Chapter 1
Introduction to the Complexity of Cell Surface and Tissue Matrix Glycoconjugates

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Abstract This chapter provides an overview of structures and functions of complex carbohydrates (commonly called glycans) that are covalently linked to proteins or lipids to form glycoconjugates known as glycoproteins, glycolipids, and proteoglycans. To understand the complexity of the glycan structures, the nature of their monosaccharide building blocks, how the monomeric units are covalently linked to each other, and how the resulting glycans are attached to proteins or lipids are discussed. Then, the classification, nomenclature, structural features, and functions of the glycan moieties of animal glycoconjugates are briefly described. All three classes of glycoconjugates are constituents of plasma membranes of all animal cells, including those of the nervous system. Glycoproteins and, particularly, proteoglycans are also found abundantly as constituents of tissue matrices. Additionally, glycan-rich mucin glycoproteins are the major constituents of mucus secretions of epithelia of various organs. Furthermore, the chapter draws attention to the incredible structural complexity and diversity of the glycan moieties of cell surface and extracellular glycoconjugates. Finally, the involvement of the glycans as informational molecules in a wide range of essential functions in almost all known biological processes, which are crucial for development, differentiation, and normal functioning of animals, is discussed.

Keywords Complex carbohydrates • *N*-Glycans • *O*-Glycans • Glycosaminoglycans • Glycoconjugates • Glycoproteins • Glycolipids • GPI anchors • Proteoglycans • Structure and functions of glycoconjugates

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1.1 Introduction

Carbohydrates are the most abundant, structurally complex, and functionally diverse organic compounds found on earth. They comprise monosaccharides (e.g., glucose), oligosaccharides (e.g., sucrose, lactose, components of glycoproteins, and glycolipids; see below), and polysaccharides (e.g., starch, cellulose, plant and microbial cell wall polysaccharides, arthropod chitin, and animal glycosaminoglycans). Carbohydrates are important dietary components for animals, including humans, and play crucial roles in energy metabolism as exemplified by glucose homeostasis. In addition to their occurrence as free molecules, when conjugated to proteins and lipids, they form glycoconjugates (glycoproteins, proteoglycans, and glycolipids) (Brooks et al. 2002; Gabius 2009; Taylor and Drickamer 2011; Varki et al. 2008). The oligo- and polysaccharides covalently conjugated to proteins and lipids are referred to as complex carbohydrates or glycans. The glycan moieties of glycoconjugates are structurally complex and are involved in a myriad of functions, which are crucial for differentiation, development, and all aspects of normal functioning of animals. For instance, they constitute the major blood group and other antigens, serve as informational molecules in cell–cell and cell–molecule interactions, function as receptors for biological processes, and assist in protein folding, targeting, and secretion. The glycan moieties of glycoconjugates are also involved in tissue organization, trafficking of lymphocytes, cell signaling, and immune regulation. In many cases, the true functions of glycan moieties are still not fully understood. Research during the past several decades and recent technological developments have deciphered the structures of thousands of glycans and substantially increased our knowledge of their biological roles. However, it is not exaggerating to state that what we know about the functions of these molecules represents only the tip of an iceberg.

Glycoconjugates occur extensively in all cells and tissues of animals, including those of the nervous system (Brooks et al. 2002; Gabius 2009; Taylor and Drickamer 2011; Varki et al. 2008). They are found in tissue matrices and extracellular fluids such as serum, spinal fluid, saliva, and are especially highly abundant in respiratory, gastrointestinal, and urogenital mucus. However, the main reason for the increased interest in these molecules is that they occur ubiquitously on cell surfaces, where they play important roles in many biological processes. The intrinsic plasma membrane glycoconjugates of cells have their glycan moieties projecting outward far and wide and thus are disposed for functional interactions. The anionic sugar residues of glycans, which impart hydrophilicity and bestow negative charges to the cell surface, are important determinants in the social behavior of cells.

In this introductory chapter, we will briefly discuss (1) the nature of the monomeric building blocks of glycans, (2) how they are linked to one another to form glycans, (3) how glycans are conjugated to proteins and lipids to form different glycoconjugates, and (4) provide an overview of the classification, key structural features, and functions of glycans. Since it is impossible to cover all aspects of glycan biology in this brief overview, readers are referred to more detailed books,

monographs, and reviews for additional information (Allen and Kisailus 1992; Bertozzi and Rabuka 2008; Brooks et al. 2002; Gabius 2009; Miljkovic 2010; Taylor and Drickamer 2011; Varki et al. 2008; Voet and Voet 2010; Yuriev and Ramsland 2012).

1.2 Monosaccharides: Building Blocks of Glycans

The structural complexity of glycans arises from the fact that monosaccharides, the monomeric units or building blocks of these molecules, have multiple functional groups and exhibit stereoisomerism (Allen and Kisailus 1992; Bertozzi and Rabuka 2008; Brooks et al. 2002; Gabius 2009; Miljkovic 2010; Taylor and Drickamer 2011; Varki et al. 2008; Voet and Voet 2010; Yuriev and Ramsland 2012). Monosaccharides (generally called sugars) are polyhydroxy aldehydes or ketones with the general formula $(CH_2O)_n$ and are referred to as aldoses or ketoses, respectively. Both aldoses and ketoses are classified into different groups based on the number of carbon atoms they contain; for example, tetroses, pentoses, and hexoses are aldoses containing 4, 5, and 6 carbon atoms, respectively. Each of these groups is further classified into D- and L-series. All D-sugars have the same stereochemistry (i.e., identical absolute configuration) as D-glyceraldehyde at the asymmetric carbon atom that is most remote (e.g., C-5 in hexoses; Fig. 1.1) from the carbonyl group. As mentioned above, a detailed discussion of the structures and stereochemistry of various sugars is beyond the scope of this chapter as it is available in the introductory chapters of biochemistry textbooks and in specialty glycobiology books (Bertozzi and Rabuka 2008; Brooks et al. 2002; Gabius 2009; Miljkovic 2010; Taylor and Drickamer 2011; Varki et al. 2008; Voet and Voet 2010). The predominant constituents of the glycan moieties of animal glycoconjugates are hexoaldoses and their derivatives. The structures and stereochemistry of the eight possible

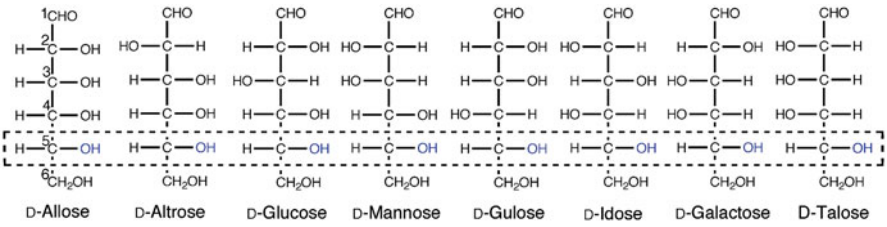

Fig. 1.1 Structures of eight possible D-aldohexoses are shown as open-chain Fischer projection formulas. They all have the same stereochemistry as that of D-glyceraldehyde at C-5, the asymmetric carbon that is most remote from the carbonyl group. The eight L-aldohexoses are the mirror images of these D-sugars. Please see refs. (Brooks et al. 2002; Taylor and Drickamer 2011; Varki et al. 2008; Voet and Voet 2010) for the structures of aldopentoses and other monosaccharides. Ketopentoses and ketohexoses are not present as constituents of the glycan moieties of animal glycoconjugates

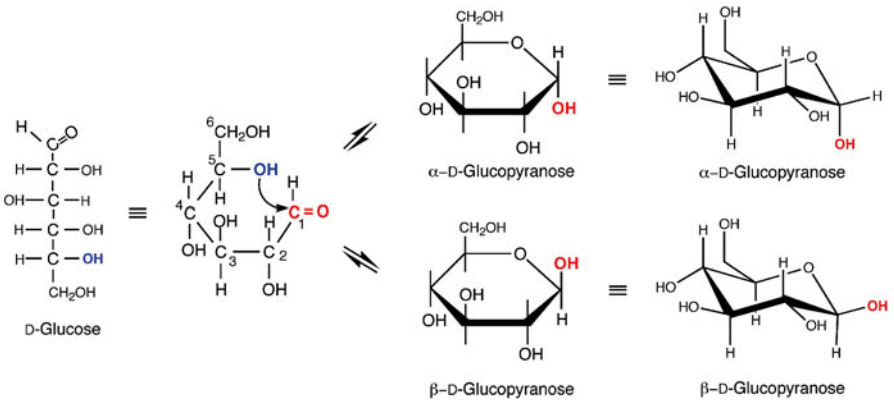

Fig. 1.2 The folding of open-chain monosaccharides (e.g., D-glucose shown here) to relieve carbon bond angle strain facilitates intramolecular nucleophilic condensation of the C-5 hydroxyl group to the carbonyl group. This intramolecular reaction results in the formation of cyclic hemiacetal structures and the creation of a chiral center at carbon atom 1 (C-1). Therefore, the majority of sugars exist primarily as two thermodynamically stable six-membered cyclic structures called α- and β-anomers. Haworth projection formulas and the chair conformational structures of α-D-glucopyranose and β-D-glucopyranose are also shown. Some pentoses and hexoses can exist as five-membered cyclic structures see refs. (Brooks et al. 2002; Taylor and Drickamer 2011; Varki et al. 2008; Voet and Voet 2010)

D-series hexoses in open-chain Fischer projection formula are depicted in Fig. 1.1; the L-series sugars are the mirror images of D-sugars. Of the eight hexoses shown in Fig. 1.1, D-galactose and D-mannose are found widely in glycoproteins. D-Glucose is present in glycolipids but is rarely found in glycoproteins. Glucose is also the biosynthetic precursor for all other sugars, which occur in nature. Similarly, there are four each of the D-series and L-series pentoses, namely, ribose, arabinose, xylose, and lyxose. Of these, xylose occurs in animals as a constituent of proteoglycans.

Because of the tetrahedral bond angle requirement for carbon, sugars are not present as highly strained open-chain linear structures. They are folded in such a way that either C-4 or C-5 hydroxyl groups come in close proximity to the electrophilic carbonyl group and react to form intramolecular hemiacetal bonds; Fig. 1.2 shows the cyclic structure of D-glucose. Thus, five or more carbon atom-containing monosaccharides exist as thermodynamically stable six-membered or five-membered cyclic structures, designated as pyranose or furanose, respectively. The formation of ring structures, which are depicted by Haworth projection formulas, results in the creation of an additional chiral center at C-1, and thus, each sugar exists as two isomers. These isomers are referred to as α- and β-anomers, and the carbonyl carbon of sugar in the ring form is the anomeric carbon. By convention, hexopyranoses in which the –OH group at C-1 and the $–CH_2OH$ group at C-5 have a *trans* or *cis* configuration when depicted by Haworth formulas are named α- and β-anomers, respectively (Fig. 1.2). In aqueous solutions the cyclic anomers are in equilibrium with open chain forms having either a free aldehyde or a keto group.

This allows sugars to display the characteristic properties of aldehydes and ketones by shifting the equilibrium toward the open chain forms. For example, all aldoses exhibit reducing property. If the anomeric hydroxyl group of a sugar in cyclic structure is locked in covalent bond formation, for example, with alcohols forming glycosides, the reducing property of the sugar is lost.

Two sugars differing in configuration at a specific carbon, other than C-1, are referred to as epimers. Thus, glucose and mannose, which differ in configuration only at C-2, are C-2 epimers. Similarly, glucose and galactose, which differ in configuration only at C-4, are C-4 epimers, and D-glucuronic acid and L-iduronic acid (see below) are C-5 epimers. Because mannose and galactose differ in configuration at more than one position, they are not epimers; they are isomers.

The ring carbon and oxygen atoms of six-membered pyranose structures of aldohexoses and their derivatives, including those that constitute glycoconjugate glycans, do not adopt coplanar structures as depicted in Haworth projection formulas (Fig. 1.2). To relieve bond angle strain and steric interactions between bulky functional groups, in most cases, sugars assume "chair" conformations with a large number of different arrangements of their various functional groups in space. The conformations in which bulky substituents occupy equatorial positions (lying in parallel to plane of the ring) are the more stable ones. The stable chair conformations of α- and β-anomers of D-glucose are illustrated in Fig. 1.2.

As mentioned above, D-galactose (D-Gal), D-mannose (D-Man), D-glucose (D-Glc), and D-xylose (D-Xyl) are the unmodified sugars that occur as constituents of glycans in animal glycoconjugates (Brooks et al. 2002; Gabius 2009; Taylor and Drickamer 2011; Varki et al. 2008). In addition, several modified forms of sugars are also common constituents of glycans. These include: 6-deoxy-L-galactose known as L-fucose (L-Fuc); the C-6 carboxyl derivative of D-Glc and L-idose (L-Ido), called D-glucuronic acid (D-GlcA) and L-iduronic acid (L-IdoA), respectively; and the *N*-acetylated forms of 2-amino-2-deoxy-D-glucose (D-GlcN) and 2-amino-2-deoxy-galactose (D-GalN), called *N*-acetyl-D-glucosamine (D-GlcNAc) and *N*-acetyl-D-galactosamine (D-GalNAc), respectively. Other types of modified sugars found in animal glycans have certain hydroxyl groups acetylated, sulfated, or phosphorylated. An unusual 9-carbon monosaccharide acid widely distributed in animals is D-neuraminic acid (5-amino-3,5-dideoxy-D-nonulosonic acid) (Yu and Ledeen 1969), which has –COOH, –C=O, and $–NH_2$ functions (Schauer 2004; Varki 1992; Varki and Schauer 2008). The amino group is either acetylated ($–NHCOCH_3$) or glycolylated ($–NHCOCH_2OH$) resulting in *N*-acetylneuraminic acid (NANA, NeuAc) or *N*-glycolylneuraminic acid (NeuGc); humans synthesize only NeuAc, but traces of NeuGc are found as a result of eating meat (Bardor et al. 2002). Some of the hydroxyl groups of these neuraminic acids are either acetylated, methylated, or sulfated forming a family of more than 20 derivatives, which are collectively called sialic acid (SA) (Schauer 2004; Varki 1992). Sialic acid occurs abundantly in brain as a constituent of glycolipids (Nagai and Iwamori 1995; Wang et al. 1998).

All these modifications of monosaccharides contribute to the incredible structural diversity of glycans. The monosaccharides and their derivatives commonly found as constituents of glycans of animal glycoconjugate are shown in Fig. 1.3; note that, of these sugars, L-iduronic acid occurs only in proteoglycans.

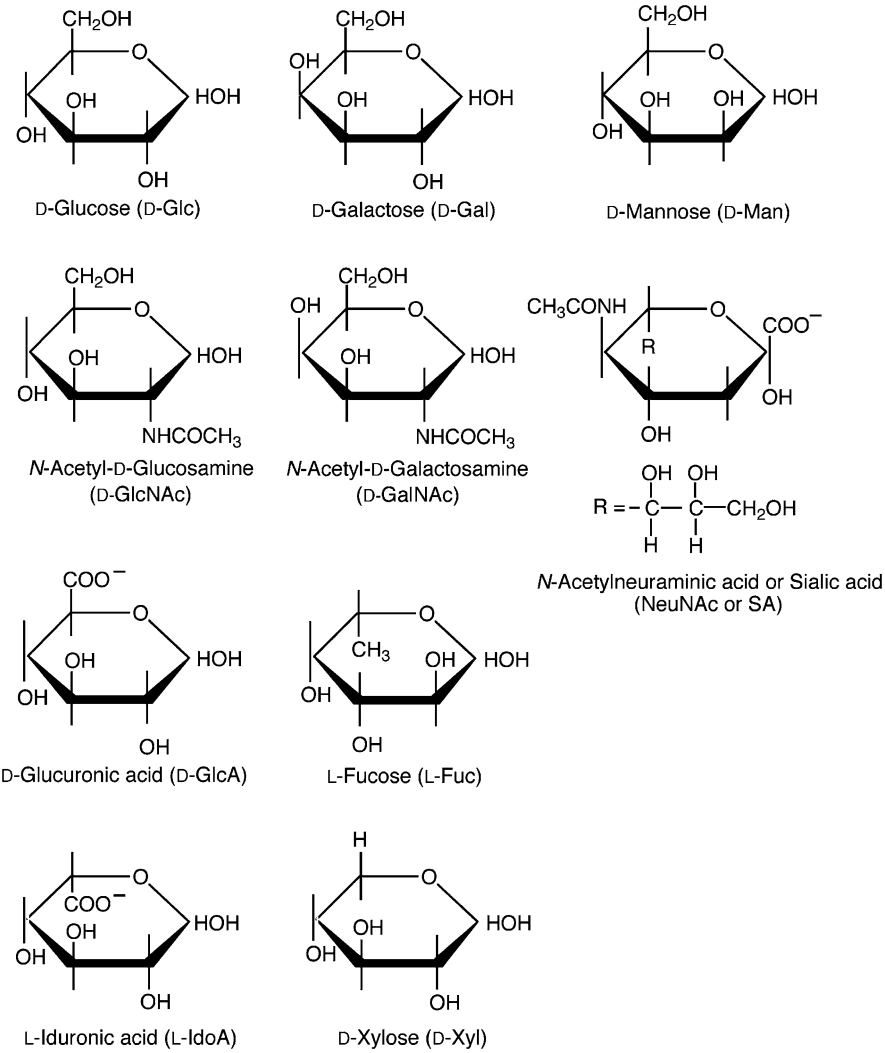

Fig. 1.3 Haworth projection formulas of monosaccharides and their derivatives found in glycans of animal glycoconjugates. Note that of these ten sugars, eight are D-sugars and two are L-sugars

1.3 Formation of Oligosaccharides

The anomeric hydroxyl group of monosaccharides is more reactive than the other hydroxyl groups and undergoes protonation, becoming a relatively good leaving group (Brooks et al. 2002; Miljkovic 2010). This makes C-1 relatively electrophilic and susceptible to attack by negatively charged or electron-rich atoms such as the oxygen of hydroxyl groups. Thus, alcohols and other compounds containing hydroxyl groups can condense with the carbonyl group of monosaccharides, and the

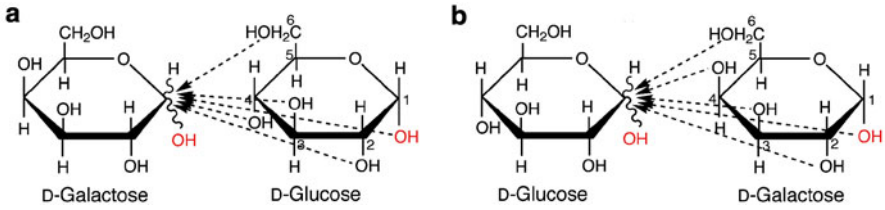

Fig. 1.4 Glycosidic bond formation between the α- or β-anomeric hydroxyl group of D-galactopyranose and the hydroxyl groups on carbons 2, 3, 4, and 6 of D-glucopyranose results in eight different disaccharides with glucose at the reducing end (**a**). When the positions of the sugars are reversed as in (**b**), eight disaccharides with galactose at the reducing end are formed. Four additional nonreducing disaccharides are formed when the anomeric hydroxyls of both sugars are involved in the interaction

bond formed by this condensation is called a glycosidic bond or a glycosidic linkage (Brooks et al. 2002; Gabius 2009; Taylor and Drickamer 2011; Varki et al. 2008). If the attacking nucleophile is a hydroxyl group of another monosaccharide, then the two monosaccharides are linked to each other, forming a disaccharide (Fig. 1.4). When a glycosidic bond is formed by involving the anomeric hydroxyl of one sugar with a non-anomeric hydroxyl of another sugar, the anomeric hydroxyl of the latter sugar is free. Therefore, this sugar retains its reducing property and is called the reducing end of the resulting disaccharide. The other monosaccharide moiety whose anomeric group is involved in the stable glycosidic bond has no reducing power and is called the nonreducing end sugar. The nonreducing and reducing ends of linear oligo- and polysaccharide chains are analogous to the N- and C-terminal amino acids of polypeptides. The α- or β-anomers of one sugar can form two different glycosidic bonds with multiple hydroxyl groups (five in the case of hexopyranoses) of a second sugar to form several different disaccharides. Thus, two molecules of the same sugar, for example, glucose, can form a total of 11 disaccharides, and two different monosaccharides can form 20 disaccharides (Fig. 1.4).

An additional complexity is involved when more than two sugars are attached to form higher oligosaccharides (Bertozzi and Rabuka 2008; Brooks et al. 2002). For example, in the case of trisaccharides that are formed from three molecules of the same sugar, D-Glc, the third molecule can be attached to either the nonreducing glucose of disaccharides, Glc–Glc, to form linear trisaccharides [Glc–Glc–Glc] or the reducing end glucose to form branched trisaccharides [Glc–(Glc)–Glc]. Thus, oligosaccharides have three ways of generating structural diversity: one is by using different hydroxyl groups, the second is by the formation of α- and β-anomeric linkages, and the third is by branching. Thus, three molecules of the same sugar can form 176 trisaccharides, and three different sugars can form 1,056 trisaccharides. Four or more different sugars can form several thousands of tetra- or higher oligosaccharides. Note that linear oligosaccharides will have one each of reducing and nonreducing sugar ends. Whereas, branched oligosaccharides will have one reducing and multiple nonreducing sugar ends and the number of nonreducing ends indicate the degree of branching.

Since several sugars (see Fig. 1.3) and their acetylated, methylated, and sulfated derivatives constitute the glycan moieties of glycoconjugates, the combinatorial

diversity of glycan structures is incredibly large and literally thousands of glycans occur in glycoconjugates (Brooks et al. 2002; Gabius 2009; Taylor and Drickamer 2011; Varki et al. 2008). Since a single glycoprotein molecule may contain hundreds of different glycans at different glycosylation sites and even one glycosylation site may contain different glycan chains, determining the structures and understanding functional roles of glycans is challenging. However, recent technological advancements, particularly mass spectrometry (Leymarie and Zaia 2012; Li et al. 2009; Nishimura 2011; North et al. 2009; Orlando 2013; Schiel 2012; Taylor and Drickamer 2011; Zaia 2010), have greatly eased structural determination efforts, and the structures of tens of thousands of glycans have been determined (Taylor and Drickamer 2011; Consortium for Functional Glycomics: http://www.functionalglycomics.org/glycomics/common/jsp/firstpage.jsp). However, as mentioned above, our understanding of the functional roles of glycans is still limited.

1.4 Classification of Glycans

The glycans of glycoconjugates (glycoproteins and proteoglycans) are classified into two major groups: (1) *N*-glycans and (2) *O*-glycans (Bhavanandan and Furukawa 1995; Brockhausen et al. 2008; Brooks et al. 2002; Fukuda 2000; Patsos and Corfield 2009; Stanley and Cummings 2008; Stanley et al. 2008; Taylor and Drickamer 2011; Zuber and Roth 2009). *N*-Glycans are linked via an *N*-glycosidic bond formed between the reducing terminal of GlcNAc and the amide nitrogen atom of asparagine (Asn) residues of proteins that are found in the sequon Asn-Xaa-Ser/Thr, where Xaa is any amino acid other than proline. Although serum glycoproteins such as alpha-1-acid glycoprotein contain exclusively *N*-linked glycans, the vast majority of glycoproteins contain both *N*- and *O*-glycans. Some examples of these are fetuin, immunoglobulin A, human chorionic gonadotropin, and many cell surface glycoproteins. *O*-Glycans are linked via an *O*-glycosidic bond formed between anomeric hydroxyl groups of sugars and the hydroxyl group of serine (Ser) or threonine (Thr) residues of proteins. The sugars involved in *O*-glycosidic linkages are predominantly GalNAc and to lesser extents Man, Fuc, Glc, and Gal. In the case of mucins and the mucin-type glycoproteins of the plasma membrane and secreted glycoproteins, the glycan substituents are linked via GalNAc. In mannan-type yeast glycoproteins, and in certain neuronal glycoproteins and proteoglycans and in a few animal muscle glycoproteins, *O*-glycans are linked through α-Man to Ser/Thr (Brooks et al. 2002; Kleene and Schachner 2004; Krusius et al. 1986; Nakamura et al. 2010; Patsos and Corfield 2009; Stanley and Cummings 2008). In proteoglycans, the glycosaminoglycan polysaccharide chains are *O*-linked via β-D-Xyl to Ser residues of proteins (see below). In proteins such as Notch, coagulation factors, and urokinase-type and tissue-type plasminogen activator, *O*-glycans are linked through α-L-Fuc to Ser/Thr (Brooks et al. 2002; Freeze and Haltiwanger 2008; Gebauer et al. 2008; Luther and Haltiwanger 2009; Patsos and Corfield 2009; Stanley and Cummings 2008). In collagens, Galβ1- monosaccharides and Glcα1-2Galβ1- disaccharides are *O*-linked to the hydroxyl group of hydroxylysines.

In various nuclear and cytoplasmic proteins, a special type of glycosylation involving single β-GlcNAc *O*-linked to Ser/Thr is found in almost all eukaryotes and believed to be involved in the regulation of signaling, transcription, and various other biological processes. This special type of glycan modification is discussed by Lagerlof and Hart in Chap. 16.

1.5 Structural Features of *N*-Glycans

All *N*-glycans have a common pentasaccharide "inner core," consisting of three Man and two GlcNAc (residues shown in blue/bold in Fig. 1.5). The occurrence of this conserved core structure is due to the involvement of common biosynthetic

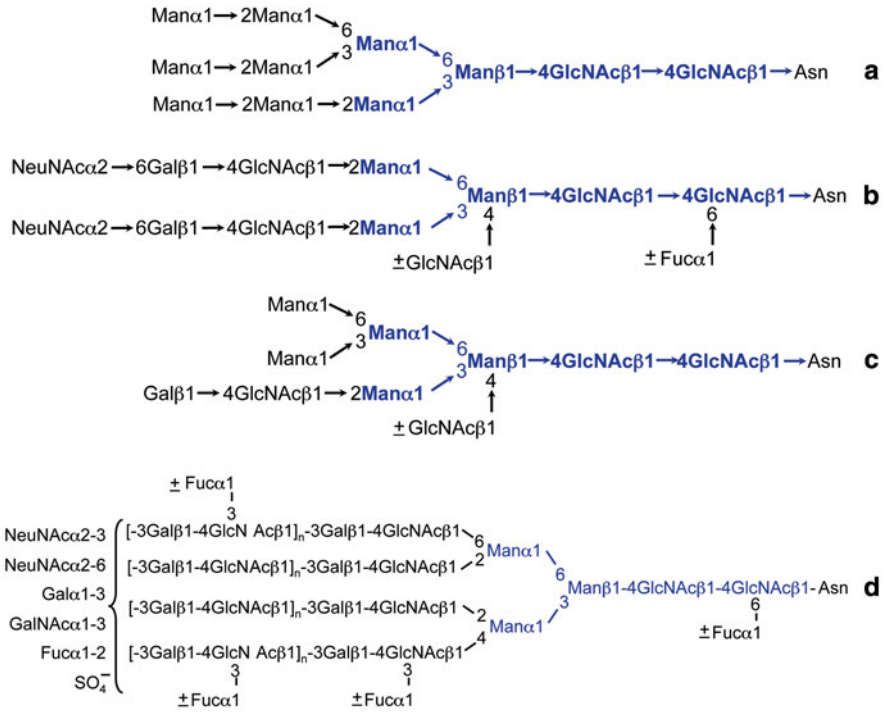

Fig. 1.5 The structural features of *N*-glycans of animal glycoproteins. Because of the involvement of a common biosynthetic lipid-linked oligosaccharide precursor, all *N*-glycans contain a common inner core structure, consisting of three D-mannose (Man) and two *N*-acetyl-D-glucosamine (GlcNAc) residues (shown in *blue/bold*). After transferring to proteins, the common oligosaccharide precursor is modified differently, but retaining the pentasaccharide core, to yield three subclasses of *N*-glycans referred to as high-mannose- or oligomannose-type (**a**), complex- or *N*-acetyllactosamine-type (**b**), and hybrid-type (**c**) *N*-glycans. The complex di-antennary *N*-glycans (**b**) may be further modified to form tri-antennary, tetra-antennary (e.g., **d**), and penta-antennary structures; $n = 2$ to ~50

pathway for all types of *N*-glycan chains found in glycoproteins (Brooks et al. 2002; Fukuda 2000; Stanley et al. 2008; Stanley and Cummings 2008; Taylor and Drickamer 2011; Zuber and Roth 2009). The *N*-glycans are classified into three subgroups based on the types of modifications peripheral to the inner core structures and are referred to as the (1) oligomannose or high mannose type, (2) complex type, and (3) hybrid type (Fig. 1.5). All these subgroups show enormous structural variations due to different types of peripheral modifications, including variation in chain lengths, attachment of different terminal sugars, incomplete addition of terminal sugars, and incomplete chain formation.

Oligomannose-type *N*-glycans contain only Man and GlcNAc residues (Fig. 1.5a), and the heterogeneity in this type of *N*-glycans is due to variations in the numbers and locations of outer Man residues linked to the two α-Man of the inner core. Complex-type *N*-glycans (Fig. 1.5b) exhibit a far wider spectrum of structural variation compared to high-mannose-type *N*-glycans (Brooks et al. 2002; Fukuda 2000; Stanley et al. 2008; Stanley and Cummings 2008; Taylor and Drickamer 2011; Zuber and Roth 2009). Typically, each of the two α-Man residues of the inner core is substituted with one or more *N*-acetyllactosamine (Galβ1-4GlcNAcβ1-) moieties, which form outer chains. Those *N*-glycans that carry two *N*-acetyllactosamine substituents—one chain on each of the α-linked Man are called complex-type di-antennary oligosaccharides (Fig. 1.5b). The glycans that contain more than one *N*-acetyllactosamine substituent on either one or both α-Man are called multi-antennary structures and are referred to as tri-, tetra-, penta-antennary based on the total number of antennas present; an example of a complex tetra-antennary structure is shown in Fig. 1.5d. In *N*-glycans of many animal glycoproteins, the *N*-acetyllactosamine moieties are elongated with repetitive sequential additions of β-GlcNAc and β-Gal, resulting in poly-*N*-acetyllactosamine chains containing two to as many as fifty or more of the repeating disaccharide, -3Galβ1-4GlcNAcβ1- (type 2 *N*-acetyllactosamine), or -3Galβ1-3GlcNAcβ1- (type 1 *N*-acetyllactosamine) units; for example, Fig. 1.5d in which $n = 2$ to ~50. The sugar chains are terminated by the substitution of β-Gal with α(2-3)- and/or α(2-6)-linked SA, α(1-2)-linked Fuc, α(1-3)-linked Gal, α(1-3)-linked GalNAc or sulfate groups. The terminal residues within one *N*-glycan may be the same sugar or two or more different α-linked sugars (SA, Fuc, Gal or GalNAc). In some *N*-glycans, terminal β-Gal residues are unsubstituted or even absent, exposing β-GlcNAc as the terminal sugar. Additionally, a wide range of different types of substitutions on the inner and subterminal type 2 *N*-acetyllactosamine moieties exist, including substitution of inner GlcNAc with α(1-3)-linked Fuc and that of β-Gal with β(1-6)-linked GlcNAc on which *N*-acetyllactosamine chains can be formed and elongated (Stanley and Cummings 2008). Type 1 *N*-acetyllactosamine (-3Galβ1-3GlcNAcβ1-) structures carrying α(1-4)-linked Fuc substitution on GlcNAc also occur (Brockhausen et al. 2008; Brooks et al. 2002; Fukuda 2000; Stanley and Cummings 2008). Moreover, β-Man of the core structure is substituted with a single GlcNAc, forming bisecting structures (Fig. 1.5). The *N*-glycans of secretory glycoproteins and erythrocyte surface proteins exhibit blood A, B, H, Lewis^a, Lewis^b, and other blood group antigenic structures (Brooks et al. 2002; Schachter and Brockhausen 1992; Cummings 1992;

Stanley and Cummings 2008). Many *N*-glycans of animal cells carry sialyl Lewis^x and related antigens, and their expression is regulated during development and differentiation; highly expressed in fetuses but rarely in adults. Sialyl Lewis^x and related structures are also highly expressed by cancer cells and therefore, are referred to as onco-fetal antigens. The hybrid-type *N*-glycans contain structural features of both oligomannose-type and complex-type oligosaccharides (see Fig. 1.5c). These glycans also show variations in the number of Man substitutions and length and substitutions of the complex-type chain, resulting in multiple structures. Because of all the different modifications mentioned above, literally tens of thousands of *N*-glycans occur as constituents of various glycoproteins.

In the central nervous system, in addition to many of the *N*-glycans described above, some unique structures are present (Bruses and Rutishauser 2000; Fukuda 2000; Gascon et al. 2007; Hildebrandt and Dityatev 2013; Kleene and Schachner 2004; Ledeen and Wu 2009; Stanley and Cummings 2008). For example, the neuronal cell adhesion molecules (N-CAMs) of developing brain contains α(2-8)-linked polysialic acid chains made up of as many as 50 or more sialic acid residues that are attached to β-Gal of one or more outer chains of *N*-glycans. This unique modification is implicated in cell migration, neurite outgrowth, and the development of nervous system. Another example of an uncommon glycan present in neural cells is the HNK-1 antigen, sulfate-3GlcAβ1-3Galβ1-4GlcNAc-, found as a terminal structure in glycan chains (Fukuda 2000; Freeze and Haltiwanger 2008; Gebauer et al. 2008; Kleene and Schachner 2004; Luther and Haltiwanger 2009; Schachter and Brockhausen 1992; Stanley and Cummings 2008). HNK-1 epitope specificity is provided by the terminal sulfate-3GlcAβ1-3Galβ1- moiety (Tokuda et al. 1998). The HNK1 antigen was originally identified in human natural killer cells and subsequently found as an antigen involved in the autoimmune disease, peripheral demyelinative neuropathy (Ariga et al. 1987). The antigen is regulated both temporally and spatially in the developing nervous system (Schwarting et al. 1987) and is found in several neuronal cell adhesion molecules, including N-CAM, myelin-associated protein, contactin, L1, and P0. HNK-1 mediates cell–cell and cell–substrate interactions (Ariga 2011; Fukuda 2000; Kizuka and Oka 2012; Kleene and Schachner 2004; Morita et al. 2008; Stanley and Cummings 2008).

1.6 Structural Features of *O*-Glycans

As discussed above, mucin-type glycans that are linked via α-GalNAc to Ser/Thr of proteins are the most abundant *O*-linked glycans in eukaryotic cells, including cells of the central nervous system (Bhavanandan and Furukawa 1995; Brockhausen et al. 2008; Brooks et al. 2002; Patsos and Corfield 2009; Schachter and Brockhausen 1992; Taylor and Drickamer 2011). The α-GalNAc *O*-linked to Ser/Thr can be substituted with β-Gal and/or β-GlcNAc or with an additional α-GalNAc residue, forming eight distinct core structures (Fig. 1.6). The wide variety of *O*-glycans found in animal cells is formed by the addition of different sugars to the core structures.

Fig. 1.6 The structures of core sugar moieties of *O*-glycans and structures of representative *O*-glycans found in animal cells and tissues

O-Glycans having core structures 1–4 are widely distributed in animal cells and tissues, and those with core structures 5–8 are rarely found (Brockhausen et al. 2008; Brooks et al. 2002; Fukuda 2000; Schachter and Brockhausen 1992; Taylor and Drickamer 2011). The unsubstituted GalNAcα1 linked to Ser/Thr constitutes the Tn antigen (Brooks et al. 2002). The substitution of α-GalNAcα1 linked to Ser/Thr with sialic acid forms NeuAcα2-6GalNAcα1, the simplest sialylated disaccharide called the sialyl Tn antigen. The core 1 structure, Galβ-1-3GalNAcα1, is known as the Thomsen–Friedenreich antigen or T antigen. The Tn and T antigens are found at low levels in normal tissues, but are highly expressed in certain tumors (Brooks et al. 2002; Varki et al. 2008). The β-Gal residue of core 1 structure Galβ-1-3GalNAcα1-Ser/Thr can be substituted with α(2-3)-linked NeuAc, and the α-GalNAc can be substituted with α(2-6)-linked NeuAc. Further, the β-Gal residue of the core 2 structure can be substituted with α(2-3)-linked NeuAc, and β-GlcNAc can be substituted with β(1-4)-linked Gal to form a type 2 *N*-acetyllactosamine structure (Fig. 1.6). Similarly, both β-GlcNAc of core 4 can be substituted with β(1,3)-linked Gal to form two branches having type 1 *N*-acetyllactosamine structure. As in the case of *N*-glycans, in both core 2 and core 4 structures, the *N*-acetyllactosamine is either terminated by the substitution of α-linked NeuAc, Fuc, Gal, and GalNAc or elongated to form polymeric *N*-acetyllactosamine chains, which are then terminated with α-linked sugars. The *O*-linked glycans of some animal mucins are also sulfated,

typically at the terminal ends and/or on internal Gal and GlcNAc. Many *O*-glycans of secretary proteins also contain the repeating units of type 1 *N*-acetyllactosamine -3Galβ1-3GlcNAcβ1- or type 2 *N*-acetyllactosamine structure, -3Galβ1-4GlcNAcβ1- structures (Brockhausen et al. 2008; Brooks et al. 2002; Patsos and Corfield 2009; Schachter and Brockhausen 1992; Taylor and Drickamer 2011). As in *N*-glycans, the inner β-Gal can be substituted with β(1-6)-linked GlcNAc to form a branched structure, which can also be elongated with *N*-acetyllactosamine moieties. In addition, the β-GlcNAc residues of type 1 and type 2 *N*-acetyllactosamine can be substituted with, respectively, α(1-4)-linked and α(1-3)-linked Fuc. These and the substitution of subterminal β-Gal with α-linked sugars results in the formation of *O*-glycans carrying the blood group A, B, H, and Lewis antigens, and development- and differentiation-specific sialyl Lewis antigens (Brockhausen et al. 2008; Brooks et al. 2002; Patsos and Corfield 2009; Schachter and Brockhausen 1992; Taylor and Drickamer 2011). All the above mentioned and various other type of modifications give rise to numerous distinct *O*-glycans found in animal glycoproteins.

Although *O*-glycans linked via α-GalNAcα1-Ser/Thr are found in most glycoproteins, they are abundantly present in two groups of glycoproteins: (1) mucins produced by epithelia of salivary glands, and the respiratory, gastrointestinal and urogenital tracts, and (2) membrane-associated mucin-like glycoproteins (Bhavanandan and Furukawa 1995; Brockhausen et al. 2008; Brooks et al. 2002; Patsos and Corfield 2009; Schachter and Brockhausen 1992; Taylor and Drickamer 2011). Epithelial mucin glycoproteins contain hundreds of α-GalNAc-linked *O*-glycans, which account for as much as 50–80 % of the mass of mucin molecules. In these mucin molecules, the *O*-glycans are found clustered at certain regions of the protein backbones. Because of the high levels of *O*-glycan substitution, mucin molecules can hold large amount of water and assume extended structures. In addition, because of the high net negative charge imparted by sialic acid and sulfate groups, mucin molecules in solution are randomly oriented, exhibiting high viscosity. This property allows mucin to serve as a protective barrier for epithelia against physical abrasion and inhibit infection by functioning as decoys for the adherence of pathogens. The latter is a double-edged sword since pathogens such as influenza virus and *Helicobacter pylori* exploit these molecules for their invasion/attachments and infect the respiratory system and stomach, respectively. Abnormality in mucin structure/function is implicated in the pathobiology of several human diseases (Brooks et al. 2002; Hennet et al. 2009; Taylor and Drickamer 2011; Varki and Freeze 2008). These include cystic fibrosis, chronic bronchitis, Crohn's disease, duodenal ulceration, colonic adenocarcinomas, infertility problems, and inflammatory ulcerative colitis.

Compared to secretory epithelial mucins, the carbohydrate content in many membrane-associated cell surface glycoproteins is relatively less because they have fewer and shorter glycan chains. Examples of such glycoproteins are the erythrocyte membrane glycophorins, and most of the cell membrane and tissue matrix glycoproteins of animal tissues, including the central nervous system. Other examples of membrane-associated, mucin-like glycoproteins are human white blood cell-associated leukosialin (CD43, sialophorin), red blood cell membrane decay-accelerating factor, low-density lipid receptor, platelet membrane-associated CD42b (glycocalicin), and human milk fat granule membrane glycoproteins. These glycoproteins contain

hundreds of shorter *O*-glycans and are present as highly extended structures, projecting far beyond the cell surface (Bhavanandan and Furukawa 1995; Brooks et al. 2002; Fukuda 2000; Taylor and Drickamer 2011). As in the case of *N*-glycans, expression of the membrane-associated cell surface *O*-glycans is markedly increased or altered in malignantly transformed cells. Because of their important role in the pathobiology of cancer there has been extensive research on this subclass of *O*-glycans. These glycans are also widely implicated as ligands for selectins, which are involved in thrombosis, inflammation, allergy, auto immunity, and cancer metastasis. For example, the endothelial cell-associated mucin-type glycoproteins such as GlyCAM-1, CD34, and MAdCAM-1 function as ligands for L-selectin, and the leukocyte membrane mucin-type glycoprotein PSGL-1 has been shown to be a ligand for P-selectin and E-selectin. Thus, these mucin-like glycoproteins are involved in recognition, binding and recruitment of immune cells to the site of injury, and in homing of the circulating lymphocytes to lymph nodes (Homeister and Lowe 2000).

It has been shown that Fuc *O*-linked to the Ser/Thr residues of EGF-like repeat-containing proteins is substituted to form GlcNAcβ1-3Fucα1-, Galβ1-4GlcNAcβ1-3Fucα1-, Galβ1-4GlcNAcβ1-3Fucα1, NeuAcα2-6 Galβ1-4GlcNAcβ1-3Fucα1-oligosaccharides, which play important roles in intracellular signaling (Brooks et al. 2002; Gebauer et al 2008; Freeze and Haltiwanger 2008; Kleene and Schachner 2004; Luther and Haltiwanger 2009; Stanley and Cummings 2008; Taylor and Drickamer 2011). Some glycoproteins and proteoglycans of the nervous system, and neuronal and muscle α-dystroglycan, in addition to having the GalNAc-linked *O*-glycans, are modified with significant levels of *O*-glycans linked via α-Man to Ser/Thr (Chai et al. 1999; Krusius et al. 1986; Nakamura et al. 2010). The α-Man that is *O*-linked to Ser/Thr is substituted with either β1-2-linked GlcNAc residues or β1-2- and β1-6-linked GlcNAc residues. These GlcNAc residues are further substituted by sequential addition of β(1-4)-linked Gal and terminated with NeuAc or 3-sulfated glucuronic acid; the latter forming the HNK-1 epitope (Ariga 2011; Fukuda 2000; Kleene and Schachner 2004; Kizuka and Oka 2012; Morita et al. 2008; Stanley and Cummings 2008). Deficiency in the *O*-Man-linked oligosaccharide modification is associated with several types of severe brain and eye abnormalities, mental retardation, severely impaired mobility, muscle weakness, reduced muscle bulk, and dystrophic muscle (Kleene and Schachner 2004; Nakamura et al. 2010).

1.7 Biosynthesis of *N*- and *O*-Glycans

One important difference in the biosynthesis of glycans compared to nucleic acids and proteins is that the synthesis of glycans is not template dependent. Instead the genetic control is exerted by the expression of enzymes called glycosyltransferases, which catalyze the biosynthesis of glycans (Brockhausen et al. 2008; Brooks et al. 2002; Cummings 1992; Stanley et al. 2008; Schachter and Brockhausen 1992; Taylor and Drickamer 2011; Zuber and Roth 2009). The glycosidic bond formation involves

the transfer of sugars from activated donors (nucleotide sugars) to acceptor monosaccharide or oligosaccharide substrates, which exist either as free molecules or as moieties linked to proteins/lipids. The activated sugars are derivatives of either uridine or guanidine diphosphate and of cytidine monophosphate in the case of sialic acids. The biosynthesis occurs at several locations, cytoplasm, lumen of the endoplasmic reticulum (ER), and lumen of the Golgi. The nucleotide sugars are formed in the cytoplasm and, for glycan biosynthesis, are transported to the Golgi by membrane transporters. In contrast, the ER membrane has no nucleotide-sugar transporters; sugar donors for glycan synthesis in the ER lumen are dolichol phosphate sugars formed by the transfer of sugars from nucleotide sugars in the cytoplasm to dolichol phosphate in the membrane. The dolichol phosphate sugars in which the sugar residues face the cytoplasmic side are then flipped to the luminal side and thus can donate sugar residues to acceptor molecules. *N*-Glycans are initially synthesized in the ER as dolichol diphosphate-linked high-mannose-type oligosaccharides containing three terminal Glc, eight Man, and two internal GlcNAc residues, and the oligosaccharide is transferred *en bloc* to the Asn residues of Asn-X-Ser/Thr motifs of polypeptide chains while the latter are still being synthesized. The oligosaccharides on proteins are then processed beginning in the ER but mostly in the Golgi to complex-type or hybrid oligosaccharides (Fig. 1.5). The *O*-glycans and glycosaminoglycans are synthesized in the Golgi by the addition of sugars sequentially after the first sugar is added to the Ser/Thr residues of proteins.

The general reaction for glycan biosynthesis is represented as the following: nucleotide-sugar donors + acceptor substrates → products + nucleotide diphosphate. The reaction is driven by the energy released by the hydrolysis of nucleoside diphosphate into nucleoside monophosphate and phosphate. Sugars are transferred from donor nucleotide sugars to different hydroxyl groups of acceptor monosaccharides, oligosaccharides, proteins, or lipids. The glycosidic linkages thus formed vary with respect to their positions and animeric configarations. The glycosyltransferases determine the types of glycosidic linkages formed by exhibiting specificity toward the donor sugars and acceptor mono- or oligosaccharide substrates. Therefore, it is the specificity of glycosyltransferases that primarily directs and controls the formation of a particular glycan. In general, one glycosyltransferase is required for the formation of each type of glycosidic linkage between two sugars in glycans, although a few exceptions exist. The glycosyltransferases involved in *N*-and *O*-glycan biosynthesis are membrane-bound enzymes and are involved in an assembly line-like process in the synthesis of specific glycans. The biosyntheses of *O*-and *N*-glycans are discussed in detail in Chap. 3 and 4, respectively.

1.8 Glycosphingolipids

Glycosphingolipids (GSLs) are a class of glycolipids that are conjugates of glycans and ceramide-containing lipids and are distributed widely in all organisms (Brooks et al. 2002; Kopitz 2009; Kundu 1992; Leeden and Wu 2009; Schnaar et al. 2008; Taylor and Drickamer 2011). They are ubiquitous plasma membrane components

and are primarily localized in the outer leaflet with their hydrophilic sugar moieties projecting outside of the cell. GSLs are abundantly found in the brain. The glycan moieties on the cell surface function as specific receptors for certain pituitary hormones, growth factors, viruses, and cholera, tetanus, and botulinum bacterial toxins. They also serve as specific determinants of cell–cell interactions and as tumor-associated antigens. Disorders of GSL catabolism are responsible for several genetic diseases, which are referred to as glycosphingolipid storage diseases. These diseases are caused by defects in specific lysosomal glycosidases, involved in the degradation of the glycan chains or in sphingolipid activator proteins. For example, Tay–Sachs and Gaucher's diseases are caused by defects in *N*-acetyl-β-hexosaminidase and β-glucosidase, respectively. The inborn errors of GSL catabolism are discussed in detail in Chap. 21.

1.9 Structural Features of the Glycan Moieties of Glycosphingolipids

In the majority of glycolipids, glycans are conjugated to the terminal (C-1) primary hydroxyl group of ceramides (Cer), which are *N*-acyl fatty acid derivatives of sphingosine. Both the fatty acid (acyl chain) and sphingosine moieties of ceramides are heterogeneous (Brooks et al. 2002; Hakomori 2003; Kopitz 2009; Kundu 1992; Leeden and Wu 2009; Schnaar et al. 2008; Taylor and Drickamer 2011). The acyl chain can be 14–26 carbons in length, saturated, unsaturated, or 2-hydroxylated. The sphingosine can be 14–20 carbons in length. The carbohydrate moieties of the GSLs consist of the following sugars: D-Glc, D-Gal, L-Fuc, D-GlcNAc, D-GalNAc, and NeuAc. The simplest GSLs are the monohexosylcerebrosides, glucocerebroside (Glcβ1-1Cer), and galactocerebroside (Galβ1-1Cer). While the Glc residue of Glcβ1-1Cer is the link sugar in complex GSLs having a wide range of short to large glycan chains, the Gal residue of Galβ1-1Cer is elaborated only to a limited degree to form GSLs with only short glycan chains and the sulfatide, 3-*O*-sulfo-Galβ1-ceramide (Kundu 1992). Thus, the glycan chains of different compositions, linkage types, and chain lengths are formed by the sequential addition of other sugars to the core Glc residue linked to Cer. Further, like in the case of glycoprotein glycans, the glycan chain lengths of glycolipids in certain cells such as erythrocytes and granulocytes are highly extended with repeating *N*-acetyllactosamine units, and inner GlcNAc are substituted with Fuc to form Lewis antigens (Fukuda et al. 1986; Fukuda and Hakomori 1982; Müthing 1996; Stanley and Cummings 2008). In addition, these chains may be terminated by one or more of α-linked NeuAc, Fuc, Gal, or GalNAc to yield various blood group and sialyl Lewis antigens (Stanley and Cummings 2008). Based on the core structures of glycan moieties that are attached to the Cer moiety, GSLs are classified into different subclasses: lacto-, lactoneo-, globo-, isoglobo-, ganglio-, muco-, and gal-series (Table 1.1). Lactosylceramide (Galβ1-4Glcβ1-1Cer) serves as the precursor for the synthesis of five families of GSLs. The GSLs that contain sialic acid are

Table 1.1 The core structures of major glycolipids found in animals

Type	Structure
Lacto	Galβ1-3GlcNAcβ1-3**Galβ1-4Glcβ1-1-ceramide**
Lactoneo	Galβ1-4GlcNAcβ1-3**Galβ1-4Glcβ1-1-ceramide**
Globo	GalNAcβ1-3Galα1-4**Galβ1-4Glcβ1-1-ceramide**
Globoneo	GalNAcβ1-3Galα1-3**Galβ1-4Glcβ1-1-ceramide**
Isoglobo	GalNAcβ1-3Galα1-3**Galb1-4Glcβ1-1-ceramide**
Ganglio	Galβ1-3GalNAcβ1-4**Galβ1-4Glcβ1-1-ceramide**
Muco	Galβ1-3Galβ1-3**Galβ1-4Glcβ1-1-ceramide**
Galacto	Galα1-4Galβ1-1-ceramide
Sulfatides	3-*O*-Sulfo-Galβ1-ceramide

The sugars highlighted in bold represent common core residues

called gangliosides. As in glycoproteins, the structures of GSLs are defined by the specificity of glycosyltransferase and their relative levels and distribution in the cells.

GSLs are present ubiquitously in cells, and the subclasses are differentially distributed in different tissues (Kundu 1992). For example, gangliosides are present at high levels in neural tissues. Among the non-sialylated GSLs, only Gal-Cer and sulfatide, SO_3-3Gal-Cer, but not Glc-Cer are present in brain and other neural tissues. However, fetal and neonatal brains contain almost equal amounts of Gal-Cer and Glc-Cer. Erythrocytes contain mainly Glc-Cer and complex GSLs having large glycan chains, whereas the kidney and intestine have substantial levels of both Glc-Cer and Gal-Cer (Kundu 1992).

Sialic acid-containing GSLs were first isolated in high yield from the brain and were thought to be exclusively present in ganglia and hence named gangliosides. However, gangliosides were subsequently found to be present throughout the body, albeit at relatively lower levels. NeuAc is the almost exclusive sialic acid in gangliosides of humans, whereas animal gangliosides contain both NeuAc and NeuGc and their acetylated and/or methylated derivatives (Brooks et al. 2002; Schauer 2004; Varki 1992; Varki et al. 2008). The gangliosides of various animal brains contain one to as many as five sialic acid residues. Gangliosides are named according to the rules proposed by Dr. Lars Svennerholm (1994). G_M, G_D, G_T, G_Q, and G_P, where G stands for ganglio, and the subscript letters define the total number of sialyl residues, indicating mono-, di- tri-, tetra- and penta-sialylated gangliosides, respectively. The numerical numbers 1, 2, 3, and 4 following these letters define 5 minus the number of neutral sugars in the molecule. The lowercase letters, *a, b,* or *c* after the numerical number define, respectively, one, two, or three SA residues on the inner Gal. For example, G_{T1b} refers to trisialyl globotetraosyl ganglioside having SAα2-8SA linked through α(1-3) glycosidic bond to the inner Gal residue. The SA residues on Gal are α(2-3)-linked, while the SA–SA glycosidic bond is α(2-8)-linked. One or more SA residues can be similarly linked to the terminal Gal. The structures of the lacto-ceramide G_M gangliosides are shown in Fig. 1.7 and examples of other gangliosides are available elsewhere (Hakomori 2003; Kopitz 2009; Kundu 1992; Schnaar et al. 2008).

An important difference in GSLs compared to glycoproteins and proteoglycans is the absence of heterogeneity at specific glycosylation sites. Glycan microheterogeneity is an inherent factor in glycoproteins and proteoglycans. Not only does the number

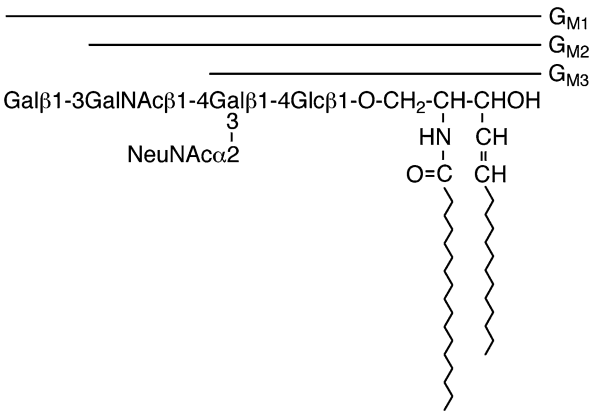

Fig. 1.7 The structures of monosialogangliosides, G_{M1}, G_{M2}, and G_{M3}, containing four, three, and two neutral monosaccharides, respectively. G_{M4} is NeuAcα2-3Galβ1-1Cer

of glycan chains attached to core proteins vary widely, there can also be heterogeneity in the glycan at a single site. For example, the single *N*-linked oligomannose chain attached to a specific Asn residue in ribonuclease varies widely in its number of mannose residues. In contrast, in GSLs such as G_{D1a} ganglioside (NeuAcα2-3Galβ1-3GalNAcb1-4(NeuAc2α-3)Galβ1-4Glcβ1-ceramide) and G_{M3} ganglioside (NeuAcα2-3Galβ1-4Glcβ1-ceramide), the glycan chains are well defined and homogenous. While there is heterogeneity in the length of fatty acyl chain and sphingosine moiety of ceramide as explained earlier, there is no glycan chain heterogeneity. In fact, if one monosaccharide is missing in the carbohydrate chain, the molecule becomes a different glycolipid.

1.10 Synthesis and Functions of the Glycan Moieties of Glycolipids

As in the case of glycoprotein *O*-glycans, glycolipids are synthesized in the Golgi by the sequential addition of sugars to the C-1 hydroxyl group of ceramide. The newly synthesized glycolipids are then transported to plasma membranes (Brooks et al. 2002; Kopitz 2009; Kundu 1992; Schnaar et al. 2008).

Like *N*- and *O*-glycans of glycoproteins, GSLs perform numerous physiological functions, including cell–cell and cell–molecule interactions that are critical for biological processes such as development, differentiation, defining cell–cell communications, cell social behavior, antigenicity, modulation of immune responses, and cell signaling. As in the case of glycoproteins, the glycan moieties of GSLs can also function as various blood group- and development- and differentiation-specific antigens such as sialyl Lewis antigen (Stanley and Cummings 2008). In the central nervous system, GSLs are involved in many tissue-specific functions, including neuritogenesis, nerve repair, inhibition of neurite outgrowth, neuromuscular formation, cell social behavior, and various other functions (Hakomori 2003; Kopitz 2009; Kundu 1992; Schnaar et al. 2008).

Alterations in GSL composition, levels of expression, and distribution appear to contribute to tumor growth and spreading. For example, high levels of G_{M2} and G_{D2} gangliosides are found in melanoma and neuroectodermal tumors, and G_{D3} ganglioside containing 9-*O*-acetyl neuraminic acid is highly expressed by melanoma tumors (Kundu 1992). Various sialyl Lewis antigen-containing GSLs are also highly expressed by teratocarcinoma, colorectal adenocarcinoma, pancreatic cancer, ovarian cancer, and other cancers. A number of monoclonal antibodies such as CA50, N-19-9, OFA-1, and OFA-2 that specifically recognize GSL glycan antigens are being used for diagnosis of cancers, including breast cancer, brain tumors, and colon cancer (Kundu 1992).

1.11 Glycosylphosphatidylinositol Anchors: A Special Group of Glycolipids

Glycosylphosphatidylinositol (GPI) anchors represent a distinct class of glycolipids and consist of glycan moieties attached to the C-6 position of the *myo*-inositol residue of phosphatidylinositol (PI) (Brooks et al. 2002; Ferguson et al. 2008; Paulick and Bertozzi 2008; Shams-Eldin et al. 2009; Taylor and Drickamer 2011). The glycan moieties of GPIs consist of a conserved trimannosyl core substituted with ethanolamine at the C-6 hydroxyl group of the terminal mannose, ethanolamine-phosphate-6Manα1-2Manα1-6Manα1-4GlcNα1-. The trimannosyl core moiety in GPIs from many sources is modified with additional sugars and one or more ethanolamine phosphate residues (Fig. 1.8). The lipid portion contains saturated or

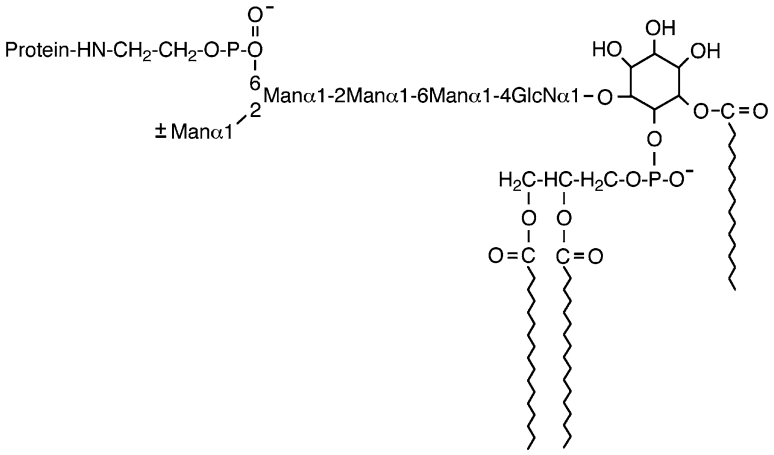

Fig. 1.8 Representative structure of a GPI anchor. The inner trimannosyl moiety is usually modified with additional sugars and ethanolamine phosphate residue(s). The lipid chains on glycerol are either acyl/acyl or acyl/alkyl chains. The chains lengths of both acyl and alkyl residues are variable and may contain unsaturated bonds (see ref. (Ferguson et al. 2008) for GPIs from many sources). GPIs from some sources carry acyl substituent, usually C16:0 at C-2 of *myo*-inositol

unsaturated fatty acyl/alkyl residues of 16–24 carbon atoms in length at the C-1 and C-2 positions of glycerol. In addition, the C-2 position of the *myo*-inositol residue is either substituted with fatty acid or unsubstituted. GPIs from different cells and species exhibit broad structural diversity due to heterogeneity in the structures of the glycan and lipid moieties (Ferguson et al. 2008).

GPIs are expressed ubiquitously by all eukaryotic cells and are found in all animal tissues. GPIs are particularly abundant in parasites, including species of the genus *Trypanosoma*, *Leishmania*, and *Plasmodium* (Ferguson et al. 2008; Shams-Eldin et al. 2009). The primary role of GPIs is to anchor certain proteins, glycoproteins, and proteoglycans of cells, including those in the brain, to the plasma membrane via formation of an amide bond between the amino group of the ethanolamine residue and the C-terminal carboxyl group of the protein. In addition to anchoring proteins to the plasma membrane, GPIs seem to be involved in many biological functions (Ferguson et al. 2008; Paulick and Bertozzi 2008; Shams-Eldin et al. 2009). GPI modification is not limited to a specific class of proteins/glycoproteins. Proteins including enzymes, receptors, cell surface antigens, cell adhesion molecules, transporters, and other functional proteins are anchored to plasma membranes via GPIs. Specific examples of GPI-anchored proteins include acetylcholine esterase and decay-accelerating factor present on the erythrocyte membrane, placental membrane alkaline phosphatase, the Thy-1 antigen of lymphocytes, the neural cell adhesion molecule and brevican proteoglycan.

Biosynthesis of GPIs occurs exclusively in the ER, although some modifications to the glycan moieties may occur after the GPI-anchored proteins move to the Golgi. In the ER, first GlcNAc is added to the membrane PI moiety on the cytoplasmic side, and after *N*-deacetylation of GlcNAc and acylation of *myo*-inositol residue at C-2, the GlcN-PI intermediate flips over to the luminal side of the ER, where Man residues donated by dolichol phosphate-Man are sequentially added. The fully assembled GPI is then transferred to the carboxyl group of the C-terminal amino acid of the acceptor protein to form an amide bond by the action of a transamidase, thereby anchoring the protein to the membrane.

1.12 Glycosaminoglycans and Proteoglycans

Glycosaminoglycans (GAGs) are linear, anionic polysaccharides consisting of alternating residues of an uronic acid or galactose and an *N*-acetylhexosamine (Bhavanandan and Davidson 1992; Buddecke 2009; Esko et al. 2008; Hascall and Esko 2008; Sasisekharan et al. 2008; Volpi 2006). An additional structural feature is that all GAGs except hyaluronic acid (see below) contain sulfate. GAGs are made up of repeating disaccharide units with or without sulfate; two such repeats of each GAG are illustrated in Fig. 1.9. Several types of GAGs occur in animals and are classified into two broad groups based on the type of *N*-acetylhexosamine present: (1) Glucosaminoglycans, containing either GlcNAc or *N*-sulfated glucosamine ($GlcNSO_3^-$), include hyaluronic acid (HA), heparan sulfate (HS), heparin, and

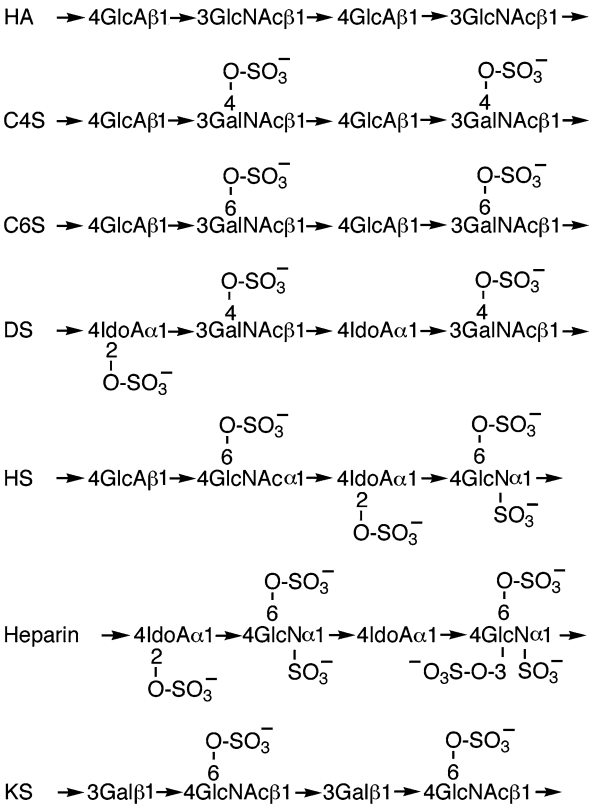

Fig. 1.9 The structural features of glycosaminoglycans (GAGs) that are commonly found in animal cells and tissues. Two repeating disaccharide units each consisting of alternating residues of an uronic acid or galactose and an *N*-acetylated or *N*-sulfated hexosamine are shown. Note that all GAGs except hyaluronic acid (HA) are sulfated

keratan sulfate (KS). (2) Galactosaminoglycans, containing GalNAc, are chondroitin sulfates (CS). In early studies, three types of galactosaminoglycans were discovered and initially named CSA, CSB, and CSC. After the structures were elucidated, these GAGs were renamed chondroitin-4-sulfate (C4S), dermatan sulfate (DS), and chondroitin-6-sulfate (C6S), respectively. DS differs from C4S and C6S in having predominantly IdoA instead of GlcA. There are several other chondroitin sulfates that can be distinguished based on the position and number of sulfate groups present, for example, chondroitin 2,6-sulfate (C2,6diS; CSD) and chondroitin 4,6-sulfate (C4,6diS or CSE) (Malavaki et al. 2008; Nandini and Sugahara 2006). In CS and DS, the sulfate groups are mainly present on GalNAc residues. However, in DS and in C2,6diS, additional sulfate groups are present at C-2 of certain IdoA and GlcA residues, respectively.

The nonsulfated GAG, HA, consists exclusively of uniformly defined repeating disaccharide units (see Fig. 1.9). In contrast, the sulfated GAGs from various tissues of different animals are highly heterogeneous with respect to uronic acid composition and sulfate content. In addition, the disaccharide moieties with different compositions are variously distributed in the polymer chains. Thus, not all repeating disaccharide units of C4S and C6S from different animal tissues are exclusively and

uniformly sulfated at C-4 and C-6, respectively. C4S from many sources contains significant amounts of nonsulfated and 6-sulfated disaccharide units. For example, bovine cartilage C4S consists of ~53 % 4-sulfated, ~39 % 6-sulfated, and ~8 % nonsulfated disaccharide units. CS from some sources, including those of the brain, have, in addition to significant levels of both C4S and C6S repeating disaccharide units, one or more elements of 2,4-di-, 3,4-di-, 3,6-di-, and 3,4,6-trisulfated disaccharide moieties (Malavaki et al. 2008; Nandini and Sugahara 2006). Some tissues such as the cornea and placenta contain uniquely low sulfated CS with 80–95 % of the disaccharides not sulfated. In placental CS, only 5–10 % of the disaccharides are sulfated exclusively at C-4, and in corneal CS, the sulfation is mainly at C-4 and to certain extent at C-6 (Achur et al. 2000, 2004). DS from different tissues, including those from nervous tissues, while containing mainly IdoA, have low to moderate levels of GlcA. As in the case of oversulfated CS, 2,4-di-, 3,6-di, 4,6-di, and 2,4,6-trisulfated disaccharide units are also found in some DS (Nandini and Sugahara 2006). The variations in uronic acid composition and the position and degree of sulfation lead to incredible structural diversity and enormous microheterogeneity in the structures of GAGs. Interestingly, as in the case of *N*- and *O*-glycans, it is this structural variation and microheterogeneity that confer the ability for GAGs to perform a wide range of biological functions. The structure and functions of GAGs of the nervous system are discussed in detail in Chap. 5.

Heparan sulfate (HS) and heparin vary substantially in the proportions of GlcA and IdoA as well as sulfate content (Bhavanandan and Davidson 1992; Buddecke 2009; Esko et al. 2008). Usually, HS contains nearly equal levels of GlcA and IdoA, whereas in heparin, >80 % of the uronic acid is IdoA and the remainder is GlcA. HS has 0.5–1.5 sulfate groups per disaccharide unit, but the average sulfate content of heparin varies from 1.5 to 2.5 sulfate groups per disaccharide moiety. In heparin, the majority of glucosamine is, in addition to having sulfate at C-6, *N*-sulfated, and some *N*-sulfated glucosamine residues are sulfated at both C-3 and C-6. Because of variations in GlcA and IdoA contents, the level of sulfate, and sulfation at different positions, including *N*-sulfation and variations in modifications at different regions of the polymer chains, both HS and heparin exhibit enormous microheterogeneity. The microheterogeneity in HS and heparin is far higher than that of CS and DS.

GAGs are ubiquitous constituents of all animal tissues and are expressed by almost all cell types of eukaryotes (Achur et al. 2000, 2004; Bernfield et al. 1999; Bhavanandan and Davidson 1992; Bishop et al. 2007; Brooks et al. 2002; Buddecke 2009; Esko et al. 2008; Hascall and Esko 2008; Malavaki et al. 2008; Nandini and Sugahara 2006; Sasisekharan et al 2008; Taylor and Drickamer 2011; Volpi 2006). CS, DS, and HA occur abundantly as gel-like ground substances in the extracellular matrix of connective tissues such as cartilage, tendon, skin, cornea, blood vessels, and umbilical cord. They also occur at substantial levels in matrices of almost all other tissues, including those of the central nervous system, and at moderate levels in the form of proteoglycans in plasma membrane. Although present at significant levels in the matrices of connective and skeletal tissues and brain (Funderburgh 2000; Krusius et al. 1986; Zhang et al. 2006), keratan sulfate (KS) is particularly

abundant in the cornea (Funderburgh et al. 1987). HS is ubiquitously found as a cell surface component and also occurs as an extracellular component in blood vessels, in the brain, and in basement membranes, particularly in the kidney, where it is involved in filtration (Bhavanandan and Davidson 1992). Heparin, on the other hand, is exclusively found in intracellular granules of mast cells that line the arterial walls. It is secreted in response to injury and functions as an anticoagulant to regulate the blood-clotting cascade (Bhavanandan and Davidson 1992).

Because of the presence of numerous hydrophilic hydroxyl groups and the high net negative charge imparted by carboxyl and sulfate groups, GAGs have the capacity to hold large amounts of water and stay as extended molecules in solution. They form highly viscous and slimy mucus-like solutions and hence their older name, mucopolysaccharides, which is still used occasionally. The ability to form gel-like substance enables GAGs to function as shock absorbers and lubricants in joints and umbilical cord and to impart resilience to tissues. GAGs also provide nutrients to cartilage and other connective tissues lacking blood vessels by their property to absorb and release extracellular fluid in the absence and presence of mechanical shear force and to regulate tissue calcification (Bhavanandan and Davidson 1992). More importantly, GAGs are involved in cell–cell and cell–molecule interactions, cell–pathogen binding, cell signaling, binding and mobilizing growth factors, chemokines and cytokines, promoting growth, and regulating immune responses (Achur et al. 2000, 2004; Bernfield et al. 1999; Bishop et al. 2007; Bhavanandan and Davidson 1992; Brooks et al. 2002; Buddecke 2009; Esko et al. 2008; Funderburgh 2000; Funderburgh et al. 1987; Hascall and Esko 2008; Iozzo and Schaefer 2010; Malavaki et al. 2008; Nandini and Sugahara 2006; Sasisekharan et al 2008; Taylor and Drickamer 2011; Varki et al. 2008; Volpi 2006; Zhang et al. 2006). Through these interactions, GAGs play important roles in biological processes such as development, differentiation, cell migration, tissue organization, cartilage and bone formation, wound healing, and in disease processes, including cancer and atherosclerosis. Furthermore, deficiencies in GAG catabolism due to lysosomal enzyme deficiencies lead to many diseases called mucopolysaccharidoses, which are discussed in detail in Chap. 5.

All GAGs, except HA, regardless of whether they are the components of various extracellular matrices or cell membranes, occur as moieties conjugated to proteins to form proteoglycans (PGs). A common tetrasaccharide core covalently attaches GAG (CS, DS, HS, and heparin) chains via xylose to the hydroxyl groups of Ser residues: **[HexNR-HexA]$_n$-GlcAβ1-4Galβ1-3Galβ1-4Xylβ1-Ser**, where HexNR is variously sulfated GlcNAc or GalNAc, HexA is nonsulfated or sulfated GlcA or IdoA, and n is number of repeating disaccharide units.

In proteoglycan biosynthesis, the addition of GlcNAc to the protein-linked tetrasaccharide core commits to the formation of heparan sulfate and heparin chains, whereas the addition of GalNAc leads to the formation of CS or DS chains. Once commitment to synthesize either CS or HS is made, presumably by the specific recognition of core proteins by the glycosyltransferase, the GAG chains are synthesized by the sequential addition of HexNAc and GlcA. Then, the sulfate residues are added, and in the case of DS, HS, and heparin, IdoA residues are formed by the

epimerization of D-GlcA to L-IdoA residues. The Ser–Gly motifs of proteins are sites for the attachment of GAG chains. If several Ser–Gly are present, the proteins are modified with multiple GAG chains. For example, the core proteins of serglycin and aggrecan contain 49 and >100 tandem repeats of Ser–Gly motifs, respectively, and all or most of these motifs are substituted with CS chains. The majority of PGs contain one type of GAG chain, CS, DS, or HS, and are designated as CSPGs, DSPGs, and HSPGs, respectively. However, some PGs contain two types of GAG chains such as CS plus HS on the same core protein molecule and thus are hybrid PGs. For example, syndecan, a cell surface PG expressed by a number of cell types, contains both CS and HS chains. KS also is found in PGs, and examples of KS containing PGs include lumican, keratocan, fibromodulin, mimecan, and aggrecan. In the case of KS, the disaccharide-containing polymer chains are built on the core residues of either *N*-linked or *O*-linked glycans in a manner similar to those of poly-*N*-acetyllactosamine chains. The KS are classified into three types, KS-I, KS-II, and KS-III (Funderburgh 2000). KS-I chains are mainly found in the cornea and are linked to proteins via the core glycan moiety of *N*-glycans to Asn residues. KS-II chains are present mainly in skeletal tissues and are linked through α-GalNAc to Ser/Thr of proteins. KS-III is present in the brain and is linked via *O*-linked β-Man residues to Ser/Thr of proteins (Krusius et al. 1986).

In many PGs, including those of the central nervous system, the GAG chains define their functions, and hence the functions of PGs are the same as those mentioned above for GAGs. In addition, the core proteins of many PGs contain functional domains, including the C-type lectin domain that interacts with HA, FGF, fibronectin III, laminin-G, and fibronectin. As such, PGs interact with cell surface and extracellular matrix proteins and these interactions define their function.

1.13 Glycans of Cell Surface Glycoconjugates Perform a Variety of Functions

The majority of animal cell surface proteins, including those on cells of the central nervous system, are modified with variable amounts of *N*- and *O*-linked glycans and/or glycosaminoglycan chains (Brooks et al. 2002; Collins and Paulson 2005; Fukuda 2000; Hattrup and Gendler 2008; Iozzo and Schaefer 2010; Schauer 2009; Springer and Gagneux 2013; Taylor and Drickamer 2011; Varki and Lowe 2008). In some instances, such as in mucin-type glycoproteins, the mass of glycans exceeds that of proteins to which they are conjugated. In addition, a significant portion of the outer head group of cell surface ceramide is modified with glycans. Thus, the *N*- and *O*-glycans of glycoproteins, the glycan moieties of glycolipids, and the chondroitin sulfate and heparan sulfate chains of proteoglycans are prominently displayed on the outer leaflet of the plasma membrane (Fig. 1.10). As mentioned earlier, because of high hydrophilicity, glycans have a large capacity to hold water and exist as bulky and highly extended molecules. The eukaryotic cell surface is thus covered with a glycan-rich zone, referred to as the glycocalyx (Weinbaum et al. 2007; Salmon and

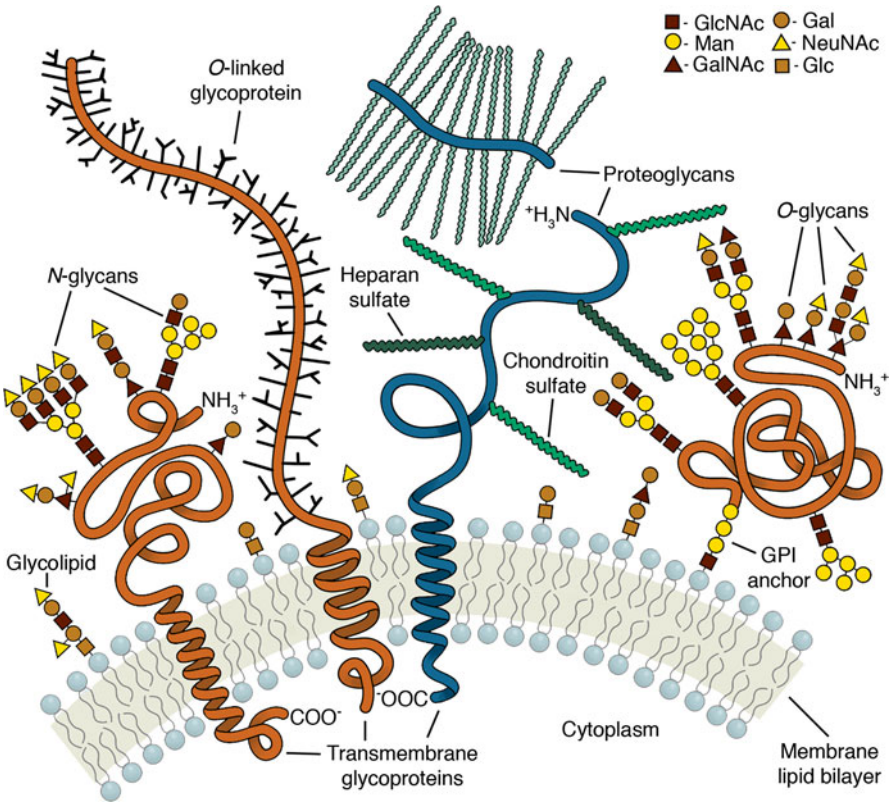

Fig. 1.10 Schematic illustration of a section of animal cell plasma membrane showing the placement of glycan moieties of glycoconjugates in the outer leaflet of the lipid bilayer. The *N*- and *O*-glycans of glycoproteins, the glycan moieties of glycolipids, and the GAG chains of integral membrane and secreted proteoglycans that together form the glycocalyx are shown. Also shown is a highly glycosylated mucin-type glycoprotein present as an extended molecule on the cell surface

Satchell 2012). Moreover, many glycan chains of glycoproteins and glycolipids carry terminal sialic acid residues and proteoglycans carry anionic chondroitin and heparan sulfate chains. Therefore, the glycocalyx is negatively charged and can be seen by electron microscopy after staining cells with a dye such as ruthenium red. The thickness of the glycocalyx is typically greater than that of the plasma membrane itself.

Because of their strategic locations, the cell surface glycans that constitute the glycocalyx play several nonspecific yet crucial physical roles. By holding large amounts of water, they help to maintain cell shape, assist tissues in their function (e.g., keeping the lungs and airways moist and open), protect epithelial barrier against mechanical damage and proteolysis, and provide an aqueous environment for biochemical interactions. They also function to stabilize protein conformation

and provide the aqueous environment essential for cell–cell and cell–matrix interactions and communication. More importantly, cell surface glycans perform numerous specific, biological functions through interactions with proteins and glycans of other cells and tissue matrices (Brooks et al. 2002; Gabius 2009; Taylor and Drickamer 2011; Varki et al. 2008). These functions are dependent on specific structural features of glycans and also on their clustering patterns and densities. The wide range of structural diversity, microheterogeneity, and distributions along protein chains enable glycans to serve as specific recognition and information molecules. Thus, they play essential and specific roles in almost all biological processes involved in fertilization and embryogenesis through the sperm–egg interactions, differentiation and development, and survival. Other biological processes in which glycans play important roles include cell migration and recruitment of cells to specific sites; turnover of cells and proteins; removal of hormones, receptors, and aged erythrocytes from the circulation; cell signaling; and immune modulation.

The glycans also play important roles in the pathogenesis of various diseases (Brooks et al. 2002; Gabius 2009; Taylor and Drickamer 2011; Varki et al. 2008). For example, when cells become transformed to a malignant state, the cell surface glycan structures and profiles are drastically altered, promoting tumor growth and metastasis. Glycoproteins expressed by most tumors have altered glycan structures and are shed into the circulation (Brooks et al. 2002; Gabius 2009; Taylor and Drickamer 2011; Varki et al. 2008a). Monoclonal antibodies recognizing the cancer-associated glycan antigens are used clinically for cancer diagnosis and management. For example, neuroblastomas express high levels of ganglioside G_{D2}, and antibodies to the glycan portion of the G_{D2} are currently being used experimentally to target drugs to these tumors (Tivnan et al. 2012). Furthermore, glycans are involved in bacterial, viral, and parasitic infections. Infectious diseases such as influenza, stomach ulcer caused by *H. pylori*, and several airway and urinary tract infections arise by the glycan-dependent recognition and binding of viruses and bacteria to host target cells and tissues (Brooks et al. 2002; Gabius 2009; Taylor and Drickamer 2011; Varki et al. 2008). For example, influenza infection involves the viral hemagglutinin-mediated attachment to cell surface sialic acid residues. The surface of the causative agent of AIDS, the human immunodeficiency virus (HIV-1), is studded with a major envelope glycoprotein (gp120). About 50 % of the molecular mass of gp120 is carbohydrate, and it is involved in the viral invasion of lymphocytes.

1.14 Summary

Carbohydrates in the form of oligosaccharides and polysaccharides (called glycans) occur widely in almost all animal cells as moieties linked to proteins or lipids to form glycoproteins, glycolipids, and proteoglycans, which are collectively called glycoconjugates. The glycan moieties are structurally highly complex, and their complexity is attributed to the multiple ways the constituent sugar residues are

linked to one another. More importantly, the glycan structural complexity arises from the variations in their sugar composition and size. The biosynthesis of these complex glycan structures is not template dependent as in the case of proteins, but instead is determined by the specificity of glycosyltransferases that catalyze their formation and the orderly manner these enzymes are organized in the biosynthetic compartments. The glycans function as informational molecules through varied and specific interactions with partner proteins and complementary glycans. As such, the wide range of glycan structural complexity is translated to a myriad of biological roles. The glycoconjugates are abundantly found in extracellular matrix and on cell surfaces, where the glycan moieties participate in numerous biological interactions, which are crucial for differentiation and development and life of the organism. Therefore, abnormal glycan metabolism leads to disease pathogenesis. Ironically, many pathogens and toxins produced by them exploit the cell surface glycans to attach and invade cells, causing debilitating and fatal disease.

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