CHAPTER 4

SPECIFICITY AND AFFINITY

The hallmark of lectins is the ability to bind carbohydrates specifically and reversibly. Understanding the properties and functions of lectins, as well as using them for diverse purposes, requires knowledge of this specificity, which is the major topic of the present chapter. Several lectins combine also with non-carbohydrate ligands, either at their carbohydrate binding sites or at sites distinct from the latter. A few others possess enzymatic activity unrelated to their carbohydrate specificity. These will be discussed briefly at the end of the chapter.

4.1 METHODOLOGY

Studies of the carbohydrate specificity of lectins are customarily performed by the hapten inhibition technique, in which different monosaccharides, oligosaccharides, or glycopeptides, are tested for their ability to inhibit either hemagglutination (see Fig. 3.1) (Rüdiger, 1993) or polysaccharide (or glycoprotein) precipitation by the lectin (see Fig. 3.2) (Goldstein, 1976). Alternately, either the carbohydrate or the lectin is immobilized in the wells of a microtiter plate and the inhibitory effect of different saccharides on the interaction of the immobilized one with its partner in solution is assayed. Using specially designed glycochips (see 3.1) with different mono- and oligosaccharides, the specificity of a lectin can be determined (Fig. 4.1). These techniques are simple, rapid and require submilligram amounts of material. They stem from the observations of Landsteiner, made in the early part of the last century, that a simple substance with a structure closely related to, or identical with, the immunological determinant group of an antigen can combine with the antibody and thereby competitively inhibit the antigen-antibody reaction. Such inhibition studies are possible also with lectins because their interaction with sugars does not result in the formation of covalent bonds and is reversible, similarly to the reaction of an antibody with an antigen (Fig. 3.2). The compound that inhibits the reaction examined at the lowest concentration is considered to have the highest affinity for the lectin and to be most complementary to its combining

site. The assay conditions used may affect the affinity of the ligand to the lectin. A putative ligand may act differently when immobilized on plastic or on a carrier such as Sepharose, expressed at the surface of a cell, or when in solution. Of biological significance is the fact that the interaction between a lectin and a ligand may be different in vitro and in a physiological setting (Sanders, W. J. et al., 1999).

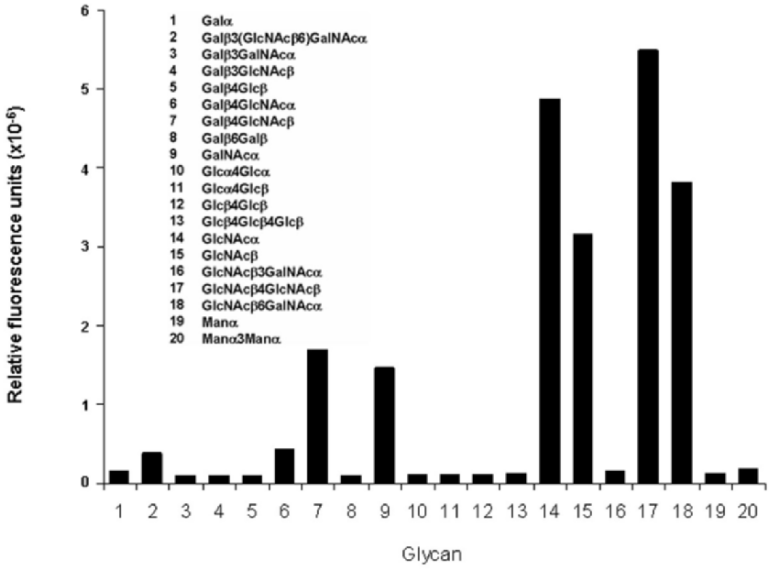

Fig. 4.1 Binding profile of wheat germ agglutinin to Glycochip^R, demonstrating the specificty of the lectin to N-acetylgalactosamine deivatives. The lectin combines also with *N*-acetylgalactosamine, but not with any other saccharides on the chip. Courtesy Dr. Ari Gargir, Glycominds Israel.

Since the lectin-carbohydrate interaction is a reversible equilibrium process, it can be described as follows:

$$L + S \Leftrightarrow LS \qquad K_a = \frac{[LS]}{[L] \times [S]}$$

(lectin + sugar ⇔ lectin-sugar complex) where L is lectin, S is carbohydrate and K_a is the association, or binding, constant of the complex formed. The reaction can be examined by physicochemical methods used for similar studies of other protein-ligand complexes (Table 4.1) (Varki, A. et al., 1999, Chapter 4), primarly by spectrophotometry, spectrofluorimetry,

Table 4.1 Quantitative methods for characterization of carbohydrate-protein interactions.

Method	*Ligand used*	*Property measured*	*Data obtained*[a]
Affinity electrophoresis	Immobilized on gel	Electrophoretic migration of lectin	T
Circular dichroism	Unlabeled	Rotation of polarized light	T
ELLA[b]	Immobilized	Lectin binding in presence of inhibitor	T
Equilibrium dialysis	Radioactive or chromophoric	Concentration of ligand	T
Fluorimetry	Nonchromophoric	Fluorescence intensity of protein	T
	Fluorescent	Fluorescence intensity or anisotropy of ligand	T
Frontal affinity chromatography	Fluorescent	Retardation of ligand on immobilized lectin[c]	c
Isothermal titration microcalorimetry	Unlabeled	Reaction heat	T
NMR (^{1}H or ^{13}C)	Unlabeled or ^{13}C labeled	NMR spectrum of ligand	T, K
Spectrophotometry	Nonchromophoric	UV absorption of protein	T,
	UV-absorbing	UV absorption of ligand	T
Stopped flow	Chromophoric	UV absorption or fluorescence of ligand	T, K
Surface plasmon resonance	Unlabeled	Refractive index	T, K
Temperature-jump relaxation	Chromophoric	UV absorption or fluorescence of ligand	T, K

[a]T, thermodynamic; K, kinetic; [b]ELLA, enzyme-linked lectin assay; [c]by this method only the association constant is calculated.

microcalorimetry (Dam, & Brewer, 2002), nuclear magnetic resonance (Poveda & Jimenez-Barbero, 1998), surface plasmon resonance (Haseley et

al., 1999) or frontal affinity chromatography (Hyrabayashi et al., 2002). By these methods, the association constants, the number of combining sites/ mole, as well as other thermodynamic and kinetic parameters of the interaction of lectins with any type of ligand can be measured accurately. This provides information on the binding process, and gives an insight into the characteristics of the combining sites of lectins. Nevertheless, for routine determination of the carbohydrate specificity of lectins, the much simpler, but less accurate, hapten inhibition technique of hemagglutination or precipitin formation is of value, in particular since association constants for the interaction of a lectin with a series of simple carbohydrates correlate well with the relative inhibitory activity of the same sugars (Fig. 4.2 and 4.3.).

The values of the association constants of a particular lectin-carbohydrate interaction obtained by different physicochemical methods are often very close and sometimes identical. For the binding of monosaccharides to lectins, they are typically in the range 10^3 to 10^4 M^{-1} (see Table 4.9), although exceptions occur. Thus, the K_a of PA-IIL for fucose is ~ 10^7 M^{-1} and that of MLL for *N*-acetylgalactosamine is ~ 10^5 M^{-1}. Oligosaccharides in most cases have a higher affinity for lectins than monosaccharides, with binding constants in the range 10^5 to 10^7 M^{-1}. This is of special significance from the functional point of view, since the native ligands of lectins are oligosaccharides of glycoproteins or glycolipids.

The association constants of lectins to mono- and oligosaccharides are of the same order of magnitude as those commonly found for the binding of haptens to antibodies and of substrates and inhibitors to enzymes. For example, the association constants for the binding of *N*-acetylglucosamine and its β4-linked di- and trisaccharides (di-*N*-acetylchitobiose and tri-*N*-acetylchitotriose) with hen egg white lysozyme are 20-50, $5x10^3$ and 10^5 M^{-1}, respectively (Chipman & Sharon, 1969), while lactose is bound to a complementary rabbit antibody with a K_a of 10^5 (Ghose & Karush, 1973). For more information on the energetics of lectin-cabohydrate interactions see section 4.5

4.2 CARBOHYDRATE SPECIFICITY

Broadly speaking, lectins can be divided into those that bind monosaccharides as well as oligosaccharides, and those that recognize oligosaccharides only (for a recent guide to the specificities of lectins see Wu, A.M. et al., 2001). It is noteworthy that almost all saccharides recognized by lectins are typical constituents of animal cell surfaces. This is perhaps a reflection of the method commonly used for lectin detection

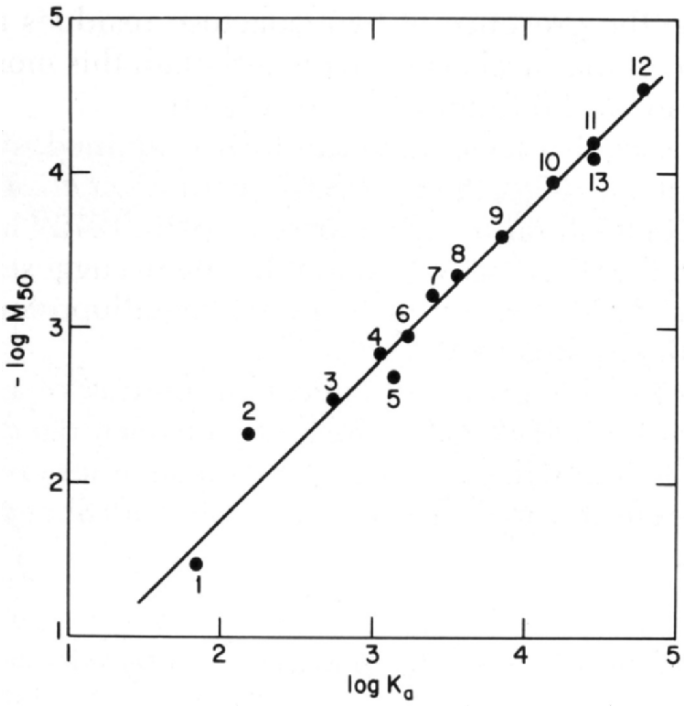

Fig. 4.2 Correlation between inhibition indices (M_{50}) and association constants (K_a) for glycosides interacting with concanavalin A. M_{50} is the molar concentration of saccharide required to give 50% inhibition of precipitation of glycogen by concanavalin A. K_a was estimated either by equilibrium dialysis, by ^{13}C-NMR spectrometry, or by difference spectrum with *p*NPαMan and competition with non-chromophoric glycosides. (1) MeβGlc; (2) *p*NPβGlc; (3) MeβFru; (4) *o*-iodophenyl βGlc; (5) maltose; (6) MeαGlc; (7) *p*NPαGlc; (8) Meα-sophoroside; (9) MeαMan; (10) *p*NPαMan; (11) *p*-chlorophenyl αMan; (12) *p*-ethoxyphenyl αMan; (13) 4-methylumbelliferyl αMan. *p*NP stands for *p*-nitrophenyl and Fru for fructose. Reproduced from Loontiens & de Bruyne, 1975; copyright 1975, with permission from Elsevier Science.

(hemagglutination), as a result of which lectins recognizing sugars not present on erythrocytes might have been overlooked.

4.2.1 Monosaccharides

Lectins specific for monosaccharides are usually classified into five specificity groups according to the monosaccharide for which they have the highest affinity, namely mannose, galactose/*N*-acetylgalactosamine,

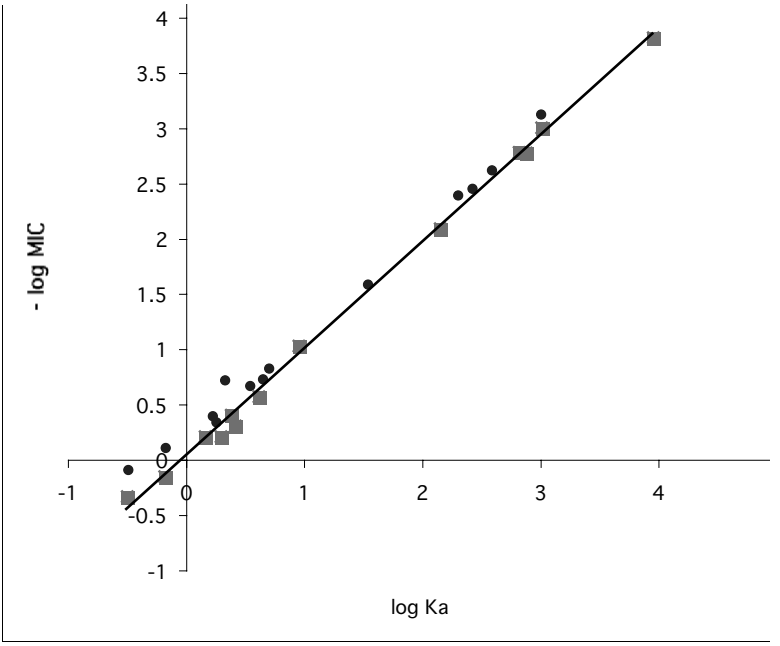

Fig. 4.3 Correlation between minimal concentration (MIC) of different linear or branched oligomannosides required for inhibition of hemagglutination of rabbit erythrocytes by concanavalin A (●) and *Dioclea grandiflora* lectin (■), and the affinity K_a for the lectins, as measured by isothermal titration calorimetry. Based on data of Dam et al., 2000; 2002.

N-acetylglucosamine, fucose and sialic acid (Table 4.2) (for the structures of the monosaccharides, see Appendix A); this is often referred to as "the primary specificity" of the lectin (Goldstein & Poretz, 1986; Goldstein et al., 1997). Lectins that possess affinity for monosaccharides other than those mentioned are rare (4.2.1.f). A small number of lectins are "promiscuous," in that they interact with monosaccharides from different specificity groups through the same combining site (4.2.3). A few exhibit dual specificity, i.e. they may combine simultaneously with different sugars (4.2.4).

The distribution in nature of lectins belonging to the various groups is not uniform. Those specific for galactose (and often also for *N*-acetylgalactosamine) appear to be most abundant and are present in all classes of organism. Lectins specific for mannose have a somewhat more limited distribution; for example, they are scarce in invertebrates. Those that interact with sialic acid alone are rare; most listed as "sialic acid-specific"

recognize this sugar only when it is at the non-reducing end of an oligosaccharide (4.2.1.e).

The lectins of the above specificity groups share some general characteristics. Thus, members of the same group may differ in their affinity for the corresponding monosaccharide or its derivatives (see below). Some interact with di-, tri- and tetrasaccharides with association constants up to several thousands fold higher than with the monosaccharide (Table 4.3). In

Table 4.2 Lectins with preference for oligosaccharides[a]

Lectin	*Specificity*		*RIA*[b]
	Saccharide		
	Mono		
Bowringia milbraedi[c]	Man	Manα2Man	15
Concanavalin A	Man	Manα3(Manα6)Man	60
		GlcNAcβ2Manα3(GlcNAcβ2-Manα6)Man	180
Codium fragile[d]	GalNAc	GalNAcα3GalNAcβ3Galα4-Galβ4Glc	125
Dolichos biflorus	GalNAc	GalNAcα3GalNAc	36
E. coli type 1 fimbriae[e]	Man	Manα3Manβ4GlcNAc	30
*Erythrina corallod*endron[f]	Gal	Fucα2Galβ4GlcNAc	30
Galectin-1[g]	Gal	Galβ4GlcNAc	650
Peanut	Gal	Galβ3GalNAc	50
Polyporous squamous[h]	Gal	Neu5Acα2,6Galβ4Glc(NAc)	2000
Scilla campanulata[i]	Man	Manα3(Manα6)Man	40
Ulex europaeus I	Fuc	Fucα2Galβ4GlcNAcβ	900
Wheat germ[j]	GlcNAc	GlcNAcβ4GlcNAcβ4GlcNAc-β4GlcNAcβ4GlcNAc	42

[a]Based on data in Goldstein & Poretz, 1986, unless otherwise stated; [b]Relative inhibitory activity of hemagglutination, as compared to that of the monosaccharide. [c]Animashaun & Hughes, 1989; [d]Wu, A. M. et al., 1997; [e]Sharon, 1987; [f](Lemieux et al., 2000); [g]see Table 4.5; [h]although classified as galactose-specific, it has the highest affinity for sialyloligosaccharides of the type listed (Mo et al., 2000); [i]Wright, L. M. et al., 1999; [j]Bains et al., 1992.

such oligosaccharides the monosaccharide for which the lectin is specific is present usually at the non-reducing end, although some (e.g., concanavalin A and WGA) recognize the complementary sugar also when it occupies an internal position.

Table 4.3 Monosaccharide specific lectins[a]

Monosaccharide	*Lectin*[b]
Man	*Allium sativum; Canavalia ensiformis*; *Crocus sativus; Dioclea grandiflora*[c]; *E.coli* type 1 fimbriae; ERGIC-53; *Galanthus nivalis*; MBLs of animals; *Pisum sativum*[c] ; *Vicia faba*[c]
Gal/GalNAc	*Arachis hypogaea*[d]*; Coprinus cinereus; Entamoeba histolytica; Erythina corallodendron*; *Dolichos biflorus*[e]; galectins; *Glycine max*; *Griffonia simplicifolia* lectin I; *Helix pomatia; Hygrophorus hypothejus; Phaseolus limensis*[e]*; Moluccella laevis*; *Polyandrocarpa misakiensis; Ptilota filicina*; *Ricinus communis*
GlcNAc	Conglutinin; *Griffonia simplicifolia* lectin II; *Tachypleus tridentatus* lectin 2; *Triticum aestivum*; *Ulex europaeus* lectin II
Fuc	*Aleuria aurentia*; *Anguilla anguilla*; *Lotus tetragonolobus*; *Pseudomonas aeruginosa* lectin II; *Ulex europaeus* lectin I, *Ulva lactuca*[f]
Sia	*Achatina fulica*[g]; *Cancer antennarius*[h]*; Hericium arinaceum*[i]*; Homarus americanus* lectin I[j]; L*imax flavus*[j]; *Scylla serrata*[k]; T*riticum aestivum*[i]

[a]For information on lectins not mentioned in text see Goldstein et al., 1997; Kilpatrick, D.C., 2000; Van Damme et al., 1998c; Wu, A.M. et al., 2001; [b]source or name of lectin; [c]binds also glucose; [d]does not bind *N*-acetylgalactosamine; [e]binds galactose very weakly; [f]Gilboa-Garber, et al., 1988); [g]binds only *9-O*-acetyl-*N*-acetylneuraminic acid; [h]binds preferentially *4*-acetyl-*N*-acetylneuraminic acid and *9-O*-acetyl-*N*-acetylneuraminic acid; [i]binds only *N*-acetylneuraminic acid; [j]binds both *N*-acetylneuraminic acid and *N*-glycolylneuraminic acid; [k]binds only *N*-glycolylneuraminic acid

Certain lectins possess anomeric specificity, i.e. they can distinguish if the configuration of the substituent at C-1 is α or β, often with pronounced preference for one of the anomers. For instance, concanavalin A, GSL-IB_4 and the lectin of *Lotus tetragonolobus* have a much higher affinity for the α-glycosides than the β-glycosides of mannose (and glucose), galactose or

fucose, respectively, whereas the galectins are specific for the β-glycosides of galactose. On the other hand, SBA and RCA are almost devoid of anomeric specificity. The anomeric preference may be affected by the nature of the aglycone, whether aliphatic or aromatic, as illustrated in Table 4.4 for the Gal/GalNAc-specific lectins.

Table 4.4 Relative affinity and anomeric preference of Gal/GalNAc-specific lectins to the corresponding monosaccharides and to their aliphatic and aromatic glycosides[a].

Lectin	*Relative affinity*[b]	*Preferred anomery of glycoside*			*Hydrophobic effect of aglycone*
		Me	*p*NP	*p*NP/Me[c]	
PNA	<0.01	α=β	α=β	1.0	-
GSL-IB_4	0.9	α	β	>4.1	+
ECorL	1.9	α	β	2.1	±
MPL	3.3	α	β	5.6	+
SJA	5.6	α=β	β	16.1	+
GSL-IA_4	25.7	α	α	1.8	±
SBA	40.0	α	α	1.8	±
VVL	52.3	α	α	0.5	±
WFL	137	α	β	22.7	+
MLL	500	β	α	40	+
DBL	>>100	α	α	1	-

[a]Modified from (Kaladas et al., 1982); [b]ratio of inhibitory activity of hemagglutination or polysaccharide precipitation by *N*-acetylgalactosamine as compared to that of galactose; [c]ratio of activity of the best *p*-nitrophenyl (*p*NP) glycoside inhibitor to that of the corresponding methyl glycoside.

Individual lectins vary considerably in their ability to react with derivatives or isomers of the monosaccharide for which they are specific. Many lectins tolerate substituents at the C-2 position of the sugar to which they bind. For instance, most of those that bind galactose interact also with *N*-acetylgalactosamine, and for this reason they are classified in a single specificity group. Concanavalin A, specific for mannose/glucose, binds also α-mannose resides substituted at the 2-OH, e.g. GlcNAcβ2Manα or Glcα2Glc (sophorose). RCA, specific for galactose, combines with Neu5Acα2,6Gal as well, although with a lower affinity than for galactose, but does not interact with Neu5Acα2,3Gal.

The configuration of the 3-OH of the monosaccharides listed in Table 4.2 affect their ability to interact with lectins. Thus, whenever tested, allose (the

3-epimer of glucose) and 3-deoxymannose (or glucose) did not interact with mannose-specific lectins (Poretz & Goldstein, 1970). The same hydroxyl was also shown to be essential for the recognition of fucose by fucose-binding lectins (Hindsgaul et al., 1982). The configuration of the 4-OH is important too, since in general galactose-specific lectins do not react with glucose (its 4-epimer) or mannose (the 2- and 4-epimer of galactose), nor do those specific for mannose bind galactose. This is not always true for lectins that recognize *N*-acetylhexosamines. For instance WGA, specific for *N*-acetylglucosamine, binds *N*-acetylgalactosamine too, albeit five times more weakly, while the *N*-acetylgalactosamine-specific HPA interacts with *N*-acetylglucosamine as well (five time more weakly than with *N*-acetylgalactosamine) (Goldstein & Poretz, 1986). The recently described tachylectin-2 of the Japanese horseshoe crab, *Tachypleus tridentatus*, binds both these *N*-acetylhexosamines, although its affinity for *N*-acetylglucosamine is 17 times higher than that for *N*-acetylgalactosamine (Beisel et al., 1999).

Occasionally, lectins combine with monosaccharides that appear structurally unrelated, but that present similar topographical features when appropriately viewed. This is the case of WGA that binds both *N*-acetylglucosamine and *N*-acetylneuraminic acid (Fig. 4.4A), and of the rat mannose-binding proteins, MBP's, that recognize fucose as well (Fig. 4.4B).

Consideration of the oligosaccharide specificity of the monosaccharide-specific lectins has revealed that many can be classified according to the type of protein-linked carbohydrate unit they recognize. Thus, PNA and the lectins of *Maclura pomifera* and *Bauhinia purpurea*, all of which exhibit a high affinity for Galβ3GalNAc (50, 80 and 7 times higher than for galactose, respectively, (Goldstein & Poretz, 1986)) react primarily with *O*-linked (mucin type) sugar units, whereas RCA and the *Polyporus squamosus* lectin (Mo et al., 2000; Zhang, B. et al., 2001) bind complex *N*-linked units.

4.2.1.a. Mannose

This was originally known as the Man/Glc specificity group, because the early studies of e.g., concanavalin A seemed to indicate that lectins specific for mannose recognize glucose too. During recent years, however, an increasing number of mannose-specific lectins that do not react with glucose have been encountered. They include members of the large family of monocot mannose-specific lectins, such as those of snowdrop and garlic, the mammalian mannose-binding proteins, as well as type 1 fimbriae of *E. coli*. Glucose is therefore no more included here in the name of the group.

The effect of substitutions at C-3 varies for different members of the mannose specificity group. For instance, the affinity of the 3-*O* methyl and benzyl ethers of glucose to concanavalin A is, respectively, 10 and 20 times weaker than that of glucose (Goldstein & Poretz, 1986). In contrast, the

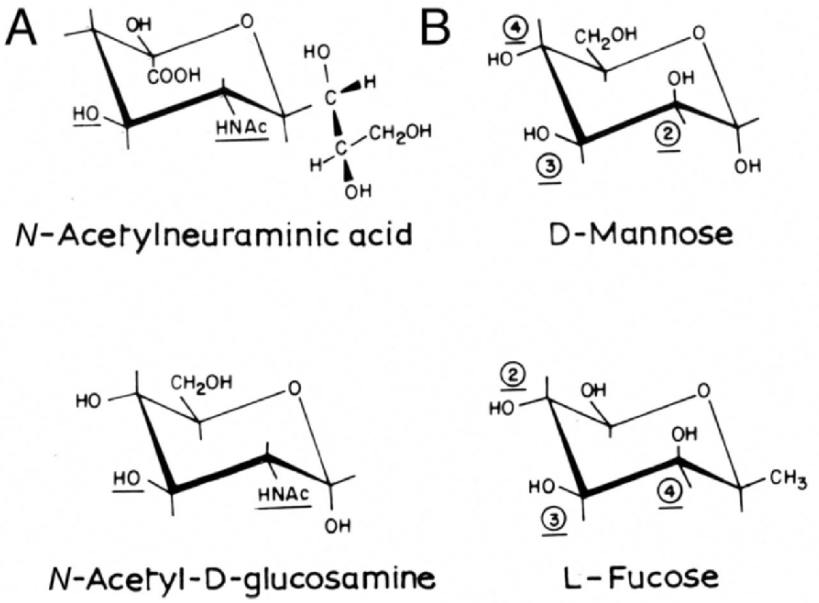

Fig. 4.4 Common structural features of *N*-acetylneuraminic acid and *N*-acetylglucosamine (A) and of mannose and fucose (B). Similarity of *N*-acetylglucosamine and *N*-acetylneuraminic acid at positions C-2 (acetamido) and C-3 (hydroxyl) of the pyranose ring is observed when the sialic acid molecule is suitably rotated. Rotation of the fucose molecule by 180° allows superimposition of its ring oxygen, 4-OH, 3-OH and 2-OH with the ring oxygen, 2-OH, 3-OH and 4-OH of mannose, respectively. Groups that thus occupy the same positions in space are underlined.

affinity of the same sugars to lentil, fava bean and pea lectins, all of which also belong to this group, is significantly higher than that of glucose (e.g., 4 and 3 times, respectively, for fava bean lectin and 16 and 6 times, respectively, for pea lectin).

Members of this specificity group illustrate another general property of lectins, namely that those with similar affinities for monosaccharides and even for certain oligosaccharides, may differ in their affinities for other oligosaccharides. For instance, Viciae lectins (e.g., of pea, lentil and *Lathyrus ochrus*) bind fucose-containing oligosaccharides (such as structure 42 in Appendix B, where the fucose is α6 attached to the asparagine-linked *N*-acetylglucosamine), as well as the corresponding glycopeptides, approximately 10-fold more tightly than the fucose-free analogues of the same oligosaccharides (Debray & Montreuil, 1991). The presence of the α6-

linked fucose is an absolute requirement for the binding to lentil lectin of oligosaccharides and glycopeptides of the *N*-acetyllactosamine and poly-*N*-acetyllactosamine type. On the other hand, interaction of the same compounds with concanavalin A is not affected by the presence of such a fucose.

Another example of this kind is that of the nine lectins (one of which is concanavalin A) from plants of the Diocleae subtribe of the Leguminosae. These lectins are specific for mannose and glucose, and unlike others of the same specificity group, they bind the trimannoside Manα3(Manα6)Man with a similar high affinity (about 100-fold stronger than methyl α-mannoside) (Debray & Montreuil, 1991; Gupta et al., 1996). Furthermore, they exhibit nearly the same pattern of specificity for different deoxy analogs of the trisaccharide, indicating that their combining sites are highly conserved (Dam et al., 1998a). However, they could be divided into two groups with respect to their affinities towards biantennary oligosaccharides, in which both non-reducing mannose residues of the above trisaccharide are substituted by β2-linked *N*-acetylglucosamine (structure 35 in Appendix B). One group, consisting of the lectins from *Canavalia brasiliensis*, *Dioclea guianensis* and *Dioclea virgata*, bind such biantennary oligosaccharides with an affinity comparable to that for the trimannoside, and includes concanavalin A with an affinity to the oligosaccharide more than twice higher. In contrast, the other five Diocleae lectins examined have a substantially (up to 60-fold) decreased affinity for the same oligosaccharides (Fig. 4.5).

The mannose-specific BMA is inhibited most effectively by oligosaccharides containing Manα2Man. Rather exceptionally, it has a high affinity for $Man_9GlcNAc$ (see structure 53 in Appendix B) but a relatively low affinity for $Man_5GlcNAc$ and $Man_6GlcNAc$ (structures 39 and 40, respectively, in Appendix B), and can discriminate between $Man_7GlcNAc$ and $Man_8GlcNAc$ (45 and 46, respectively, in Appendix B), all intermediates in the processing of Asn-linked oligosaccharides (Animashaun et al., 1993). The mannose-specific lectins from monocot plants, all of which bind this monosaccharide equally well, differ markedly in their interaction with mannose oligosaccharides. For instance, GNA has the highest affinity for Manα3Man, the lectins from the daffodil and twayblad bind preferentially Manα6Man and Manα3Manα3Man, respectively (Van Damme et al., 1998b), whereas the garlic lectin favors α2-linked mannose, especially when attached to the α6 arm of the pentamannoside [Manα6(Manα3)-Manα6](Manα3)Man (Bachhawat et al., 2001).

Rather bizarre carbohydrate-binding properties are exhibited by the banana (*Musa acuminata*) lectin (Goldstein et al., 2001). It was originally classified as Man/Glc-specific, since among others it precipitated branched α-

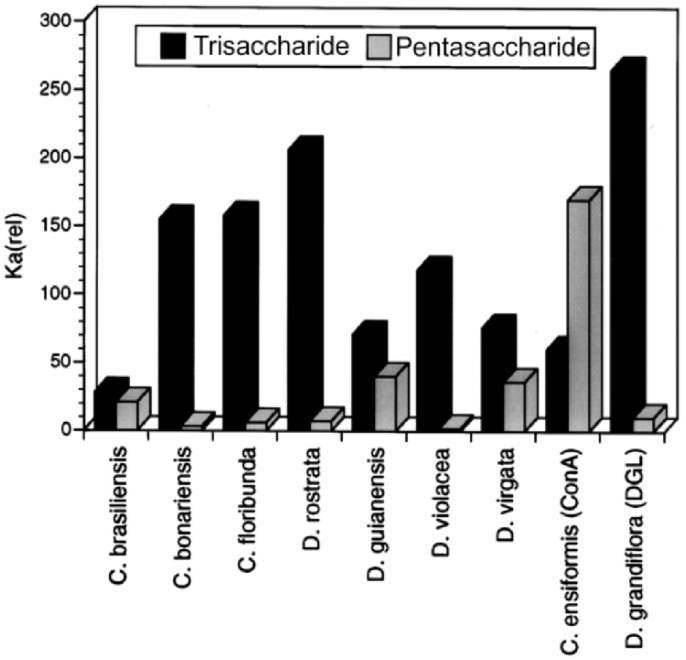

Fig. 4.5 .Plot of the ratio of K_a values of the nine Diocleae lectins for the trisaccharide Manα3(Manα6)Man and the pentasaccharide GlcNAcβ2Manα3-(GlcNAcβ2Manα6)Man, relative to those of MeαMan for the same lectin. reproduced with permission from Dam et al., 1998a; copyright 1998 The American Society for Biochemistry & Molecular Biology.

mannans and α-glucans, and these precipitations were inhibited by mannose and glucose. However, unlike concanavalin A, this lectin recognized 3-*O*-methyglucose as well as internal 3-*O*-substituted α-glucose units, as found for example in nigeran, but it also binds the reducing glucosyl residues of β3-linked glucose oligosaccharides, namely laminaribiose (Glcβ3Glc) and its higher homologs. In addition it combines with β6-linked glucosyl end groups that occur in many β3/β6-linked polysaccharides. It is thus the first plant lectin known to recognize internal Glcα3 bonds, as well as the first to bind laminaribiose.

Of the oligosaccharides examined as inhibitors, the type 1 fimbrial lectins of *E. coli* and *Klebsiella pneumoniae* interacted best with Manα3Manβ4GlcNAc (30 times better than MeαMan) (Sharon & Lis, 1997). In contrast, several Salmonella species examined bound the above trisaccharide weaker than MeαMan. Although similar in their monosaccharide specificity, the lectins of *E. coli* and *K. pneumoniae* differed in

affinity for aromatic mannosides. Thus, *p*-nitrophenyl α-mannoside was 30-fold more effective as inhibitor of type 1 fimbriated *E. coli* than MeαMan, but only 4 times more effective than MeαMan as inhibitor of the similarly fimbriated *K. pneumoniae.*

4.2.1.b. Galactose/N-acetylgalactosamine.

As mentioned, a considerable number of lectins that bind galactose interact also with *N*-acetylgalactosamine, and for this reason they are classified in a single specificity group. Within this group, the ratio of affinities for the two sugars varies widely (Table 4.4). On one end is PNA that does not bind *N*-acetylgalactosamine at all, and on the other are *Moluccella laevis* lectin, the affinity of which for this sugar is 500 times higher than that for galactose (Lis & Sharon, 1994) and DBL that binds *N*-acetylgalactosamine almost exclusively. In between are lectins such as ECorL, that binds the acetamido sugar 2-3 times better than galactose, and SBA that binds the former monosaccharide some 40 times better than the latter. Of the two subunits A and B that form the five isolectins A_4, A_3B, A_2B_2, AB_3 and B_4 of GSL-I (cf. 3.2.1), the A subunit is specific for α-*N*-acetylgalactosamine but binds also α-galactose, while the B subunit recognizes only the latter monosaccharide and agglutinates blood type B erythrocytes. A lectin that binds *N*-acetylgalactosamine but does not recognize galactose is amaranthin; it recognizes, however, the disaccharide Galβ3GalNAc and its α-linked glycosides with an affinity 350 times higher than *N*-acetylgalactosamine (Transue et al., 1997).

Replacement of the acetyl group in *N*-acetylgalactosamine, or its α and β methyl glycosides, by dansyl (2-dimethylaminonaphthalene 8-sulfonate, or Dns, Fig. 4.6) to give *N*-dansylgalactosamine, increases the affinity of the

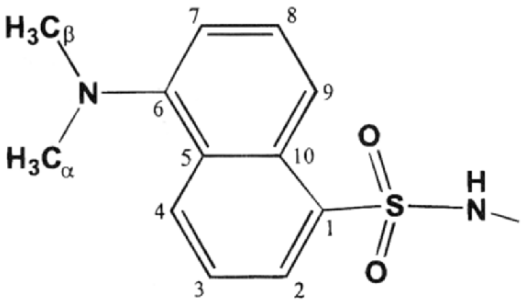

Fig. 4.6 The 2-dimethylaminonaphthalene 8-sulfonate (dansyl) group

ligand for ECorL and for the very similar *Erythrina cristagalli* lectin (ECL)

by about 250 times (Arango et al., 1993; DeBoeck et al., 1984a) and for SBA by 20 times. (DeBoeck, et al., 1984b). This high affinity is strictly sugar specific since, as shown for ECorL, the bound *N*-dansylgalactosamine can be displaced from the lectin with galactose (Kinzy et al., 1992) and, moreover, the lectin does not interact with *N*-dansylglucosamine. The high affinity of *N*-dansylgalactosamine and its glycosides attest to the presence at the primary combining site of the lectins of a hydrophobic binding region, as revealed also by X-ray crystallography (see 6.1.2.a). This site accommodates other bulky substituents attached to the 2-position (e.g. fucose) as well.

An important family of galactose-specific lectins are the galectins. All its members bind β-galactosides such as lactose and *N*-acetyllactosamine, but do not react with *N*-acetylgalactosamine (Table 4.5). They differ, however, in

Table 4.5 Carbohydrate specificity of three rat lung galectins[a]

Carbohydrate	*Relative inhibitory activity*[b]		
	Galectin-1	*Galectin-3*	*Galectin -5*
Gal	1.0	1.0	1.0
Galβ4Glc	130	100	60
Galβ4GlcNAc	650	700	66
Galβ3GlcNAc	155	270	60
Galβ3GalNAc	5	7	60
Galα3GalαOMe	5	6	18
GalNAcβ3GalαOMe	4	40	60
GalNAcα3(Fucα2)Galβ4Glc[c]	40	2500	120

[a](Leffler & Barondes, 1986; Sparrow et al., 1987); [b]the inhibitory activity of galactose was arbitrarily set as 1; [c]according to more recent literature, the affinity of galectin-3 for this tetrasaccharide is almost 100 times higher than that of galectin-1 (Henrick et al., 1998).

their affinity for substituted galactosides. Galectin-3 in particular reacts with oligosaccharides bearing such substituents with increased affinity (Henrick et al., 1998), among them the tetrasaccharide GalNAcα3(Fucα2)Galβ4Glc; as a result, galectin-3 is also blood type A-specific. Several of the galectins examined, among them chicken liver galectin (CG-16) (Wu, A.M. et al., 2001), and the recently characterized 16-kDa galectin from the nematode *C. elegans* (Ahmed et al., 2002) interact with Galβ3GlcNAc, although in most cases with a lower affinity than with *N*-acetyllactosamine (Table 4.5).The *C. elegans* galectin combines also with GalβGalNAcα and GalβGalNAcβ.

Of the many *N*-acetyllactosamine- (and poly-*N*-acetyllactosamine-) containing glycoproteins and glycolipids of animals, galectin-1 binds only a

limited number. High-affinity ligands include the laminins, the lysosome-associated membrane proteins (LAMPs)-1 and -2, as well as CD45, the transmembrane protein phosphotyrosine phosphatase of T cells (Perillo et al., 1998). The term "counter-receptor" has been proposed for such glycoproteins (Crocker & Feizi, 1996).

The Charcot-Leyden crystal (CLC) protein (cf. 5.2.1) presents a special case; it was designated as galectin-10 on the basis of its sequence homology with other galectins, although it has no affinity for β-galactosides (Swaminathan, G. J. et al., 1998). Instead it binds mannose, but only when in crystal.

4.2.1.c. N-Acetylglucosamine

WGA, like almost all other cereal lectins, binds *N*-acetylglucosamine and its β4-linked oligomers with an affinity that increases markedly with chain length of the latter up to four residues. It also binds *N*-acetylgalactosamine, although more weakly than *N*-acetylglucosamine (see 4.2.1), and is one of the rare lectins that bind *N*-acetylneuraminic acid in free form. The ability of WGA to bind *N*-acetylneuraminic acid derives from structural similarity of this monosaccharide to *N*-acetylglucosamine (Fig. 4.4).

4.2.1.d. Fucose

Agglutination of blood type H(O) erythrocytes and precipitation of H(O) blood group substances by the fucose specific lectins of the eel (AAA) and of *Lotus tetragonolobus* (LTA) were found, many years ago, to be inhibited by the 2-*O*-methyl, 3-*O*-methyl and 2,3-di-*O*-methyl derivatives of the rarely occurring D-fucose (but not by the unsubstituted monosaccharide) as well, if not better, than by their L-enantiomorphs (Springer, G. F. & Williamson, 1962). This apparent conflict with well-established principles of stereospecificity was resolved at the time by examining three-dimensional models, which showed that rotation of 2-*O* or 3-*O*-methyl-D-fucose by 180° about its major axis would align these sugars with methyl α-L-fucopyranoside in such a way that centers of hydrogen bonding and hydrophobicity would become virtually superimposable (Kabat, 1962). Very recently the ability of AAA to bind 3-*O*-methyl galactose was shown, on the basis of the three-dimensional structure of the lectin–fucose complex, to be due to the topologically equivalent positions of the axial 4-OH and the small hydrophobic group at C3 of above monosaccharide and those of the correponding groups of fucose (Bianchet et al., 2002).

Both LTA and *Aleuria aurantia* lectin (also fucose-specific) bind Le^x, while the latter lectin interacts with sLe^x as well (Haselhorst et al., 2001).

4.2.2 Sialic acids

Most lectins classified as sialic acid-specific do not bind the free sugar but only when it is either α2,3- or α2,6-linked to monosaccharides, usually galactose, or to disaccharides such as lactose or *N*-acetyllactosamine. Of the limited number of lectins that do bind the free sugar, the majority recognize almost exclusively *N*-acetylneuraminic acid or its 9- and/or 4-*O*-acetyl derivatives, while often lectins, such as those from the Indian horseshoe crab (Mohan et al., 1982) and the marine crab *Scylla serrata* (Mercy & Ravindranath, 1992), from the fungus *Hericium arinaceum* (Kawagishi et al., 1994) and from the leaves of mulberry (*Morus alba*) (Ratanapo et al., 1998) react preferentially with *N*-glycolylneuraminic acid (a sialic acid that differs from *N*-acetylneuraminic acid by the presence of a hydroxyl on the *N*-acyl substituent); a few lectins (e.g. that of *Homarus americanus*) bind both types of sialic acid. A narrow specificity for 9-*O*-acetyl-*N*-acetylneuraminic acid is exhibited by the lectins of the marine crab *Cancer antennarius* and the snails *Achatina folica* and *Cepaea hortensis* (see Table 3.4).

4.2.2.a. Unusual specificities

Some lectins are specific for monosaccharides other that those that define the five groups discussed above. One example is the intracellular membrane-bound P-type lectins specific for mannose-6-phosphate, a recognition marker of lysosomal enzymes (see 5.2.). Another is human serum amyloid P component (SAP) (see 5.2.4), a lectin specific for the 4,6-cyclic pyruvate acetal of galactose (4,6-*O*-[(*R*)-carboxyethylidene] galactose); this uncommon carbohydrate is present in certain algal polysaccharides, in a marine sponge and a yeast, but not in bacteria or higher organisms. These and additional lectins with uncommon specificities are listed in Table 4.6.

Table 4.6 Lectins with unusual monosaccharide specificities

Monosaccharide recognized[a]	*Lectin*[b]	*Ref.*
Gal6*P*	C-Reactive protein	(1)
4,6-*O*-[(*R*)-carboxyethylidene]-galactose	Serum amyloid protein	(2)
Gal*f*[c]	Human intelectin	(3)
GalU	*Aplysia depilans*	(4)
GalN/ GlcN	Ym1	(5)
ManN	*Selenocosmia huvena* venom	(6)
ManNAc	Lung surfactant protein A	(7)
Man6*P*	Man6*P* receptors	(8)
	Giardia lamblia	(9)

Table 4.6 Lectins with unusual monosaccharide specificities

Monosaccharide recognized[a]	*Lectin*[b]	*Ref.*
L-Rha	*Periplaneta americana*	(10)
	Trout eggs	(11)

[a]For structures of the monosaccharides see Appendix A; [b]name or source; [c]galactofuranose; (1) (Culley et al., 2000); (2) Hind et al., 1985; (3) Tsuji et al., 2001; (4) Wu, A. M. et al., 2000; (5) Chang et al., 2001; (6) Lü et al., 1999; (7)Haurum et al., 1993; (8) Sahagian et al., 1981; (9) Ward et al., 1990; (10) Kubo, T. et al., 1993; (11) Tateno et al., 1998.

4.2.3 Oligosaccharides

As mentioned, certain lectins interact with oligosaccharides only; examples are listed in Table 4.7. The most prevalent of these are specific for oligosaccharides with terminal non-reducing sialic acid, (generally *N*-acetylneuraminic acid). Lectins that recognize oligosaccharides terminating with other monosaccharides are less common, while lectins recognizing oligosaccharide constituents of glycosaminoglycans are rare.

Table 4.7 Oligosaccharide-specific lectins[a]

Oligosaccharide	*Lectin*[b]
Sialyl-	
Neu5Acα2,3Gal[β4Glc(NAc)]$_{0,1}$	*Helicobacter pylori*; influenza virus (birds and horse); *Heterometrus granulomanus*; *Maackia amurensis* leukoagglutinin; *Mycoplasma pneumoniae*; murine polyoma virus; *Neisseria gonorrhoea*; Newcastle disease virus; *Plasmodium falciparum*;siglec-1, 4a and 8
Neu5Acα2,6Gal[β4Glc(NAc)]$_{0,1}$	*Carcinoscorpus rotunda*; influenza virus (human strains); *Sambucus nigra;* siglec-2 and 3
Neu5Acα2,6GalNAc	Siglec-5 and 6
Neu5Acα2,3Galβ3GalNAc	*Escherichia coli* S; *Maackia amurensis* hemagglutinin
Neu5Acα2,3Galβ3(Neu5Acα2--6)GalNAc	Murine polyoma virus
Neu5Gcα2,3Galβ4GlcNAc	*Escherichia coli* K 99
Neu5Gcα2,3Galβ4GlcNAc/-GalNAc	*Polyporus squamosus*
Neu5,9Ac$_2$α2,3Galβ4(GlcNAc)	Influenza virus C; coronavirus

Table 4.7 Oligosaccharide-specific lectins[a]

Oligosaccharide	*Lectin*[b]
Neu5Acα2,8Neu5Ac	*Escherichia coli* S; Sendai virus; siglec-5, 7 and 11
Neu5Acα2,3Galβ4(Fucα3)–-GlcNAc	E- and P-selectins
Neu5Acα2,3(6-sulfo)Galβ-4(Fucα3)GlcNAc	L-selectin
Galactosyl-	
Galα3Gal	*Marasmius oreades*
Galβ3GalNAc	*Agaricus bisporus; Agaricus campestris; Vicia graminea*
Galα4Glc	*E. coli* P; *Streptococcus pneumoniae*
Galβ4GlcNAc-[c]	E-PHA
[d]	L-PHA
Galβ(Fucα3)4GlcNAc	Flagellar lectin of *Pseudomonas aeruginosa*
***N*-Acetylglucosaminyl-**	
$(\beta 4GlcNAc)_{2\text{-}4}$	*Aaptos papillata*; *Datura stramonium; Lycopersicon esculentum; Phytolacca americana; Solanum tuberosum*
Glucosyl-	
Glcα3Manα2[e]-	Calnexin; calreticulin; EDEM
β3-Glucans	*Bombyx mori*; *Blaberus discoidalis*; dectin-1; *Musa acuminata*
Mannosyl-	
Oligomannosides[f]	Cyanovirin-N

[a]These lectins do not bind monosaccharides; [b]source or name of lectin; [c]structure 36 in Appendix B; [d]43 in Appendix B; [e]55 in Appendix B; [f]53 in Appendix B

4.2.3.a. Sialic acid oligosaccharides

Several oligosaccharides with sialic acid at their non-reducing end serve commonly as lectin ligands: (*i*) Neu5Acα2,3/6Gal or Neu5Acα2,6GalNAc as such, or preferentially when they are attached β4 to glucose or *N*-acetylglucosamine and occasionally β3 to *N*-acetylgalactosamine and (*ii*) Neu5Acα2,3Galβ4(Fucα3)GlcNAc (sLe^x). Many lectins with specificity of the first type occur in animals and viruses, but rarely in other organisms. Only a few of them have been found in plants, for instance in the bark of several species of *Sambucus* trees, the best characterized of which is that from

Sambucus nigra, highly specific for α2,6-linked *N*-acetylneuraminic acid. The two *Maackia amurensis* lectins recognize sialic acid linked α2,3 (but not α2,6) to galactose: the leukoagglutinin (MAL) preferentially binds Neu5Acα2,3Galβ4GlcNAc (Knibbs et al., 1991), whereas the hemagglutinin (MAH) displays higher affinity for the disialylated tetrasaccharide, Neu5Acα2,3Galβ3(Neu5Acα2,6)GalNAc (Konami et al., 1994). However, ascribing specificity for sialic acid to plant lectins may be incorrect; thus MAL should perhaps be classified as galactose-specific, since its affinity to the latter monosaccharide is also noticeably enhanced by an acidic group other than sialic acid, such as sulfate, that is linked to the 3-*O* position of galactose (Bai et al., 2001).

E. coli expressing the S-fimbrial surface lectin combines with *N*-acetylneuraminic acid residues on integral membrane glycoproteins, as well as on gangliosides, preferentially in the form of Neu5Gcα2,3Gal- and Neu5Acα2,8Neu5Ac-. This lectin binds also sulfated glycolipids, apparently through a different fimbrial subunit than that which interacts with sialic acid (Prasadarao et al., 1993). The lectin released from merozoites of *Plasmodium falciparum* is specific for α2,3-linked *N*-acetylneuraminic acid; its ligand on human erythrocytes is glycophorin A (Sim et al., 1994).

Specificity for Neu5Acα2,3/6Gal is expressed by the hemagglutinins of numerous viruses, notably of influenza, as well as several others, such as Sendai, Newcastle disease, and polyoma viruses. Over 100 strains of influenza virus were examined for their ability to bind to enzymatically modified erythrocytes carrying terminal *N*-acetylneuraminic acid attached to galactose either by an α2,3- or α2,6 linkage. Differences in specificity with respect to this linkage were correlated with the species origin of the virus. Human isolates preferentially agglutinated resialylated erythrocytes containing the Neu5Acα2,6Gal sequence, while avian and equine isolates exhibited preference for Neu5Acα2,3Gal. Strains of influenza C virus (as well as coronaviruses) do not bind *N*-acetylneuraminic acid but recognize its 9-*O*-acetylated derivative ($Neu5,9Ac_2$), nor do any of the human strains recognize *N*-glycolylneuraminic acid, and therefore are not infectious for example to ducks that express this sialic acid on their cell surfaces. However, a human virus with a mutant hemagglutinin that aquired the ability to recognize *N*-glycolylneuraminic acid bound to duck cells and was able to cause infection in the birds (Ito et al., 2000) (see also Fig. 11.17).

The two strains of murine polyoma virus examined differed in their specificity for sialic acid oligosaccharides: those that form large plaques bound oligosaccharides terminating in Neu5Acα2,3Gal, whereas the small plaque strains also tolerated branched structures with a second, α2,6-linked, sialic acid, e.g., Neu5Acα2,3Galα3(Neu5Acα2,6)GalNAc (Freund et al., 1991). These strains also differed in their ability to form tumors in mice: the

small plaque strains produced few, if any, tumors, while the large plaque strains were highly tumorigenic.

A systematic study of the human siglecs (Brinkman-Van der Linden & Varki, 2000) has shown that they all require the carboxyl group of *N*-acetylneuraminic acid for ligand binding and that in addition to distinguishing between the linkage of this monosaccharide to the underlying galactose, their ability to recognize ligands depends also on other structural parameters of the latter (Table 4.8). With the exception of siglec-9, the presence of a fucose α3-linked to the *N*-acetylglucosamine of Neu5Acα2,3Galβ4GlcNAc (as in sLe^x) decreases significantly the interaction of such oligosaccharide with the siglecs that recognize α2,3-linked *N*-acetylneuraminic acid. Furthermore, some siglecs recognize Neu5Acα2,6GalNAc, in addition to Neu5Acα2,6Gal. Siglec-6 is the only member of this group of lectins that does not require the glycerol side chain of sialic acid for interaction with this sugar.

Table 4.8 Specificities of the human siglecs[a]

Siglec	*Oligosaccharides recognized*					*Essential group of sialic acid*	
	Siaα2,6 Lac(NAc)	*Siaα2,3 Lac(NAc)*	*sLe^x*	*Siaα2,6 GalNAc*	*Siaα2,8 Sia*	*Glycerol side chain*	*Carboxyl group*
1	+	++	-	-	[b]	+	+
2	++	-	-	+	-	+	+
3	++	+	-	+	-	Var.[c]	+
4a	-	+	-	-	-	+	+
5	+	+	-	+	+	Var.[c]	+
6	-	-	-	+	-	-	+
7[d]	+	+	ND	ND	ND	ND	ND
8[e]	+	++	ND	ND	ND	ND	ND
9[f]	+	+	+	±	ND	+	+
10[g]	+	+	ND	ND	ND	ND	ND
11[h]	±	±	ND	ND	+	ND	ND

[a]Based on Brinkman-Van der Linden & Varki, 2000; [b] - according to Brinkman-Van der Linden & Varki, 2000 and + according to earlier work (Collins et al., 1997); [c]variable; [d]Nicoll et al., 1999; [e]Floyd et al., 2000; [f]Angata & Varki, 2000; [g]Munday et al., 2001; [h]Angata et al., 2002

Specificity for other sialic acid oligosaccharides, such as sLe^x, is exhibited by the three selectins L, P and E. These lectins interact also with

sLe^a [Neu5Acα2,3Galβ3(Fucα4)GlcNAc], the positional isomer of sLe^x; shortening the glycerol side chain of the sialic acid in these ligands by periodic acid/borohydride treatment did not affect binding of either of the selectins. The selectins differ, however in their fine specificity (Feizi & Galustian, 1999). L-Selectin is highly specific for the monosulfated sLe^x derivative sialyl-(6-sulfo)-Le^x, in which the sulfate on the 6-OH of *N*-acetylglucosamine (structure 11 in Appendix B), while P and E selectins recognize both sulfated and unsulfated sLe^x. An even better ligand for L-selectin than the above mentioned 6-sulfated sLe^x is the rarely occurring neuraminyl form of the latter oligosaccharide, in which the amine of neuraminic acid is free and not *N*-acetylated as almost always found in nature (Komba et al., 2001). It is however unlikely that the neuraminic acid oligosaccharide can be a natural ligand for L-selectin, since it is highly unstable; this sugar is now known to be a transient metabolic intermediate in the transformation of sialyl-(6-sulfo)-Le^x to its stable cylic form that no longer binds selectins (Kannagi, 2002).

Like the galectins, the selectins too bind preferentially only a few cell surface glycoproteins, mostly mucins, that carry sLe^x , sLe^a and sialyl-(6-sulfo)-Le^x. Well studied examples are the L-selectin ligand GlyCAM-1 (glycosylation-dependent cell adhesion molecule-1) and the P-selectin ligand PSGL-1 (P-selectin glycoprotein ligand-1). The latter interacts with the other two selectins as well (McEver & Cummings, 1997). GlyCAM-1, a mucin secreted from high endothelial venules of peripheral lymph nodes, binds L-selectin only if it carries several branched *O*-glycans that are sialylated, fucosylated and sulfated, and that are linked to the protein backbone via the *N*-acetylglucosamine of the core-2 trisaccharide GlcNAcβ6(Galβ3)-GalNAcα. PSGL-1 is a homodimeric type 1 leukocyte membrane glycoprotein of 402 residues. To bind P-selectin, it must be sulfated on any one of three clustered tyrosines (Tyr46, Tyr48 or Tyr51) and *O*-glycosylated at Thr57 with an oligosaccharide containing sLe^x that is β6-linked to the *N*-acetylgalactosamine of the core-1 disaccharide Galβ3GalNAcα-Thr, forming the structure: Neu5Acα2,3Galβ4(Fucα3)GlcNAcβ6-(Galβ3)-GalNAcαThr (Liu, W. et al., 1998; Nicoll et al., 1999). A synthetic glycosulfopeptide (GSP-6), modeled after the N-terminal domain of PSGL-1, with three sulfated tyrosine residues together with the above saccharide (Fig. 4.7) bound tightly to immobilized P-selectin with a K_a of 3 x 10^6 M^{-1} (Leppänen et al., 1999). In contrast, binding of L-selectin does not depend on tyrosine sulfation as previously proposed, but has an absolute requirement for the 6-sulfated sLe^x. Neither tyrosine sulfation, nor sLe^x sulfation, is required for binding of E-selectin (Kannagi, 2002).

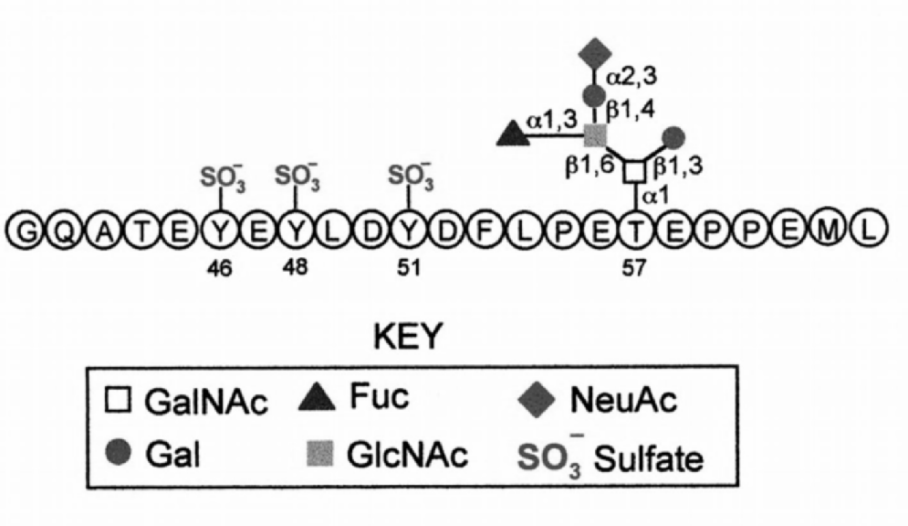

Fig. 4.7 Structure of GSP-6. Reprinted with permission rom Leppänen et al., 1999; copyright 1999 The American Association for Biochemistry & Molecular Biology.

4.2.3.b. Galactose oligosaccharides

Galactose serves as ligand for numerous lectins, almost all of which interact with galactose-terminated oligosaccharides with a higher affinity than with the monosaccharide; some of these do not combine with monosaccharides to any significant extent. Prominent examples of the latter are E-PHA and L-PHA, two isolectins specific for Galβ4GlcNAc-terminating complex oligosaccharides of slightly diffrent structures (Table 4.7). Lectins that recognize only galactosyl disaccharides, such as Galβ3GalNAc, Galα4Gal (galabiose) or Galα3Gal are rare. One specific for the first disaccharide just mentioned is that of *Vicia graminea*; it is distinct from PNA and jacalin that also have a high affinity for the same disaccharide, but bind galactose as well. *Vicia graminea* lectin interacts best with clusters of this disaccharide that are *O*-linked on neighboring hydroxyamino acids, indicating that it recognizes carbohydrate sequences together with the amino acid or peptide to which the latter are linked. Other Galβ3GalNAc-specific lectins that recognize the amino acid or peptide to which the disaccharide is linked are those from the mushrooms *Agaricus bisporus* (Presant & Kornfeld, 1972) and *Arthrobotrys oligospora* (Rosen, S. et al., 1996). The only known lectin that is highly specific for Galα3Gal has been found in the mushroom *Marasmius oreades* (see 3.2.3).

A flagellar component of *Pseudomonas aeruginosa* recognizes Galβ(Fucα3)4GlcNAc (Le^x) (Scharfman et al., 2001). *E. coli* P fimbriae recognize galabiose not only when it is in the free form or attached to other carbohydrates, but also when it occupies an internal position in oligosaccharides (Karlsson et al., 1994); (see also Table 8.3). The same holds also for *Propionibacterium granulosum* that binds specifically Galβ4GlcβCer (lactosyceramide), as well as GalNAcβ3Galβ4GlcβCer and Galα3Galβ4GalβGlcCer, in which the lactose occupies an internal position. (Strömberg et al., 1988). On the other hand, the closely related *P. freundreichii* binds only lactosylceramide. It is noteworthy that these two bacterial lectins species recognize separate epitopes on the lactose of lactosylceramide. The surface lectin of *Strep. pneumoniae* too interacts with internal oligosaccharides: it recognises GlcNAcβ3Galβ4GlcGalβ–4GlcNAcβ3Galβ4Glc and Neu5Acα2,6Galβ4GlcNAcβ3Galβ4Glc, (Barthelson et al., 1998; Idänpään-Heikkilä et al., 1997).

4.2.3.c. N-Acetylglucosamine oligosaccharides

Specificity for β4-linked oligosaccharides of *N*-acetylglucosamine, such as those present in chitin, is found almost exclusively in lectins of the Solanaceae family (potato, tomato and *Datura stramonium*). The affinity of these lectins to such oligosaccharides increases from the dimer to tetramer; the latter having typically association constants 10-20 times higher than the former and 1000 times higher than the monosacchride (Goldstein & Poretz, 1986).

4.2.3.d. Glucose oligosaccharides.

Calnexin and calreticulin, lectins found in the endoplasmic reticulum, recognize glucose only when it is part of the *N*-linked intermediates of protein glycosylation (e.g., structure 55 in Appendix B) (Helenius & Aebi, 2001; Parodi, 2000). A few invertebrate lectins posses the uncommon specificity for β3-glucans. First reported in larval hemocytes of the wax moth, *Galleria mellonella*, such lectins also occur in the silk worm, *Bombyx mori*, in several cockroaches, e.g., *Blaberus discoidalis* (Kilpatrick, D.C., 2000) as well as in banana (see 4.2.1.a)**.** A novel lectin, designated dectin-1, that binds β3 and β6 glucans has been isolated from dendritic cells (Ariizumi et al., 2000; Willment et al., 2001).

4.2.3.e. Mannose oligosaccharides

The recently described cyanovirin-N, an antiviral protein from the cyanobacterium *Nostoc ellipsosporum*, binds strongly branched oligo-mannosides with 8 or 9 units (structure 53 Appendix B), but not their smaller

homologs (Bolmstedt et al., 2001). It also binds the dimannoside Manα2Man albeit with lower affinity. The lectin has two carbohydrate combining sites per molecule that exhibit differing affinities for the disaccharide with K_a of 7.2 (± 4) x 10^6M^{-1} and 6.8 (±4) x 10^5M^{-1} for the high and low affinity sites, respectively (Bewley & Otero-Quintero, 2001).

4.2.3.f. Fucose oligosaccharides

The presence of a nonreducing fucose residue in certain oligosaccharides increases to varying extents their affinity to several lectins not classified as fucose-specific. Among these lectins are those of *Erythrina corallodendron* and *E. cristagalli*, of different Viciae species, as well as the selectins.

4.2.3.g. Glycosaminoglycans

Numerous proteins bind glycosaminoglycans, and although most are not considered lectins, they deserve to be mentioned here. The majority of these interact with heparin/heparan sulfate, several bind hyaluronan and only relatively few are known to interact with chondroitin sulfate or keratan sulfate (Rostand & Esko, 1997; Varki, A. et al., 1999, p. 435-437). L- and P-selectin interact with heparin and heparin-derived oligosaccharides (Wang, L. et al., 2002), whereas certain microorganisms (e.g., *Bordetella pertussis*, *Neisseria gonorrhoeae* and *Plasmodium falciparum)* bind to host cells surface via heparan sulfate. Proteins that combine with the latter glycosaminoglycan include annexins (Ishitsuka et al., 1998; Satoh et al., 2000), chemokines (Lortat-Jacob et al., 2002) and many viral surface proteins (Spillmann, 2001). HIV envelope glycoprotein gp120 recognizes sulfated polysaccharides, such as heparin (Rider, 1997) and dextran sulfate (Mbemba et al., 1994).

The numerous hyaluronan binding lectins (now termed hyaladherins) (Day & Prestwich, 2002) include two membrane glycoproteins, CD44 and RHAMM (receptor for hyaluronic acid and motility), as well as the four lecticans (cf. Table 3.3).

4.2.4 Promiscuous lectins.

Some lectins are unusual in that they interact with carbohydrates from different monosaccharide specificity groups at the same combining site. WGA that can combine with both *N*-acetylglucosamine and *N*-acetylneuraminc acid may be included in this group. Other examples are UEA-II that recognizes both galactose and *N*-acetylglucosamine (Loris et al., 2000) and the two closely related Gal/GalNAc-specific lectins from the bulbs of the Dutch iris (*Iris hollandica)*, that recognize mannose too (Hao et al., 2001). Furthermore, according to a recent report, the galactose-specific jacalin binds mannose as well (Barre et al., 2001).

Several animal lectins exhibit a relaxed specificity. The mannose specific-MBP, MMR, conglutinin and bovine collectin-43 bind fucose and *N*-acetylglucosamine as well, although more weakly (Kilpatrick, 2000). A similar broad pattern of recognition, but in a different order of preferred affinities (fucose>glucose>mannose), is shown by the lung surfactant proteins A and D. The binding of both mannose and fucose to these lectins can be rationalized by the topographical similarity of the two monosaccharides (Fig. 4.3). The Kuppfer cell receptor and the cockroach lectin recognize both fucose and *N*-acetylgalactosamine.

4.2.5 Lectins with dual specificities

The lectins discussed hitherto contain carbohydrate combining site(s) of a single specificity in the same subunit, as evidenced by the fact that the different ligands can compete with each other for binding to these lectins. However, rare cases of lectins possessing two distinct sites (in the same subunit), with dissimilar specificities, have been described (Table 4.9). Such lectins, sometimes referred to as exhibiting dual specificity, can bind different ligands simultaneously.

Table 4.9 Lectins with dual specificities

Lectin source	*Specificities*	*Ref.*
Arthrobotrys oligospora	I. Galβ3GalNAc; II. sulfated glycoconjugates	(1)
Psalthyrella velutina	I. Heparin/pectin; II. *N*-acetylglucosamine-containing neoglycoproteins	(2)
Tulipa spp.	I. Mannose; II. *N*-acetylgalactosamine	(3)[a]
Xanthosoma sagittifolium L	I. α3-Linked oligomannosides; II. triantennary Galβ3/4GlcNAc–terminated oligosaccharides	(4)

(1) Rosen, S. et al., 1996; (2) Ueda et al., 1999; (3) Van Damme, et al., 1996; (4) Mo et al., 1999;. [a]The lectin of tulip is very unusual in that when tested with rabbit erythrocytes, its agglutinating activity could be inhibited only by a mixture of methyl α-mannoside and *N*-acetylgalactosamine, whereas agglutination of human erythrocytes was inhibited by the latter monosaccharide, by lactose, fucose or galactose.

The two molecular forms of MMR, both of which are encoded by the same DNA, represent a special case of dual specificity. One of the forms is expressed on macrophages and binds mannose, *N*-acetylglucosamine and fucose. The other form, found on hepatic endothelial cells, binds GalNAc-4-SO_4-terminated ligands through a different combining site. Four classes of

sulfated oligosaccharides have been identified as ligands of this form: those of pituitary hormones (Fiete et al., 1998; Roseman & Baenziger, 2000), chondroitin sulfates A and B, sulfated blood group determinants and sulfated glycolipids (Feizi, 2000; Leteux, 2000).

4.3 ROLE OF OLIGOSACCHARIDE CONFORMATION.

Oligosaccharides are flexible molecules that may assume different shapes, due to the considerable freedom of rotation around the glycosidic bonds connecting their monosaccharide constituents. This flexibility leads to conformational heterogeneity. Molecular modeling of di- and oligosaccharides, as well as their study in solution by high resolution NMR, revealed that lectins select a single conformer which best fits their combining sites but that is not necessarily the most populated one in solution (Imberty & Perez, 2000; von der Lieth & . 1998). Upon binding, the rotational freedom of the saccharide becomes restricted, resulting in a decrease in the entropy of the system (see 4.5). For example, of the two conformers of Galβ2Gal that predominate in solution, each of the two galactose-specific lectins, galectin-1 and mistletoe (*Viscum album*) agglutinin, selects a different one (Fig. 4.8) (Siebert & Gabius, 1999). Molecular dynamic simulations of Galα3Gal in solution and in complex with GSL-IB_4 revealed that the lectin recognizes the lowest energy conformation of the disaccharide (Tempel et al., 2002).

Another example is the pentasaccharide core, Manα6(Manα3)Manβ4-GlcNAcβ4GlcNAc, present in all asparagine-linked carbohydrates. In this compound the α6-linked mannose can form two rotational isomers relative to the C5-C6 bond of the β4-linked mannose. The prevalence of either of the two isomers depends on the substitution pattern of this mannose. In particular, attachment to the mannose of a β4-linked (bisecting) *N*-acetylglucosamine fixes the orientation of the α6-linked mannose (and consequently of the α6 arm or antenna of the oligosaccharide) into one of two possible conformations (Fig. 4.8) and markedly decreases the binding of the oligosaccharide to concanavalin A as compared to the one devoid of the bisecting *N*-acetylglucosamine.

Because of their flexibility, oligosaccharides that differ in their chemical structure may have substantial topographic features in common, as a result of which they will bind to the same lectin (Spohr & Lemieux, 1985). On the other hand, different lectins with similar specificities may recognize different epitopes on the same oligosaccharide (Fig. 4.9). It means that the same oligosaccharide may carry different messages that can be decoded by different lectins.

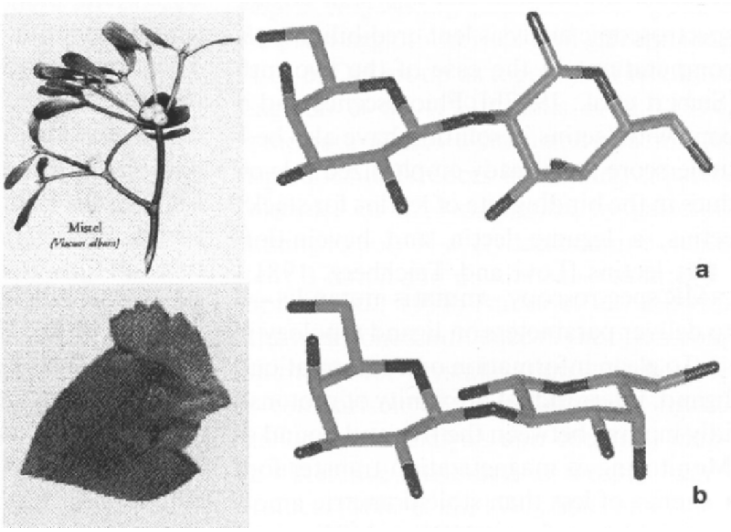

Fig. 4.8 Illustration of the geometry of the low-energy conformers of Galβ2Gal serving as ligand either for the mistletoe agglutinin (a) or for the avian galectin (b). The position of the non-reducing galactose units is deliberately kept constant to depict the remarkable topological difference. Reproduced with permission from Siebert & Gabius, 1999; copyright 1999 Deutsche Apotheker Zeitung.

4.4 EFFECT OF MULTIVALENCY

The most striking features of the lectin-monosaccharide interactions are that they are relatively weak (usually in the millimolar range), and often show relaxed specificity, when compared, for example, with that of enzyme-substrate interactions. Thus, a lectin may bind different sugars that have little in common except the orientation of a hydroxyl and an acetamide as in *N*-acetylglucosamine and *N*-acetylneuraminic acid or a few hydroxyls, for instance mannose and fucose (Fig. 4.3). Nevertheless, lectins exhibit high affinity and specificity for oligosaccharides of cell surface glycoproteins and glycolipids, a prerequisite for their function as recognition molecules in biological processes. It has therefore been suggested that multiple protein-carbohydrate interactions cooperate in each recognition event to give the necessary functional affinity (or avidity) and specificity (Drickamer, 1995b; Brewer, 1996; Lee, R. T. & Lee Y.C., 2000; Monsigny et al., 2000; Lundquist & Toone, 2002;). There are several possible ways, either alone or together, by which this is achieved: (a) ligand multivalency; (b) an extended binding region capable of interaction with more than just a single

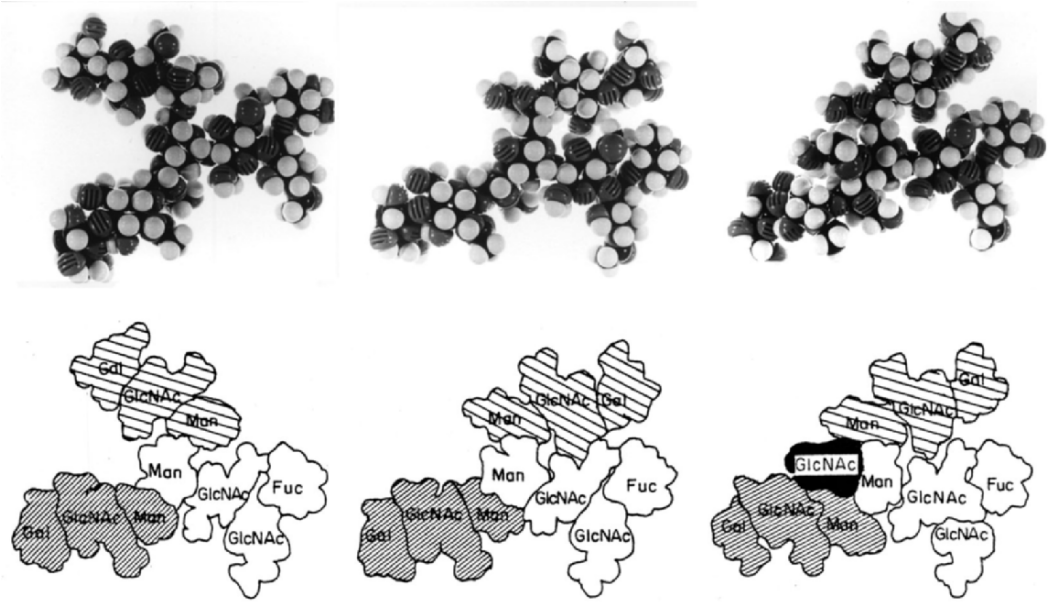

Fig. 4.10 Space-filling model (top) and silhouettes (bottom) of the complex oligosaccharide (structure 54 in Appendix B) (left and center) and of the same oligosaccharide with a bisecting *N*-acetylglucosamine (structure 52 in Appendix B) (right). The unbisected structure can adopt two orientations around the α6-linkage, whereas the bisected analogue can adopt only a single orientation about this linkage. Fine shaded area, α3 arm; striped area, α6 arm; black area; bisecting *N*-acetylglucosamine. Modified from Rademacher et al., 1986. (Photographs of the model courtesy Dr. R.A. Dwek, University of Oxford).

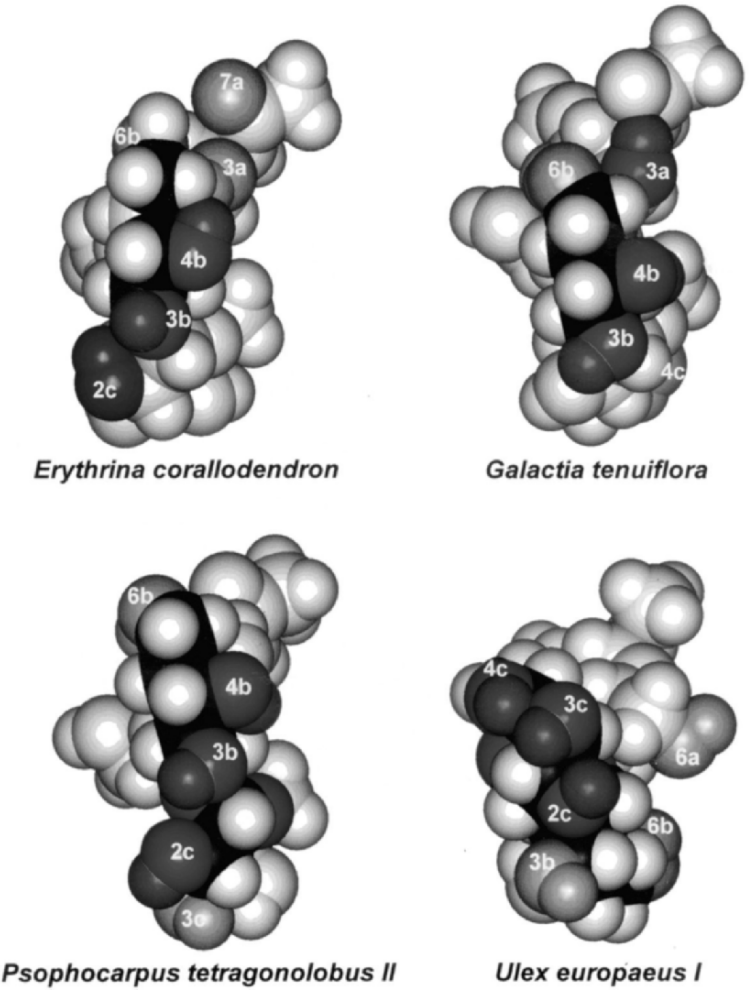

Fig. 4.9 Different lectins recognize different epitopes on the same oligosaccharide. The oligosaccharide shown is Fucα2Galβ4GlcNAc, where GlcNAc is the a unit, Gal the b unit and Fuc the c unit. The figure demonstrates how this trisaccharide is viewed from slightly different angles by identically positioned combining sites of four legume lectins. The numbers refer to the OH (or NHAc) groups of the constituent sugars of the trisaccharide; e.g. 3a is 3-OH of GlcNAc and 7a is its NHAc. Reproduced with permission from Lemieux et al., 2000; copyright 2000 Laser Pages Publishing Ltd.

monosaccharide residue of an oligosaccharide, as seen in concanavalin A or LOL-I and (c) clustering of several identical binding sites by formation of protein oligomers that can combine simultaneously with different, and

appropriately spaced, arms of a branched oligosaccharide, to separate carbohydrate chains of the same glycoprotein, or to carbohydrate chains on different glycoproteins (or glycolipids) on a cell surface; (d) combination of (a) and (c).

The effect of protein multivalency on the affinity of lectins is illustrated by the GSL-I isolectins (see 4.2.1.b), the A subunit of which is specific for α-*N*-acetylgalactosamine, the blood type A determinant. The AB_3 isolectin, that is monovalent for α-*N*-acetylgalactosamine, binds type A erythrocytes with an association constant (K_a = 7.5 x 10^5 M^{-1}) virtually identical to the K_a for MeαGalNAc; the association constants of the isolectins then increase with their valency to K_a = 1.42 x 10^7 M^{-1} for A_3B, which is however the same for A_4 (K_a = 1.2 x 10^7 M^{-1}) (Knibbs et al., 1998).

A synthetic polymer carrying multiple mannose residues exhibited a 10^5-fold higher affinity for concanavalin A than methyl α-mannoside (Mortell et al., 1996). About the same increase in affinity (from K_a of 5x10^4 to 0.4-2.0x10^8 M^{-1}) was observed with the human MMR and a series of lysine-based cluster mannosides when the number of mannose residues per molecule increased from two to six (Biessen et al., 1996). On the average, expansion of the cluster with the addition of a single α-mannose resulted in a 10-fold increase in affinity. Similarly, the affinity of rat HBP for aminotris-(hydroxymethyl)-methane, to which three lactose residues were attached, was 100-fold higher than for the same compound derivatized with a single lactose (Lee, R. T. et al., 1984). By varying the structure of the scaffold and introducing flexible linkers of different length between the scaffold and the carbohydrate, trivalent lactose derivatives with 1000-fold higher affinity than lactose were generated (Lee, Y.C. & Lee, R.T., 1995). Clustered or dendritic *N*-acetylglucosamine-based synthetic analogs with valencies of 2, 4, and 8 on a scaffold of multibranched polylysine were reported to possess 5-fold, 25-fold and 170-fold enhanced affinities, respectively, for WGA (Zanini & Roy, 1997).

Two neoglycoproteins, one with 40 galactose residues, and the other with 39 *N*-acetylgalactosamine residues per mole of bovine serum albumin, were 4700 and 140,000 times more potent inhibitors, respectively, of hemagglutination by the *N*-acetylgalactosamine-specific *E. histolytica* lectin, than *N*-acetylgalactosamine (Adler et al., 1995).

Another class of polyvalent carbohydrate ligands are the so-called starburst glycodendrimers (Roy, 1996). They represent tree-shaped, monodisperse molecules with the carbohydrate at the outer periphery. Such "sugar ball"-like compounds expose a moderate or high density of carbohydrates on their surfaces and enhance the affinities to lectins by several

orders of magnitude. An impressive recent example is that of an oligovalent ligand named STARFISH, (Fig. 4.11) tailored to fit simultaneously the five carbohydrate binding sites of the galabiose-specific, lectin-like, Shiga toxin (Kitov et al., 2000). It exhibited a 10^6-fold higher affinity to the toxin over that of the univalent ligand, Galα4Galβ4Glc.

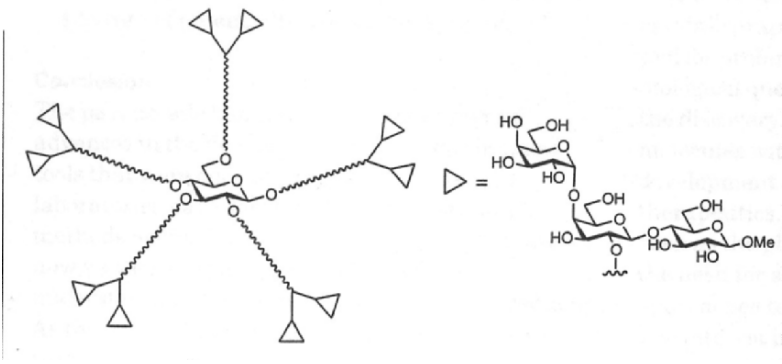

Fig. 4.11 STARFISH dendrimer. Schematic presentation of bridged dimers of the trisaccharide Galα4Galβ4Glc coupled to a pentavalent scaffold, made of a functionalized glucose molecule, to give a pentameric presentation of the trisaccharide dimers. Reproduced from Williams & Davies, 2001; copyright 2001, with permision from Elsevier Science.

The findings described have led to the general acceptance of the "cluster glycoside" effect, defined as “an affinity enhancement over and beyond what would be expected from the concentration increase of the determinant sugar in a multivalent ligand” (Lee, Y.C. & Lee, R.T., 1995; Lindquist & Toone, 2002). These studies also emphasize the importance not only of the number of carbohydrate residues of the ligand but also that of their spacing and orientation, as well as their display. In nature, the proper spacing and presentation of the ligands is achieved by displaying them on suitable carrier proteins (Fig. 4.12), as already mentioned in connection with the galectins (4.2.1.b) and selectins (4.2.2.a).

In addition to affecting the affinity of lectin-carbohydrate interactions, ligand multivalency may alter their specificity. Thus, whereas concanavalin A binds MeαMan with a 4-fold higher affinity than MeαGlc, it discriminated between polyvalent derivatives of the corresponding monosaccharides with an up to 160-fold difference in affinity (Mortell et al., 1996). The effect of ligand clustering on the specificity of lectins was also demonstrated with a solid-phase carbohydrate library of approximately 1300 related di- and trisaccharides attached to beads, so that each bead contained clusters of a

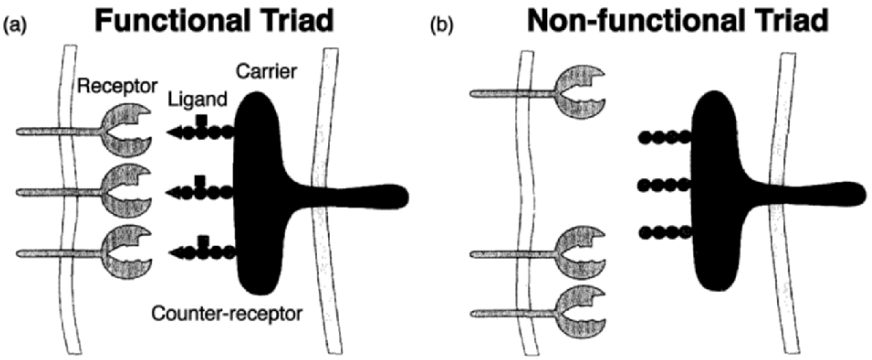

Fig. 4.12 Schematic representation of receptor-ligand assemblies. a) Functional. b) Non-functional. The oligosaccharide ligands must be effectively presented on protein or lipid carriers, which together serve as functional counter-receptors. Reproduced from Crocker & Feizi, 1996; copyright 1996, with permission from Elsevier.

single carbohydrate species (Liang et al., 1996). Out of this pool, *Bauhinia purpurea* lectin (specific for *N*-acetyllactosamine) picked out beads carrying two particular disaccharides, namely *N-p*-nitrobenzoyl- and *N*-isovaleroyl-lactosamine, respectively. In solution, however, these disaccharides had no higher affinity to the lectin than *N*-acetyllactosamine. Therefore, the amplified affinity and specificity of the lectin to the beads carrying these two disaccharides appears to be due to their polyvalent presentation. An even more dramatic effect of ligand density was the switch in the preference of *Bauhinia purpurea* lectin for Galβ3GalNAcβR over Galα3GlcNAcαR when the disaccharides were in solution to a preference for the latter disaccharide over the former when they were immobilized (Horan et al., 1999). Since immobilized carbohydrates simulate clusters on cell surfaces, the above results can be seen as mimicking the selectivity of lectins in biological systems.

The disposition of the individual binding sites of a polymeric lectin plays an important role in defining with which multivalent saccharide it can interact strongly. The ability of different members of the plant legume lectin family to form tetramers through different types of dimer-dimer contacts, thereby presenting their combining sites in different arrangements, may represent another means of diversifying their preference for particular ligands (Drickamer, 1995b).

The increase mentioned earlier in the affinity of rat HBP for multivalent ligands relative to a monovalent one can be seen as an example of the effect of clustered binding sites of the receptor on affinity. The ~10^6-fold increase in affinity that is observed in the binding to the menbrane-bound lectin of a triantennary or tetraantennary oligosaccharide (structures 49 and 56 respectively**,** in Appendix B), as compared to that for a monoantennary one (Fig. 4.13), is due to the steric organization of the subunits of the receptor that allows the three terminal galactose residues of the oligosaccharide to bind simultaneously to the combining sites of the subunits (Lee, Y.C. et al., 1983).Indeed, this phenomenon is much less pronounced when detergent-solubilized lectin is used.

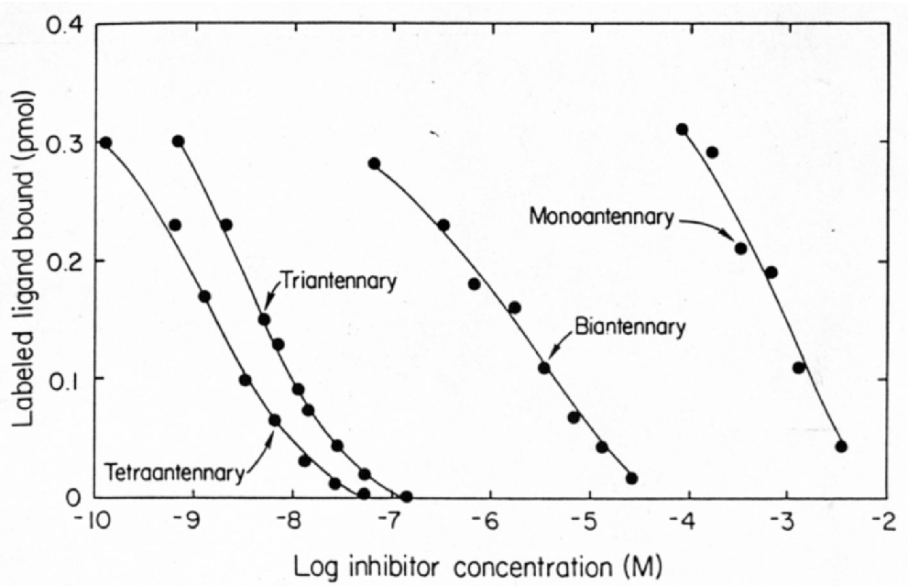

Fig. 4.13 Inhibition of binding of ^{125}I-Tyr-asialotriantennary glycopeptide to rabbit hepatocytes. The monoantenaary ligand used was Galβ4GlcNAcβ6Man. For the struture of the biantennary, triantennary and tetraantennary ligands see structures 41, 49 and 56, respectively in Appendix B. Reproduced with permission from Lee, Y.C. et al., 1983; copyright 1983 Journal of Biological Chemistry

Sugars that are good inhibitors for a given lectin in solution, do not always bind to the same lectin when it is immobilized. Comparison of the elution pattern of various glycopeptides from columns of concanavalin A-Sepharose with their association constants with the lectin in solution revealed that only those glycopeptides with constants in the range of 4.5 x 10^6 to 25 x 10^6 M^{-1} are retained on the columns, while glycopeptides with association constants

of 4 x10^6 M^{-1} or less are not retained (Baenziger & Fiete, 1979). This is perhaps not surprising, since the orientation of the combining sites of an immobilized lectin may differ from that of the same lectin in solution.

The multivalency of both lectins and oligosaccharides provide them with a potential to form diverse arrays when in combination (Brewer, 1996). Linear arrays are obtained, for example, with divalent oligosaccharides and dimeric lectins, in which identical binding sites are located at the two sites of the lectin dimer (see also 6.2.1 and Fig. 6.31). Lectins with more than two combining sites, as found in tetrameric SBA, form with divalent oligosaccharides cross-linked, three-dimensional lattices, resulting in precipitation of the lectin-oligosaccharide complexes (Fig. 4.14). Homogenous precipitates, with distinct lattice patterns, are formed even in solutions of a single such lectin in the presence of a mixture of two oligosaccharides or, for that matter, of a single oligosaccharide and two lectins with similar specificity. These findings point to the presence of long-range order and well-defined geometry in the cross-linked complexes and thus possibly a new source of specificity for lectins, namely the ability to selectively cross-link and aggregate glycoproteins in mixed systems. This has important implications for the interaction of lectins with cells, where they are confronted with large, nearly planar arrays of oligosaccharides, and may also account for the biological activities of lectins, for which cross-linking and clustering of receptors is required (Sacchettini et al., 2001).

4.5 ENERGETICS OF PROTEIN-CARBOHYDRATE INTERACTIONS

For a long time the main tool for the study of the specificity of lectins and of their affinity for ligands was inhibition of hemagglutination or of polysaccharide precipitation. However, to obtain the complete thermodynamic profile of a binding interaction, more accurate and sensitive physicochemical techniques are required (Table 4.1). Such methods provide data on changes in free energy, ΔG, in heat of binding (or enthalphy), ΔH, and in entropy of binding, ΔS, as well as heat capacity changes, ΔCp, of the lectin-carbohydrate interactions (Table 4.10). These parameters, in combination with information from high resolution X-ray crystallography (cf. Chapter 6), are essential for the understanding of carbohydrate-based biological recognition (Burkhalter et al., 2000; Dam & Brewer, 2002; Toone, 1994). ΔH and ΔS for a given complex establish the magnitude and sign of ΔG. Greater negative ΔH values for the binding of an oligosaccharide relative to the negative ΔH values for that of the corresponding monosaccharides indicate the existence of an extended combining site. By monitoring changes in the thermodynamic parameters of binding that result from the replacement of a particular hydroxyl in the ligand with hydrogen or fluorine (as in deoxy-

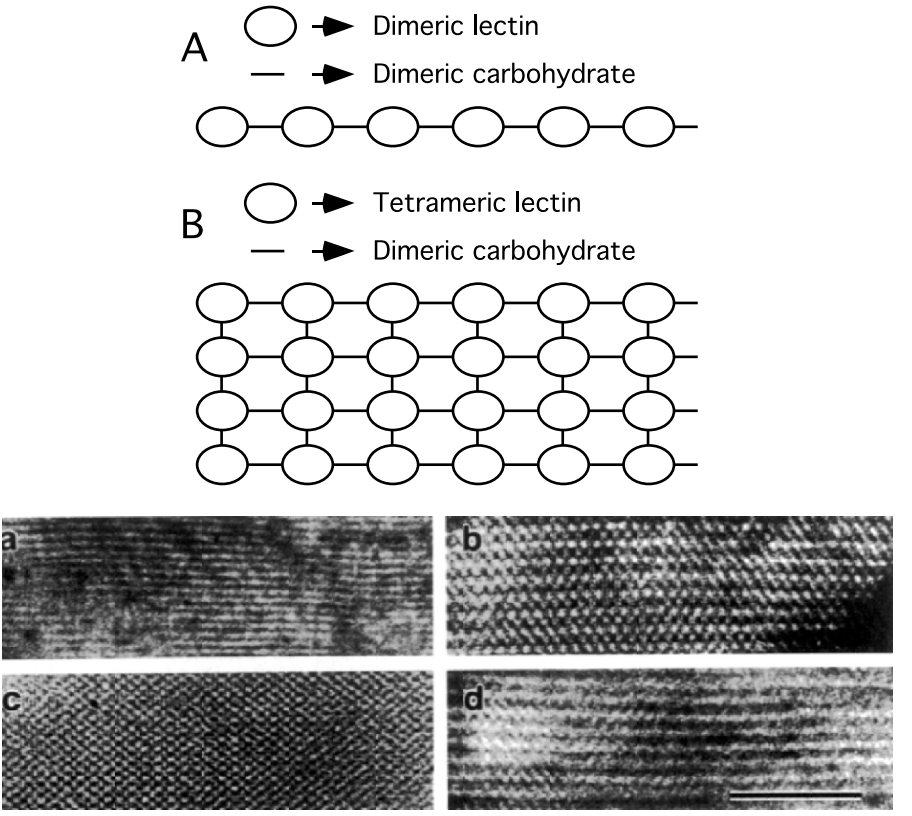

Fig. 4.14 (A). Schematic diagram of a cross-linked complex between a tetravalent lectin and a divalent carbohydrate. The lectin is represented by a circle and the carbohydrate by a line. (B). Negative stain electron micrographs of the precipitates of soybean agglutnin with different biantennary pentasaccharides a, b, c, and d (structures 30, 31, 32 and 33, respectively in appendix B). Reproduced with permision from Gupta et al., 1994; copyright 1994 American Chemical Society.

or fluorodeoxy derivatives of mono- or oligosaccharides), or its conversion to a methoxy group, the contribution of that hydroxyl can be assessed (Gupta et

Table 4.10 Thermodynamic parameters of lectin-carbohydrate interactions[a]

Lectin	*Carbohydrate*	*Ka* $10^{-3}\ M^{-1}$	$-\Delta G$, *kcal/mol*	$-\Delta H$, *kcal/mol*	$-T\Delta S$, *kcal/mol*	ΔCp, *kcal/mol/deg*
Concanvalin A	MeαMan	8.2	5.3	8.2	2.9	-90

Table 4.10 Thermodynamic parameters of lectin-carbohydrate interactions[a]

Lectin	*Carbohydrate*	*Ka* 10^{-3} M^{-1}	*-ΔG, kcal/mol*	*-ΔH, kcal/mol*	*-TΔS, kcal/mol*	*ΔCp, kcal/ mol/deg*
	Manα6-(Manα3)Man	490	7.8	14.4	6.6	-110
ECorL	MeαGal	1.4	4.3	5.2	0.85	
	MeβGal	0.7	3.9	4.3	0.47	
	GalNAc	1.3	4.3	5.5	1.2	
	MeβGalNAc	1.3	4.3	6.8	2.5	
	Galβ4Glc	1.9	4.5	9.8	5.4	
	Galβ4GlcNAc	9.7	5.4	11.3	5.9	
	MeαGalNDns	350	7.6	5.5	-2.1	
Galectin-1[b]	Galβ4Glc	5.6	5.2	8.8	3.6	
Galectin 1[c]	Galβ4GlcNAc	6.2	7.6	6.6	1.4	-90
Lentil	MeαMan	0.8	3.9	4.1	0.2	
MBL-A	MeαMan	1.0	3.8	4.7	0.9	
	MeαGlcNAc	1.0	3.8	5.2	1.4	
MBL-C	MeαMan	1.0	3.8	5.1	1.3	
	MeαGlcNAc	1.0	3.8	4.7	0.9	
Pea	MeαMan	1.6	4.4	5.9	1.5	
SBA	MeαGal	1.0	4.1	9.1	4.9	
	MeβGal	0.5	3.7	10.6	6.9	-94
	GalNAc	9	5.4	9.5	4.1	
	MeαGalNAc	24	6.0	10.7	4.5	
	MeβGalNAc	22	5.9	13.9	8.0	-100
	GalNDns	590	3.7	7.9	4.3	
	Galβ4Glc	0.2	3.1	5.5	2.4	
	Galβ4GlNAc	0.5	3.9	8.2	4.3	
WGA	GlcNAc	0.4	3.7	6.1	2.4	
	$(GlcNAc)_2$	5.3	5.1	15.6	10.5	
	$(GlcNAc)_3$	11	5.5	19.4	13.9	
WBA-II	Gal	1.2	4.2	5.8	1.6	
	MeαGal	6.6	5.2	5.6	0.4	
	MeβGal	1.0	4.1	4.7	0.6	
	GalNAc	7.2	5.3	d6.7	1.4	

Table 4.10 Thermodynamic parameters of lectin-carbohydrate interactions[a]

Lectin	*Carbohydrate*	*Ka* 10^{-3} M^{-1}	*-ΔG, kcal/mol*	*-ΔH, kcal/mol*	*-TΔS, kcal/mol*	*ΔCp, kcal/ mol/deg*

[a]Representative data taken from Toone, 1994; for additional data, see Burkhalter et al., 2000 and Dam, T. K. & Brewer, 2002; [b]from sheep spleen; [c]recombinant lectin from Chinese hamster ovary cells.

al., 1997; Lemieux, 1996; Lemieux et al., 2000)). There is however no way to evaluate the contribution of individual hydrogen bonds (Janin, 1997). This is clearly illustrated by a comparison of the thermodynamic parameters of binding of the trimannoside Manα6(Manα3)Man to concanavalin A, with those of the interaction of the lectin with a series of mono-, di-, and trideoxy analogues of the ligand (Dam et al., 1998b). The results obtained were in agreement with the X-ray crystal structure of the complexes of the above trisaccharide with concanavalin A and with *Dioclea grandiflora* lectin with respect to the hydroxy groups involved in binding (Dam et al., 1998c) (cf. 5.1.1.). However the free energy and enthalpy contribution of the individual groups was not linear, indicating that the differences measured are due not only to the loss of hydrogen bonds but also to differential contributions of other factors, such as protein and solvent effects, and also the effects of the bonds on each other.

The overall process of binding of a ligand to a lectin involves the encounter of a solvated polyhydroxylated carbohydrate and a solvated combining site. When the ligand presents a surface complementary to the combining site, water can be displaced and binding occurs. The differences between the hydrogen bond energies of the protein-water and the carbohydrate-water complexes (solute-solvent interactions) on the one hand, and those between the protein and the carbohydrate (solute-solute interactions) on the other, are an important contributing factor to the net binding energy. Additional contributions derive from changes in protein conformation, van der Waals bonds, hydrophobic interactions, as well as entropic effects (Toone, 1994; Lemieux, 1996; Burkhalter et al., 2000; Dam, et al., 2000). It has been postulated that water molecules at the combining site of lectins are in a special perturbed state of higher energy than bulk water, and that the release of this water to the bulk provides a driving force for sugar complexation by the lectin (Lemieux, 1996).

Calorimetric data reveal that protein-carbohydrate interactions are enthalpy driven, and that in almost all cases the enthalpy of binding is more negative than, or equal to, the free energy of binding. Enhancement of the affinity of lectins for multivalent ligands may however be due to the relatively positive TΔS contribution with respect to monovalent sugar binding (Dam et

al., 2000). A significant fraction of the enthalpy of complexation arises from solvent reorganization. The binding data also show a strong linear enthalpy-entropy compensation, as illustrated by the interaction of WBA II and oligosaccharides (Fig. 4.14). Thus, the unfavorable loss in entropy resulting from changes in rotational degrees of freedom as torsion angles around the glycosidic bonds are frozen upon binding is compensated by an advantageous change in enthalpy due to the removal of ligated water. The values of ΔC_p for lectin-carbohydrate binding, a term generally thought to reflect changes in solvent structure during binding, are small (<100 cal mol^{-1} $degree^{-1}$) and negative (Table 4.10).

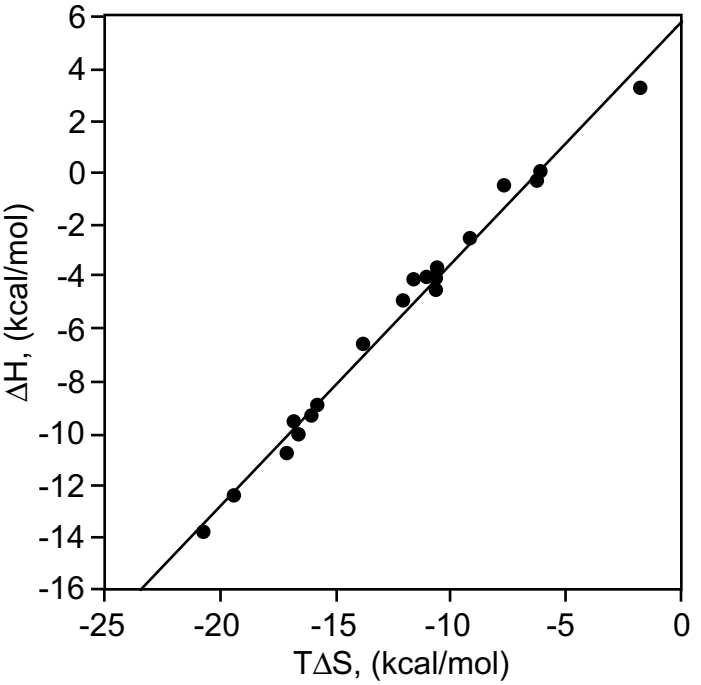

Fig. 4.15 Enthalpy-entropy compensation plot of the binding of H-type-II-OMe trsaccharide and it various derivatives to WBA II. The slope of this plot is 0.90 and the correlation coefficient is 0.98. Reproduced with permission of Federation of European Biochemical Societies from Srinivas et al., 1999.

4.6 NON-CARBOHYDRATE LIGANDS

During the last decade a number of peptides have been shown to mimic carbohydrates and bind to lectins. Thus, non-glycosylated peptides that

contain the Tyr-Pro-Tyr motif combined with concanavalin A, with an affinity close to that of methyl α-mannoside (Oldenburg et al., 1992; Scott et al., 1992; Kaur et al., 1997). Since the peptides competed with the binding of the mannoside to the lectin, it was suggested that they interact with it at the primary carbohydrate binding site, by hydrogen bonding with the hydroxyl groups of the tyrosines (which mimic sugar hydroxyls) and by hydrophobic interactions with an aromatic side chain of these peptides (that mimic structurally the carbons of the pyranose ring), thus presenting a case of true glycomimetics. Mannose inhibitable interactions with non-glycosylated proteins have also been observed with the mannose-specific type 1 fimbrial lectins of *E. coli* (Sokurenko et al., 1994). It is not known however whether these reactions occur via the carbohydrate binding site proper. Other examples are listed in Table 4.11.

Table 4.11 Peptide mimics of carbohydrates recognized by lectins

Carbohydrate	*Peptide mimic*	*Lectin*	*Ref.*
Meα-Man	DVFYP	Concanavalin A	
	YPYASGS		Oldenburg et al., 1992
	MYWYPY		Scott et al., 1992
Galα3Gal	DAHWESWL	GSL-IB_4	Sandrin et al., 1997
	SSLRGF	GSL-IB_4	Kooyman et al., 1996
NLc4Cer[a]	VPPYFTLMY	RCA	Taki et al., 1997
sLe^x	DITWDQLW DLMK	E-selectin	Martens, 1995
sLe^x	IELLQAR	E-selectin	Fukuda & Fukuda, 2000

[a]Galβ4GlcNAcβ3Galβ4GlcCer

Several additional compounds combine with lectins at distinct sites that are however remote from the carbohydrate binding site. For instance, lima bean lectin and concanavalin A interact stoichiometrically with hydrophobic ligands such as indoleacetic acid with low affinity, and with 1,8-anilinonaphtalene-sulfonic acid with a somewhat higher affinity (K_a = 3.9 x 10^3 M^{-1} (Roberts, D. D. & Goldstein, 1983). These compounds bind at a single site per lectin subunit, that in lima bean lectin is located about 30Å from the carbohydrate binding site. Certain plant lectins have a specific and high-affinity binding site for adenine and its *N*-6-derivatives, with association constants of 10^5-10^6 M^{-1}. Adenine binding sites are found mostly in tetrameric lectins, among them E-PHA, L-PHA, SBA, LBL and DBL, but also in some dimeric ones, such as DB58 and WBA-II (Srinivas et al., 2000).

Quite unusually, there is only one (or two) such site(s) per lectin molecule, and not one per subunit. Several lectins [pea lectin (Bhanu et al., 1997), jacalin (Komath et al., 2000a) and snake gourd lectin (Komath et al., 2000b)] combine with porphyrins at a site distinct from that for carbohydrates. It is not known, whether the porphyrin site is related to any of the hydrophobic sites mentioned earlier. However, concanavalin A binds porphyrins at the carbohydrate-combining site (Goel et al., 2001).

Specific interactions with non-carbohydrate ligands have also been observed between the Man6*P* receptor and insulin-like growth factor II (see 5.2.3); between siglec-6 and leptin (Patel et al., 1999) and between the N-terminal region of galectin-3 and RNA (Wang, L. et al., 1995). Other examples are the interaction of the slime mold lectin with a receptor on the surface of aggregating *D. discoideum* via the tripeptide Arg-Gly-Asp (Springer, W. R. et al., 1984), and of the hydrophobic domain of membranal endocytic lectins and the lipids of cellular membranes, a prerequisite for the insertion of the lectin into the membrane (Barondes, 1988).

4.7 LECZYMES

On rare occasions lectins were shown to possess enzymatic activity unrelated to their carbohydrate specificity; such lectins are referred to as leczymes (Nitta, 2001). Thus, the lectins from the eggs of the frogs *Rana castebiana* and *Rana japonica* exhibit RNase activity (Nitta et al., 1993), while the LNP lectin from the roots of *Dolichos biflorus* acts as an apyrase that catalyzes the hydrolysis of phosphoanhydride bonds in nucleosides of di- and triphosphates (Roberts, N. J. et al., 1999). Ricin and ricin-related lectins are also leczymes with RNA *N*-glycosylase activity.