9 Use of Complex Carbohydrates as Targets and Ligands for Imaging

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9.1 Introduction

Complex carbohydrates were long considered to be biological compounds that lack any biological specificity, serving merely as structural components of cells, of the extracellular matrix, and of connective tissues, as mucins on epithelial surfaces, or as a source of energy. Only within the last three decades has it become evident that complex carbohydrates form a highly diverse and complex class of biomolecules that are almost ubiquitous in any living organism and that mediate a large number of biological functions, ranging from purely structural roles to such complex processes as cell–cell interactions operative in the immune system or during embryonic development (for selected monographs and reviews see Rademacher et al. 1988; Kobata 1992; Varki 1993; Lis and Sharon 1993; Fukuda and Hindsgaul 1994; Montreuil et al. 1995). Moreover, inherited and acquired carbohydrate disorders have been discovered to play key roles in the etiology and progression of various types of disease, including metabolic disorders, immunological diseases, infections, and malignancy (selected references: Kornfeld 1986; von Figura and Hasilik 1986; Hakomori 1989; Etzioni et al. 1992; Furukawa and Kobata 1991; Muramatsu 1993; Ofek and Sharon 1990; Jaeken et al. 1993). Apart from contributing to the understanding of basic pathological mechanisms, studies on glycosylation changes in disease have led to the discovery of distinct carbohydrate structures as diagnostic markers of disease processes and to the development of novel diagnostic procedures and of new diagnostic and therapeutic agents (Young et al. 1991; Turner 1992; Muramatsu 1993; Mulligan et al. 1993; Parekh and Edge 1994). It is the aim of this chapter to briefly review the structures and functions of complex carbohydrates, of glycosylation changes in disease, and to describe the use of carbohydrate epitopes as targets and ligands for diagnostic and therapeutic agents.

9.2 Structures, Classification, and Distribution of Complex Carbohydrates

Complex carbohydrates are characterized by a high degree of structural diversity, which is one of the molecular bases for their biological functions (Fig. 1). The first source of structural diversity derives from the

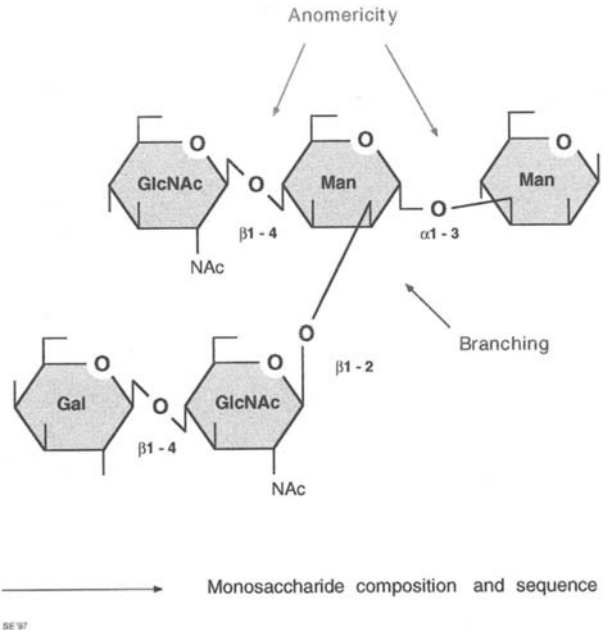

Fig. 1. Determinants of structural variability of complex carbohydrates

chemical nature of the oligosaccharide monomers in that monosaccharides can be arranged in various compositions and sequences within an oligo- or polysaccharide. For a long time, oligo- and polysaccharides were thought to be constituted by D-galactose, D-mannose, D-glucose, L-fucose, D-xylose, L-arabinofuranose; the amino sugars *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine; and sialic acids. Novel analytical methods have led to the discovery of additional monosaccharides which are components of complex carbohydrates (for review, see Vliegenthart and Montreuil 1995). As an example, according to present knowledge, the group of sialic acids that has pivotal biological functions comprises 35 different members (Schauer 1991). Secondly, as monosaccharides usually have three or four free hydroxyl groups, the oligosaccharide monomers may be linked to each other at different positions. In addition to different linkages between sugars arranged in a linear se-

Table 1. Types of glycoconjugates in eukaryotic cells

Type	Glycans	Distribution
Glycoproteins	*N*-and *O*-linked mono-, di- and oligosaccharides	
Plasma/serum glycoproteins		Plasma/serum, extracellular fluids, effusions
Membrane glycoproteins		Cell surface membrane and intracellular membranes
Matrix glycoproteins		Connective tissues, extracellular matrix
Glycoprotein hormones		Plasma/serum
Cytoplasmic/nucleoplasmic glycoproteins		Cytoplasm, nucleus
Proteoglycans	Glycosaminoglycans	Connective tissue, extracellular matrix, cell surface membrane
Glycolipids	Mono-, di-, oligosaccharides	
Cerebrosides Sulfatides Gangliosides		Cell membranes

quence, this raises the possibility of branching (Fig. 1). Thirdly, since *O*-glycosidic linkages may be either in α or β position, each disaccharide can exist in two anomeric conformations. The structural diversity is even extended by a significant but limited motional flexibility of oligosaccharide conformation (Homans 1995). As a result the various monosaccharides in complex carbohydrates, at least theoretically, may exhibit a much greater structural variability than proteins or nucleic acids and may encode biological information.

Although an astronomical number of carbohydrate structures are theoretically conceivable, certain restrictions have been observed which form the basis for several levels of classification. Complex carbohydrates are normally linked covalently to proteins and lipids, constituting the family of glycoconjugates (Table 1). In *glycoproteins* mono-, di-,

oligo-, or polysaccharides are covalently bound to a protein. Individual glycoproteins often carry several carbohydrate units which can be attached at different positions of the polypeptide by either *N*-linkage or *O*-linkage (Vliegenthart and Montreuil 1995). *Proteoglycans* are a subclass of glycoproteins distinguished by the nature, quantity, and arrangement of their sugar side chains, which are termed glycosaminoglycans. Glycosaminoglycans are much longer (100–200 monosaccharide residues) than glycans in other glycoproteins and form long, unbranched chains composed of characteristic disaccharide repeats. Glycosaminoglycans contain anionic residues such as sulfate and uronic acid, giving them a highly negative overall charge. With respect to the basic disaccharide units four different types of glycosaminoglycans can be distinguished: (1) hyaluronan, (2) chondroitin and dermatan sulfate, (3) heparan sulfate and heparin, and (4) keratan sulfate. Except for hyaluronan are all found attached to proteins. Although composed of repetitive motifs, glycosaminoglycans of a given proteoglycan exhibit marked heterogeneity within as well as between the individual oligosaccharide chains. This is caused by sulfate substitution and epimerization (Kjellen and Lindahl 1991).

The *glycolipids* constitute a second class of glycoconjugates. Glycolipids are constituents of the outer surface of the plasma membrane, but are also present in distinct intracellular membrane compartments. In glycolipids of animal cells one glycan composed of D-galactose, D-glucose, *N*-acetyl-D-galactosamine and *N*-acetylneuraminic acid is linked to ceramide. Simple glycolipids with only one neutral sugar residue forming their headgroup are called cerebrosides. The most complex of the glycolipids are gangliosides, with their glycans containing one or more negatively charged sialic acid residues (for review, see Hannun and Bell 1989; Hakomori 1990).

Glycosylphosphatidylinositol (GPI) membrane anchors represent a third, smaller class of glycoconjugates. They are complex glycophospholipids covalently bound to the C-terminus of a variety of externally oriented plasma membrane proteins (Cross 1990; Ferguson and Williams 1988). The GPI moiety is partly embedded in the outer surface of the plasma membrane and serves to anchor various proteins to the external surface of eukaryotic cells. GPI-anchored proteins include cell-specific antigens, parasite coat proteins, enzymes, cell adhesion molecules, receptors, and transporters. Although, apart from the anchor-

ing function, the exact biological role is not yet known, it is assumed that the GPI anchor may be related to transmembrane signaling, sorting to the apical surface of polarized epithelial cells, and clathrin-independent endocytosis via caveolae. GPI anchors are composed of a lipid portion and a tetrasaccharide which is linked glycosidically to the 6-hydroxyl group of phosphatidylinositol. The terminal sugar is linked via a phosphodiester bond to ethanolamine, which in turn is linked to the α-carboxyl group of the C-terminal amino acid of the protein. GPI anchors exhibit species- and tissue-specific variations in the lipid portion and in sugar substituents joined to the tetrasaccharide core. These additional sugars give rise to a structural diversity in the carbohydrate moiety of GPI anchors.

This chapter will focus on complex carbohydrates attached to the peptide chains of "classical" glycoproteins by either *N*-glycosyl linkages to asparagine or by *O*-glycosyl linkages to serine, threonine, hydroxylysine, or hydroxyproline. "Classical" *N*-linked oligosaccharides have an *N*-acetylglucosaminyl-asparagine bond, with the *N*-acetylglucosaminyl residue being the innermost sugar of a common pentasaccharide core unit Manα1–6(Manα1–3)Manβ1–4GlcNAcβ1–4GlcNAc (Fig. 2). Different mono- and oligosaccharides may be attached to this core. According to the type of the branches linked to the trimannosyl core, three different types of *N*-linked glycans are distinguished (for references, see Kobata 1992; Vliegenthart and Montreuil 1995). Oligomannosyl or high mannose type glycans only have D-mannose residues linked α1–3, α1–6, or α1–2 to the trimannosyl core. By contrast, complex-type glycans have outer antennae composed of *N*-acetyl-D-glucosamine, D-galactose, L-fucose, and sialic acid. Due to branching at the two α-mannosyl residues of the trimannosyl core, complex-type glycans may have up to five outer branches or antennae (Fig. 2). This basic structural diversity of complex *N*-glycans is extended by (a) repeating disaccharide *N*-acetyllactosamine units Galβ1–4GlcNAcβ1–3 in the outer antennae, giving rise to so-called poly-*N*-acetyl lactosamine structures, (b) branching of the *N*-acetyllactosamine repeats to form Galβ1–4GlcNAcβ1–6 (Galβ1–4GlcNAcβ1–3)Gal structures, and (c) numerous variations of the outer antennae, including sulfation and presence of *N*-acetyl-D-galactosamine. A third type of *N*-glycans, termed the hybrid type, has outer branches of the complex type linked to the Manα1–3 arm and D-mannose residues linked to the Manα1–6 arm of

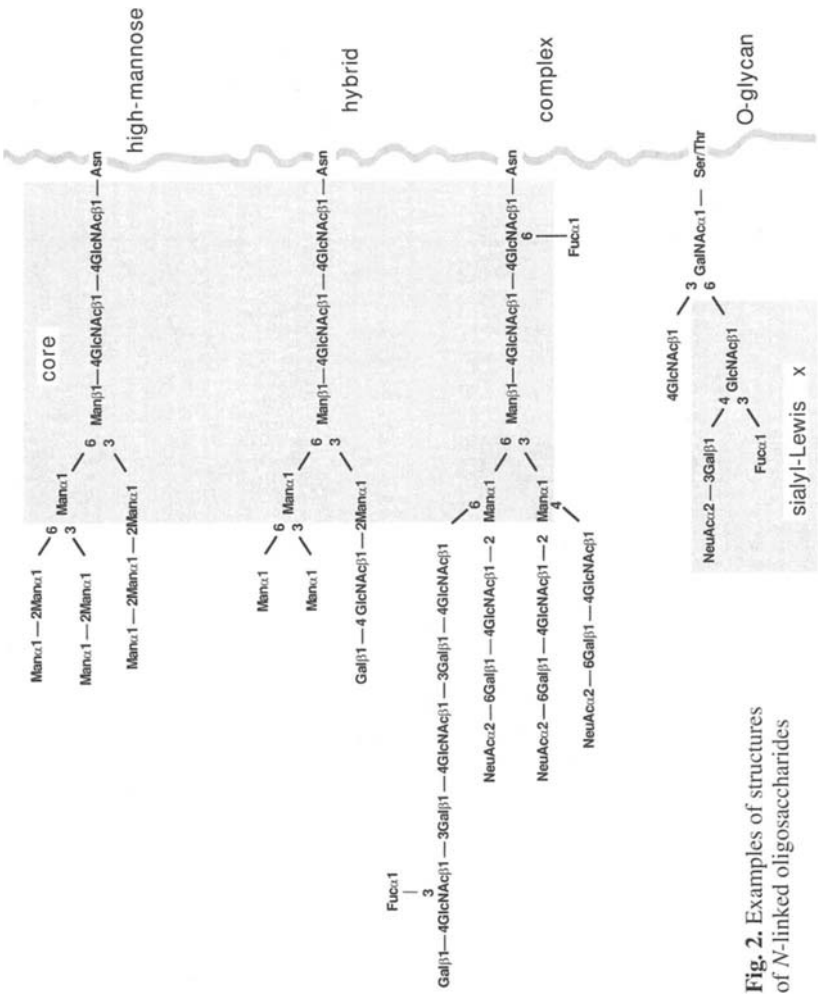

Fig. 2. Examples of structures of *N*-linked oligosaccharides

the trimannosyl core. Frequently, two additional substitutions (d) L-fucose α1-6-linked to the innermost *N*-acetyl-D-glucosaminyl residue of the common pentasaccharide and (e) a "bisecting" *N*-acetyl-D-glucosaminyl residue β1-4-linked to the β-mannosyl-residue of the trimannosyl core are found in both the complex- and hybrid-type *N*-glycans (Fig. 2).

"Classical" *O*-linked glycans attached to serine or threonine residues can be constituted by a single monosaccharide GalNAc-Ser/Thr, disaccharides such as NeuAcα2–6GalNAc-Ser/Thr, or larger oligosaccharides containing D-GalNAc, D-Gal, D-GlcNAc, L-fucose, and sialic acid. Although *O*-linked glycans lack a common core structure, several cores such as (a) Galβ1–3GalNAc-Ser/Thr, (b) GlcNAcβ1–6(Galβ1–3)GalNAc-Ser/Thr, (c) GlcNAcβ1–3GalNAc-Ser/Thr, and (d) others are frequently found on *O*-glycosylated glycoproteins (for review, see Schachter and Brockhausen 1992). These di- or trisaccharide cores can be extended by attaching D-galactose and *N*-acetyl-D-glucosamine residues and by addiing D-galactose, *N*-acetyl-D-galactosamine, L-fucose, and sialic acid as terminal sugars. Apart from these "classical" structural groups of *N*- and *O*-linked glycans, several novel carbohydrate structures have been identified on glycoproteins, including (a) glycosaminoglycans *N*-linked to asparagine on cell surface glycoproteins (Roux et al. 1988), (b) 4-(SO_4) *N*-acetyl-D-galactosamine terminal on *N*-linked oligosaccharides of glycoprotein hormones (Green et al. 1986), (c) *O*-linked-L-fucose and D-glucose on glycoproteins having epidermal growth factor (EGF) homology domains such as tissue plasminogen activator (Harris and Spellman 1993), (d) *O*-linked D-glucose with two additional D-xylose residues (Nishimura et al. 1989), (e) *N*-acetyl-D-galactosamine linked via a phophodiester linkage to complex-type *N*-glycans (Hayes and Varki 1993), and (f) *N*-acetyl-D-glucosamine *O*-linked to serine or threonine of cytoplasmic or nucleoplasmic proteins (Hart et al. 1989).

The biological importance of complex carbohydrates is indicated by their widespread distribution in the organism and by the prevalence or even exclusive presence of distinct types of complex carbohydrates at certain histological and subcellular sites. Extracellular glycoproteins are present in the different tissues and compartments of the organism, including (a) the majority of plasma/serum proteins glycosylated with *N*-linked oligosaccharides, (b) glycoproteins and proteoglycans of con-

nective tissues and of the extracellular matrix in all parenchymal tissues, and (c) *O*-linked sialylated and sulfated oligosaccharides on mucus glycoproteins on the epithelial surfaces of the respiratory, urogenital, and gastrointestinal tracts. At the cellular level, glycoproteins and glycolipids are the predominant components of the cell surface, constituting the glycocalyx that faces the extracellular microenvironment and is in contact with neighboring cells. In the cell's interior the majority of membrane proteins facing the lumen of the endoplasmic reticulum, the Golgi apparatus, and the lysosomes are glycosylated. Very recently, carbohydrate residues on proteins have also been identified in subcellular compartments so far regarded as being devoid of glycoprotein glycans, including *O*-linked *N*-acetyl-D-glucosamine on proteins in the cytoplasm, the nucleoplasm, and the nuclear pore complex (Hart et al. 1989), dermatan sulfates in the nucleus (Hiscock et al. 1994), and, though a matter of controversy glycoproteins in mitochondria (Gasnier et al. 1992).

9.3 Biosynthesis and Postbiosynthetic Modifications

Principally, complex carbohydrates differ from nucleic acids and proteins in that they are not synthesized on a template. Whereas the sequence of the monomeric constituents of DNA, RNA, and proteins is encoded by the base sequence, complex carbohydrates are secondary gene products formed by the sequential and coordinated action of numerous enzymes and transport systems. These include glycosyltransferases that transfer monosaccharides or oligosaccharides from nucleotide sugar donors, dolichylphosphate sugar donors, or the oligosaccharide donor dolichylpyrophosphate-$Glc_3Man_9GlcNAc_2$ to either the phosphorylated polyprenol dolichylphosphate, glycosylation sites on proteins, or acceptor sites on lipid- or protein-bound glycans. Moreover, an array of glycosidases may remove sugar residues from oligosaccharide intermediates during the biosynthetic process. As has been described in several comprehensive reviews (Kornfeld and Kornfeld 1985; Schachter 1986, 1995; Hemming 1995; Verbert 1995; Brockhausen 1995), this coordinated action of glycosyltransferases and processing glycosidases creates the basis for the formation of the wide variety of *N*-linked and *O*-linked glycans. Biosynthesis of both types of

glycans takes place in the endoplasmic reticulum, the Golgi apparatus, and the *trans*-Golgi network during transport of newly synthesized glycoproteins to the cell surface.

9.3.1 Biosynthesis of *N*-Linked Oligosaccharides

The biosynthesis of the *N*-linked oligosaccharides of glycoproteins proceeds in severals steps (Fig. 3). Briefly, first a lipid-linked precursor oligosaccharide $Glc_3Man_9GlcNAc_2$ is assembled by stepwise transfer of *N*-acetyl-D-glucosamine, D-mannose, and D-glucose residues to the phosphorylated polyprenol dolichylphosphate in the endoplasmic reticulum. After completing the assembly of the dolicholpyrophosphate bound $Glc_3Man_9GlcNAc_2$, the precursor oligosaccharide is co-translationally transferred by oligosaccharyltransferase to the asparagine residues of suitable sequons Asn-X-Ser/Thr (X can be any amino acid except proline) on the nascent polypeptide chains. Once transferred to the polypeptide chain, the precursor oligosaccharide is subject to stepwise trimming reactions that include removal of glucose and mannose residues by glucosidases I and II and several mannosidases in the endoplasmic reticulum and the *cis*-Golgi to generate $Man_5GlcNAc_2$. At this level of glycoprotein biosynthesis the two inner glucose residues seem to be of particular importance for promoting folding, oligomeric assembly, and quality control of the newly synthesized glycoproteins (Helenius et al. 1997) (see below). Whereas these early steps of oligosaccharide processing are almost identical for all newly synthesized glycoproteins, subsequent steps localized in the Golgi give rise to the numerous individual glycan structures found on mature glycoproteins. The $Man_5GlcNAc_2$ intermediate is the preferred substrate for *N*-acetylglucosaminyltransferase I of the medial Golgi compartment generating $GlcNAcMan_5GlcNAc_2$. This structure can be acted upon by various additional enzymes, including *N*-acetylglucosaminyltransferase III and *N*-acetylglucosaminyltransferase IV, or can move from the medial to the *trans*-Golgi to form different types of hybrid structures. Alternatively, two additional mannose residues can be removed from $GlcNAcMan_5GlcNAc_2$ by mannosidase II in the medial Golgi to form $GlcNAcMan_3GlcNAc_2$, which can be acted upon by *N*-acetylglucosaminyltransferase II, III, or IV or by α6-fucosyltransferase, or can

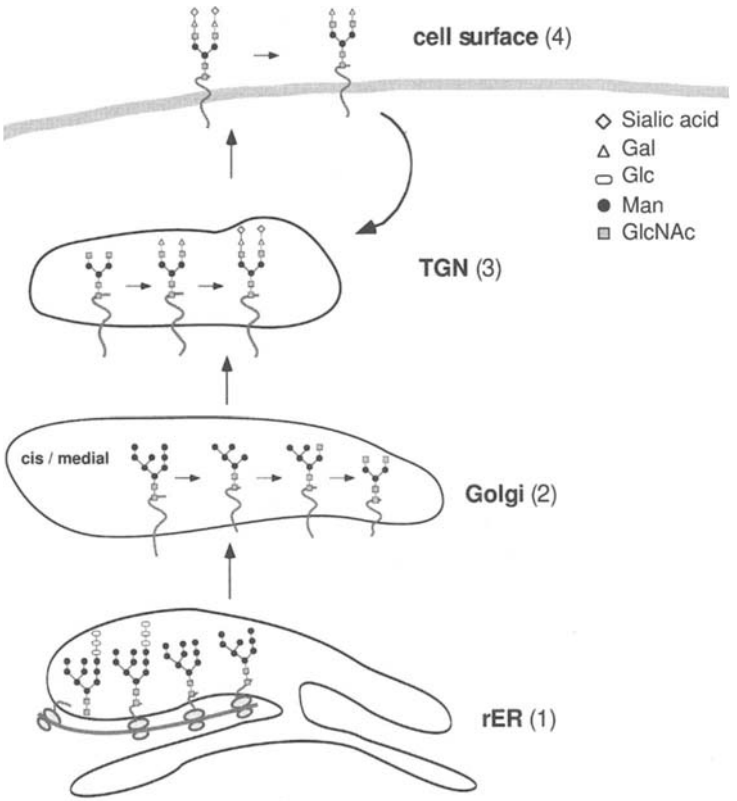

Fig. 3. Biosynthesis of *N*-linked oligosaccharides. *1*, Endoplasmic reticulum (*rER*): cotranslational transfer of $Glc_3Man_9GlcNAc_2$ from DolPP to Asn-X-Ser/Thr acceptor sites on the nascent polypeptide chain by oligosaccharide transferase. Removal of the three glucose and of mannose residues by processing glucosidases and mannosidases. *2*, Golgi apparatus: removal of additional mannose residues by mannosidases I and II, transfer of *N*-acetyl-D-glucosamine residues by GlcNac-transferases I, II, III and IV. Transfer of galactose and fucose residues by galactosyl- and fucosyltransferases. 3, *Trans*-Golgi network (*TGN*): transfer of sialic acid residues by sialytransferases. *4*, Cell surface: stepwise removal of sialic acid, fucose, galactose and mannose residues by exoglycosidases

enter the *trans*-Golgi where the outer chains are extended by galactosyl-, fucosyl-, and sialyltransferases. Since the multiple processing enzymes may act upon the oligosaccharide substrates in various combinations, this nontemplate assembly line is perfectly suited to generate the wide variety of oligosaccharide structures found in secretory and membrane glycoproteins.

9.3.2 Biosynthesis of *O*-Linked Oligosaccharides

The biosynthesis of *O*-linked glycans differs from that of *N*-linked oligosaccharides in that no common lipid-linked oligosaccharide precursor is formed. Instead, the innermost D-GalNAc residue is directly transferred from UDP-GalNAc to Ser/Thr residues on the polypeptide, followed by elongation of the glycan by stepwise transfer of additional sugars. Moreover, *O*-linked glycans are not processed by glycosidases during the biosynthetic process. Similar to the assembly of the outer antennae of *N*-linked glycans, assembly of the different types of *O*-linked glycans occurs along various pathways.

Glycosylation of cell surface glycoproteins, in addition, may be modulated postbiosynthetically, in that sugar residues can be removed from the termini of glycans as well as being re-transferred to preformed glycoproteins on the cell surface or in the Golgi apparatus. These *postbiosynthetic processing reactions* are presumed to present a pathway by which cell surface carbohydrates on preformed glycoproteins are modified in response to endogenous or exogenous stimuli without the necessity for breakdown and de novo synthesis of glycoproteins (Tauber et al. 1994).

9.3.3 Factors that Influence Oligosaccharide Biosynthesis

Despite a certain structural microheterogeneity, individual glycosylation sites on a glycoprotein tend to have oligosaccharides exhibiting characteristic structural features. Hence, mechanisms must be operative that control the formation of distinct oligosaccharide structures at a given glycosylation site of a glycoprotein (Table 2). Cellular factors that guide formation of oligosaccharide structures include (a) the location of

Table 2. Factors that control oligosaccharide biosynthesis

Cellular factors	Polypeptide factors
Expression of glycosyltransferases and glycosidases	Protein conformation
Substrate levels	Quarternary structure
Newly formed glycans	
Nucleotide sugars	Membrane anchoring
Routes and kinetics of intra- and intercompartimental transport	Position of glycosylation sites, steric accessibility of glycans

the various glycosidases and glycosyltransferases in the subcompartments of the endoplasmic reticulum and the Golgi apparatus constituting a glycan "assembly line" (Roth 1995), (b) the activity and substrate specificity of the glycosyltransferases, (c) the concentration of nucleotide sugar donors in the lumen of the Golgi apparatus regulated by specific antiport-transport systems (Abeijon and Hirschberg 1992), and (d) the routes and the velocity of intracellular transport of the biosynthetic intermediates along the assembly line. The level of expression of enzymes and antiport–transport systems is mainly under genetic control at the transcriptional and translational level (Glick 1995). This explains why protein glycosylation is markedly influenced by the genetic program of cells as is reflected by differences in glycosylation between species, individuals, and cell types, as well as by changes in glycosylation during development and differentiation and in response to genetic defects (for review, see Kobata 1992). In addition, the biosynthetic machinery may also be influenced by the cellular and extracellular "microenvironment," e.g., by conditions affected by nutrition or by metabolic diseases, although little is known about these nongenetic influences. Apart from cellular factors, oligosaccharide processing is also influenced by the polypeptide backbone of newly synthesized glycoproteins, i.e., by the conformation and the quarternary structure, which have an impact on the steric accessibility of the glycans for processing enzymes (Hubbard 1988).

9.4 Biological Functions of Glycoprotein Glycans

The oligosaccharides of glycoproteins may have numerous biological functions including rather general roles that do not depend on distinct carbohydrate structures such as their role in protein folding, conformational stability and solubility, more specific roles related to distinct biophysical features of the glycans such as barrier and structural functions, and highly specific interactions with receptors having binding specificity for refined shapes formed by the oligosaccharide ligand. As outlined in a recent comprehensive review (Varki 1993), it is difficult to predict specific rules for the functions an oligosaccharide may have on a glycoprotein, since these depend on the type of glycoprotein, the structure of the oligosaccharide, and the biological context.

9.4.1 Role in Protein Folding, Conformational Stability, and Solubility

As has been shown for numerous soluble and membrane-bound glycoproteins, glycosylation contributes to the folding of newly synthesized proteins in the endoplasmic reticulum, helps to stabilize the conformation of glycoproteins once the proteins have folded properly, and are essential to maintain solubility once soluble glycoproteins have been secreted. Moreover, there is ample evidence that glycans may protect glycoproteins from proteolytic cleavage by either masking proteolytic cleavage sites or conferring intramolecular stability to glycoproteins. Although there are many examples of proteins where folding, conformation, and solubility do not depend on glycosylation, these stabilizing and protective roles of glycoprotein glycans seem to be of general importance. As discovered recently, the $Glc_{1-3}Man_9GlcNAc_2$ glycans on newly synthesized glycoproteins play a key role in protein folding in that they interact with two molecular chaperones, calnexin and calreticulin, in the endoplasmic reticulum, providing a mechanism for promoting folding, oligomeric assembly, and quality control for newly synthesized glycoproteins (Helenius et al. 1997). The importance of this folding and quality control mechanism is highlighted by the observation that inhibition of deglucosylation causes a blockade in the secretion of many secretory proteins (Gross et al. 1983). It is tempting to speculate

that the fact that except for albumin almost all plasma proteins are *N*-glycosylated is related to this mechanism.

9.4.2 Barrier and Structural Functions

A second type of function is dependent on biophysical properties generated by the oligosaccharides in conjunction with their polypeptide backbones.This function is pivotal in gel-forming mucins that protect epithelial surfaces in the gastrointestinal, respiratory, and reproductive tracts, in mucin regions of cell surface receptors, and in the three-dimensional structure of the extracellular matrix. Mucins are very large glycoproteins, with a molecular mass of up to several million containing 50%–80% of complex carbohydrate per molecular weight. The glycans are *O*-glycosidically attached to the polypeptide, are tightly packed, and protrude from the molecule, resulting in a "bottle brush"-like overall shape. Due to their negative charge and their tight attachment to the protein backbone, the sialylated and sulfated glycans confer a rigid, rod-like structure upon the glycoprotein monomers. The monomers are linked to each other both covalently by disulfide bonds and noncovalently by glycan–glycan interactions to form a highly viscous gel that provides both a protective barrier and a sliding surface on the epithelia (Allan and Garner 1980). Mucin regions are also present on cell surface receptors such as the LDL receptor where they result in the formation of rodlike molecular domains assumed to extend the distance of the ligand binding site from the cell surface (Jentoft 1990).

9.4.3 "On–Off" and "Tuning" Functions

A third type of oligosaccharide function has been termed "on–off" or "tuning," describing that oligosaccharides may modulate the biological activity of various glycoproteins (Varki 1993). Examples of "on–off" functions include the glycosylation of glycoprotein hormones such as human chorionic gonadotropin (HCG) which is required for stimulation of adenylate cyclase, but not for binding of the hormone to its receptor (Sairam 1989), modulation of the binding activity of the neural cell adhesion molecule N-CAM by polysialylation (Roth et al. 1992), and

Table 3. Biological functions of complex carbohydrates: oligosaccharide-receptor interactions

Type of Binding	Ligand	Receptor	Function
Individual glycoproteins to			
Intracellular receptors	Phosphorylated oligomannosidic *N*-glycans of lysosomal hydrolases	Mannose 6-phosphate receptors	Routing of newly synthesized lysosomal enzymes to lysosomes (Kornfeld 1986; von Figura and Hasilik 1986)
Cell surface receptors	Plasma glycoproteins with terminal β Gal	Asialoglycoprotein receptor of hepatocytes	Clearance of plasma glycoproteins (Ashwell and Harford 1982)
Cells and particles to			
Phagocytes	Cells (erythrocytes, lymphocytes, tumor cells) with exposed terminal β Gal/β GalNAc residues	Macrophage Gal/GalN receptor	Clearance of senescent erythrocytes (Lee et al. 1990; Roos et al. 1983)
	Cells (protozoa, yeast) with exposed terminal D-mannose residues	Macrophage Man/Fuc receptor	Phagocytosis of pathogens (Ezekowitz et al. 1991; Stahl 1992)
	Particles with exposed β Gal residues	Surface immobilized C-reactive protein	Opsonization, phagocytosis (Kempka et al. 1990; Köttgen et al. 1992)
Pathogens (viruses, bacteria, protozoa, fungi)	Terminal sialic acids on host cell gangliosides and glycoproteins	Viral hemagglutinins (e.g., influenca virus, rota virus, enterovirus)	Binding of viruses to the host cell: infection (Markwell and Paulson 1980; Rogers et al. 1986)
	Oligosaccharides of host cell gangliosides and glycoproteins	Bacterial adhesion (e.g., *Escherichia coli, Neisseria gonorrhoeae*), fungal receptors (e.g., *Cryptococcus neoformans, Candida albicans*), receptors of protozoa (e.g., *Plasmodium falciparum*)	Infection of host cells (Ofek and Sharon 1990; Jimenez-Lucho et al. 1990; Orlandi et al. 1992)

the modulation of receptor-binding affinity and biological activity of hematopoietic growth factors (Cebon et al. 1990), among others.

9.4.4 Oligosaccharide–Receptor Interactions

By virtue of their structural diversity, the glycans of both glycoproteins and glycolipids are extremely well suited to serve as recognition structures that interact specifically with different types of receptors that have binding sites for distinct structural oligosaccharide epitopes. Oligosaccharide–receptor interactions have been shown to be pivotal for numerous biological processes (Table 3). These include (a) the sorting of proteins during intracellular trafficking such as transport of newly synthesized lysosomal hydrolases from the Golgi complex to lysosomes (Kornfeld 1986; von Figura and Hasilik 1986) and that of secretory glycoproteins to the apical surface (Scheiffele et al. 1995) and (b) selective binding of oligosaccharides to sugar-specific receptors termed mammalian lectins, which has been shown to mediate the clearance of plasma glycoproteins and senescent erythrocytes from the circulation by binding to two types of galactose receptors exposed on hepatocytes and Kupffer cells, respectively (Ashwell and Harford 1982; Roos et al. 1983). Other receptors, including those with binding specificity for mannose and fucose expressed on macrophages (Stahl 1992; Ezekowitz et al. 1991), a circulating core-specific lectin (Colley et al. 1988), as well as C-reactive protein immobilized onto cell surfaces (Kempka et al. 1990; Köttgen et al. 1992), are assumed to be involved in the clearance of cell debris and pathogens. In contrast, viruses, bacteria, fungi, and protozoa utilize complex carbohydrates exposed on the surface of host cells for binding, i.e., for the first step in infection (for examples see Table 3).

9.5 Carbohydrate-Mediated Cell Adhesion

Carbohydrate–lectin interactions mediated by selectins and their ligands are pivotal for the adhesion of leukocytes and platelets to endothelial cells under shear stress that initiates the recruitment of leukocytes to sites of acute and chronic inflammation (for selected reviews, see Lasky 1992; Bevilacqua and Nelson 1993; Kansas 1996).

9.5.1 Selectin–Carbohydrate Interactions

The carbohydrate-binding selectin family of adhesion molecules comprises the three related type I transmembrane cell surface proteins L-, E- and P-selectin, all of which are encoded by genes which are in close proximity to each other on human (and mouse) chromosome 1. L-selectin is expressed by leukocytes, while E-selectin is expressed by chronically activated endothelial cells and P-selectin by acutely activated endothelium and platelets. All three selectins have an N-terminal lectin domain which is capable of carbohydrate binding in a Ca^{2+}-dependent fashion, followed by an EGF homology domain and a variable number of tandem short consensus repeat domains homologous to domains found in various complement-regulating proteins. The carbohydrate–selectin interaction tethers flowing leukocytes to stationary cells of the vessel wall, which is comprised mainly of activated or specialized endothelium but also arrested leukocytes and platelets (Ley et al. 1991, 1993). This results in a rapid loss of relative velocity, which is a prerequisite for an ensuing definitive leukocyte arrest mediated by protein–protein interactions between members of the immunoglobulin and integrin superfamilies (von Andrian et al. 1992). In addition, the continuous recirculation of lymphocytes through the blood into peripheral lymph nodes is completely dependent on L-selectin (L-selectin has therefore been referred to as "peripheral lymph node homing receptor"). Lymphocyte homing to mucosa-associated lymphatic tissue involves L-selectin and α4-integrins in an overlapping rather than sequential fashion.

9.5.2 Carbohydrate Selectin Ligands

All selectin-binding molecules contain carbohydrates bearing a strong negative charge due to modification by neuraminic acid, sulfate, or phosphate residues. In vitro, the three selectins bind two related fucose-containing acidic oligosaccharides, sialyl Lewis X (NeuAc α2–3Galβ1-4(Fucα1–3)GlcNAc; CD15s] (Fig. 2) and sialyl Lewis A [NeuAc α2–3Galβ1-3(Fucα1–4)GlcNAc] (Foxall et al. 1992). Clustering of the carbohydrate moieties appears to be important for high-avidity binding to selectins: Tetravalent sialyl Lewis X is much more efficient in block-

ing lymphocyte adherence to activated endothelium than di- or trivalent sialyl Lewis X (Turunen et al. 1995). Murine lymph node endothelium L-selectin ligands contain sulfated sialyl Lewis X derivatives (Hemmerich et al. 1994) but the relative importance of 6-sulfo sialyl Lewis X (sulfate→6GlcNAc) and 6'sulfo sialyl Lewis X (sulfate→^{6}Gal) remains controversial.

9.5.3 Glycoprotein Selectin Ligands

Selectin-binding complex carbohydrates are presented by various protein scaffolds. With the notable exception of E-selectin ligand 1 (ESL-1), all selectin-binding glycoproteins with known amino acid sequence of the protein core (GlyCAM-1, CD34, MAdCAM-1 und PSGL-1) belong to the sialomucin family, characterized by copious *O*-linked carbohydrate side chains (Shimizu and Shaw 1993). GlyCAM-1 is a secreted L-selectin-binding sialomucin isolated from murine lymph node endothelial cells which bears 6- and 6'-sulfated sialyl Lewis X and which may act as a soluble L-selectin antagonist (Hemmerich et al. 1995). Lymph node endothelial cells contain another L-selectin binding sialomucin, CD34, which has a protein core identical to that found in hematopoietic stem cells. Due to differential glycosylation, only lymph node endothelial cell-derived CD34 binds to L-selectin. The sialomucin MAdCAM-1 also displays L-selectin binding capacity but only when isolated from mesenterial lymph nodes and not from endothelioma cells. Of the sialomucins mentioned, only CD34 has been shown to be also present in human peripheral endothelium, but it remains unclear whether it has any role in mediating leukocyte extravasation into areas of inflammation. The leukocytes themselves may bind all three selectins: ESL-1 binds E-selectin via *N*-linked carbohydrate moieties (Steegmaier et al. 1995), CD162 binds P- and L-selectin (PSGL-1=P-selectin glycoprotein ligand-1) (Guyer et al. 1996). Leukocyte–leukocyte binding mediated by PSGL-1–L-selectin interaction is pivotal in amplifying leukocyte recruitment in a snowball-like manner after the first leukocytes have adhered to the vessel wall (Walcheck et al. 1996). In addition to glycoprotein ligands, selectins may also bind to distinct glycosaminoglycans. Cultivated endothelial cells contain L-selectin-binding heparansulfate proteoglycans (Norgard-Sumnicht et al. 1993). The rele-

vance of these binding sites may be inferred from the observation that heparinase treatment of activated arterial endothelial cells reduces L-selectin-dependent monocyte binding by 80% (Giuffré et al. 1987). Binding of L-selectin chimeras to sialyl Lewis X can be effectively inhibited by ΔUA2Sα1–4GlcNS6Sα1–4IdoA2Sα1–4GlcNS6S and related heparin tetrasaccharides. Intravenous administration of these heparin tetrasaccharides reduces extravasation of neutrophils into areas of acute inflammation similar to the way anti-L-selectin monoclonal antibodies do. As a corrollary, certain batches of heparin exert anti-inflammatory effects in animal models of transplant rejection, adjuvant arthritis, or experimental autoimmune encephalitis (Lider et al. 1989). The anti-inflammatory effects presumably reside in the ability to interfere with leukocyte extravasation but are unrelated to the anticoagulant properties of the heparin batches used.

9.5.4 Therapeutic Inhibition of Carbohydrate–Selectin Interaction

Aberrant or overshooting leukocyte extravasation may do more harm than good. Therefore, inhibition of leukocyte extravasation has been attempted in animal models of various diseases by means of monoclonal antibodies, soluble selectin chimeras, bacterial or synthetically produced lectin-domain-analogous peptides, sialyl Lewis X glycoconjugates, sulfated polysaccharides such as dextran sulfate or fucoidin (which binds both L- and P-selectin), glycyrrhetin-C-fucoside, and chemically synthesized saccharomimetics such as inositol polyphosphates (for review, see Parekh and Edge 1994). The three disorders studied most extensively are postischemic reperfusion injury, acute respiratory distress syndrome (ARDS), and acute meningoencephalitis.

9.5.4.1 Postischemic Reperfusion Injury

Transient ischemia followed by reperfusion elicits inflammatory changes both locally and in the lung. The associated tissue damage in both areas can be reduced by intravenous administration of selectin-blocking monoclonal antibodies, sialyl Lewis X oligosaccharides or fucoidin (Seekamp et al. 1994). For example, myocardial necrosis in cats subjected to coronary ischemia and reperfusion can be reduced by

as much as 50% by sialyl Lewis X oligosaccharides or anti-L-selectin monoclonal antibodies (Buerke et al. 1994).

9.5.4.2 ARDS

Selectin inhibition also yields favorable effects in ARDS-like pulmonary damage induced by experimental sepsis, cobra venom factor, or immune complex formation in that L-selectin antagonists effectively reduce leukocyte infiltration, plasma exudation, and pulmonary hemorrhage (Mulligan et al. 1993).

9.5.4.3 Meningoencephalitis

As shown by experiments with cyclophosphamide-induced neutropenia, neutrophils play no major role in the process of eliminating bacteria from the central nervous system during acute meningoencephalitis. On the contrary, reducing leukocyte extravasation into the brain has a favorable impact on the sequelae of bacterial meningoencephalitis. After intracisternal injection of bacterial polysaccharide toxins, pleocytosis and the accumulation of lactate and serum proteins are ameliorated by intravenous administration of selectin antagonists, such as fucoidin or selectin-binding prokaryotic peptides (Angstwurm et al. 1995).

In conclusion, selectin-mediated leukocyte extravasation is pivotal in acute inflammation, appears to occur in chronic inflammation, and may be important for transplant rejection and autoimmune diseases. However, animal data for these situations are scarce in comparison with those for acute neutrophil-mediated disorders. Fortunately, even prolonged selectin–carbohydrate inhibition appears to have little impact on the ability to fend off viral or bacterial intruders. Since the role of selectin–carbohydrate binding is limited to conditions of shear, the extravasation of leukocytes at pathologically low flow rates and the function of already extravasated leukocytes at work in the surrounding tissue is not altered by therapeutic inhibition of the selectin–carbohydrate interaction. Therefore, this approach holds promise both for acute and chronic leukocyte-mediated diseases and awaits phased clinical studies.

9.6 Changes of Glycosylation in Disease

Owing to the improved techniques and methods for analyzing oligosaccharide structures and functions and for unraveling the biosynthetic pathways of glycoprotein glycans, an increasing number of inherited and acquired defects in glycosylation have been described. Reflecting the diverse biological functions of protein glycans, defects in glycosylation may have highly variable consequences in the diseased organism, ranging from lethal aberrations to almost undetectable morphological and functional disorders. Congenital diseases resulting from aberrant protein glycosylation are very rare in humans. These include the lysosomal storage disorders mucolipidosis II (I-cell disease) and mucolipidosis III (Pseudo-Hurler polydystrophy) (Kornfeld 1986; von Figura and Hasilik 1986), congenital dyserythropoietic anemia type II (Fukuda 1991), paroxysmal nocturnal hemoglobinuria (Rosse 1990), carbohydrate-deficient glycoprotein syndrome (Jaeken et al. 1993), and leukocyte adhesion deficiency II (Etzioni et al. 1992), among others. Children suffering from leukocyte adhesion deficiency II are unable to synthesize the sialyl Lewis X and A ligand of E-selectin, resulting in a severe impairment of neutrophil extravasation and, as a consequence, in recurrent severe infections. As compared to primary congenital disorders acquired secondary aberrations in protein glycosylation are more frequent. Patients with rheumatoid arthritis (Parekh et al. 1985), tuberculosis and Crohn's disease (Parekh et al. 1989) have serum IgG molecules with a higher percentage of oligosaccharides on the Fc part which lack terminal galactose residues, resulting in the exposure of terminal N-acetylglucosamine. Decreased galactosylation of IgG presumedly results from a reduced activity of B-cell βF255-galactosyltransferase (Axford et al. 1987); this has been suggested to be of relevance for the pathogenesis of rheumatoid arthritis (Axford 1991) and may be an aid in differential diagnosis (Young et al. 1991). Chronic alcohol consumption causes elevation of serum transferrin isoforms termed carbohydrate-deficient transferrin (CDT), in which the terminal trisaccharides are deficient (Stibler 1991). Incomplete glycosylation of CDT is presumedly caused by a depression in the activities of glycosyltransferases involved in the biosynthesis of the transferrin oligosaccharides (Xin et al. 1995). CDT was proven to be a sensitive marker of alcohol abuse (Stibler 1991). Chronic liver disease has been shown to be associated with an

Table 4. Targeting of drugs, genes and contrast media to the liver

Drug targeting	Gene targeting	Imaging
Anti-neoplastic drugs	Plasmids (Stankovics et al. 1994)	Scintigraphy (Kudo et al. 1992)
Anti-viral drugs (Biessen et al. 1994)	Antisense oligonucleotides	Magnetic resonance imaging (Reimer et al. 1991a,b)
Lipid-lowering drugs		
Tri-antennary Gal-glycans	Asialoorosomucoid-poly-lysine	^{99m}Tc-DTPA-galactosyl HSA
Lactosylated poly-L-lysine	Asialo-alpha-1-acid glycoprotein	Arabino galactan/ pyrrolidinoxyl radicals
Gal-carboxymethyl-dextran		
Lactosaminated HSA		Asialofetuin iron oxid nanoparticles
(2-Hydroxypropyl)-methacrylamide Copolymers-Galoxide		Arabino galactan iron nanoparticles

DTPA, diethylenetriamine–penta acetic acid; HSA, human serum albumin

elevated sialylation of fibrinogen (Martinez et al. 1978). Furthermore, acute and chronic inflammation are associated with specific alterations in the glycosylation of acute-phase proteins suggested to represent a humoral feedback response of the hepatic acute-phase reaction to dampen the selectin-mediated cellular inflammatory reaction (van Dijk et al. 1994). During the past three decades numerous studies have demonstrated that malignant transformation is associated with structural alterations of the carbohydrate groups of glycoproteins and glycolipids. Structural changes of cell surface carbohydrates are exhibited by essentially all experimental and naturally occurring malignomas regardless of type, cause, or stage of development (for review, see Warren et al. 1978; Hakomori 1989; Reading et al. 1988). Structural changes include an elevation of tri- and tetra-antennary *N*-linked glycans, increased poly-*N*–acetyllactosamines, and "bisecting" *N*-acetyl-D-glucosamine residues, the neo-expression of Lewis antigens, and increased sialylation and fucosylation (selected references: Ogata et al. 1976; Yamashita et al.

1984; Kim et al. 1986; Vischer and Reutter 1978). Tumor-associated carbohydrate structures have been shown to be related to metastasis and to tumor immunology (Takano et al. 1994). For example, the expression of increased amounts of sialyl Lewis X and sialyl Lewis A structures known to be ligands for E-selectin expressed on endothelial cells has been suggested to be involved in the attachment of blooD-borne metastatic tumor cells to endothelial cells and their subsequent extravasation (Sawada et al. 1993). This assumption is supported by the observation that inhibitors of N-glycan processing such as swainsonine and castanospermine inhibit metastasis of experimental tumor cells (Dennis 1986). With respect to immunological mechanisms, distinct carbohydrate antigens have been shown to suppress natural killer cell cytotoxicity for tumor cells, thus contributing to the poor prognosis of tumors expressing these antigenic epitopes (for review, see Muramatsu 1993). Anti-carbohydrate antibodies and lectins have been shown to be diagnostically useful to phenotype tumor cells or to monitor serum levels of tumor-associated antigens (for review, see Reading et al. 1988; Muramatsu 1993).

9.7 Carbohydrates as Ligands for Targeting and Imaging

Distinct types of cells, including macrophages, hepatocytes, endothelial cells, and leukocytes express on their surface nonenzymatic, sugar-binding proteins termed lectins, which mediate endocytosis, phagocytosis, and cell adhesion, among other biological functions (see above). The specific binding of carbohydrate ligands to lectins has been utilized to specifically target various types of cargo to these cells. In particular, two galactose-specific receptors, i.e., the asialoglycoprotein receptor that is expressed on hepatocytes and internalizes glycoproteins and small particles exposing galactose, and the galactose-particle receptor on Kupffer cells with preferential binding affinity for large galactose-exposing particles, have been used for targeting drugs, antisense oligodeoxynucleotides, plasmids, and contrast media to the liver (Table 4). For example, lactosylated poly-L-lysine has been successfully used as a carrier for targeting anti-viral drugs to liver parenchymal cells (Biessen et al. 1994), and asialoglycoprotein/polylysine/DNA complexes were employed for in vivo gene delivery to the liver (Stankovics et al. 1994).

Based on the same strategy, imaging of the asialoglycoprotein receptor with technetium-99m galactosyl human serum albumin (Kudo et al. 1992) or with arabinogalactan-coated ultrasmall superparamagnetic iron oxide (Reimer et al. 1991a) was used to quantitatively assess liver function. Moreover, magnetic resonance asialoglycoprotein receptor imaging using arabinogalactan-coated ultrasmall superparamagnetic iron oxide was shown to be suitable to differentiate between primary liver tumors and functional liver tissue (Reimer et al. 1991b). Targeting to the reticuloendothelial system in liver, spleen, and bone marrow has been attempted using carriers exposing D-mannose and L-fucose residues (Gonsho et al. 1994) or by engineering a mannose glycoform of the proteins to be delivered (Brady et al. 1994).

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