Human Splenic Galaptin: Carbohydrate-Binding Specificity and Characterization of the Combining Site†

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ABSTRACT: A galactose-binding lectin (galaptin) from human spleen has been purified to homogeneity by affinity chromatography on asialofetuin–Sepharose. The carbohydrate-binding specificity of galaptin has been investigated by analyzing the binding of galaptin to asialofetuin in the presence of putative inhibitors. An enzyme-linked immunosorbent assay (ELISA) was developed that involved adsorption of asialofetuin to microtiter plates. Galaptin bound to asialofetuin was detected with polyclonal rabbit anti-galaptin serum followed by goat anti-rabbit IgG–peroxidase conjugate. The concentrations of inhibitors giving 50% inhibition of galaptin binding relative to controls were graphically determined and normalized relative to galactose or lactose. These analyses revealed that galaptin has a combining site at least as large as a disaccharide. The disaccharides having non-reducing-terminal β-galactosyl residues linked (1,3), (1,4), and (1,6) to Glc or GlcNAc are better inhibitors than free Gal. GalNAc, either free or glycosidically linked, appears to have no affinity for the lectin. The nitrophenyl galactosides are better inhibitors than methyl galactosides, indicating the occurrence of hydrophobic interactions. The data indicate that OH groups at C-4 and C-6 of Gal and the OH at C-3 of GlcNAc in Galβ(1,4)GlcNAc are important for lectin sugar interaction. Our data support the hypothesis that endogenous receptors for galaptin are most likely lactosaminoglycan moieties.

A family of galactoside-binding lectins (galaptins) with subunit mass of about 14 kDa occurs in a variety of animal tissues (Simpson et al., 1978; Barondes, 1981, 1986). These lectins are related both antigenically (Childs & Feizi, 1979a; Carding et al., 1984) and structurally (Okyama et al., 1986; Gitt & Barondes, 1986; Southan et al., 1987). They require thiols but not divalent cations for their hemagglutinating activity (Barondes, 1986).

This class of lectins preferentially binds disaccharides (lactose and its derivatives) and oligosaccharides rather than monosaccharides (galactose and its glycosides) (Waard et al., 1976). Various investigations of carbohydrate recognition by calf heart lectin (Childs & Feizi, 1979b; Abbott et al., 1988; Merkle & Cummings, 1988), rat lung lectin (Leffler & Barondes, 1986), and human lung lectin (Sparrow et al., 1987) show that type 1 [Galβ(1,3)GlcNAc] and type 2 [Galβ(1,4)GlcNAc] oligosaccharide backbone sequences are potent inhibitors of their binding, although different forms of human and rat lung lectins differed significantly relative to each other in carbohydrate-binding specificity (Leffler & Barondes, 1986; Sparrow et al., 1987). Galaptins from spleen of several animals and from human spleen and leucocytes have been isolated, and their carbohydrate-binding properties were studied by using various saccharides and erythrocytes (Allen, 1986; Allen et al., 1987). Although these lectins were similar in their reactivity toward different saccharides, significant differences were noted in their reactivity toward erythrocytes.

In order to adequately understand the putative functions of galaptins, it is necessary to thoroughly characterize their carbohydrate-binding requirements and specificities. The binding activity of galaptins has been assayed most frequently either by agglutination of trypsinized rabbit erythrocytes or by binding of radiolabeled galaptin to the same erythrocytes or to some form of immobilized desialylated fetuin. We describe here a new ELISA utilizing desialylated fetuin adsorbed to the wells of microtiter ELISA plates. In this assay, galaptin was preincubated with a variety of synthetic and commerically available saccharides, followed by binding to desialylated fetuin and immunodetection of the bound galaptin. The binding specificity of human splenic galaptin was studied and the results are presented here.

Materials and Methods

Materials

BSA,[1] DTT, thimerosal, fetuin, galactose and its simple glycosides [Me, S-*i*-Pr, phenyl, *o*- and *p*-nitrophenyl], GlcN, GalNAc, Fuc, Lac, *p*-aminophenyl lactoside (**27**), LacNAc (**32**), sialyllactose from bovine milk (**28**), melibiose (**23**), thiodigalactoside (**41**), raffinose (**24**), stachyose (**25**), and GlcNAc were obtained from Sigma Chemical Co., St. Louis, MO. 2-Acetamido-1-(L-β-aspartamido)-1,2-dideoxy-β-D-glucose was obtained from Vega Inc., Tucson, AZ. Goat anti-rabbit IgG–peroxidase conjugate and ABTS peroxidase substrate reagent were obtained from Kirkegaard & Perry Laboratories, Gaithersburg, MD. Other saccharides were synthetic glycosides for which the syntheses have been described in numerous publications [e.g., Matta et al. (1981), Abbas et al. (1982, 1987), Kohata et al. (1984), and Thomas et al. (1989)]. $GlcNAc_n$ was prepared as before (Allen et al., 1980). *N*-1-(1-Deoxylactitol)–BSA (mole ratio 7:1) was prepared by reductive amination (Schwartz & Gray, 1977). *p*-Aminophenyl-Lac–BSA (mole ratio 8:1) was prepared by

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[1] Abbreviations: BSA, bovine serum albumin; DTT, dithiothreitol; ME, mercaptoethanol; PBS, 0.05 M Na_2HPO_4/0.10 M NaCl; ABTS, 2,2′-azinobis(3-ethylbenzothiazolinesulfonate); Gal, galactose; Glc, glucose; Fuc, L-fucose; Fru_f, fructofuranoside; GalNAc, *N*-acetylgalactosamine; AllNAc, *N*-acetylallosamine (differs from GlcNAc by epimerization at 3-OH); Lac, lactose; LacNAc, *N*-acetyllactosamine; GlcN, glucosamine; GlcNAc, *N*-acetylglucosamine; $NO_2(o)$, *o*-nitro; $NO_2(p)$, *p*-nitro; UDP, uridine diphosphate. All sugars except Fuc are in the D configuration; all except Fru_f are in the pyranose form.

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diazotization (McBroom et al., 1973). Asialofetuin was prepared by hydrolysis of fetuin in 0.1 N H_2SO_4 for 70 min at 80 °C. The hydrolysate was dialyzed and lyophilized prior to use.

Methods

Preparation of Galaptin. Galaptin was purified from human spleen as described [Sharma et al., 1990 (preceding paper in this issue)]. The purified galaptin was equilibrated with 10 mM Tris/25 mM NaCl/10 mM mercaptoethanol buffer, pH 7.3, and was adsorbed in 1-mg aliquots to DEAE-Sephacel (Pharmacia) in the presence of 0.1 M lactose/50% glycerol. Galaptin was then stored at −20 °C until used. Galaptin was recovered from DEAE-Sephacel by washing with the preceding Tris buffer followed by elution with 10 mM Tris/200 mM NaCl/10 mM mercaptoethanol buffer, pH 7.3. Hemagglutinating activity was assayed as before (Allen et al., 1987). Protein was assayed with a commercial Coomassie blue dye binding reagent. Rabbit anti-galaptin serum was as described in the preceding paper.

Inhibition Assay by ELISA. Immulon I micro ELISA plates (Dynatech Laboratories, Chantilly, VA) were coated with asialofetuin in 100 mM Na_2CO_3/0.02% NaN_3 buffer, pH 9.6 (2.0 μg/100-μL well), at 37 °C for 3 h. The plates could then be stored at 4 °C for several days prior to further processing. After complete aspiration of the asialofetuin solution, the wells were fixed with 2% formalin in PBS (150 μL) for 45 min at RT. The wells were washed three times with buffer A (10 mM Na_2HPO_4/120 mM NaCl/0.01% thimerosal/0.05% Tween 20, pH 7.3) and then blocked with 0.4% BSA in buffer A (200 μL) for 30 min at 37 °C. The blocking solution was aspirated and the wells were washed three times with buffer B (buffer A containing 1.0 mM DTT). For inhibition studies, 18 μg of galaptin in 60 μL of buffer B was mixed with an equal volume of inhibitor in buffer B and the mix was preincubated for 1 h at 37 °C. A 100-μL aliquot of the mix was then added to the wells of the blocked ELISA plates containing adsorbed asialofetuin. The plates were then incubated for 1 h at 37 °C. After being washed with buffer B, the wells were fixed again with 2% formalin to prevent loss of bound galaptin. The wells were then washed with buffer A. Rabbit anti-galaptin serum (100 μL of a 1:5000 dilution in 5% BSA/buffer A per well) was added to the wells and the plates were incubated for 1 h at 37 °C. The wells were then washed with buffer A and incubated with 100 μL of goat anti-rabbit IgG–peroxidase conjugate (2.0 μg/mL in 1% BSA/buffer A). Following incubation for 1 h at 37 °C, the wells were washed with buffer A. Substrate, 100 μL, was added and the plates were incubated for 15 min at 37 °C. The reaction was stopped by addition of either 50 μL of 20% SDS or 100 μL of 200 mM citric acid. The plates were read at 405 nm on a plate reader. For all assays, the outermost wells of the plates were not utilized.

To assay concentration-dependent inhibition, inhibitors were 2-fold serially diluted prior to being mixed with galaptin. Concentrations of inhibitors giving 50% inhibition of galaptin binding, I_{50} (relative to galaptin controls), were graphically determined. For daily assays, a complete Lac inhibition curve was always obtained and all I_{50} values were normalized relative to the corresponding Lac I_{50} value.

The pH optimum for galaptin binding to plastic-adsorbed asialofetuin was determined. Galaptin, 18 μg in 60 μL of buffer B, was mixed with 60 μL of various buffers (100 mM), and 100 μL of this mix was used in the galaptin-binding assay as described above. The buffers used were citrate–phosphate, pH 4.5–6.0; phosphate, 6.5–8.0; and carbonate–phosphate, pH 8.5–9.5. All of these buffers contained 1.0 mM DTT and 100 mM NaCl.

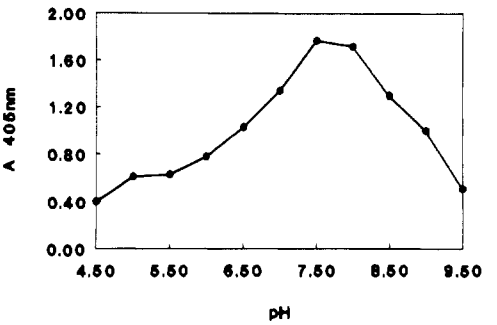

FIGURE 1: pH-dependent binding of galaptin to asialofetuin. Binding was determined by ELISA as described under Methods.

Results and Discussion

Human splenic galaptin was isolated as a 30-kDa dimer composed of two identical 14.5-kDa subunits [Allen et al., 1987; Sharma et al., 1990 (preceding paper in this issue)]. Freshly isolated galaptin had a hemagglutinating specific activity (titer mg^{-1} mL^{-1}) of 6×10^3. Hemagglutinating activity and asialofetuin binding activity were stable for several weeks under the conditions of storage described here. However, isolated galaptin in solution appeared to have a propensity to adsorb to glass and plastic, leading to significant losses in protein and lectin activity. This loss could be prevented by using BSA as a cosolute.

The ELISA described here was arrived at after several preliminary experiments to optimize conditions. The inclusion of formalin fixation procedures was necessary to obtain consistent results. Although galaptin was isolated by affinity chromatography on asialofetuin–Sepharose, plastic-adsorbed asialofetuin was an inefficient ligand for binding galaptin. Galaptin input was 15 μg/well for ELISA; however, only 5 ng of galaptin was bound under optimal conditions. Anomalous behavior of asialofetuin–galaptin interaction was also noted by Merkle and Cummings (1988). Interestingly, replacement of asialofetuin by Lacβ–$OC_6H_4NH_2(p)$–BSA did not lead to improved binding of galaptin.

The binding activity of galaptin as a function of pH is shown in Figure 1. Optimal activity occurred at pH 7.5 with a sharp decline below pH 7.5 and above pH 8.0.

The carbohydrate-binding specificity of galaptin was determined by analyzing the binding of the lectin to asialofetuin in the presence of a series of putative inhibitors. Representative inhibition curves are shown in Figure 2. The data are summarized in Tables I–III.

The data revealed that galaptin has a combining site at least as large as a disaccharide. The disaccharides having nonreducing-terminal β-galactosyl residues linked (1,3), (1,4), and (1,6) to Glc or GlcNAc are better inhibitors than free Gal. From a previous study (Allen et al., 1987), it was known that the hemagglutinating activity of splenic galaptin could be inhibited by Gal and its derivatives. Glc, GlcNAc, Manα–OMe, and L-Fuc, all of which have equatorial OH groups at C-4, were inactive, indicating the importance of 4-OH in binding. This is true for other mammalian thiol-dependent Gal-binding lectins (Leffler & Barondes, 1986; Sparrow et al., 1987). The α-galactosides tested (**2**, **6**, and **7**) had considerably higher inhibitory activity compared to their β anomers (**3**, **8**, and **9**). This fact was also noted for galaptins from other sources (Allen, 1986; Leffler & Barondes, 1986; Sparrow et al., 1987). However, when the non-reducing-terminal Gal is glycosidically linked to another saccharide

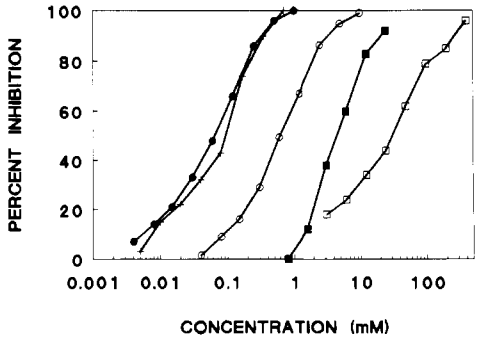

FIGURE 2: Inhibition of galaptin binding to asialofetuin by various saccharides. Binding was determined by ELISA as described under Methods. Gal, (□); Galα–$OC_6H_4NO_2(p)$ (**7**), (■); Lac (**26**), (○); LacNAc (**32**), (●); Galβ(1,3)GlcNAcβ(1,3)Galβ(1,4)Glc (**38**), (+).

Table I: Inhibition of Galaptin Binding to Asialofetuin by a Series of Saccharides[a]

no.	compound	rel act.[b,c]
1	Gal	1
2	Galα–OMe	0.8
3	Galβ–OMe	0.5
4	Galβ–S-*i*-Pr	2.5
5	Galβ–OC_6H_5	0.74
6	Galα–$OC_6H_4NO_2(o)$	4.9
7	Galα–$OC_6H_4NO_2(p)$	7.3
8	Galβ–$OC_6H_4NO_2(o)$	1.25
9	Galβ–$OC_6H_4NO_2(p)$	1.38
10	Galβ–$SC_6H_4NO_2(p)$	1.78
11	1-azido-βGal	30% inhibn at 15 mM
12	UDP–Gal	NI <40 mM
13	UDP	NI <50 mM
14	GalNAc	0.26
15	GalNAcα–OMe	24% inhibn at 15 mM
16	GalNAcα–OC_6H_5	5% inhibn at 7.5 mM
17	GalNAcα–$OC_6H_4NO_2(o)$	8% inhibn at 7.5 mM
18	GalNAcβ–OBzl	13% inhibn at 15 mM
19	MeO–2Galβ–OMe	0.6
20	Fucα(1,2)Galβ–$OC_6H_4NO_2(o)$	0.35
21	GlcN	NI at <100 mM
22	Fuc	NI at <100 mM
23	Galα(1,6)Glc	0.51
24	Galα(1,6)Glcβ(1,2)Fru_f	0.35
25	Galα(1,6)Galα(1,6)Glcβ(1,2)Fru_f	1.3
26	Galβ(1,4)Glc	50.0

[a] Inhibition is expressed as I_{50} of each compound relative to galactose I_{50} (35 mM). [b] NI, no inhibition. [c] GlcNAc, $GlcNAc_n$ when n = 2, 4, 7, or 8, and GlcNAc-*N*-Asp gave no inhibition at 50, 15, and 20 mM, respectively.

rather than a hydrophobic residue, galaptin has a pronounced binding preference for the β anomers (compare **42** and **43**). The increased inhibitory activity of *o*- and *p*-nitrophenyl galactosides (**6**–**9**) over their corresponding methyl galactosides (**2** and **3**) suggests that hydrophobic interaction between the aglycon and galaptin may also occur. The inhibitory activities of **27**, **31**, and **35** compared to those of **26**, **30**, and **34** are also increased, apparently due to hydrophobic interaction. The isopropyl group (in **4**) appeared to contribute to binding affinity, whereas the thioglycosides showed a slight enhancement (compare **9** with **10**). The nitro group on the aromatic ring may also play a role in enhancing binding affinity of the galactosides (compare **5** with **6**–**10**). UDP–Gal was a very poor inhibitor relative to the other α-galactosides analyzed. Apparently the charged phosphate groups counteract or prevent a hydrophobic interaction between the galaptin binding site and uracil.

Melibiose (**23**) and raffinose (**24**) gave slightly less inhibition than Galα–OMe, while stachyose (**25**) inhibited the lectin 4 times as much as raffinose and 2.5 times as much as melibiose. This is not unusual because raffinose was found to be coiled into segments by hydrogen-bonded helices with one turn per molecule (Berman, 1970), thus preventing access of Gal of raffinose to the lectin binding site. With stachyose, the terminal Gal linked α(1,6) is not expected to be part of a helical segment and would be expected to show better inhibitor activity than raffinose, as was found.

Table II: Inhibition of Galaptin Binding to Asialofetuin by a Series of Saccharides[a]

no.	compound	rel act.[b]
26	Galβ(1,4)Glc	1
27	Galβ(1,4)Glcβ–$OC_6H_4NH_2(p)$	2.5
28	NeuAcα(2,3)Galβ(1,4)Glc	0.8
29	GlcNAcβ(1,3)Galβ(1,4)Glc	0.25
30	GlcNAcβ(1,6)Galβ(1,4)Glc	NI <15 mM
31	GlcNAcβ(1,6)Galβ(1,4)-Glcβ$OC_6H_4NO_2(p)$	40% inhibn at 15 mM
32	Galβ(1,4)GlcNAc	10.2
33	MeO–2Galβ(1,4)GlcNAc	15.6
34	Galβ(1,3)GlcNAcα–OMe	2.7
35	Galβ(1,3)GlcNAcβ–$OC_6H_4NO_2(p)$	4.3
36	Fucα(1,2)Galβ(1,3)GlcNAcβ–$OC_6H_4NO_2(p)$	4.3
37	Galβ(1,3)GlcNAcβ(1,3)Galβ–OMe	14% inhibn at 15 mM
38	Galβ(1,3)GlcNAcβ(1,3)Galβ(1,4)Glc	5.6
39	Galβ(1,3)GlcNAcβ(1,3)Galβ(1,3)-GalNAcα–OBzl	5.0
40	Galβ(1,6)GlcNAcβ–$OC_6H_4NO_2(p)$	0.23
41	Galβ1–S–1βGal	3.2
42	Galα(1,3)Galβ–OMe	0.11
43	Galβ(1,3)Galβ–OMe	0.58
44	Galβ(1,3)GalNAc	0.05
45	Galβ(1,3)AllNAc	NI <7.5 mM
46	Galβ(1,6)AllNAc	NI <7.5 mM

[a] Inhibition is expressed as I_{50} of each compound relative to lactose I_{50}, which varied from 0.55 to 0.72 mM. [b] NI, no inhibition.

Table III: Inhibition of Galaptin Binding to Asialofetuin by Glycoconjugates

glycoconjugate	concn[a]
fetuin	3.0 mg/mL
asialofetuin	0.59 mg/mL
N-1-(1-deoxylactitol)–BSA	NI <1.2 mM[b]
Lacβ–$OC_6H_4NH_2(p)$–BSA	0.08 mM[b]

[a] Concentration required to give 50% inhibition. [b] Expressed as concentration of Lac and 1-deoxylactitol residues.

When the OH at C-2 of Gal is replaced by an acetamido group, as in GalNAc and its derivatives (Table I), the sugar is no longer active as an inhibitor. *O*-Methyl substitution at C-2 of Gal (as in **19**) does not affect ligand binding. Indeed, methyl substitution at C-2 of Gal, as in MeO–2Galβ(1,4)-GlcNAc (**33**), enhances binding compared to Galβ(1,4)-GlcNAc (**32**).

Table II shows the inhibitory activity of disaccharides and higher order oligosaccharides and their derivatives relative to lactose. The most potent disaccharides are Galβ(1,4)GlcNAc (LacNAc, **32**) and 2′-OMe-substituted LacNAc (**33**), which show 10 and 15 times more activity than lactose, respectively. Glycosides of Galβ(1,3)GlcNAc (**34** and **35**) are 2–4 times less potent than LacNAc, while the activity of the glycoside of Galβ(1,6)GlcNAc (**40**) is 44 times less than LacNAc and is even 4 times less than Lac.

From the analysis of carbohydrate recognition by six soluble lectins, three from rat lung (Leffler & Barondes, 1986) and three from human lung (Sparrow et al., 1987), it has been proposed that these galaptins recognize and bind one side of the Lac/LacNAc molecule as shown in Figure 3. This is based on the fact that substitution at the 4- and 6-OH groups of the Gal residue in Lac/LacNAc is not allowed for efficient binding, whereas substitution at 2′- and 3′-OH did not affect

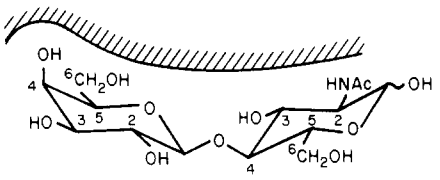

FIGURE 3: Region of LacNAc postulated to interact with galaptin (shaded area).

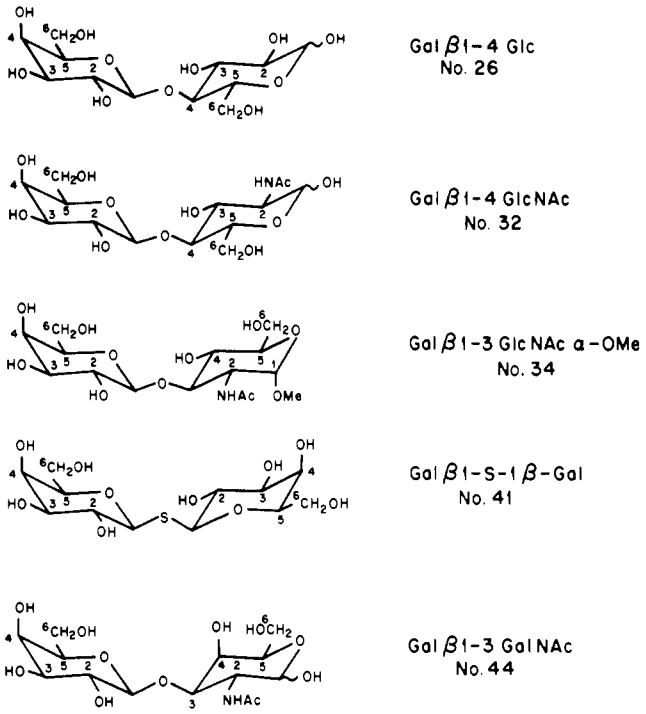

FIGURE 4: Schematic representation of the preferred conformations of some saccharides. The conformations for **26**, **32**, **34**, and **44** are based on NMR analysis and potential energy calculation (Thogensen et al., 1982; Sabesan et al., 1984; Bock et al., 1985). For **41**, the depicted conformation is hypothetical and is taken from Leffler and Barondes (1986).

the binding significantly. Substitution of the Glc 3-OH by Fuc abolished the binding affinity, and the presence of a 2-acetamido group on Glc increased the affinity relative to Lac binding. X-ray crystallographic examination of LacNAc showed that it exists in a preferred conformation that is common to all β(1,4)-linked disaccharides, in which the planes of the two sugar rings face opposite directions and the 3-OH of GlcNAc is hydrogen-bonded to the ring –O– of Gal (Longchambon et al., 1981). This conformation was also indicated from NMR and molecular modeling studies (Lemieux et al., 1980). In this conformation, all of the above mentioned important groups are located on the far side of the molecule as shown in Figure 3. Similarly, the known conformation of Galβ(1,3)GlcNAc places the GlcNAc 4-OH equatorial and at the same relative position as the GlcNAc 3-OH of LacNAc (Figure 4). As shown in Figure 4, all the disaccharides and their derivatives that show similar or higher activity than Lac have a hydroxyl group corresponding to the spatial orientation (equatorial) of 3-OH of Lac. The axial 4-OH of GalNAc in Galβ(1,3)GalNAc (**44**) (Figure 4) appears to significantly hamper ligand binding. Since galaptin binding occurs on the side of Lac/LacNAc as depicted in Figure 3, the substitution of 2-OH on the Gal residue does not alter ligand binding affinity (compare **36** to **35**). However, methyl substitution at this position did increase binding affinity somewhat (compare **33** with **32**). In the case of the monosaccharide, substitution at C-2 of Gal by a bulky Fuc residue

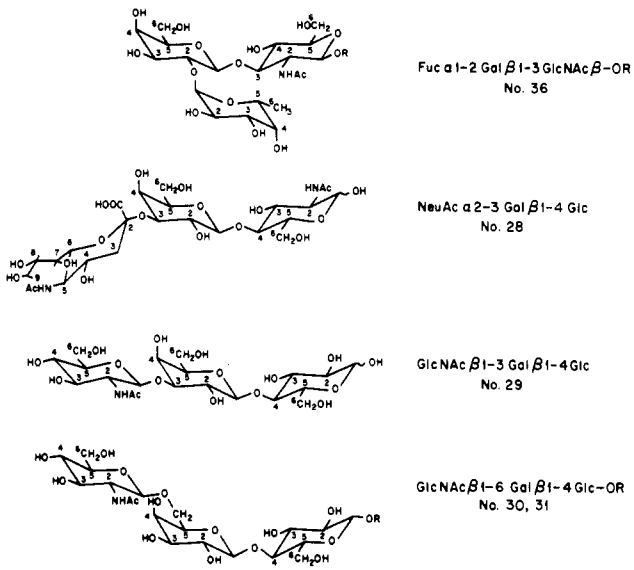

FIGURE 5: Proposed solution conformations of some oligosaccharides. The structures shown for **29** and **36** are based upon the conformation of blood group B oligosaccharides (Lemieux et al., 1985). The structure for **28** is based on conformational information for ganglioside GM_1 given in Sabesan et al. (1984). For **30** and **31**, the conformation is hypothetical and assumes similar glycosidic bond torsion angles as for the other compounds. For **36**, R = $C_6H_4NO_2(p)$; for **30**, R = H; for **31**, R = $C_6H_4NO_2(p)$.

(as in **20**) may interfere with hydrophobic interaction, and thus **20** becomes a weaker inhibitor than **5** and **8**–**10**. Substitution of the Lac 3′-OH by NeuAc (as in **28**) also does not significantly affect binding, whereas substitution with GlcNAc (as in **29**) results in slightly lower inhibitory activity compared to that of Lac. These results are commensurate with the structures depicted in Figure 5.

In **30** and **31**, GlcNAc, by virtue of its β(1,6) linkage to Gal, would block the top faces of the molecules (Figure 5), resulting in the loss of recognition by lectin. From previous studies of lectins from rat and human lung (Leffler & Barondes, 1986; Sparrow et al., 1987) and bovine heart (Childs & Feizi, 1979b; Abbott et al., 1988), it was not known whether substitution of Gal 6-OH prevented binding by masking the top face of the Lac molecule or if 6-OH is itself important for binding. To evaluate these two possibilities, we oxidized the CH_2OH of the Gal residue in Lac with galactose oxidase and found that the oxidized product was a poor inhibitor (data not shown). This finding leads us to the preliminary conclusion that the 6-OH of the Gal residue is itself important for lectin binding.

The planar orientation of disaccharides may be important for high-affinity binding to galaptin. Galα(1,6)Glc (**23**), Galβ(1,3)AllNAc (**45**), Galβ(1,6)AllNAc (**46**), and Galβ-(1,6)GlcNAcβ–$OC_6H_4NO_2(p)$ (**40**) (Figure 6) are similar to Lac/LacNAc (Figure 4) in that the Gal 4-OH groups are axial and the reducing-terminal 4-OH groups are equatorial. However, in contrast to Lac/LacNAc, the Gal residues for **23**, **40**, and **46** project upward from the plane occupied by their reducing termini. Likewise, these compounds are poor inhibitors relative to Lac/LacNAc.

Of interest is the observation that all of the Galβ(1,3)-GlcNAc derivatives are of similar inhibitory reactivity regardless of the residues at the GlcNAc terminus (**34**–**36**, **38**, and **39**) with the exception of **37** [Galβ(1,3)GlcNAcβ(1,3)-Galβ–OMe], which was found to be a very poor inhibitor. Two separate lots of this compound yielded the same results. It is difficult to explain this observation but we assume it to be related to the three-dimensional structure of the compounds.

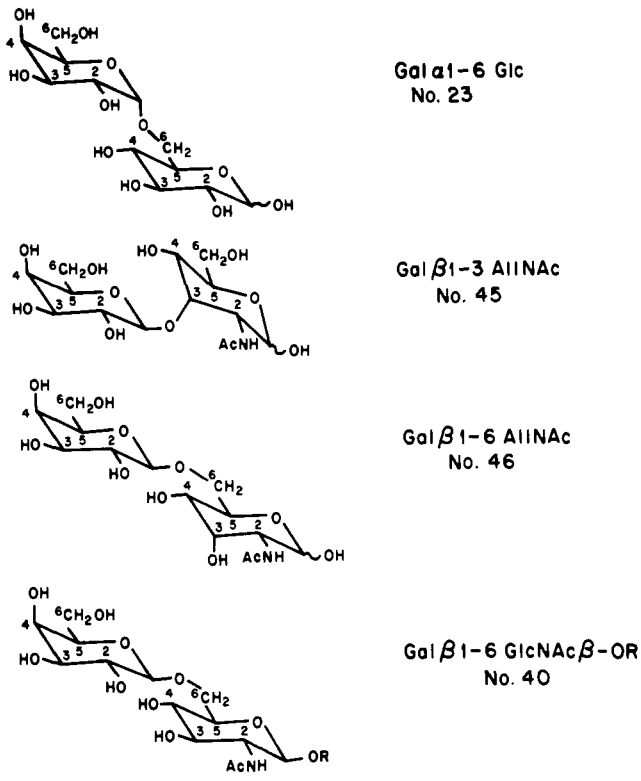

FIGURE 6: Proposed conformations of disaccharides that are weak inhibitors of galaptin. All the conformations are hypothetical and assume similar glycosidic bond torsion angles as for the structures in Figures 4 and 5. For **40**, R = $C_6H_4NO_2(p)$.

Abbott et al. (1988) also showed that extension of the Gal–GlcNAc sequence did not alter inhibitory activity for bovine lectin.

N-1-(1-Deoxylactitol)–BSA was found to be less inhibitory than Lac, presumably due to opening of the Glc ring upon reduction of the Schiff base. Lacβ–$OC_6H_4NH_2(p)$–BSA was only 4 times more active than Galβ(1,4)Glcβ–$OC_6H_4NH_2(p)$, suggesting the absence of a significant cluster effect for multivalent ligand interaction with galaptin. This has been studied in greater detail and will be presented elsewhere (Lee et al., 1990). Although plastic-adsorbed asialofetuin was inefficient in binding galaptin, it was 5 times more potent than native fetuin as an inhibitor. The low binding efficiency reported here agrees with the observation of Merkle and Cummings (1988) that asialofetuin oligosaccharides bind poorly to insolubilized calf heart lectin. The data presented here are in general agreement with data presented by others for rat and human lung and bovine heart lectins. The importance of Galβ[1,3(4)]GlcNAc sequences in lectin binding is clearly demonstrated. Our observations suggest that anomeric specificity of the combining site may be modified by hydrophobic interactions. It will be worthwhile, therefore, to evaluate the interaction of galaptin with glycolipids.

The function of galaptin remains unknown, as well as the identity of endogenous receptors. However, a detailed knowledge of the galaptin binding-site specificity will aid in the eventual characterization of these receptors.

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