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Carbohydrate–Protein Interactions: Basis of Glycobiology

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Introduction

Glycobiology[1] has become a popular topic in recent years when frontiers of biological sciences are discussed. Indeed, there are a number of important biological phenomena which depend on carbohydrate–protein interactions. For example, heparin, a sulfated polysaccharide composed of glucuronic acid, iduronic acid, and glucosamine, plays an important role in the blood coagulation system.[2] Delivery of newly synthesized lysosomal enzymes from the Golgi apparatus to lysosome requires the recognition of mannose 6-phosphate on these enzymes by a specific receptor.[3] In the preimmune defense system of the human body, there is a "mannose-binding protein (MBP)" (which actually has a much broader specificity than the name implies) which interacts with invading agents through their surface carbohydrates and promotes opsonization or initiates complement fixation.[4] Pituitary hormones contain unique oligosaccharides which are required for manifestation of activity and regulation of their circulation.[5] Selectins, a group of carbohydrate-binding proteins residing in cell membranes, are known to mediate the initial recognition of immunologically important events such as lymphocyte routing and neutrophil and monocyte recruitment to the injury site.[6] Increasingly, carbohydrate–protein interactions are viewed as important mechanisms for biological information transfer between cells and cell-substratum. Beyond a large number of phenomenological examples such as those listed above, there are two fundamental reasons for promoting such a view. It is a well-known fact that most cells are covered with carbohydrates, and these carbohydrates as well as those on some circulating glycoproteins appear to be modulated depending on the physiological status, such as during developmental processes and oncological transformation. Secondly, unlike proteins and nucleic acids, carbohydrates, due to the presence of multiple functional groups (most commonly hydroxyl groups) on each monomeric unit, are capable of forming many different combinatorial structures, including branched ones, from relatively small numbers of sugar units. Each structure could potentially carry a specific biological message, thus widening the spectrum of reactivity that is possible from a limited number of monomers.

Carbohydrate-binding proteins, excluding enzymes and immunoglobulins, are generally called "lectins". Lectins were first discovered in plants more than 100 years ago, and they have been tremendously useful as tools for chemistry and biology that involve carbohydrate recognition, e.g., differentiating cell types by cell surface carbohydrates. Some lectins are also receptors; i.e., they can bind carbohydrate-containing ligands and transport the bound ligands to different destinations inside the cells. One of the most active cell surface receptors, the hepatic lectin family, will be discussed in detail later.

How To Study Carbohydrate–Protein Interactions

Investigation of carbohydrate–protein interactions can be approached from two different angles. One is from the side of proteins, for which currently available

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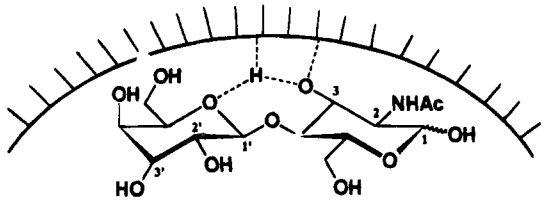

Figure 1. *N*-Acetylactosamine binding at the galectin binding site.

molecular biological approaches are extremely useful. For instance, a carbohydrate-binding protein can be cloned and overexpressed, so that it can be crystallized for X-ray crystallographic studies. Site-directed mutagenesis can provide mutants which are valuable in understanding the mode of interaction between carbohydrates and lectins.

In the other approach, which mostly depends on synthetic organic chemistry, carbohydrates are manipulated to bring about structural changes which facilitate the understanding of binding specificity and other fundamental properties of carbohydrate–protein interactions.[7] For example, galectins[8] (formerly known also as galaptin, β-galactoside-binding protein, S-Lac, etc.) are lactose- or *N*-acetyllactosamine-binding proteins ubiquitously present in many tissues, and they appear to have multiple functions both inside and outside the cells. An example of chemical manipulation of the ligand to gain insight into the binding mechanism of a galectin is described below. It was known that a substitution on the 3-OH of the *N*-acetylglucosamine residue in *N*-acetyllactosamine, a good ligand for galectins, leads to the loss of activity. Crystallographic studies showed that the conformation of *N*-acetyllactosamine or lactose is as shown in Figure 1, in which the 3-OH of *N*-acetylglucosamine is believed to form a hydrogen bond to the ring O of the galactosyl residue. One can surmise, therefore, that substitution at the 3-OH may prevent such a derivative from maintaining this conformation, leading to a loss of binding. However, when we prepared the 3-deoxy derivative of *N*-acetyllactosamine, it did not bind to galectin well, although its conformation as determined by NMR (NOE) measurements remains the same as shown in Figure 1. Thus a logical conclusion is that the 3-OH of *N*-acetylglucosamine must be directly involved in the binding to galectin.[9] Eventually, X-ray crystallographic studies of lactose interacting with galectins showed that indeed the 3-OH is within hydrogen-bonding distance of Arg 49, Glu 68, and Arg 70 in the binding site.[10,11]

To study carbohydrate–protein interactions, or the action mechanism of lectins, hemagglutination assay is a convenient first method to employ. Since erythrocytes are endowed on their cell surface with many kinds of carbohydrate groups, lectins with different sugar specificities can often mediate hemagglutination, if the lectins are multivalent. The carbohydrate structure to which a lectin specifically binds can be probed with a panel of sugars as inhibitors. Results of the hemagglutination-inhibition test will often be immediately useful for the next step of investigation, which is isolation and purification of the lectin. An inhibitory sugar or its derivatives conjugated to a solid support would serve as a useful affinity medium for this lectin.

Once a lectin is isolated, its protein nature including its primary, secondary, and tertiary structures can be studied with standard protein techniques. The extent of lectin–carbohydrate interaction is usually determined in two ways: (1) by isolating the complex formed between the lectin and a tagged (e.g., radiolabeled or fluorescence-labeled) ligand and determining the amount of the "tag" in it or (2) by measuring a physical parameter, such as UV absorption, fluorescence, or the NMR spectrum, that changes upon lectin–ligand interaction. In recent years, availability of sensitive and sophisticated microcalorimeters has allowed direct determination of thermodynamic parameters of lectin–carbohydrate interactions.

For the first type of determination which requires separation of the lectin–ligand complex from free ligand, immobilization of lectin or ligand to microtiter wells is a convenient and sensitive method. Agarose beads and other derivatizable media are also popular for immobilization. A recently developed device (e.g., BIAcore) also adopts the immobilization approach and utilizes the surface plasmon phenomenon to determine the equilibrium association as well as kinetic parameters.[12] If a lectin is already in a solid phase or in a bound form, such as on the cell surface or on the plasma membrane, separation of the lectin–ligand complex is as easy as rapid centrifugation or filtration. For a soluble lectin, one can separate the lectin–ligand complex by precipitating it by ammonium sulfate or other precipitants under conditions that do not precipitate the ligand. This method, originally developed by Hudgin et al.[13] for the determination of hepatic lectin–ligand association, works well with the receptor-type lectins as well as MBPs that exist as large aggregates.

Among the methods that depend on the change in physical properties upon lectin–ligand interaction, fluorometry is very sensitive and versatile. If the innate fluorescence of a protein changes upon binding of carbohydrate, it can be used to determine the association constant.

If the change in the innate fluorescence of protein is not large, a fluorescent ligand (e.g., 4-methylumbelliferyl glycoside) may be devised to obtain the fluorescence change upon binding. Yet another method for using fluorometry is to utilize the anisotropy of the

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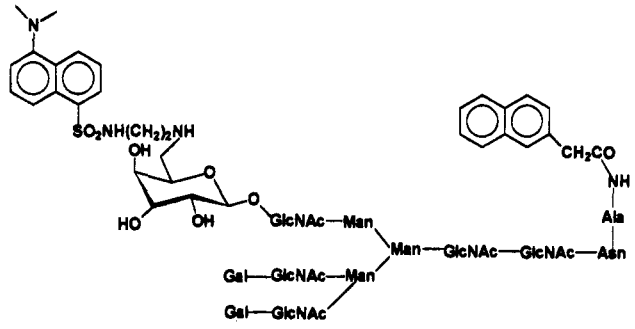

Figure 2. Doubly fluorescent labeled triantennary glycopeptide for energy transfer studies.

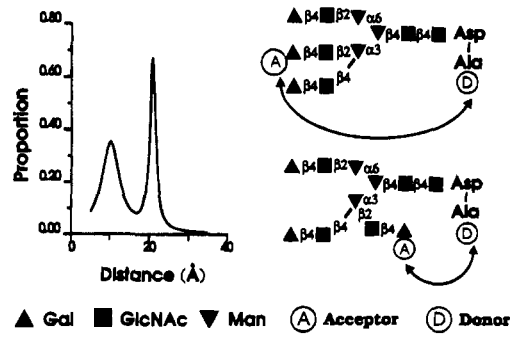

Figure 3. Graph showing the distribution of donor–acceptor distance of the glycopeptide depicted.

bound ligand as compared to the freely rotating ligand. This is quite attractive when the ligand is small so that its binding to protein will result in a large decrease in its motion in solution which causes vectorial dependence of fluorescence (i.e., anisotropy).

Fluorometry can be useful not only for measurement of binding affinity but also for conformational analysis of oligosaccharides which are often ligands for lectins. During the course of our studies of animal lectins, it became apparent that binding of branched oligosaccharides by mammalian hepatic lectins requires exquisite spatial arrangement of the target sugar.[7,14] Therefore, we attempted to determine fluorometrically the conformation of the best ligand, which is a Gal-terminated triantennary structure as shown in Figure 2, by attaching a naphthyl group to the N-terminus of the peptide portion and a dansyl group to one of the three galactosyl residues.[14] Figure 2 shows only one of such isomeric derivatives. (For the procedure of attaching a dansyl group to a modified galactose, see the section Glycoside Cluster Effect.) Since the emission wavelength of the naphthyl group coincides with the excitation wavelength of the dansyl group, irradiation of the naphthyl group (at 280–290 nm) results in the emission of the dansyl group (at 520–530 nm) when the donor and acceptor fluorophores are placed within a certain distance. The extent of such energy transfer can be used to determine the distance between the fluorescent probes.[15]

Conformational analysis by fluorescence energy transfer is not limited to the static measurement as described above. By measuring the time course of fluorescence decay (lifetime measurement), further information on the flexibility of each of the branches was obtained (Figure 3). The figure shows that the particular branch (bearing Gal 6) being probed existed in two different conformational states, indicating the flexibility of this branch. The branch bearing Gal 6′ also appears flexible by the same kind of measurement. However, the branch of Gal 8 showed no evidence of comparable flexibility.[14] Moreover, it was found that the branch flexibility varies greatly with temperature. Surprisingly, when the two unmodified branches are trimmed by successive digestion with glycosidases to the branching mannose, leaving only the originally dansyl-modified branch intact, the branch flexibility disappears,[16] suggesting that the flexibility of each of the branches is greatly dependent on the presence of the neighboring branches.

Animal Lectins

Animal lectins can be classified into four groups, of which the first two are dominant and will be discussed briefly below: (1) C-type lectins, (2) S-type lectins (or galectins), (3) P-type lectins, and (4) others. Though originally classified on the basis of binding characteristics (e.g., the C-type lectins are so named because of their requirement of calcium for binding activity), the classification is now based on a polypeptide motif present in each group that is conserved among the lectins. A minimum polypeptide structure that is required for binding carbohydrate ligands (which is ca. 135 amino acids for C-type and ca. 130 amino acids for galectins) is called the carbohydrate recognition domain (CRD[17]). A CRD can exist alone in nature or in tandem with other CRDs or with other protein domains such as transmembrane domain, collagen domain, and epidermal growth factor (EGF) domain. X-ray crystallographic structures of two C-type CRDs[18,19] and two galectins[10,11] have been published. These studies showed that CRDs within a group adopt the same protein folding, and the folding pattern of the two groups is completely different. Interestingly, the C-type CRDs can display different monosaccharide specificities. For instance, the mammalian hepatic lectins (MHLs) are specific for Gal/GalNAc,[13] the chicken hepatic lectin (CHL) is specific for GlcNAc,[20] and the alligator hepatic lectin (AHL) is specific for Man/L-fucose.[21] In contrast to this, all galectins thus far examined recognize only the lactose- and *N*-acetyllactosamine-containing carbohydrates.

C-Type Cectins

We have been studying the sugar-binding properties of some C-type lectins, such as MHL, CHL, MBP, and AHL, mostly by manipulating their ligand structures and assessing their effect on the binding affinity. MHL (of which we studied rat and rabbit hepatic lectins, RHL and RbHL) and CHL are lectins present respectively on mammalian and chicken hepatocyte

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cell surfaces as well as on their inner membranes. They are endocytic receptors capable of internalizing and degrading Gal/GalNAc- and GlcNAc-containing glycoproteins, respectively. MHL is also known as asialoglycoprotein receptor (ASGP-R), and one of its functions is thought to be that of removal of the desialylated and thus Gal-exposed senescent serum glycoproteins from circulation. Recently, we have isolated and purified a lectin from alligator liver that is specific for Man/L-Fuc. Characteristics of this lectin suggest it to be the hepatic lectin of alligator homologous to MHL and CHL. There are two types of mannose-binding proteins, the serum type and the liver type. The monomers of these two types are quite homologous to each other, but the serum-type MBP exists as much bigger oligomers (ca. 18-mer) compared to the liver type (hexamer). As mentioned in the Introduction, serum-type MBP is an important component in the preimmune defense against invading microorganisms. At the moment, we do not know the function of the liver-type MBP. In the following sections of this Account, we discuss only the serum-type MBP.

The MBPs recognize a wide variety of monosaccharides, such as Man, ManNAc, GlcNAc, L-Fuc, and others such as heptulose derivatives found on microorganisms. This broad specificity is an important asset for the functioning of MBP as a defense molecule. From our studies on the ligand-binding specificity and other binding characteristics of MBP and hepatic lectins, the following properties were shown to be shared by these lectins.[22]

1. These lectins recognize mostly terminal monosaccharide units. The binding site appears to be of a trough type that allows the presence of large substituents both on the hydroxyl group of C-1 (i.e., aglycon) and that on C-6. Other C-type lectins, such as selectins, appear to contain a positively charged secondary interaction site which is thought to interact with the negatively charged group (sialic acid or sulfate) of selectin ligands.[23]

2. With all the lectins studied thus far, 3-deoxy and 4-deoxy derivatives of the parent sugar have little binding affinity to the lectin, suggesting that 3-OH and 4-OH provide the major binding force of the interaction with the lectin-binding site. Other substituents, i.e., 2-OH, 2-NHAc, and 6-OH, have a much smaller or little contribution to the binding energy. The interactions of the vicinal 3- and 4-OH groups with the lectin is especially important for MBP. The broad specificity of this lectin, accommodating Man, ManNAc, GlcNAc, Glc, and L-Fuc at its binding site, indicates that both 2- and 6-substituents are unimportant, and the interaction with the lectin depends primarily on 3- and 4-OH groups. The crystallographic structure in fact showed that 3- and 4-OH of Man not only ligate directly to a calcium ion (required for binding) but also form an extensive network of H-bonding[18] (Figure 4).

3. Binding affinity of these lectins for individual monosaccharide units is rather weak. For instance, rat and chicken hepatic lectins bind GalNAc and GlcNAc, respectively, with an affinity expressed as a K_d of ca. 0.1 mM, while that of Man for MBP is ca. 1 mM. However, when neoglycoproteins bearing a large number of determinant sugar residues are used as ligand, the binding affinity increases geometrically with a linear increase in the number of sugar residues[22] (Figure 5). Apparently, binding of multiple determinant sugar residues is a requisite for generating strong interactions for these lectins. This important phenomenon, termed glycoside cluster effect, will be discussed in greater detail below.

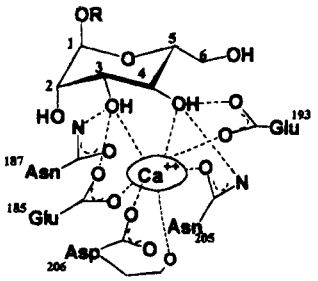

Figure 4. Hydrogen-bonding and calcium coordination bonding patterns of a mannoside bound at the MBP binding site.

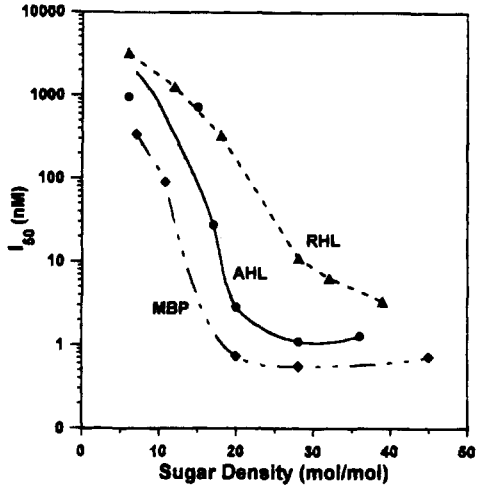

Figure 5. Increase in binding affinity of sugar-derivatized bovine serum albumin (BSA) molecules with increasing level of sugar coupled. Note that the I_{50} value (y-axis) is inversely proportional to the binding affinity and is plotted on a logarithmic scale (▲) RHL binding Gal-BSA; (●) AHL binding Man-BSA; (◆) MBP binding Man-BSA.

Glycoside Cluster Effect

As mentioned above, MHL, CHL, AHL, and MBP exhibit a phenomenal glycoside cluster effect for appropriately derivatized bovine serum albumin (BSA) neoglycoproteins, although the degree of dependency of affinity increase on valency (Figure 5) varied from lectin to lectin. A strong glycoside cluster effect obviously requires two partners: a lectin with clustered sugar binding sites and a multivalent ligand that can present sugars with proper orientation and spacing.

Hepatic lectins are type II transmembrane proteins that have a strong tendency to associate. Their C-terminal ends contain CRD which faces the outside of the cell. To the CRD is attached a stalk (or neck) region, followed by a transmembrane segment and a short cytosolic tail at the N-terminus (See Figure 6). Detergent-solubilized and affinity-purified hepatic lectins appear to exist as hexamers, and there are

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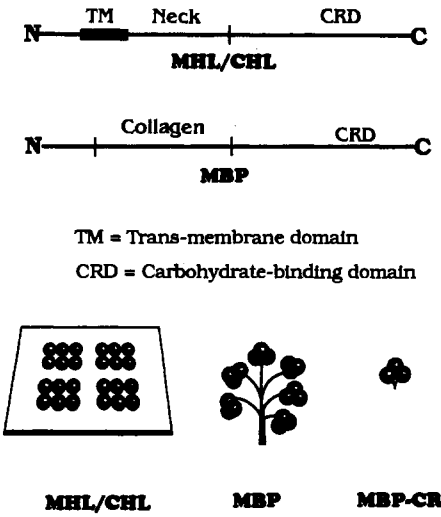

Figure 6. Top: Monomer constructs of hepatic lectins and MBP. Bottom: Oligomeric states of these lectins.

apparently two binding sites per subunit.[24] On rat or rabbit hepatocyte surface, MHL may further associate into tightly packed aggregates (Figure 6). This is shown by the fact that the binding of a protein ligand (asialoorosomucoid) at saturation appears to eclipse a number of potential binding sites, which are no longer available to the protein ligand but are still capable of interacting with a small ligand.[25]

The MBP monomer is essentially a CRD to which a collagenous stalk is attached, thus the smallest MBP assembly is likely to be a trimer. In the native serum-type MBP, six or more such trimers are assembled further to form a larger aggregate structure resembling a bouquet of flowers, with the CRDs spread apart rather than in a tight cluster (Figure 6). This structure is very similar to that of C1q, the first component of the complement fixation complex. Collagenous domains of both C1q and MBP contain one disruption of the collagen triplet (Gly-Xaa-Yaa) which apparently allows the stalk to bend into the bouquet form.[26] Interestingly, bacterially expressed MBP-CRD containing a short connecting piece but no collagen domain exists as a trimer as well.[27] An X-ray crystallographic study[18] and equilibrium binding studies (unpublished data) suggest that there is one sugar-binding site per subunit of MBP. This means that a cluster of only three sugar-binding sites is capable of binding a protein highly modified with a mannose derivative, such as $Man_{28}BSA$, with an affinity of K_d = ca. 1 nM (Figure 5). This neoglycoprotein, having 28 Man residues (on the average) on the surface, must be able to present three properly placed Man residues that can interact with three MBP-CRDs simultaneously.

Small di- and trivalent ligands can also manifest a cluster effect, if sugars are placed at proper distances. We have used two synthetic schemes to obtain such cluster glycosides (Figure 7). In the first method, three hydroxyl groups of aminotris(hydroxymethyl)-methane were chemically glycosylated to various

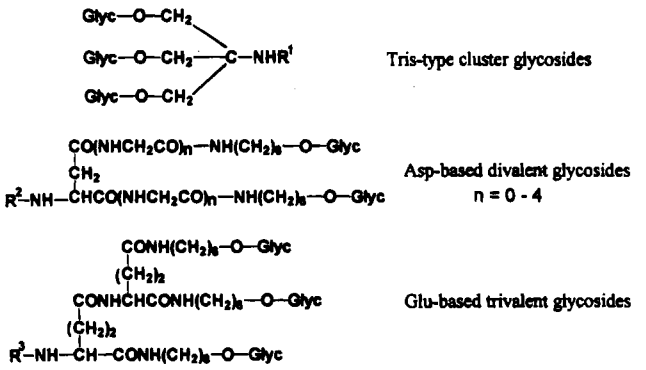

Figure 7. Structures of synthetic cluster glycosides.

Table 1. Binding Affinities of Various Cluster Glycosides for C-Type Lectins

	I_{50} (μM)			
	RHL	CHL[b]	AHL	MBP
Glyc[a]	14	440	17000	1100
bis-Glyc		160		1700
NAcYD(ah[c]-Glyc)		15	600	900
NAcYD(G-ah-Glyc)	0.03	9	400	120
NAcYD(GG-ah-Glyc)		28	700	900
YEE(ah-Glyc)	0.004	0.17	200	550
tris-Glyc		85		900

[a] Glyc = GalNAc for RHL, GlcNAc for CHL, Man for AHL and MBP. [b] CHL was assayed with isolated hepatocytes. Other lectins are all soluble preparations. [c] ah = 6-aminohexyl.

degrees, yielding derivatives containing one to three sugar residues per molecule.[28] In this type of glycosides, sugars are placed close together[29] and do not have total freedom of orientation. Another group of glycoside cluster ligands was designed to have longer arms with flexibility. Asp (D) and Glu (E) were utilized to provide di- and tribranching through their COOH groups which are coupled to ω-aminoalkyl glycosides, usually 6-aminohexyl glycosides.[29–31] As shown in Figure 7, the branch arm was varied in length by attaching up to four Gly (G) residues to the 6-aminohexyl group. Table 1 shows the affinity of such small cluster glycosides for RHL, CHL, AHL, and MBP measured by inhibition assay. The inhibitor concentration that causes 50% reduction in the binding of a tagged reference ligand (I_{50} value) under properly designed conditions is very close to the Kd value and is inversely proportional to the binding affinity. As shown in the table, clustered glycosides based on Asp and Glu exhibited impressive cluster effect toward hepatic lectins, while the tris-type ligands containing simple monosaccharides hardly showed any glycoside clustering effect, suggesting that sugars in the latter type are too close together (0.9 nm maximum) to span two sugar-binding sites. Most impressive is $YEE(ah\text{-}GalNAc)_3$, which had affinity for RHL 500-fold higher than GalNAc. However, its Man counterpart did not show any appreciable extent of affinity enhancement for MBP, suggesting that distances between sugar-binding sites of hepatic lectins and MBP are quite different. $NAcYD(GGGG\text{-}ah\text{-}Man)_2$, which is the divalent ligand with the longest arm thus far made, still did not show much affinity enhancement (unpublished results).

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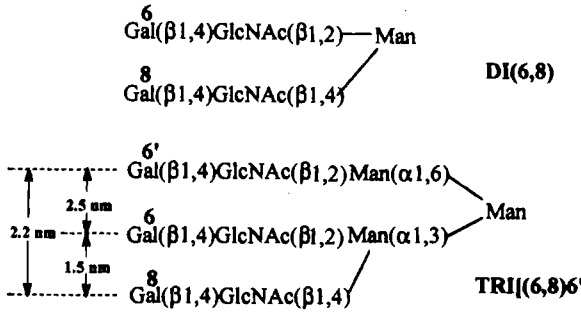

Figure 8. The most potent di- and trivalent oligosaccharides for MHL.

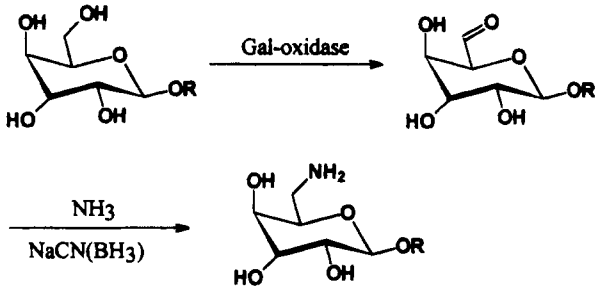

Figure 9. Reaction scheme for converting a 6-OH of a galactoside to a 6-amino group.

Although the flexible cluster glycosides exhibit an impressive affinity enhancement toward hepatic lectins, certain oligosaccharides were found to manifest phenomenal degrees of glycoside cluster effect toward rat and rabbit hepatic lectins on the cell surface.[32] When various divalent and trivalent complex-type oligosaccharide structures were tested, K_d values of divalent oligosaccharides were in the range 1−40 μM and those of trivalent structures in the range 10−100 nM.[33] The two structures that gave the highest affinity in each group are shown in Figure 8. Since Gal and simple Gal glycosides bind to the lectin with K_d = ca. 1 mM , the maximum affinity that can be generated by concerted binding of two and three such sugar residues would be 1 μM and 1 nM, respectively. Therefore, the two oligosaccharide structures shown in Figure 8 appear to represent structures with near optimal placement of Gal residues. NMR studies[34] of the triantennary oligosacchride TRI[(6,8)6'] suggest that in its preferred conformation the three terminal Gal residues are separated from one another with spatial distances of ca. 1.5, 2.2, and 2.5 nm as shown in Figure 8. This orientation of three Gal residues is likely to be complementary to the arrangement of sugar-binding sites on the rat hepatocyte surface.[35]

This contention was further strengthened by our photoaffinity-labeling studies of RHL on hepatocyte surface.[36] A set of photoaffinity-labeling reagents was prepared from a bovine fetuin glycopeptide having the same terminal structure as TRI[(6,8),6'] (Figure 8) by modifying the Gal residues at C-6 by using the scheme shown in Figure 9, eventually yielding a structure similar to that in Figure 2. This approach was taken since the presence of a large substituent on 6-OH of Gal does not affect the binding affinity significantly.[37] In this scheme (Figure 9), the CH_2OH group was first converted to CHO by galactose oxidase and then to CH_2NH_2 by reductive amination. A photoactivatable group was then attached to the amino group. The oxidation conditions of the triantennary glycopeptide were maximized for the production of monooxidized isomers, which were separated by HPLC as hydrazones. The aldehydo function was regenerated from each hydrazone, and the ensuing reactions were carried out on each of the separated isomers, resulting in three isomeric photoaffinity-labeling glycopeptide reagents.[36]

All MHLs are composed of two types of subunits: MHL-1, which is smaller (40−43 kDa) but more abundant, and MHL-2, which is larger (54−60 kDa) and less abundant. They are quite homologous in the peptide sequence and differ mainly in the neck region of the monomer. It has been amply documented that both types of subunits have to be present on the cell surface in order to have a functional receptor.[38,39] Photoaffinity labeling experiments with the three isomeric glycopeptides resulted in two of the isomers labeling only RHL-1 and the third isomer labeling only RHL-2/3.[36] (Only the rat has two forms of the larger subunits. RHL-2 and RHL-3 are believed to be differently glycosylated on the same protein backbone.) The results suggest that two types of RHL subunits are organized in a sterically specific and rigid orientation, so that the three terminal Gal residues of the glycopeptide bind simultaneously to these sites in a stereospecific manner.

Although CHL functions similarly to MHL as far as endocytic and degradative activities toward GlcNAc-terminated neoglycoproteins are concerned,[40] little is known about its subunit organization on hepatocyte surface, nor do we know much about circulating chicken serum glycoproteins. We have not found any natural oligosaccharide structure that can bind to CHL with an affinity of K_d < 10 nM.[36] The recent discovery that a reptilian (alligator) hepatic lectin recognizes Man (L-Fuc) hints an evolutionary progression of the complex-type *N*-glycoside structures now found in mammalian species. The change in the sugar specificity of hepatic lectins, going from the outer sugar residue (Gal) to inner sugar residue (Man) of the complex-type oligosaccharide structure as one descends the evolutionary tree, suggests that oligosaccharide chains in avian and reptilian glycoproteins may be structurally and biosynthetically more abbreviated than the mammalian counterparts. Indeed many insect cells are known not to make any complex-type oligosaccharide chains.[41]

As mentioned earlier, MBP has a built-in flexibility due to a break in the collagenous stalk. This may

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mean that there is no preferred carbohydrate ligand of definitive structure for this lectin. The fact that MBP is a general defense molecule important in preimmune combating of foreign agents dictates that MBP not only recognizes many different kinds of monosaccharides but also copes with the ligand sugar residues oriented in various geometrical arrangements, some of which may be quite far apart from each other.

Perspectives

In this Account, we have concentrated on C-type lectins whose subunits exist in clusters or aggregates enabling them to exhibit very strong binding forces given a suitable multivalent ligand. However, there are many other lectins, including some C-types, that do not exist in clusters. A prime example is galectin-1, which exists as dimers with a sole binding site of each subunit facing away from each other.[10] While galectin-1 interaction with its ligands probably does not generate a strong binding force (e.g., K_d in the nanomole/liter range as hepatic lectins manifest), these lectins may be well suited to serve as modulators of biological events, such as a role suggested for this lectin as a modulator in the developmental processes. Immunological processes are another area where carbohydrate–protein interaction is often implicated. For instance, Mac-2 (a galectin) accumulates on the cell surface when a macrophage is stimulated.[42] Three known selectins (E-, P-, and L-selectins) are all involved in proper routing of immunologically important cells.[6] Study of this type of carbohydrate–protein interaction is probably more difficult, because not only are the interactions likely to be weaker but also these events are often fleeting and involve small numbers of cells or molecules. However, we can expect from these studies to provide even more exciting new insights into the role carbohydrates play in a wide variety of biological events.

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