning of several human and murine fucosyltransferase genes. He reported that the genes for the blood group H-dependent α2-fucosyltransferase from humans with a hh/Sese or hh/SeSe genotype (para-Bombay) or with a hh/sese genotype (Bombay) had stop codons in the normal open reading frame sequence. These people lack an active H-dependent α2-fucosyltransferase and cannot make red blood cell H antigen. Lowe also found a secretor-dependent open reading frame homologous to the H-dependent α2-fucosyltransferase gene downstream of the H gene but has not as yet expressed this putative Se gene. Lowe has now cloned five human α3-fucosyltransferase genes named Fuc-transferases III to VII. These are grouped into three families according to their structural similarities and chromosomal localizations (III, V and VI on chromosome 19; IV on chromosome 11; VII on chromosome 9). The properties and tissue distributions of these enzymes were discussed.

Gerald Hart reviewed his work on the *O*-linked GlcNAc residues (to Ser or Thr) found on a variety of intracellular cytoplasmic and nuclear proteins. He reviewed the ever-increasing body of evidence which suggests that certain amino acid residues on a variety of phosphoproteins can exist either in the phosphorylated or glycosylated state. The O-GlcNAc residue may therefore play an important regulatory role. Hart discussed the role of O-GlcNAc residues on RNA polymerase II, transcription factors, neurofilament protein, oncogene proteins and estrogen receptors. The GlcNAc-transferase which makes the O-GlcNAc structure and a neutral cytosolic O-N-acetylglucosaminidase have been purified.

Jeffrey Esko described studies on the factors which determine the chain type and fine structure of glycosaminoglycans synthesized in animal cells. Mutant cells deficient in xylosyltransferase were fed various β-xylosides which acted as primers for chondroitin sulphate and heparan sulphate assembly. He found that the β-xyloside aglycone had a strong influence on the relative rates of synthesis of the two glycosaminoglycans. Esko also carried out site-directed mutagenesis at six potential glycosaminoglycan attachment sites of rat betaglycan (TGF-β Type III receptors). Expression of the mutated core proteins in Chinese hamster ovary cells showed that only two of the sites were glycosylated and identified a neighbouring Trp residue essential for heparan sulphate assembly.

Michael Ferguson discussed the complex biosynthetic pathway for the glycosylphosphatidylinositol (GPI) membrane anchor in the sleeping-sickness parasite *Trypanosoma brucei*. The variable surface glycoprotein (VSG) of this parasite has a GPI anchor. VSG provides an essential defence system for the trypanosome. The de-*N*-acetylase which converts phosphatidylinositol-bound GlcNAc to phosphatidylinositol-bound glucosamine has been purified. The inhibition of GPI anchor synthesis by mannosamine has been shown to be due to the incorporation of mannosamine into an inhibitory analogue.

The second and third sessions on Protein-Carbohydrate Interactions were chaired respectively by Colin Hughes, Michael Ferguson, Helen Muir and Ten Feizi. Ten Feizi reviewed the utility of using neoglycolipids to decipher the oligosaccharide 'area codes' recognized by a variety of endogenous lectins. Feizi discussed the use of this technique for determining the ligands recognized by selectins.

Steven Rosen described the natural ligands present on lymph node high endothelial venules (HEV) which are recognized by L-selectin on leukocytes. These ligands are two sulphated, fucosylated and sialylated glycoproteins (about 50 and 90 kDa) with a large number of *O*-glycan chains. The gene for the 50 kDa protein (GlyCAM-1) has been cloned. The 90 kDa protein (Sgp90) has been shown to be CD34. The sulphate moieties on both these proteins are essential for binding both to L-selectin and to an antibody known as MECA 79 which stains lymph node HEV.

Brian Brandley discussed the potential use of analogues of the oligosaccharide ligand of E-selectin (sialyl-Lewis X) as anti-inflammatory agents. The initial strategy was to replace sialic acid with sulphate, acetate or lactate groups. Three animal models of acute lung injury were used to show that some of these analogues had beneficial anti-inflammatory effects *in vivo*.

Samuel Barondes discussed the Galectin family, a family of soluble galactoside-binding lectins which are also called S-lectins. Four galectins have been well-characterized, cloned and expressed: galectin-1 (L-14-I), galectin-2 (L-14-II), galectin-3 (L-29) and galectin-4 (L-36). Less is known about galectin-5 (L-18). Barondes reported the crystal structure of galectin-2 which was determined in collaboration with Jim Rini. Galectins are secreted in an atypical manner which does not involve the endoplasmic reticulum or Golgi apparatus. Blebs form under the plasma membrane and burst to release galectin molecules to the extracellular space. Transgenic mice with null mutations of the L-14 gene show a normal phenotype and the functions of these interesting proteins remain unknown.

Thomas Rademacher reported that some growth factors trigger the 'flash activation' of a glycan-specific phospholipase which releases extracellular inositolphosphoglycans that enter the cell to mediate signal transduction. Rademacher suggested that insulin may act via this second messenger pathway. The structural characterization of the inositolphosphoglycans is under way.

Ulf Lindahl discussed the mechanism whereby heparan sulphate binds basic fibroblast growth factor (bFGF). Using *in vitro* binding assays, Lindahl showed that the 2-O-sulphate group on iduronate and the *N*-sulphate group on glucosamine are essential for binding to bFGF but that the 6-O-sulphate group on glucosamine can be removed without affecting binding. A minimal pentasaccharide binding sequence was identified. A dodecasaccharide heparin fraction containing *N*-sulphate, 2-O-sulphate and 6-O-sulphate groups was reported to be the minimal structure required for promotion of the mitogenic activity of bFGF.

Klaus Bock discussed some of the uses for synthetic glycopeptides, e.g. the effect of the peptide moiety on oligosaccharide conformation and the ability of the peptide-bound oligosaccharide to serve as a substrate for glycosyltransferases. He then described a semi-automated solid-phase synthesis procedure that has been used in his laboratory for the relatively rapid synthesis of glycopeptides. He illustrated the utility of this technology with a study of the binding requirements of the mannose-6-phosphate receptor. The study showed that a synthetic pentapeptide with two mannose-6-phosphate groups was an excellent inhibitor of oligosaccharide binding to the receptor.

Raymond Dwek discussed several examples in which glycosylation is relevant to disease. He reported the correlation between the presence of under-galactosylated glycoforms of IgG and the severity of the disease in human rheumatoid arthritis and in an animal model of arthritis (collagen-induced arthritis). Inhibition of the α-glucosidases involved in *N*-glycan processing has been shown to cause an increase in the amount of high mannose *N*-glycans on gp120 from the human immunodeficiency virus (HIV) and also to prevent some of the biological effects of HIV on lymphocytes *in vitro*. Different glycoforms of tissue plasminogen activator vary in their fibrinolytic activities. Glycosylation appears to play a role in antigen presentation in Class I MHC-antigen complexes.

Dianna Bowles reviewed several examples in which the information content of glycans plays a signalling role in plants. The glycans may originate from the plant or from pathogenic or symbiotic organisms that interact with the plant. These signalling glycans are often oligosaccharides formed by enzyme-induced fragmentation of larger polysaccharides. The signals mediated by these glycans may function in the defence of the plant against infection, or may control the growth and development of the plant, or may mediate a symbiotic relationship. Jean-Claude Promé described the highly species-specific interactions that occur between legumes and symbiotic rhizobia in the process of atmospheric nitrogen fixation. The conversion of nitrogen into ammonia requires the formation of a specialized plant organ called the nodule. The symbiotic process eventually results in the activation of a set of rhizobial genes called the nod genes which promote the biosynthesis of sulphated lipo-oligosaccharide signals called the Nod factors. The structures of many of these oligosaccharides have been determined. They are specific both for the plant and the rhizobium and induce nodule formation.

Session 4 on the Crystallography of Protein-Carbohydrate Recognition was chaired by Nobel Laureate Sir Aaron Krug. Detailed structures were presented for several carbohydrate-binding proteins and their complexes with carbohydrate ligands, as follows: Christian Cambillau

(*Lathyrus ochrus* lectin), Boaz Shaanan (*Erythrina corallodendron* lectin), Christine Wright (Wheat Germ Agglutinin), Kurt Drickamer (mannose-binding protein, a C-type lectin), Osnat Herzberg (Galectin-1, an S-type lectin), David Bundle (four bacterial polysaccharide-specific antibodies), and Tom Blundell (pentameric serum amyloid P component, a carbohydrate-binding pentraxin). It is beyond the scope of this brief summary to describe the detailed structural properties of these various proteins. One common theme is that the carbohydrate epitope in direct contact with the protein is usually quite small (three residues or less) and that water molecules play an important role in mediating the interaction between the oligosaccharide and the protein. To quote Bundle, 'water is a multiplier of hydrogen bonds'. However, Bundle reported an unusual antibody that requires nine sialic acid residues for binding. Another interesting finding is that although there is no obvious primary sequence homology between the galectins and the legume lectins, it is possible to superimpose the three-dimensional structures of either galectin-1 or -2 on either *Lathyrus ochrus* lectin or pea lectin. Shaanan reported that the *N*-glycan on *Erythrina corallodendron* lectin was seen very clearly due to stabilization of the oligosaccharide conformation by interaction with the protein. This type of stabilization is believed to play an important role in glycosyltransferase substrate specificity. Finally, the specificity of a lectin for its carbohydrate ligand is controlled by a relatively small number of amino acids and can be experimentally manipulated by site-directed mutagenesis.

Harry Schachter

Glycobiology Group Colloquium on Advances in Structural Glycobiology, in honour of Professor Akira Kobata, held during the 649th meeting of the British Biochemical Society, Imperial College, London, December 20 1993.

The Colloquium was organized by Anne Dell, R. Colin Hughes and H. R. Morris. The meeting opened with a warm tribute by Colin Hughes to Akira Kobata and his many contributions to glycobiology. Akira Kobata gave the opening lecture on the use of immobilized lectin columns for the fractionation and structural analysis of oligosaccharides. Kobata reviewed the specific carbohydrate ligand requirements for several lectins: concanavalin A (Con A), the phytohaemagglutinins (E_4-PHA and L_4-PHA), *Datura stramonium* agglutinin (DSA), *Aleuria aurantia* lectin (AAL), *Allomyrina dichotoma* lectin (Allo A-II) and *Psathyrella velutina* lectin (PVL). He illustrated the use of some of these lectins in the fractionation of IgG oligosaccharides obtained by hydrazinolysis. PVL detects terminal GlcNAc and can be used in an ELISA assay for under-galactosylated IgG in rheumatoid arthritis patients.

H. R. Morris gave a clear and concise over-view of the various mass spectrometry methods that are available at Imperial College. He gave many examples of the use of ES-MS to detect very small differences in protein molecular weights and of both ES-MS and FAB-MS for the analysis of oligosaccharides. He ended his talk with a description of a new MS instrument designed at Imperial College which uses focal plane detection with a maximum wide angle array detector. This instrument has a femtomole sensitivity and a 15 kDa mass range.

Hans Vliegenthart reviewed the use of proton-NMR for the structural analysis of both *N*- and *O*-glycans. He pointed out that with the available instrumentation this technique has a nanomole sensitivity. Several interesting new structures were reported. For example, human urinary plasminogen activator has a single *N*-glycan with a GalNAcβ1-4GlcNAc antenna that can be substituted with Fucα1-3GlcNAc, 4-O-sulphate-GalNAc or NeuNAcα2-6GalNAc moieties. This protein also has an *O*-linked fucose residue. Vliegenthart gave several examples of extreme oligosaccharide micro-heterogenity which caused great difficulties in fractionation, i.e., recombinant erythropoietin produced in Chinese hamster ovary cells, equine chorionic gonadotropin and porcine zona pellucida glycoproteins.

John T. Gallagher briefly reviewed the structure and function of the heparan sulphates and pointed out that these glycosaminoglycans were probably involved in the control of cell growth and cell adhesion. He used heparitinase to obtain sulphated fragments of heparan sulphate and used an affinity column carrying basic fibroblast growth factor (bFGF) to isolate heparan sulphate fragments which bind bFGF. He identified a 14-residue oligosaccharide which had a high affinity for bFGF. Activation of bFGF was optimal with a 14–16 residue oligosaccharide and 2-O-sulphate iduronate and *N*-sulphate glucosamine were essential for activity. Fragments smaller than 10 residues were not effective in activating bFGF.

Peter Albersheim reviewed the structures of five plant cell wall polysaccharides that are present in all plants: rhamnogalacturonan I, rhamnogalacturonan II, homogalacturonan, xyloglucans and arabinoglucans. Although rhamnogalacturonan II is small (molecular weight of 4600), it has an extremely complex structure with 11 different sugars in a variety of linkages. Whereas there does not appear to be any variation in the structure of rhamnogalacturonan II from one plant to the next, fragmentation of xyloglucans from different plants shows different patterns. There is evidence to indicate that the fragmentation of these ubiquitous plant polysaccharides releases oligosaccharides required for the control of plant growth and development and in defence against infection. For example, plants produce endoglucanases which release anti-fungal oligosaccharides whereas fungi produce endoglucanase inhibitors.

Richard Cummings reported studies on the substrate specificity of GlcNAc-transferase V. Although only certain

specific glycoproteins such as the LAMPs (lysosome associated membrane glycoproteins) carry GlcNAc-transferase V-initiated antennae and associated poly-N-acetyllactosamine chains, Cummings concluded that the polypeptide moiety of the glycoprotein substrate does not directly determine GlcNAc-transferase V substrate specificity. He suggests rather that steric accessibility may be an important control factor. Cummings also discussed the ability of the S-type lectin L-14 to recognize poly-N-acetyllactosamine chains and described studies on the cloning, biosynthesis, subcellular distribution and atypical secretion of Chinese hamster ovary L-14.

Harry Schachter