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Binding of synthetic sulfated ligands by human splenic galectin 1, a β-galactoside-binding lectin

Howard J. Allen*, Hafiz Ahmed and Khushi L. Matta

Department of Gynecologic Oncology, Roswell Park Cancer Institute, Buffalo, New York 14263, USA

The carbohydrate-binding site of galectin 1, a vertebrate β-galactoside-binding lectin, has a pronounced specificity for the βGal(1 → 3)- and βGal(1 → 4)GlcNAc sequences. The binding inhibition study reported herein was carried out to determine whether sulfation of saccharides would influence their binding by galectin 1. The presence of 6′-OSO_3- on LacNAc greatly reduces the inhibitory potency relative to LacNAc. 3′-OSO_3-LacNAc, 3′-OSO_3-Galβ(1 → 3)GlcNAcβ1-OBzl and 3-OSO_3-Galβ1-OMe are more potent inhibitors than the non-sulfated parent compounds. Surprisingly, 2′-OSO_3-LacNAc showed over 40 fold less inhibitory potency relative to LacNAc. Ovarian carcinoma A121 cells were shown to synthesize sulfated macromolecules that bind to galectin 1. Modulation *in vivo* of saccharide sulfation may lead to modulation of galectin 1 interaction with glycoconjugates; hence, sulfation could play a role in modulating lectin functions.

***Keywords*: galectin; lectin; sulfated saccharides; binding specificity; β-galactoside-binding**

***Abbreviations*: BSA, bovine serum albumin; Gal, galactose; Glc, glucose; Fuc, fucose; GalNAc, *N*-acetylgalactosamine; Lac, lactose (Galβ1 → 4Glc); LacNAc, *N*-acetyllactosamine (Galβ1 → 4GlcNAc); GlcNAc, *N*-acetylglucosamine; NeuAc, *N*-acetylneuraminic acid; Bzl, benzyl. All saccharides studied, except L-fucose, are in the D configuration and in the pyranose form.**

Introduction

The galectins are a group of endogenous animal lectins generally characterized as being soluble, thiol-dependent, cation-independent and inhibitable by lactose [1]. These lectins have generally been referred to as S-LAC lectins [2] and are somewhat similar to the group of lectins previously termed galaptins by Harrison and Chesterton [3]. The galectins have recently been reviewed by Kasai and Hirabayashi [4]. One of these lectins, which is now termed galectin 1, is usually isolated as a native ∼30 kDa dimer composed of identical ∼14.5 kDa subunits [5]. The occurrence of this lectin in mammalian cells and tissues is quite ubiquitous [6, 7]. It occurs not only in a wide variety of cell types but also in the extracellular matrix [8]. Glycoconjugates that bind galectin 1 in a lactose-inhibitable manner are present on lymphocytes [9], on carcinoma and mesothelial cells [10], and in extracellular matrix [10]. The function of galectin 1 remains unknown [4].

The carbohydrate-binding specificity of galectin 1 isolated from human [11–13], rat [14], and bovine [15–18] sources has been characterized. These studies show that galectin 1 has a pronounced specificity for the Galβ(1 → 3)- and Galpβ(1 → 4)GlcNAc sequences with no apparent affinity for GalNAc residues. Except for hydrophobic aglycones, residues at the reducing end of Gal-GlcNAc sequences have little affect on inhibitory potency of oligosaccharides for galectin 1. Multivalency of ligands also do not significantly increase the inhibitory potency of saccharides [12]. These data suggest that galectin 1 has a binding site that accommodates primarily the Galβ(1 → 3) GlcNAc and Galβ(1 → 4)GlcNAc residues.

Molecular modeling analyses based on binding-site specificity data lead to the prediction that the galectin 1 carbohydrate binding-site interacts primarily with one surface of the Galβ(1 → 4)GlcNAc or Galβ(1 → 3)GlcNAc sequence [11–13]. In agreement with this prediction, substitutions at 3′-O with αNeuAc [11, 13, 14, 16], βGlcNAc [13,14], αGal [13, 16], or αGalNAc [11, 14] reduce only slightly (⩽ 4 fold) the inhibitory potency of ligands relative to the parent compound. Substitution at 2′-O with αFuc also yields compounds with good galaptin-binding activity [11, 13, 14, 16].

Glycoconjugates uniquely correlated with galectin 1 function have not been isolated; however, glycoconjugates that do bind to galectin 1 in a carbohydrate-dependent manner

* To whom correspondence should be addressed. Tel: 716-845-5725; Fax: 716-845-3458; E-mail: hallen@hsl.buffalo.edu

have been identified. These glycoconjugates include tissue fibronectin [19], laminin [19–21], carcinoembryonic antigen [22], lamp-1 [22, 23], and lamp-2 [22]. Although galectin 1 binds these glycoconjugates and modulates cell adhesion *in vitro* to some of them [19, 24], binding affinities are relatively low with affinity constants being in the order of 10^6 M [21]. The binding affinity of galectin 1 to cell surface glycoconjugates is also in the order of 10^6 M [9].

It is possible that higher order binding affinities between galectin 1 and lactosamine-containing glycoconjugates may depend on the presence of unique carbohydrate-linked substituents. One substituent not previously studied for its effect on saccharide binding to galectin 1 is the sulfate residue. Sulfation of carbohydrates may impart unique biological properties to a variety of glycoconjugates [25]. For example, sulfate has been reported to be present in human lysosomal enzymes [26], in thyroglobulin [27], in pancreatic carcinoma cell surface glycoproteins [28], in pituitary glycoprotein hormones [29], as well as in glycosaminoglycans [30] and sulfatides [31]. Sulfate is also a component of GlyCAM-1 [32] and appears to be important for the binding of saccharides by L-selectin [33].

The study reported herein was carried out to determine whether sulfation of saccharides would influence their binding by galectin 1.

Materials and methods

Galectin 1 was isolated from human spleen by affinity chromatography on lactose-Sepharose followed by DEAE Sephacel chromatography [34]. Galectin 1 was alkylated with iodoacetamide to eliminate the thiol requirement for retention of carbohydrate-binding activity [10].

Galectin-binding inhibition assays were carried out by a previously described ELISA procedure [35]. In brief, galectin was coupled to horseradish peroxidase with 0.1% glutaraldehyde. The conjugate (12 ng) was mixed with an equal volume (60 µl) of saccharide of varying concentrations in duplicate. After incubation for 60 min at 4 °C, the conjugate-ligand mixture (100 µl) was added to ELISA plate wells containing adsorbed asialofetuin (2 µg per 100 µl) that had been post-fixed with 2% formaldehyde. The blocking agent was 0.4% BSA/0.05% Tween 20. The plates were incubated for 60 min at 4 °C, washed with Tween 20 buffer, and bound peroxidase was assayed with diammonium 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate) as described [35]. The assays were repeated two to three times.

The synthesis of the sulfated saccharides has been previously reported [36].

Endogenous sulfated macromolecules were obtained by incubating A121 ovarian carcinoma cells [37] with $[^{35}S]O_4^{2-}$ as previously described [38]. Radiolabeled macromolecules released to the culture medium were collected for affinity chromatography on polygalectin-Sepharose. Polygalectin was prepared by polymerization of dimeric galectin 1 with 0.1% glutaraldehyde [9] and it was repurified by affinity chromatography on a lactose-Sepharose column [9]. Polygalectin was coupled to CNBr-activated Sepharose 4B (3.2 mg per ml gel) according to Allen and Johnson [39].

Galectin was polymerized for affinity chromatography to minimize the possibility that steric interference from the Sepharose might prevent binding of radiolabeled glycoconjugates, as appeared to be the case for asialofetuin glycopeptide binding by galectin [15].

Galectin-binding $^{35}SO_4$-labelled glycoconjugates released to the culture medium by A121 cells were obtained by affinity chromatography on polygalectin-Sepharose. The culture medium was concentrated by ultrafiltration (membrane cut-off 10 kDa) and was dialyzed against 0.6 M NaCl, 0.05 M K_2H PO_4, 0.05% NaN_3, pH 7.3. The dialyzed medium (1.25×10^6 cpm) was applied to a polygalectin-Sepharose column (0.8×2 cm) equilibrated with the preceding buffer at 4 °C. After extensive washing with buffer, the column was eluted with 0.1 M lactose in buffer. The lactose eluate was reduced and alkylated prior to electrophoresis on 8% acrylamide gels.

SDS-PAGE and fluorography were carried out as previously described [34, 40] following buffer exchange to sample buffer via centrifugal filtration.

Results and discussion

The inhibition of galectin 1 binding to asialofetuin by synthetic ligands is shown in Figure 1. The data are summarized in Table 1. The I_{50} values for Lac (**1**) (0.33 mM) and LacNAc (**2**) (0.06 mM) are similar to those previously reported [13] (0.64 mM and 0.06 mM, respectively) for a differently formatted ELISA.

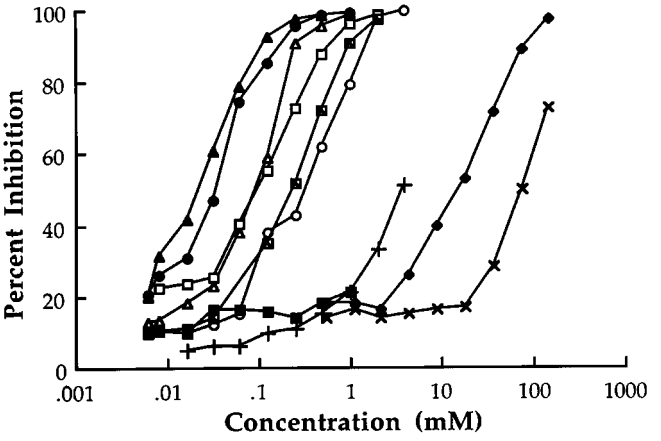

Figure 1. The inhibition of galectin-peroxidase conjugate binding to asialofetuin by saccharides. Binding inhibition assays were carried out by the ELISA described in Materials and Methods. Galβ1 → 4Glc (○), Galβ1 → 4GlcNAc (△), 3′-OSO_3Galβ1 → 4GlcNAc (▲), 6′-OSO_3Galβ1 → 4GlcNAc (■), Galβ1 → 4(6-OSO_3) GlcNAc (●), 2′-OSO_3Galβ1 → 4GlcNAc (+), Galβ1 → 3GlcNAcβ1-OBzl (⧅), 3′-OSO_3Galβ1 → 3GlcNAcβ1-OBzl (□), Galβ1-OMe (×), 3-OSO_3Galβ1 → OMe (◆).

Table 1. Inhibition of galectin-peroxidase conjugate binding to asialofetuin by saccharides

No.	*Compound*	I_{50}[a] *(mM)*	*Relative activity*[b]
1	Galβ1 → 4Glc	0.33	1
2	Galβ1 → 4GlcNAc	0.06	5.5
3	2′-OSO_3Galβ1 → 4GlcNAc	2.75	0.12
4	3′-OSO_3Galβ1 → 4GlcNAc	0.02	16.5
5	6′-OSO_3Galβ1 → 4GlcNAc	$\gg 1$[c]	$\ll 1$
6	Galβ1 → 4(6-OSO_3)GlcNAc	0.03	10.0
7	Galβ1 → 3GlcNAcβ1 → OBzl	0.19	1.7
8	3′-OSO_3Galβ1 → 3GlcNAcβ1-OBzl	0.08	4.1
9	Galβ1-OMe	53.0	0.006
10	3-OSO_3Galβ1-OMe	15.0	0.022

[a] The concentration of saccharide required to give 50% inhibition of binding. I_{50} values were obtained from plots as shown in Figure 1.
[b] The I_{50} of each compound relative to lactose I_{50}.
[c] 10% or less inhibition was obtained at 1.0 mM.

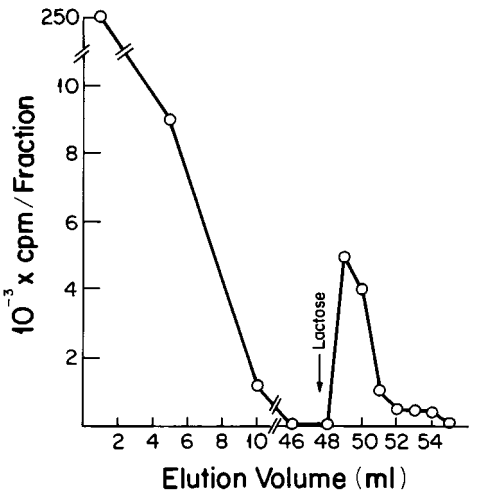

Figure 2. Polygalectin-Sepharose affinity chromatography of [^{35}S]O_4-labelled macromolecules released to culture medium by A121 ovarian carcinoma cells. The ultrafiltered and dialyzed sample was applied to a 1 ml affinity column. The column was washed and eluted with lactose as described in Materials and Methods. 1.25×10^6 cpm were loaded on the column. The wash-thru fraction contained 1.04×10^6 cpm. The lactose-eluted fraction contained 1.1×10^4 cpm. The fraction volume was 1.0 ml.

The presence of 6′-OSO_3- on LacNAc (**5**) has a pronounced affect in that it greatly reduces the inhibitory potency relative to LacNAc (**2**). This is commensurate with previous observations which show that substitution at 6′-OH with GlcNAc [13] or NeuAc [16] significantly reduces inhibitory potency. The oxidation of lactose with galactose oxidase also reduces inhibitory potency [13].

In some cases, sulfation increases the inhibitory potency relative to parent ligands. 3′-OSO_3- on LacNAc (**4**) increases the inhibitory potency three-fold. 3′-OSO_3- on Galβ(1 → 3)GlcNAcβ1-OBzl (**8**) increases the inhibitory potency two-fold relative to the parent compound (**7**), and 3-OSO_3 on Galβ1-OMe (**10**) increases the inhibitory potency over three-fold relative to Galβ1-OMe (**9**). This increase of inhibitory potency due to sulfation is of interest since similar substitution with NeuAc and GalNAc somewhat reduces inhibitory potency [11, 13, 14, 16].

Substitution at the 6-O position of LacNAc was predicted to have little affect on inhibitory potency relative to the parent compound. However, sulfation at the 6-O position (**6**) increased inhibitory potency about two-fold.

A surprising result was obtained when sulfate was placed at the 2′-O position of LacNAc (**3**). Inhibitory potency was reduced over 40-fold relative to LacNAc (**2**) whereas fucosylation at the 2′-O position only slightly reduces inhibitory potency [11, 13, 14, 16]. It was found previously that methylation of the 2′-O position slightly increased inhibitory potency [13].

It is possible that the neutral and somewhat hydrophobic character of the methyl group and fucosyl group have little impact on the conformation of LacNAc. It is also possible that hydrogen bonds form between one of the hydrogens of these constituents linked 2′ and the 6-OH hydrogen, thus stabilizing the LacNAc conformation. In contrast, the presence of the highly charged 2′-sulfate group may significantly alter the conformation of LacNAc and account for the low inhibitory activity of compound **3** relative to LacNAc.

The results obtained with the synthetic ligands indicated that some endogenous galectin-binding glycoconjugates may be sulfated. To gain preliminary evidence to suggest whether this might be the case, sulfated macromolecules released to medium by A121 ovarian carcinoma cells were chromatographed on a polygalectin-Sepharose column. A radiolabelled fraction was eluted from the column with 0.1 M lactose (Figure 2). The results of SDS-PAGE/fluorography showed that this fraction consisted of a high molecular weight glycoconjugate ($\geqslant$ 200 kDa) (Figure 3). These results are similar to those obtained with *Ricinus communis* agglutinin I [38]. These data do not identify the sulfated constituent within the galectin-bound and lactose-eluted macromolecule(s). It may be that tyrosine or some other non-carbohydrate constituent is biosynthetically sulfated. However, many O-linked oligosaccharides in mucins are sulfated [41] and it will be of interest to identify sulfation patterns of macromolecules that bind to galectin 1.

The data presented here show that sulfation of LacNAc and Galβ(1 → 3)GlcNAc, particularly of the Gal residue, can influence the interaction of those residues with galectin. Several β-galactoside-binding lectins co-exist in a variety of cells and tissues [2, 42]. It is possible that differences in binding specificities of these lectins are present relative to the influence of sulfation. If that is the case, modulation of saccharide sulfation may lead to modulation of lectin interaction with glycoconjugates; and hence, sulfation could play a role in modulating lectin functions.

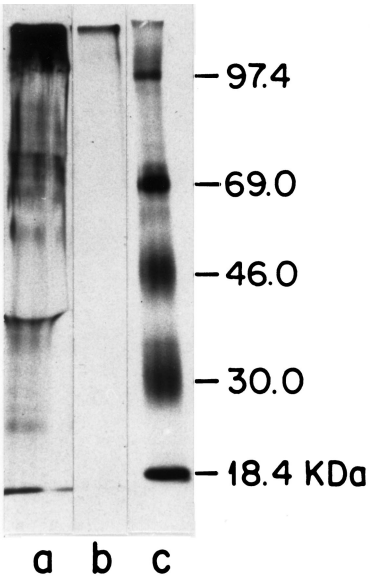

Figure 3. SDS-PAGE fluorography of [^{35}S]O_4-labelled glycoconjugates eluted from polygalectin-Sepharose with 0.1 M lactose. The polygalectin affinity column load and the lactose-eluted fraction shown in Figure 2 were analyzed as described in Materials and Methods. (a) Affinity column load; (b) lactose-eluted fraction; (c) molecular weight markers.

Since the β-galactoside-binding lectins are thought to play a role in metastasis [43], it will be of interest to compare the nature of the galectin-binding sulfated glycoconjugates for normal and malignant cells to see if these are altered as a result of transformation, as appears to be the case for colorectal neoplasia [44].

Yuen *et al.* [45] reported the presence of 3′-OSO_3-Galβ1- in an oligosaccharide that can bind E-selectin. Their data suggest that sulfate may replace the requirement for sialyl residues in E-selectin receptors. Those data support the notion that metabolic sulfation may be a regulatory mechanism for modulating lectin-receptor interaction.

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References

1 Barondes SH, Castronovo V, Cooper DW, Cummings RD, Drickamer K, Feizi T, Gitt MA, Hirabayashi J, Hughes C, Kasai K, Leffler H, Liu FT, Lotan R, Mercurio AM, Monsigny M, Pillai S, Poirier F, Raz A, Rigby PW, Rini JM, Wang JL (1994) *Cell* **76**: 597–8.
2 Leffler H, Masiarz FR, Barondes SH (1989) *Biochem* **28**: 9222–9.
3 Harrison FL, Chesterton CJ (1980) *FEBS Lett* **122**: 157–65.
4 Kasai K, Hirabayashi J (1996) *J Biochem* **119**: 1–8.
5 Allen HJ, Cywinski M, Palmberg R, DiCioccio RA (1987) *Arch Biochem Biophys* **256**: 523–33.
6 Harrison FL (1991) *J Cell Sci* **100**: 9–14.
7 Allen HJ, Karakousis C, Piver MS, Gamarra M, Nava H, Forsyth B, Matecki B, Jazayeri A, Sucato D, Kisailus E, DiCioccio R (1987) *Tumor Biol* **8**: 218–29.
8 Allen HJ, Sucato D, Gottstine S, Kisailus E, Nava H, Petrelli N, Castillo N, Wilson D (1991) *Tumor Biol* **12**: 52–60.
9 Ahmed H, Sharma A, DiCioccio RA, Allen HJ (1992) *J Molec Recog* **5**: 1–8.
10 Allen HJ, Sucato D, Woynarowska B, Gottstine S, Sharma A, Bernacki RJ (1990) *J Cell Biochem* **43**: 43–57.
11 Sparrow CP, Leffler H, Barondes SH (1987) *J Biol Chem* **262**: 7383–90.
12 Lee RT, Ichikawa Y, Allen HJ, Lee YC (1990) *J Biol Chem* **265**: 7864–71.
13 Ahmed H, Allen HJ, Sharma A, Matta KL (1990) *Biochem* **29**: 5315–19.
14 Leffler H, Barondes SH (1986) *J Biol Chem* **261**: 10119–26.
15 Merkle RK, Cummings RD (1988) *J Biol Chem* **263**: 16143–9.
16 Abbott WM, Hounsell EF, Feizi T (1988) *Biochem J* **252**: 283–7.
17 Zhou Q, Cummings RD (1990) *Arch Biochem Biophys* **281**: 27–35.
18 Solomon JC, Stoll MS, Penfold P, Abbott WM, Childs RA, Hanfland P, Feizi T (1991) *Carbohydr Res* **213**: 293–307.
19 Ozeki Y, Matsui T, Yamamoto Y, Funahashi M, Hamako J, Titani K (1995) *Glycobiol* **5**: 255–61.
20 Castronovo V, Luyten F, van den Brule F, Sobel ME (1992) *Arch Biochem Biophys* **297**: 132–8.
21 Zhou Q, Cummings RD (1990) *Arch Biochem Biophys* **281**: 27–35.
22 Ohannesian DW, Lotan D, Lotan R (1994) *Cancer Res* **54**: 5992–6000.
23 Do KY, Smith DF, Cummings RD (1990) *Biochem Biophys Res Commun* **173**: 1123–8.
24 van den Brule FA, Buicu C, Baldet M, Sobel ME, Cooper DN, Marschal P, Castronovo V (1995) *Biochem Biophys Res Commun* **209**: 760–7.
25 Hooper LV, Manzella SM, Baenziger JU (1996) *FASEB J* **10**: 1137–46.
26 Braulke T, Hille A, Hattner WB, Hasilik A, Von Figura K (1987) *Biochem Biophys Res Commun* **143**: 178–85.
27 Spiro RG, Bhoyroo VD (1988) *J Biol Chem* **263**: 14351–8.
28 Sunblad G, Kajiji S, Quaranta V, Freeze HH, Varki A (1988) *J Biol Chem* **263**: 8897–903.
29 Green ED, Baenziger JU (1988) *J Biol Chem* **263**: 36–44.
30 Giacoletto KS, Rumbarger T, Schwartz BD (1989) *Meth Cell Biol* **32**: 207–30.
31 Roberts DD, Rao CN, Liotta LA, Gralnick HR, Ginsburg V (1986) *J Biol Chem* **261**: 6872–7.
32 Hemmerich S, Butcher EC, Rosen SD (1994) *J Exp Med* **180**: 2219–26.
33 Koenig A, Jain R, Vig R, Norgard-Sumnicht KE, Matta KL, Varki A (1997) *Glycobiol* **7**: 79–93.
34 Sharma A, Chemelli R, Allen HJ (1990) *Biochem* **29**: 5309–14.
35 Ahmed H, Allen HJ, DiCioccio RA (1991) *Carbohydr Res* **213**: 321–4.

36 Chandrasekaran EV, Jain RK, Matta KL (1992) *J Biol Chem* **267**: 19929–37.
37 Crickard K, Niedbala MJ, Crickard U, Yoonessi M, Sandburg AA, Okugama K, Bernacki RJ, Satchidanand SK (1989) *Gynecol Oncol* **32**: 163–73.
38 Allen HJ, Gamarra M, Piver MS, Sharma A, Ahmed H, Johnson EAZ (1991) *Cancer Biochem Biophys* **12**: 23–31.
39 Allen HJ, Johnson EAZ (1980) *Biochim Biophys Acta* **600**: 320–31.
40 Allen HJ, Gottstine S, Sharma A, DiCioccio RA, Swank RT, Li H (1991) *Biochem* **30**: 8904–10.
41 Schachter H, Brockhausen I (1992) In *Glycoconjugates*: *Composition*, *Structure*, *and Function* (Allen HJ, Kisailus EC, eds) pp 263–332. New York: Marcel Dekker, Inc.
42 Sharma A, DiCioccio RA, Allen HJ (1992) *Glycobiol* **2**: 285–92.
43 Lotan R (1992) In *Glycoconjugates*: *Composition*, *Structure*, *and Function* (Allen HJ, Kisailus EC, eds) pp 635–71. New York: Marcel Dekker, Inc.
44 Yamachika T, Nakanishi TH, Inada K, Kitoh K, Kato T, Irimura T, Tatematsu M (1997) *Virch Archiv* **431**: 25–30.
45 Yuen CT, Lawson AM, Chai W, Larkin M, Stoll MS, Stuart AC, Sullivan FX, Ahern TJ, Feizi T (1992) *Biochem* **31**: 9126–31.

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