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Thermodynamic binding studies of galectin-1, -3 and -7

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The carbohydrate binding specificities of the galectin family of animal lectins has been the source of intense recent investigations. Isothermal titration microcalorimetry (ITC) provides direct determination of the thermodynamics of binding of carbohydrates to lectins, and has provided important insights into the fine carbohydrate binding specificities of a wide number of plant and animal lectins. Recent ITC studies have been performed with galectin-1, galectin-3 and galectin-7 and their interactions with sialylated and non-sialylated carbohydrates. The results show important differences in the specificities of these three galectins toward poly-N-acetyllactosamine epitopes found on the surface of cells.
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Abbreviations: **ITC, isothermal titration microcalorimetry; CRD, carbohydrate recognition domain; ThiodiGal, β-Thiodigalactopyranoside; LacNAc, N-acetyllactosamine.**

Introduction

Galectins are a family of animal lectins defined by common consensus sequences and structures, and possess specificities for β-Gal and LacNAc residues in oligosaccharides [1–3]. Fourteen members of the family have currently been identified in mammals and designated galectin-1 through -14, and more are likely to be found since homologies have already been reported in the literature [4–6]. Progress has been made in determining some of the biological activities of mammalian galectins which include roles in the regulation of inflammation, and modulators of cell adhesion, cell proliferation and cell death [7,8].

The structures of the mammalian galectins can be identified as prototype (galectin-1, -2, -5, -7 , -10 and probably -11, -12, -13 and -14) which exist as monomers or homodimers consisting of one carbohydrate-recognition domain (CRD); chimera type (galectin-3) that contains a nonlectin N-terminal short sequence segment followed by 8–129 amino acid long collagen-like repeats connected to the C-terminal of about 140 amino acids; and tandem-repeat type (galectin-4, -6, -8 and -9) composed of two carbohydrate recognition domains (CRD) domains in a single polypeptide chain connected by a linker peptide [1–3]. The X-ray crystal structures of galectin-1 [9,10], galectin-2 [11], galectin-7 [12], galectin-10 [13] and CRD domain of galectin-3 [14] have been reported. Studies of the carbohydrate binding specificities of the galectins have been largely restricted to galectin-1 [15,16] and galectin-3 [16–18] with conflicting data reported for galectin-10, the Charcoat-Leyden crystal protein [13,19]. These studies generally show specificities of the galectins for LacNAc residues, with higher affinities reported for some naturally occurring oligosaccharides. Indeed, there is evidence that the galectins recognize polylactosamine chains with high affinity (cf. [20]), and that these chains are receptor sites on the surface of cells (cf. [21]). However, thermodynamic data for mammalian galectins binding to sialylated and nonsialylated LacNAc oligomers has not been available until recently.

Considerable insight has been gained on the biological properties of specific galectins. Galectin-1 has been implicated in a variety of biological processes in multicellular organisms including inflammation, development, mRNA splicing, differentiation, and cell adhesion [1–3]. Galectin-1 has also recently been demonstrated to play a role in the immune system by mediating apoptosis of activated T cells with CD7 as target [22]. Galectin-3 has also been shown to exhibit roles in regulating inflammation, cell growth and cell adhesion [3,23]. Galectin-3, unlike galectin-1, possesses anti-apoptotic effects in a variety of cells, and its expression has been shown to

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correlate with the metastatic potentials of certain cancers [24]. Galectin-7 is reported to be marker for stratified epithelia [25]. It has been shown that the galectin-7 gene is a transcriptional target of the tumor suppressor protein p53 and may play a role in the proapoptotic function of p53 [26]. Expression of galectin-7 is elevated in chemically induced rat mammary carcinomas [27] and its mRNA is detected frequently in human breast and colon cancer cell lines [28]. Thus, Galectin-7 appears to be another member of the galectin family with regulatory activity for cell survival. Hence, determining the fine carbohydrate binding specificities of these three galectins is important in understanding their biological functions.

Recently, new insights have been reported on the carbohydrate binding specificities of galectin-1, galectin-3 and galectin-7 using isothermal titration microcalorimetry (ITC) and hemagglutination inhibition analysis [29]. The results demonstrate important differences in the binding specificities of the three galectins.

Thermodynamic binding data

Table 1 shows ITC data for bovine heart galectin-1, murine recombinant galectin-3, and human recombinant galectin-7 binding to carbohydrates in Figure 1. Table 2 shows hemagglutination inhibition data for the three galectins. The data in both tables indicate that, in general, galectin-7 possesses weaker affinity for LacNAc as the primary epitope, relative to the other two galectins. For example, Table 1 shows that the K_a value for galectin-7 binding LacNAc-II is nearly 6-fold lower than the corresponding value for galectin-1, and nearly 11-fold weaker than that of galectin-3. Due to the weaker affinity of galectin-7 for carbohydrates, most of the ITC binding data for galectin-7 were obtained at 287 K and compared to data for galectin-1 and galectin-3 at the same temperature.

Thermodynamic binding data of galectin-1 and -3 to disaccharides

The thermodynamics of binding of bovine heart galectin-1 and recombinant murine galectin-3 to simple disaccharides are shown in Table 1. The disaccharide LacNAc exists as two isomers, LacNAc-I and -II, which differ in their linkage between Gal and GlcNAc. LacNAc-I possesses a β(1–3) linkage and LacNAc-II a β(1–4) linkage. Previous studies of the binding of human galectin-1 [16] and rat galectin-3 [30] to an asialofetuin-Sepharose column showed similar inhibitory activities for LacNAc-I and LacNAc-II. ITC data at 300 K in Table 1 reveals that there is little difference in the K_a values of galectin-1 for LacNAc-I (0.90×10^4 M^{-1}) *versus* LacNAc-II (1.1×10^4 M^{-1}). The enthalpies of binding (ΔH) are -7.9 kcal mol^{-1} and -9.2 kcal mol^{-1}, respectively. Likewise, galectin-3 shows little difference in its K_a values for LacNAc-I (0.82×10^4 M^{-1}) *versus* LacNAc-II (1.9×10^4 M^{-1}), with ΔH values of -6.4 kcal mol^{-1} and -7.5 kcal mol^{-1}, respectively. These results confirm similar affinities of both galectins for type I and II isomers of LacNAc. These results agree with those of Schwarz *et al*. [31] for bovine spleen galectin-1, and Surolia and coworkers [20] for recombinant human galectin-3.

Galectin-1 from a number of sources [32–34] have previously been shown to bind to β-thiodigalactopyranoside (ThiodiGal) with affinities comparable to LacNAc-II. Galectin-3 from human [16] and rat lung [30] have also been shown to bind to ThiodiGal. ITC data in Table 1 shows that bovine heart galectin-1 and recombinant murine galectin-3 bind to ThiodiGal at 300 K with nearly the same K_a values as LacNAc-II (factor of two difference for galectin-1). The enthalpies of binding ($-\Delta H$) of galectin-1 to the two disaccharides are similar, as are the $-\Delta H$ values for galectin-3 binding the two sugars. Thus, bovine spleen galectin-1 and recombinant murine galectin-3 share similar binding thermodynamics to ThiodiGal. These results are similar to those recently reported by Surolia's group [20] for recombinant human galectin-3.

Thermodynamic binding data of galectin-1 and -3 to LacNAc oligomers

The thermodynamics of binding at 300 K of bovine heart galectin-1 and murine galectin-3 to dimeric (DiLacNAc) and trimeric (TriLacNAc) oligomers of LacNAc-II are shown in Table 1. Galectin-1 possesses K_a values of 0.74×10^4 M^{-1} and 2.2×10^4 M^{-1} for Di- and TriLacNAc, respectively, as compared to 1.1×10^4 M^{-1} for LacNAc-II. Hence, the K_a values for Di- and TriLacNAc are similar to that of LacNAc-II. The ΔH values for the three carbohydrates are also similar: -9.2 kcal mol^{-1} for LacNAc-II; -11.4 kcal mol^{-1} for DiLacNAc; and -10.1 kcal mol^{-1} for TriLacNAc. Furthermore, the n values for all three carbohydrates, the number of binding sites per monomer of protein, are all close to one, indicating that all three oligosaccharides bind to the same site in the protein. The n values are also an indication of the valencies of the carbohydrates [35], and, therefore, all three oligosaccharides are monovalent, even though Di- and TriLacNAc have two and three LacNAc residues, respectively. Hemagglutination inhibition data with Di- and TriLacNAc also shows similar affinities of galectin-1 for all three carbohydrates (Table 2).

These data indicate that galectin-1 possesses a binding site that recognizes primarily one LacNAc residue, even when binding oligomers of LacNAc. ^{13}C NMR studies by Pierce and coworkers [36] of the C2S galectin-1 mutant from chinese hamster ovary cells binding to specifically ^{13}C labelled oligomers of LacNAc also concluded that galectin-1 bound primarily to the nonreducing terminal LacNAc residues of the oligomers. The X-ray crystal structure of bovine spleen galectin-1 in complex with LacNAc-II also shows only one mole of bound carbohydrate [10].

Lacto-N-tetraose (Galβ(1-3)GlcNAcβ(1-3)Galβ(1-4)Glc) has been shown to bind well to human galectin-1 [16], and possesses both a nonreducing terminal LacNAc-I moiety and a nonreducing terminal lactose moiety. Hemagglutination inhibition data demonstrate that bovine heart galectin-1 binds

Table 1. Thermodynamic binding parameters of bovine heart galectin-1, murine recombinant galectin-3 and human recombinant galectin-7 with carbohydrates [29]

Sugars	K_a[a] *($M^{-1} \times 10^{-4}$)*	$-\Delta H$[b] *(kcal mol^{-1})*	$-\Delta G$[c] *(kcal mol^{-1})*	$-T\Delta S$[d] *(kcal mol^{-1})*	n[e] *(sites/monomer)*
		Bovine Heart Galectin-1			
		Temp. 300 K			
LacNAc-I	0.90	7.9	5.4	2.5	0.97
LacNAc-II	1.1	9.2	5.5	3.7	1.01
ThiodiGal	1.8	9.8	5.8	4.0	1.04
DiLacNAc	0.74	11.4	5.3	6.1	0.94
TriLacNAc	2.2	10.1	5.9	4.2	0.94
Lacto-N-tetraose	1.0	9.7	5.5	4.2	0.94
		Temp. 287 K			
Lactose	0.61	9.7	5.0	4.7	0.94
LacNAc-II	2.4	8.7	5.7	3.0	0.99
2,3-Sialyl LacNAc	2.3	8.5	5.7	2.8	1.08
		Galectin-3			
		Temp. 300 K			
LacNAc-I	0.82	6.4	5.3	1.1	0.92
LacNAc-II	1.9	7.5	5.9	1.6	1.01
ThiodiGal	0.98	7.8	5.5	2.3	1.08
DiLacNAc	3.5	8.8	6.2	2.6	1.07
TriLacNAc	6.3	9.8	6.6	3.2	0.92
2,6-Sialyl-DiLacNAc	3.2	9.7	6.2	3.5	0.98
Lacto-N-tetraose	2.6	5.0	6.1	1.1	0.95
		Temp. 287 K			
LacNAc-II	3.7	9.7	6.0	3.7	0.95
2,3-Sialyl LacNAc	3.3	8.3	5.9	2.4	0.96
2,6-Sialyl Lacto-N-neotetraose	2.1	6.4	5.6	0.8	1.05
2,6-Sialyl DiLacNac	4.8	10.1	6.1	4.0	0.94
Trisaccharide[f]	0.88	7.7	5.2	2.5	0.96
		Galectin-7			
		Temp. 300 K			
Lactose	0.22	10.6	4.6	6.0	1.03
LacNAc-II	0.17	11.8	4.4	7.4	0.99
		Temp. 287 K			
ThiodiGal	0.40	10.0	4.7	5.3	1.08
Lactose	0.49	10.5	4.8	5.7	1.02
LacNAc-II	0.63	11.3	5.0	6.3	1.07
DiLacNAc	0.74	12.3	5.1	7.2	1.08
TriLacNAc	0.65	11.4	5.0	6.4	1.07

[a]Errors in K_a range from 1–7%; [b]errors in ΔH are 1 to 4%; [c]errors in ΔG are less than 2%; [d]errors in TΔS are 1% to 7%; [e]errors in n are less than 4%. [f]GlcNAcβ(1-3)Galβ(1-4)Glc

to lacto-N-tetraose as well as LacNAc-II (Table 2). ITC data shows a K_a value of 1.0×10^4 M^{-1} for galectin-1 binding to lacto-N-tetraose (Table 1) which is similar to that of LacNAc-II and DiLacNAc (Table 1). In addition, there are two potential binding moieties in lacto-N-tetraose, the nonreducing terminal LacNAc-I moiety and the reducing internal lactose moiety. However, the n value for the tetrasaccharide is close to one. These data support the conclusion that the CRD of bovine heart galectin-1 accommodates only one LacNAc or lactose residue in the above carbohydrates. Similar data were obtained for lacto-N-neo-tetraose by hemagglutination inhibition data (Table 2).

Table 1 shows ITC data for galectin-3 binding to Di- and TriLacNAc. The K_a values for binding LacNAc-II, Di- and TriLacNAc are 1.9×10^4 M^{-1}, 3.5×10^4 M^{-1} and 6.3×10^4 M^{-1}, respectively. The ΔH values for LacNAc-II, Di- and Tri-LacNAc are -7.5 kcal mol^{-1}, -8.8 kcal mol^{-1} and -9.8 kcal mol^{-1}, respectively. Hence, there is a factor of three increase in the affinity of galectin-3 from LacNAc-II to TriLacNAc, and a 2.3 kcal mol^{-1} increase in $-\Delta$H values. Hemagglutination inhibition results also agree with the ITC data for the relative affinities of galectin-3 for all three carbohydrates (Table 2). The n values for galectin-3 binding to Di- and TriLacNAc are

Lactose - Galβ(1-4)Glc~OH

Methyl β-Lactose - Galβ(1-4)GlcβOCH$_3$

LacNAc-I - Galβ(1-3)GlcNAc~OH

LacNAc-II - Galβ(1-4)GlcNAc~OH

Methyl β-LacNAc-I - Galβ(1-3)GlcNAcβ(1-)OCH$_3$

Methyl β-LacNAc-II - Galβ(1-4)GlcNAcβ(1-)OCH$_3$

ThiodiGal - Galβ(1-1)βGal

DiLacNAc - Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAc~OH

TriLacNAc - Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAc~OH

Lacto-N-tetraose - Galβ(1-3)GlcNAcβ(1-3)Galβ(1-4)Glc~OH

Lacto-N-neo-tetraose - Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)Glc~OH

2,3-Sialyl LacNAc - Neu5Acα(2-3)Galβ(1-4)GlcNAc~OH

2,6-Sialyl LacNAc - Neu5Acα(2-6)Galβ(1-4)GlcNAc~OH

2,6-Sialyl DiLacNAc - Neu5Acα(2-6)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAc~OH

2,6-Sialyl Lacto-N-neo-tetraose - Neu5Acα(2-6)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)Glc~OH

"Trisaccharide" - GlcNAcβ(1-3)Galβ(1-4)Glc~OH

Figure 1. Structures of carbohydrates.

also close to one (Table 1), as for LacNAc-II, suggesting that all three oligosaccharides bind to the same single site in the protein. The X-ray crystal structure of the CRD domain of human galectin-3 shows one lactose molecule bound per monomer of the protein [14]. The *n* values of one for Di- and TriLacNAc therefore indicate that these oligomers are monovalent in binding to galectin-3, even though they possess two and three LacNAc residues, respectively.

Table 1 shows that galectin-3 binds lacto-n-tetraose with a K_a value in between that of LacNAc-II and DiLacNAc. The ΔH value for lacto-*n*-tetraose binding to galectin-3 is much lower (-5.0 kcal mol^{-1}) relative to that of LacNAc-II (-7.5 kcal mol^{-1}) and Di- and TriLacNAc. The *n* value for galectin-3 binding lacto-*n*-tetraose is one which indicates that the tetrasaccharide binds to one site in the protein, as observed for the other carbohydrates in Table 1. Similar data were obtained for lacto-N-neo-tetraose by hemagglutination inhibition data (Table 2).

Binding of sialylated LacNAc analogs

Galectin-1 from a number of sources is known to bind 2,3-sialyl lactose as well as lactose [16,30,37]. Hemagglutination inhibition data in Table 2 shows that galectin-1 from bovine heart binds to 2,3-sialyl LacNAc as well as to LacNAc-II. Table 1 shows that the K_a value at 287 K for galectin-1 binding

Table 2. Relative inhibitory potencies of different sugars for bovine heart galectin-1 and recombinant murine galectin-3 mediated hemagglutination of rabbit erythrocytes at 21°C [29]

Saccharide	*Galectin-1*[a,b]		*Galectin-3*[a,b]	
Lactose	0.2	(2.2)	0.2	(2.2)
LacNAc-I	0.8	(0.51)	0.8	(0.51)
LacNAc-II	1.0	(0.41)	1.0	(0.41)
DiLacNAc	0.7	(0.58)	1.4	(0.29)
TriLacNAc	1.2	(0.35)	2.3	(0.17)
Lacto-N-tetraose	1.1	(0.37)	0.6	(0.70)
Lacto-N-neo-tetraose	1.2	(0.35)	0.6	(0.70)
2,3-Sialyl LacNAc	0.6	(0.70)	0.6	(0.70)
2,6-Sialyl LacNAc	NI[d]	(4.8)	NI[d]	(4.8)
2,6 Sialyl DiLacNAc	0.1	(3.9)	1.2	(0.34)
2,6-Sialyl- lacto-N-neo-tetraose	0.1	(3.9)	1.6	(0.25)
"Trisaccharide"[c]	NI[d]	(3.1)	0.4	(1.0)

[a]With respect to LacNAc-II; [b]concentration (mM) of sugar required for inhibition is shown in parenthesis; [c]GlcNAcβ(1-3)Galβ(1-4)Glc~OH; [d]NI = not inhibitory.

to 2,3-sialyl LacNAc (2.3×10^4 M^{-1}) is nearly the same as that for LacNAc-II (2.4×10^4 M^{-1}). Table 1 also shows that the $-\Delta H$ values for galectin-1 binding to the two oligosaccharides (-8.5 and -8.7 kcal mol^{-1}, respectively) are also nearly the same. Galectin-1 from a number of sources is known not to bind to 2,6-sialyl lactose [16,30]. Hemagglutination inhibition data in Table 2 confirm that bovine heart galectin-1 does not bind to 2,6-sialyl LacNAc.

Galectin-3 from rat lung has been shown to bind 3-fold more weakly to 2,3-sialyl lactose than to lactose [30]. Human galectin-3 exhibits nearly the same relative affinities for the two oligosaccharides in the inhibition assay [16]. Table 2 presents hemagglutination inhibition data indicating that murine galectin-3 binds 2,3-sialyl LacNAc as well as LacNAc-II. Lack of precipitation of a neogylcoprotein with this sugar epitope [17] is thus not due to insufficient binding. ITC data in Table 1 indicate that the K_a value of 3.3×10^4 M^{-1} for 2,3-sialyl LacNAc at 287 K compared to LacNAc-II (3.7×10^4 M^{-1}) at that temperature for galectin-3. The ΔH values are -8.3 kcal mol^{-1} and -9.7 kcal mol^{-1}, respectively, for 2,3-sialyl LacNAc and LacNAc-II underscoring that substitution of the non-reducing end Gal unit is tolerated.

Hemagglutination inhibition data in Table 2 shows that murine galectin-3 does not bind to 2,6-sialyl LacNAc, as has been previously demonstrated for this protein from human [16] and rat lung [30].

Since neither galectin-1 nor galectin-3 binds 2,6-sialyl LacNAc, 2,6-sialyl DiLacNAc was synthesized to determine if either galectin could bind to the internal (reducing end) LacNAc residue. While hemagglutination inhibition data in Table 2 show that galectin-1 binds 12-fold more weakly to 2,6-sialyl DiLacNAc than to LacNAc-II, galectin-3 binds 2,6-sialyl DiLacNAc as well as LacNAc-II. ITC data in Table 1 confirm these observations by showing that galectin-3 possesses nearly the same K_a value for 2,6-sialyl DiLacNAc (3.2×10^4 M^{-1}) as for LacNAc-II (1.9×10^4 M^{-1}). These results indicate binding of galectin-3 but not galectin-1 to the reducing LacNAc residue of 2,6-sialyl DiLacNAc.

Similar results for galectin-3 binding to 2,6-sialyl lacto-N-neo-tetraose were obtained. 2,6-Sialyl lacto-N-neo-tetraose differs from 2,6-sialyl DiLacNAc in having a type I LacNAc moiety at the nonreducing end of the molecule and a lactose moiety at the reducing end of the molecule. Hemagglutination inhibition data in Table 2 shows that galectin-3 binds 2,6-sialyl lacto-N-neo-tetraose nearly as well as LacNAc-II, and 16-fold better than galectin-1. ITC data obtained at 287 K shows that the K_a value for 2,6-sialyl lacto-N-neo-tetraose of galectin-3 is 2-fold less than for LacNAc-II. These results demonstrate that galectin-3 recognizes the internal lactose moiety in 2,6-sialyl lacto-N-neo-tetraose.

The trisaccharide GlcNAcβ(1-3)Galβ(1-4)Glc which is present in 2,6-sialyl lacto-N-neo-tetraose and Lacto-N-tetraose was synthesized to further test the ability of galectin-3 but not galectin-1 to bind to "internal" lactose residues in oligosaccharides. Table 2 shows hemagglutination inhibition data that indicates that galectin-1 does not bind to the trisaccharide. However, Table 2 demonstrates that galectin-3 binds to the trisaccharide as nearly well as to lactose. ITC results at 287 K in Table 1 show that galectin-3 possesses a K_a value ~4-fold less than LacNAc, and only ~2-fold less than 2,6-sialyl-lacto-N-neo-tetraose which has a reducing end lactose moiety. The ΔH value for galectin-3 binding to the trisaccharide is -7.7 kcal mol^{-1} *versus* -6.4 kcal mol^{-1} for 2,6-sialyl-lacto-N-neotetraose. These findings support the conclusion that galectin-3 but not galectin-1 can bind well to internal LacNAc or lactose residues in the above oligosaccharides.

The X-ray crystal structure of the CRD domain of human galectin-3 [14] shows differences in the amino acids near the binding site of the lectin with respect to galectin-1. Specifically, Ser29 in galectin-1 is replaced by Arg144 in galectin-3, and Leu31 in galectin-1 is replaced by Ala146 in galectin-3 and further modelling with the CRD of hamster galectin-3 implicated Arg 139, Glu 230 and Ser 232 as relevant for a putative extended binding site [38]. These differences may account for the differences in specificities between galectin-3 and galectin-1.

Thermodynamics of binding of galectin-7 to carbohydrates

ITC data in Table 1 at 300 K shows that galectin-7 possesses a K_a of 0.17×10^4 M^{-1} for LacNAc-II which is nearly 6-fold lower than that for galectin-1 (1.1×10^4 M^{-1}) and nearly 11-fold weaker than that of galectin-3 (1.9×10^4 M^{-1}). The ΔH value for galectin-7 binding LacNAc-II (-11.8 kcal mol^{-1}) is greater than that of galectin-1 (-9.2 kcal mol^{-1}) and galectin-3 (-7.5 kcal mol^{-1}). Conversely, $T\Delta S$ of galectin-3 binding LacNAc-II is more unfavorable (-7.4 kcal mol^{-1}) than that of galectin-1 (-3.7 kcal mol^{-1}) and galectin-3 (-1.6 kcal mol^{-1}). Hence, galectin-7 has a lower K_a for LacNAc-II than galectin-1 and galectin-3 because of its unfavorable entropy of binding. As a consequence of the weaker affinity of galectin-7, most of the ITC data for the lectin were obtained at 287 K and compared to data for galectin-1 and galectin-3 at the same temperature.

Galectin-1 from human [16], rat lung [30], bovine spleen [39] and the homologue of avain liver [34] all show 4–5 fold increases in affinity for LacNAc-II relative to lactose. Similarly, galectin-3 from rat and human lung [16,30] both bind LacNAc with higher affinity than lactose. Bovine heart galectin-1 in the present study also binds LacNAc-II with ~4-fold higher affinity than lactose (Table 1), as does murine galectin-3 (Table 2). ITC data in Table 1 shows that galectin-7 at 300 K binds lactose with nearly the same K_a (0.22×10^4 M^{-1}) as LacNAc-II (0.17×10^4 M^{-1}). The same relative results are obtained at 287 K (Table 1). Table 1 shows that at 287 K bovine heart galectin-1 has a K_a for lactose of 0.61×10^4 M^{-1} which is similar to the K_a of galectin-7 (0.49×10^4 M^{-1}) for lactose. Thus, galectin-7 binds lactose with essentially the same affinity as galectin-1. These results indicate a difference in specificity of galectin-7 and galectin-1

and -3 for lactose and LacNAc. Similar data were obtained by hemagglutination inhibition measurements (Table 2).

ITC data at 287 K (Table 1) show that there is little difference in the specificity of galectin-7 binding to ThiodiGal as compared to lactose and LacNAc-II, and that their $-\Delta H$ values are similar for all three disaccharides. These results are similar to those for galectin-1 and galectin-3 in Table 1.

Table 1 shows the binding thermodynamics of Di- and TriLacNAc to galectin-7 at 287 K. Little difference exists in the K_a values of LacNAc-II (0.64×10^4 M^{-1}), Di- (0.74×10^4 M^{-1}) and TriLacNAc (0.65×10^4 M^{-1}). The same is true for the ΔH values of the carbohydrates. Hemagglutination inhibition data also agree these findings (Table 3). The value of n for all three carbohydrates is essentially one, indicating that they are likely to bind to the same site in galectin-7, and that all three oligosaccharides are univalent. These results indicate that galectin-7 recognizes one predominant LacNAc moiety in binding to LacNAc oligomers, as observed for galectin-1 and galectin-3. The X-ray crystal structure of human galectin-7 shows one LacNAc-II molecule bound in the B monomer of the protein [13], in agreement with these findings (access to the A monomer is blocked in soaking experiments by crystal packing contacts).

Hemagglutination inhibition data in Table 3 demonstrates that galectin-7 binds to 2,3-sialyl LacNAc but not to 2,6-sialyl LacNAc. These findings are similar to those for galectin-1 and galectin-3. The hemagglutination inhibition data shows that galectin-7 binds to 2,6-sialyl DiLacNAc nearly as well as LacNAc-II. These results indicate that galectin-7 recognizes the internal (reducing end) LacNAc moiety of 2,6-sialyl DiLacNAc, as does galectin-3. Data in Table 3 also reveals that galectin-7 binds to 2,6-sialyl lacto-N-neo-tetraose slightly better than LacNAc-II. Binding of galectin-7 to lacto-N-tetraose and lacto-neo-N-tetraose are also similar to that of LacNAc-II (Table 3). Galectin-7 also recognizes the trisaccharide moiety, GlcNAcβ(1-3)Galβ(1-4)Glc$\sim$OH, which is present in lacto-N-tetraose and lacto-neo-N-tetraose. These results indicate that galectin-7 like galectin-3, but not galectin-1, can recognize internal LacNAc or lactose residues in the above carbohydrates. The X-ray crystal structure of human galectin-7 shows conservation of the carbohydrate binding residues of galectin-1, however, there are significant changes in the binding pocket due to shortening of a loop structure in the region [13]. These differences may relate to the observed differences in specificity of galectin-7 relative to galectin-1 and -3.

Table 3 also provides relative binding data for a variety of mono- and oligosaccharides to galectin-7. These data indicate that galectin-7 is specific for lactose and LacNAc determinants.

Table 3. Relative inhibitory potencies of different sugars for recombinant human galectin-7 mediated hemagglutination of rabbit erythrocytes at 21°C [29]

Saccharides	*Relative inhibitory potency*[a,b]	
Galactose	0.02	(66)
Methyl β-Galactopyranoside	0.02	(86)
Lactose	0.9	(1.8)
Methyl β-Lactose	0.8	(2.0)
LacNAc-I	1.6	(1.0)
Methyl β-LacNAc-I	2.3	(0.7)
LacNAc-II	1.0	(1.6)
Methyl β-LacNAc-II	0.5	(3.3)
ThiodiGal	0.5	(3.5)
Trisaccharide[c]	0.7	(2.3)
DiLacNAc	0.7	(2.3)
TriLacNAc	1.4	(1.2)
Lacto-N-tetraose	1.2	(1.4)
Lacto-N-neo-tetraose	1.5	(1.1)
2,3-Sialyl LacNAc	0.3	(5.0)
2,6-Sialyl LacNAc	NI[d]	(7.2)
2,6-Sialyl Lacto-N-neo-tetraose	2.1	(0.8)
2,6-Sialyl DiLacNAc	1.2	(1.4)

[a]With respect to LacNAc-II; [b]concentration (mM) of sugar required for inhibition is shown in parenthesis; [c]GlcNAcβ(1-3)Galβ(1-4)Glc$\sim$OH; [d]NI = not inhibitory.

Summary

ITC and hemagglutination inhibition data demonstrate that galectin-7 possesses 6- to 11-fold weaker affinities for carbohydrates with LacNAc epitopes as compared to galectin-1 and galectin-3. Thus, galectin-3 differs in its carbohydrate binding specificity, in this regard, from galectin-1 and -3. The results also show that galectin-1 binds primarily to the nonreducing terminal LacNAc residues of oligosaccharides, while galectin-3 and galectin-7 bind to nonreducing terminal LacNAc residues as well as internal LacNAc (and lactose) residues of oligosaccharides. These differences in the specificities of the three galectins may relate, in part, to differences in their biological activities [40].

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