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Chapter 8.2
[Glycoproteins]: Properties

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1 Introduction

Whereas Chap. 8.1 provided an overview of the structures of glycoproteins and glycopeptides occurring in nature, this chapter focuses on the biological roles of their carbohydrate units. However, the frequently asked question "What is the function of glycosylation?" is actually as unreasonable as the question "What is the function of proteins?" would be. Today, we know there is no unifying function for the carbohydrates present in glycoproteins. They rather span the complete spectrum from being relatively unimportant to being crucial for the survival of an organism. Moreover, the same glycan may have different functions at different locations on a given protein, or in different cells or developmental stages of an organism. Thus, each glycoprotein has to be studied individually in order to unravel the roles of its glycans. The aim of this chapter is to indicate some general principles of protein glycosylation.

2
Biological Roles of Glycans in Glycoproteins

Diverse approaches are being employed in order to uncover the roles of carbohydrates contained in glycoconjugates [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12]. They include the localization of specific glycans using lectins or antibodies, the modification of glycans by glycosyltransferases and glycosidases, and the use of inhibitors of glycan biosynthesis or processing. Natural or synthetic ligands can be used to identify specific carbohydrate receptors. Finally, the methods of molecular biology provide a powerful tool to study glycan function. Thus, it is possible to generate cell mutants with altered glycosyltransferase expressions. Alternatively, recombinant glycoproteins can be expressed in different cells with different glycosylation properties. Chinese hamster ovary (CHO) cells, for example, are not able to generate sialic acid α-2$\rightarrow$6 linkages and galactose α-1$\rightarrow$3 linkages and bacteria like *Escherichia coli* produce completely non-glycosylated proteins.

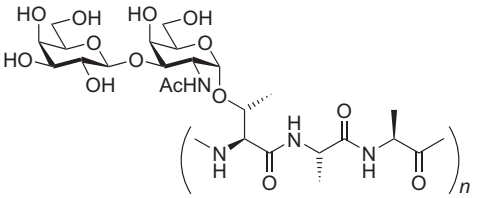
In a crude scheme, the biological functions of glycans can be classified into two groups. Thus, the carbohydrates can modify intrinsic properties of a protein by altering its size, charge, solubility, accessibility, structure, or dynamic properties. On the other hand, the glycans themselves may be specifically recognized by carbohydrate-binding proteins and thus participate in adhesion processes and signal transduction.

2.1
Modulatory and Structural Roles of Glycans

In the following sections some modulatory and structural roles of glycans are discussed. However, it should be kept in mind that the chosen classification of the roles of glycans is somehow arbitrary since the individual effects often cannot be separated from each other. Thus, varied stability or biological function of a protein upon glycosylation is often a result of altered structural parameters caused by the carbohydrates.

2.1.1
Modulation of Physicochemical Properties

The modification of physicochemical properties of proteins by attached carbohydrates is often observed, especially in glycoproteins with high carbohydrate content [1, 7, 12, 13]. Sialylated or sulfated glycans, e.g., change the overall charge of a protein and increase its solubility. This is important for the highly glycosylated mucins (high sialic acid content) and the highly sulfated proteoglycans. Contained in mucous secretions of most epithelial cells, they can provide a gelation function due to their ability to retain water. Thus, they function as lubricants and protection for epithelial surfaces and mediate transport. Examples include synovia, buffering excessive gastric acid, and transport of chyme. In the



Scheme 1. Structure of the glycotripeptide repeating unit -[Gal(β-1→3)GalNAc(α-1→O)]Thr-Ala-Ala- of antifreeze glycoproteins

extracellular matrix, proteoglycans provide elasticity and tensile strength. Furthermore, both mucins and proteoglycans act as adhesion molecules in numerous cell-cell, cell-matrix, and cell-microbe recognition events.

Antifreeze glycoproteins (AFGPs) [14, 15, 16] circulate in the blood of Antarctic fish and enable them to avoid freezing in their perpetually icy environment where the temperature is frequently as low as –1.9 °C. These mucin-type O-glycoproteins are composed of repeats of the glycotripeptide unit -[Gal(β-1→3) GalNAc(α-1→-O)]Thr-Ala-Ala- (Scheme 1). Eight distinct fractions of these proteins have been isolated (AFGP 1–8) with the number of glycotripeptide repeats ranging from 50 to 4. In smaller-sized AFGPs, proline replaces some of the alanine residues following threonine. Antifreeze glycoproteins function in a non-colligative manner by binding to and inhibiting the growth of ice crystals that enter the fish and maybe also by preventing the nucleation of ice crystals. Studies revealed that chemical modification (acetylation, periodate oxidation, and complexation with borate) or removal of the sugar residues results in a loss of antifreeze activity [15]. Recently, a novel antifreeze glycoprotein has been isolated from *Pleuragramma antarcticum* which was shown to be a proteoglycan [15]. Beside these antifreeze glycoproteins, several non-glycosylated antifreeze proteins are known [14, 15, 16].

2.1.2
Protective and Stabilizing Functions

There is little doubt that the "coating" of oligosaccharides on many glycoproteins can protect the peptide backbone from recognition by proteases and antibodies [1, 7, 12, 13]. An example is the decay accelerating factor (DAF, CD55) which is proteolyzed upon removal of its *O*-linked sugars [17]. Decoration of the surfaces of most types of cells with different kinds of glycoconjugates gives rise to the so-called glycocalyx which can present a substantial physical barrier. Glycosylation can also alter the heat stability of proteins which has been shown for two different β-1→3/4 glucanases from *Bacillus* species. Expression in *Saccharomyces cerevisiae* resulted in heavily glycosylated enzymes (carbohydrate content of about 45%) which were significantly more heat stable than their non-glycosylated counterparts expressed in *Escherichia coli* [18]. Such findings are of special in-

terest for the industrial use of proteins. Other examples of the stabilizing function of carbohydrates [19, 20] are ovine submaxillary mucin [21], an isolated tailpiece from human serum immunoglobulin M [22], RNase B [23, 24, 25], human CD2 [26], and the protease inhibitor PMP-C [27]. In these cases, the glycans had little overall effect on the conformation of the proteins but rather globally decreased the dynamic fluctuations of the glycoproteins, as revealed by NMR spectroscopy. The stabilizing function can be achieved even by a single carbohydrate unit [26, 27].

2.1.3 *Modulation of Biological Activity*

There are many examples for the ability of carbohydrates to modulate the biological activity of functional proteins [1, 7, 12]. Bovine pancreas ribonuclease (RNase) for example occurs in unglycosylated (RNase A) and glycosylated (RNase B) forms, the latter being a set of nine different glycoforms of the high mannose type ($Man_{5-9}[GlcNAc]_2$) with respect to the only *N*-glycosylation site (Asn-34) of the protein [7]. Using double stranded RNA as substrate, it was shown that RNase A is more than three times as active as RNase B [25]. Furthermore, enzyme activities of several glycoforms of RNase, prepared by exomannosidase treatment of naturally occurring RNase B, have been determined and may be ranked in terms of decreasing activity as: RNase A>RNase ($Man_0[GlcNAc]_2$)>RNase ($Man_1[GlcNAc]_2$)>RNase ($Man_5[GlcNAc]_2$)>RNase B. These differences in activities were attributed to an overall increase in dynamic stability of the protein upon glycosylation (as demonstrated by NMR spectroscopic determination of proton exchange rates of the various glycoforms) and to steric hindrance between the oligosaccharides and the RNA substrate which is also supported by molecular modeling [28].

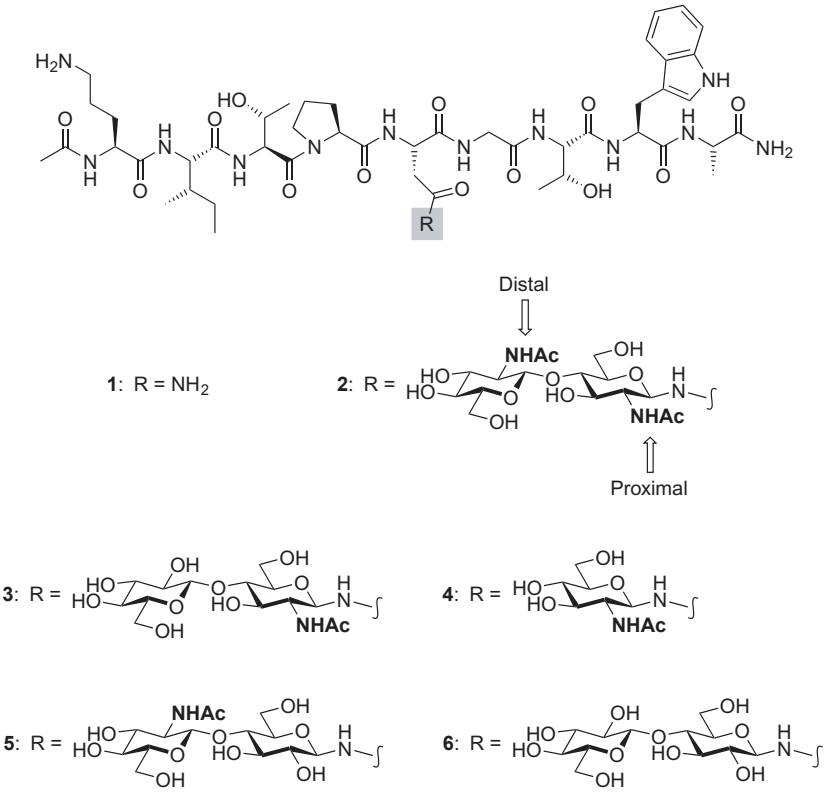
Tissue plasminogen activator (tPA) is another enzyme whose activity is affected by glycosylation [7]. tPA is a serine protease which converts plasminogen into plasmin which itself is a serine protease with fibrinolytic activity. There are two major classes of glycoforms of naturally occurring tPA. Type I contains three *N*-linked glycans at Asn-117, Asn-184, and Asn-448, whereas type II has only two, at Asn-117 and Asn-448. Variable occupancy of Asn-184 affects the population of glycoforms at Asn-448. Plasminogen is also a mixture of two major glycoforms containing one *O*-glycan and one *N*-glycan (type 1) or one *O*-glycan (type 2). For an efficient proteolytic activity of tPA, formation of a ternary complex with plasminogen and fibrin is required. Rate of formation and turnover of the complex is dependent on the glycosylation site occupancy of both tPA and plasminogen [29]. Thus, clot lysis occurs 2–3 times faster with type II tPA in combination with type 2 plasminogen versus participation of type I tPA and type 1 plasminogen. Both the *N*-glycan linked to Asn-184 in type I tPA and the *N*-glycan contained in type 1 plasminogen reduce the enzymatic activity of the serine proteases. In contrast to this tuning function of the *N*-glycans, it has been shown that the *O*-glycan contained in plasminogen is crucial for its proteolytic activity.

Glycosylation of a ligand can also mediate such an on-off or switching effect. Deglycosylation of the hormone human β-chorionic gonadotropin (β-HCG), for example, leads to a species which is still able to bind to its receptor with similar affinity but fails to stimulate adenylate cyclase [1]. In most cases, however, such effects of glycosylation are incomplete, i.e., the carbohydrates provide a means of tuning the primary function of the proteins. From numerous examples studied, it was concluded that the relatively large *N*-linked glycans generally downmodulate the activities of enzymes and signal molecules whereas *O*-glycosylation can result in both a down-regulation and an up-regulation [10, 30, 31].

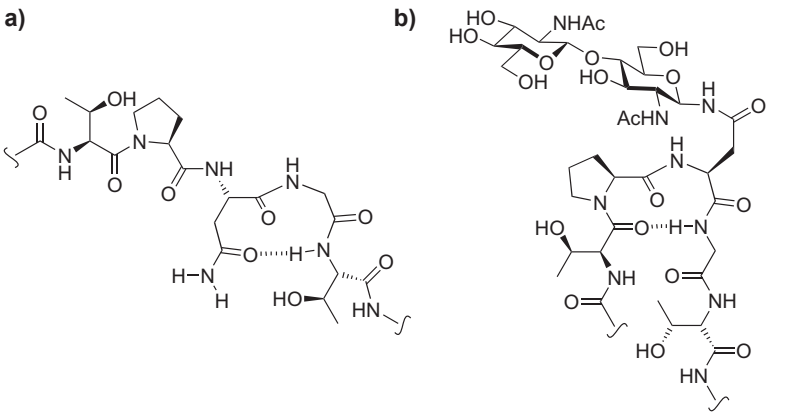
2.1.4 *Influence on Peptide Secondary Structure*

Glycosylation can affect protein structure in several capacities [7, 9, 10, 19, 20, 32]. Structural roles of glycans associated with protein stability and the regulation of protein function have already been mentioned in the preceding sections. Oligosaccharides attached to matrix molecules like collagens and proteoglycans are important in the physical maintenance of tissue structure, porosity, and integrity. As *N*-glycosylation is a co-translational process, it is believed to play a major role in the initiation of correct folding of the nascent polypeptide chain in the rough endoplasmic reticulum [9, 20, 32, 33, 34]. Indeed, numerous examples exist in which removal of certain *N*-glycosylation sites by site-directed mutagenesis results in improper folding of glycoproteins [9, 32, 35, 36, 37, 38, 39]. The impact of *N*-glycosylation on the conformation of model peptides was nicely demonstrated by Imperiali et al. in a series of publications using time-resolved fluorescence resonance energy transfer (FRET) [40] and NMR techniques [41, 42]. The examined peptides were derived from the A282-A288 sequence (Ile-Thr-Pro-Asn-Gly-Ser-Ile) of the hemagglutinin glycoprotein from influenza virus containing the critical Asn-A285 glycosylation site. This sequence represents a β-turn surface loop in the native protein which is of considerable interest as this motif is a common feature among the *final* structures of glycosylation sites in many glycoproteins.

In the FRET study [40], fluorescently labeled analogues of peptide **1** and glycopeptide **2** (Scheme 2) with a dansyl group at N^{δ} of Orn were examined. The study revealed that glycosylation of **1** with a chitobiosyl moiety (→**2**) promoted the adoption of a more compact peptide secondary structure. Subsequent 2D ^{1}H-NMR investigations in aqueous solution [41] supported this analysis and indicated that peptide **1** adopts an open and extended Asx-turn conformation prior to glycosylation whereas glycopeptide **2** exhibits a compact type I β-turn conformation (Scheme 3), quite similar to that observed in the final native protein structure. Both the FRET and NMR studies provide direct evidence that glycosylation of peptide **1** with chitobiose, a disaccharide representing the first two *N*-acetylglucosamine residues of the native tetradecasaccharide (cf. Chap. 8.1), induces a conformational switch in the peptide backbone. This observation is important with respect to the role glycosylation plays in the correct folding of glyc-



Scheme 2. Peptide **1** and glycopeptides **2–6** synthesized in order to probe the influence of asparagine-linked glycosylation on peptide secondary structure [40, 41, 42]



Scheme 3. Glycosylation-induced conformational switching from an **(a)** Asx-turn to a **(b)** type I β-turn [41]

oproteins. It suggests that *N*-linked glycosylation may serve as a critical trigger to help the polypeptide chain to adopt a conformation that is populated in the native folded protein, but not in the nascent unmodified sequence.

Despite the strong influence of glycosylation on peptide conformation, no specific interactions between the chitobiose moiety and the peptide backbone were detected in the NMR analysis in aqueous solution. Therefore, it was proposed that the conformational change observed upon glycosylation results from either a steric effect in which the disaccharide alters the conformational space available to the peptide or from a modulation of the local water structure that influences the environment that the peptide experiences [41]. To address the important question if chitobiose may be replaced with other disaccharides, glycopeptides **3**–**6**, containing the saccharides Glc(β-1→4)GlcNAc, GlcNAc, GlcNAc(β-1→4)Glc, and Glc(β-1→4)Glc, respectively, were examined (Scheme 2) [42]. In all cases, less well-ordered peptide conformations as compared to **2** were determined by NMR analysis. The study revealed that the *N*-acetyl group of the proximal sugar is critical for maintaining a β-turn conformation. Surprisingly, the *N*-acetyl group of the distal sugar also plays an important role in rigidifying both the saccharide and the peptide.

An influence of *N*-glycosylation on peptide conformation has also been observed by Danishefsky et al. upon attachment of the non-natural trisaccharide Gal(β-1→6)Gal(β-1→6)GlcNAc β-*N*-glycosidically to the Asn side chain of the model peptide H-Ala-Leu-Asn-Leu-Thr-OH [43]. Whereas the unglycosylated peptide failed to manifest any appreciable secondary structure, the glycopeptide was assumed to exist in an equilibrium between an ordered and a random state. When carried out at –12 °C using a 90:10 mixture of H_2O/acetone-d_6, NMR analysis revealed nuclear Overhauser effect (NOE) cross-peaks between the methyl of the sugar *N*-acetyl group and backbone amide protons.

In contrast to *N*-glycosylation, *O*-linked glycosylation is an entirely post-translational and post-folding event in mammalian cells. Nevertheless, *O*-glycans may have major impact on the secondary, tertiary, and quaternary structure of fully folded proteins [10]. Otvos et al. showed that glycosylation distorts the α-helicity of an epitope on the rabies virus glycoprotein, and that *O*-glycosylation of threonine is more effective in perturbing the secondary structure of the peptide than *N*-glycosylation of asparagine [44]. In several NMR studies it has been shown that the peptide backbone of model peptides responds to *O*-glycosylation as evidenced by changes in sequential amide-amide NOE interactions [45, 46, 47, 48, 49]. The examples include the mucin-type GalNAc(α-1→O)Thr [45, 46, 47] as well as the GlcNAc(β-1→O)Thr linkages [48, 49]. In all cases, the NMR data, which were supplemented in part by CD and fluorescence measurements [47, 48], molecular modeling calculations [47, 48, 49], and chemical evidence [49], were indicative for a glycosylation-induced conformational change from a random structure to a turn-like structure. The conformational response is further modulated by whether the sugar component is a mono-, di-, or oligosaccharide [46, 49].

2.2
Involvement of Glycans in Recognition Events

Oligosaccharides have an enormous information-storing potential, being substantially higher than that of oligopeptides and oligonucleotides [50]. Thus, it is not surprising that the carbohydrates contained in glycoconjugates, beside their role in modulating intrinsic properties of glycoproteins, can act as recognition markers in numerous physiological and pathological processes. Responsible for deciphering the encoded information are carbohydrate-binding proteins. They can be subdivided into enzymes involved in sugar utilization and glycoconjugate turnover, antibodies, and carbohydrate-binding proteins which are neither enzymes nor immunoglobulins. The latter have been referred to as lectins [51]. Carbohydrate-lectin interactions play a crucial role in many cellular recognition processes including clearance of glycoproteins from the circulatory system, control of intracellular traffic of glycoproteins, bacterial and viral adhesion to host cells, recruitment of leukocytes to inflammatory sites, cell interactions in the immune system, and tumor metastasis, just to name a few. In the following sections some of these processes will be highlighted.

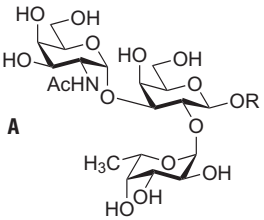
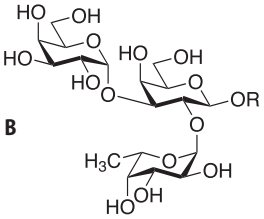
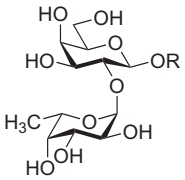
2.2.1
Blood Group Antigens

The discovery of the ABO blood group system 100 years ago by Landsteiner et al. [52] was based on the observation that humans could be divided into different groups according to the presence or absence of serum constituents that would agglutinate red cells isolated from other humans. Although they were not aware of the underlying glycan basis, the work of Landsteiner and colleagues laid the basis for the safe transfusion of blood from one individual to another [53]. Today, we know that the agglutinating serum constituents are antibodies and that their cognate antigens are oligosaccharides whose structures are genetically polymorphic [12, 54, 55, 56]. The first discovery of these antigens on the surface of human erythrocytes led them to be classified as "blood-group antigens". However, they are also found in human secretions and mucosal tissues and it was suggested that they are more accurately defined as "histo-blood group antigens" [57].

The A, B, and H antigens are formed by sequential action of distinct glycosyltransferases on different precursor saccharides. According to the nature of these peripheral disaccharide core structures on which the blood group antigens are synthesized, different types are distinguished. In type-1 structures, the antigen synthesis starts from Gal(β-1→3)GlcNAc(β). Similarly, modification of Gal(β-1→4)GlcNAc(β), Gal(β-1→3)GalNAc(α), and Gal(β-1→3)GalNAc(β) leads to type-2, type-3, and type-4 antigens, respectively. Fucosylation of these precursors by an α-1→2 fucosyltransferase encoded by the *H* or the *Secretor* locus produces the blood group H determinant represented by the disaccharide Fuc(α-1→2)Gal(β). Further glycosylation by an α-1→3-*N*-acetylgalactosaminyltrans-

ferase (corresponding to the *A* allele of the *ABO* locus) or an α-1→3-galactosyltransferase (corresponding to the *B* allele of the *ABO* locus) then leads to GalNAc(α-1→3)[Fuc(α-1→2)]Gal(β) (blood group A determinant) and Gal(α-1→3)[Fuc(α-1→2)]Gal(β) (blood group B determinant), respectively. The ABO classification is based on the presence or absence of the A and B antigens and the two antibodies anti-A and anti-B which always occur in the plasma when the corresponding antigen is missing (Table 1). With rare exceptions the H antigen is expressed on the cells of all blood group O individuals, but in persons belonging to phenotypes A, B, and AB there is complete or partial masking of H activity. Despite the vast accumulated knowledge of serology, chemistry, and genetics of the blood group structures, it is still not possible to assign a clearly defined physiological function to the *ABO* locus.

Table 1. The ABH(O) system of antigens on erythrocytes and antibodies and glycosyltransferases in plasma[a]

Genotype	Phenotype	Blood group antigens on red cells (minimal determinant structure)	Antibodies in plasma	Glycosyltransferases in plasma
AA AO	A	 **A**	anti-B	α-1→3-GalNAcT
BB BO	B	 **B**	anti-A	α-1→3-GalT
AB	AB	**A** and **B**	–	α-1→3-GalNAcT, α-1→3-GalT
OO	O	 **H**	anti-A, anti-B	–

[a] Abbreviations: GalNAcT, *N*-acetylgalactosaminyltransferase; GalT, galactosyltransferase

2.2.2 *Classification of Lectins*

Although lectins [8, 12, 58, 59, 60, 61, 62, 63] were first described more than 100 years ago in plants, it was not until the 1960s that they were recognized to be present throughout nature including the animal kingdom and the microbial world. Originally, they were classified according to their monosaccharide binding specificity. However, with the advent of molecular cloning, a more consistent classification based on amino acid sequence homology and evolutionary relatedness emerged [64]. Whereas the biological function of plant lectins is still unclear, their use as biochemical tools has made an enormous contribution to the understanding of the structures and functions of carbohydrate structures in animal cells. Today, we have a much more detailed picture of the function of animal lectins than of plant lectins. Table 2 gives an overview of the current classification of animal lectins [12, 61].

C-Type lectins were named after their requirement of calcium ions for recognition. They are all characterized by an extracellular carbohydrate recognition domain (CRD) and bind a diversity of sugars. However, not all calcium-requiring lectins are C-type lectins, as exemplified by calnexin and calcireticulin which recognize glucose residues on newly synthesized *N*-glycoproteins. Galectins, formerly named S-type lectins due to their dependency on free thiols for full activity, are soluble β-galactoside-specific lectins that combine preferentially with lactose and *N*-acetyllactosamine. P-Type lectins bind mannose 6-phosphate as

Table 2. Classification of animal lectins according to known sequence homologies[a] [12, 61]

Lectin class	Defining structural motif	Carbohydrate ligand	Calcium dependence
C-type (includes selectins, collectins, endocytic lectins)	conserved CRD	variable	yes (most)
Galectins (formerly S-type)	conserved CRD	β-galactosides	no
P-type	unique repeating motif	mannose 6-phosphate on high-mannose-type *N*-glycans	variable
I-type (includes Siglec family)	immunglobulin-like CRD	variable (Siglecs: sialic acids)	no
Calnexin, calcireticulin, calmegin	homology with each other	glucosylated high-mannose-type *N*-glycans in the ER	yes
Hyaluronan-binding proteins	homologue CRD	hyaluronan chains	no
Frog egg lectins	sequence homology		yes

[a] Abbreviations: CRD, carbohydrate recognition domain; ER, endoplasmic reticulum

their ligand and I-type lectins share a common immunoglobulin-like CRD. Another class of evolutionarily very ancient circulating soluble lectins are the pentraxins. They are characterized not so much by primary sequence homologies, but by a pentameric arrangement of their subunits and a probable role in the primary host immune response.

Multivalency appears to play an important role in lectin-mediated interactions [65] since many lectins are found to recognize individual carbohydrate epitopes only with low affinity. Indeed, most lectins are either intrinsically multivalent because of their defined multisubunit structure or by virtue of having multiple CRDs within a single polypeptide or they can become functionally multimeric by non-covalent association or by clustering on cell surfaces. High-avidity binding can result from multiple interactions of adequately presented low-affinity single sites, and this appears to be a common mechanism of modulating lectin function *in vivo* [59, 66].

2.2.3
Clearance and Targeting of Glycoproteins

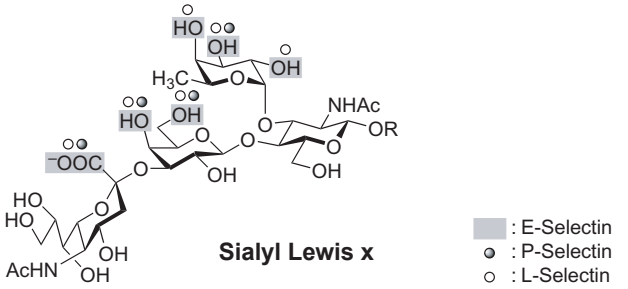
Some effects of glycosylation on the stability of proteins have already been mentioned in Sect. 2.1.2 and they can presumably affect their half-life in single cells. In the intact organism, recognition of glycan structures by certain receptors can result in removal of the glycoconjugate or even a whole cell from the circulation. This was first observed in the late 1960s by Ashwell and coworkers who serendipitously found that desialylation of glycoproteins resulted in significantly shorter serum half-lives [67, 68]. This led to the characterization of the asialoglycoprotein receptor (a hepatic lectin of the C-type) [69], the prototype of an animal lectin, which not only recognizes desialylated, i.e., galactose terminated, *N*-linked oligosaccharides but also GalNAc structures as they occur on some *O*-linked glycans. These findings were traditionally interpreted as representing a physiological clearing mechanism for glycoproteins [69] which has, however, not yet proven beyond doubt [70]. Another example of a receptor responsible for glycoprotein clearing is the GalNAc-4-sulfate receptor. Rapid removal of luteinizing hormone, for example, which contains this (*N*-linked) sugar is important to generate a circadian rhythm [71].

The mannose 6-phosphate (Man6*P*) receptors are the best understood examples of receptors responsible for intracellular trafficking of glycoproteins [12]. These P-type lectins mediate the routing of lysosomal enzymes to their final destination in the lysosomes. Two such receptors are known, one cation-dependent and of low molecular mass (ca. 45 kDa) which has been crystallized [72], the other cation-independent and of high molecular mass (ca. 275 kDa). The targeting is mediated by recognition of Man6*P* residues on oligomannose-type *N*-glycans of lysosomal enzymes by the Man6*P* receptors. A defect in the synthesis of the Man6*P* markers, caused by a deficiency of GlcNAc-phosphotransferase (the first enzyme in the mannose phosphorylation pathway), results in I-cell disease (also called mucolipidosis II or MLII), an inherited lysosomal storage disease.

2.2.4
Leukocyte Trafficking

The leukocyte trafficking to inflammatory sites is a highly regulated multistep process, referred to as the inflammatory cascade [73]. The initial events, the tethering and rolling of leukocytes along the vascular endothelium, are mediated by the interaction of a family of adhesion molecules, termed selectins, and their carbohydrate-containing ligands [12, 74, 75]. The cascade begins with the release of cytokines and other signaling molecules at the site of injury that stimulate the transient expression of E- and P-selectin on the endothelium surface. These C-type lectins bind to their ligands displayed on the circulating leukocytes and promote leukocyte adhesion to the stimulated endothelial cells. L-Selectin is constitutively expressed on leukocytes, and it recognizes its ligands on endothelial cells. The rolling leads to activation of integrins on the leukocytes that interact with their counter-receptors on endothelial cells (e.g., intercellular adhesion molecule-1, ICAM-1) and promote firm adhesion. This stronger interaction then allows emigration or extravasation of the leukocytes into the underlying damaged tissue. However, if too many leukocytes are recruited to the site of injury, normal cells can also be destroyed. This occurs in the condition known as septic shock, in chronic inflammatory diseases such as psoriasis and rheumatoid arthritis, and in the reperfusion tissue injury that occurs following a heart attack, stroke or organ transplant.

The selectins are membrane-bound proteins comprising five domains: a cytosolic tail that may play a role in signal transduction, a transmembrane domain, several complement regulatory domain repeats, an epidermal growth factor (EGF) domain, and an N-terminal, calcium-dependent C-type carbohydrate recognition domain (CRD). Both the EGF domain and the CRD are required for ligand binding, although the site of binding has been localized to the CRD. As has been demonstrated in numerous studies, the tetrasaccharide sialyl Lewis x (sLe^x) Neu5Ac(α-2→3)Gal(β-1→4)[Fuc(α-1→3)]GlcNAc (Scheme 4) is the minimum structure recognized by all three selectins with modest specificity and



Scheme 4. Sialyl Lewis x, Neu5Ac(α-2→3)Gal(β-1→4)[Fuc(α-1→3)]GlcNAc, the minimum structure being recognized by E-, P-, and L-selectin. Indicated are the functional groups that have been shown to be crucial for binding to the selectins [75]

affinity. However, the interaction of the selectins with their natural macromolecular ligands which contain sLe^x and modifications thereof as terminating structures is of much higher affinity. Obviously, additional interactions are important for high-affinity binding. The natural ligand for L-selectin (GlyCAM-1), for example, contains sLe^x sulfated at Gal-6, GlcNAc-6, or both positions and in the natural ligand for P-selectin, PSGL-1, the 19 amino acid N-terminus of the protein which contains several sulfated tyrosine residues beside the *O*-linked, sLe^x-bearing glycan was found to be critical for binding. In addition, it may well be that multivalent interactions between the selectins clustered on cell surfaces with multiple sLe^x residues presented on the highly glycosylated mucin-type counter-receptors contribute to high-affinity binding *in vivo*. Inhibition of the selectin-ligand interactions is an attractive strategy for treating inflammation-related diseases [76, 77, 78]. As such, sLe^x has been intensively used as lead structure for development of anti-inflammatory drugs both in industry and academic laboratories [75].

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