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Oligo-*N*-acetyllactosaminoglycans bearing Galβ1-4(Fucα1-3)GlcNAc sequences reveal lower affinities than their nonfucosylated, or α(1-2) fucosylated counterparts for immobilized wheat germ agglutinin

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Relative affinities of several fucosylated and nonfucosylated oligo-*N*-acetyllactosaminoglycans for immobilized wheat germ agglutinin (WGA) were studied using a chromatographic technique. α(1-3) Fucosylation of the *N*-acetylglucosamine unit(s) in mono- and biantennary saccharides of the Galβ1-4GlcNAc-R type strongly reduced the WGA-affinity. In contrast, α(1-2) fucosylation of the nonreducing galactose unit(s) of the saccharides did not reduce the affinity.

Keywords: oligo-*N*-acetyllactosaminoglycans, alditols, WGA-agarose chromatography, Lewis-x, H-antigens, SSEA-1, *in vitro* biosynthesis, α(1-2) fucosylation

Poly-*N*-acetyllactosaminoglycan-related saccharides containing linear GlcNAcβ1-6Gal-sequences bind to wheat germ agglutinin (WGA) with high affinity, while isomeric saccharides containing GlcNAcβ1-3Gal-sequences show much lower affinities [1–5]. The early work of Allen *et al.* [6] has shown that methylation of the hydroxyl group at C-3 of *N*-acetylglucosamine methyl glycosides destroys the WGA-affinity. It was therefore of interest to study the effect of α(1-3) fucosylation of the C-3 position of penultimate *N*-acetylglucosamine units in some glycans that possess medium or high WGA affinities in the nonfucosylated form. The results of our present affinity chromatography experiments show that all tested saccharides bearing a Galβ1-4(Fucα1-3)GlcNAc group have much lower affinities for immobilized WGA than their nonfucosylated, or α(1-2) fucosylated counterparts.

Materials and methods

Chromatographic methods

WGA-affinity chromatography was performed on a small column of agarose-bound WGA (1.65 mg WGA per ml of 4% beaded agarose; Pharmacia, Sweden) as described [1, 5]. Briefly, the column was equilibrated with 10 mM sodium phosphate buffer, pH 7.1, containing 0.9% NaCl and 0.02% NaN_3. Fractions of 0.55 ml were collected and assayed for radioactivity. Fractions 1–40 were eluted with the equilibration buffer and fractions 41–70 with the buffer containing 0.2 M *N*-acetylglucosamine. The affinity of saccharides is presented as a difference, $\Delta = V_e - V_0$, where V_e is the peak fraction of the saccharide and V_0 is the peak fraction of galactose. A slow decrease of Δ values has been evident during the three year period of use of the same affinity column. The V_e of rapidly eluting oligosaccharides was measured by co-chromatography experiments with either [^{3}H]Gal ([^{14}C]oligosaccharides) or [^{14}C]Gal ([^{3}H]-oligosaccharides).

Paper chromatography was carried out on Whatman III Chr paper using the upper phase of n-butanol:acetic acid: water, 4:1:5 by vol (solvent A). The mobilities of saccharides are presented in relation to lactose (R_{Lact}), maltotriose (R_{MT}), maltotetraose (R_{MTet}), maltopentaose (R_{MP}) and maltoheptaose (R_{MH}), which were all purchased from Sigma (St. Louis, MO, USA).

Acid hydrolysis

Complete and partial removal of fucose residues was performed with an adaptation of a mild acid hydrolysis procedure described by Hounsell *et al.* [7]: the saccharides were dried in a 10 ml screw cap tube and 100 µl of 0.02 M

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H_2SO_4 was added. Incubation was conducted at 100°C for various periods of time to obtain a suitable degree of cleavage. The reaction was stopped by cooling on ice and by adding 100 µl of 0.1 M sodium acetate. The hydrolysate was desalted (see below) and the products were purified by paper chromatography.

Enzymatic methods

Galactosylation was performed with either UDP-Gal or UDP-[6-^{3}H]Gal (Amersham International, UK) and bovine milk galactosyltransferase (EC 2.4.1.22) (Sigma) as described [5].

α(1-2)-Fucosyltransferase reactions were performed with GDP-fucose (BioCarb, Lund, Sweden) and a β-galactoside α(1-2)-fucosyltransferase purified from porcine submaxillary glands as described [8, 9].

Cleavage with jack bean β-galactosidase (EC 3.2.1.23, Sigma) was conducted in a 45 µl reaction mixture containing 2.2 U ml^{-1} of the enzyme, 0.05 M sodium citrate, pH 4.0. Incubations were performed at 37°C overnight (unless otherwise indicated). The reactions were stopped by heating in boiling water for 3 min. Cleavage with *Diplococcus pneumoniae* was conducted as described [2].

Cleavage with jack bean β-*N*-acetylhexosaminidase (EC 3.2.1.30, Sigma) was conducted in a 45 µl reaction mixture containing 3.3 U ml^{-1} of the enzyme, and 0.05 M sodium citrate, pH 4.0, and 55 mM γ-galactonolactone. Incubations were performed overnight at 37°C. The reactions were stopped by heating in boiling water for 3 min.

Desalting

All enzymatic reaction mixtures and pooled saccharides were desalted by passing them through a mixed bed of Dowex AG1 (AcO^-) and Dowex AG50 (H^+), and then lyophilized.

Preparation of radiolabelled saccharides

The structural formulae of the key saccharides in the present experiments are presented in Table 1.

[6-^{3}H]Galβ1-4GlcNAcβ1-3([6-^{3}H]Galβ1-4GlcNAcβ1-6)Galβ1-4Glc (**11**) was synthesized as follows: Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4Glc (BioCarb) was digested with jack bean β-galactosidase, the reaction mixture was desalted, and the saccharides were galactosylated with UDP-[6-^{3}H]Gal and bovine milk galactosyltransferase. The labelled hexasaccharide was purified by paper chromatography (R_{MH} = 1.20, R_{MP} = 0.57).

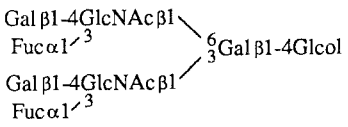
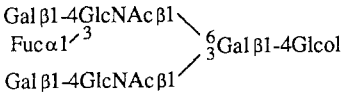
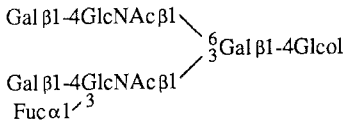
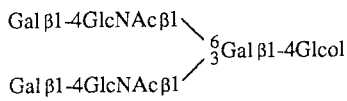
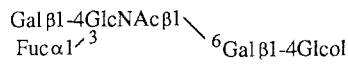
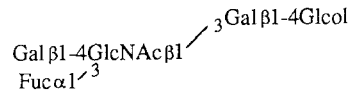
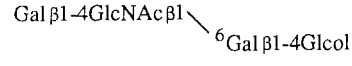
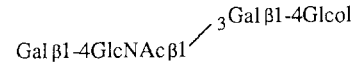
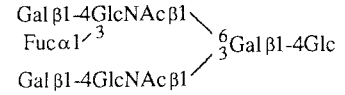
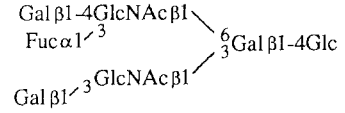
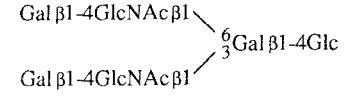
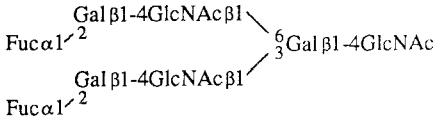
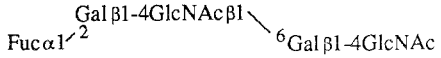
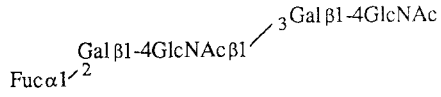
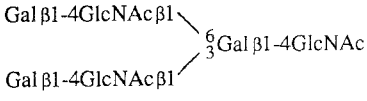
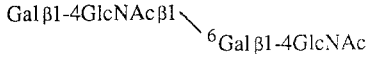
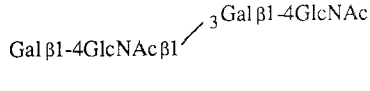
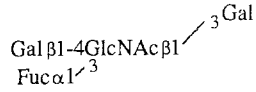
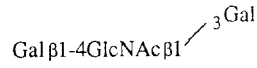
A portion of the hexasaccharide **11** was reduced with $NaBH_4$ essentially as described by Rasilo and Renkonen [10], and the hexasaccharide alditol Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4Glc-ol (**4**) was further purified with a small column of Dowex AG1 (OH^-).

[6-^{3}H]Galβ1-4GlcNAcβ1-3Galβ1-4[1-^{14}C]Glc-ol (**8**) was synthesized as follows: Galβ1-4[1-^{14}C]Glc (Amersham International, UK) was incubated with UDP-GlcNAc and human serum as described by Yates and Watkins [11]. The trisaccharide GlcNAcβ1-3Galβ1-4[1-^{14}C]Glc (R_{MT} = 1.00, R_{Lact} = 0.75) obtained was purified by paper chromatography, desalted, and galactosylated with UDP-[6-^{3}H]Gal and bovine milk galactosyltransferase. The galactosylation product was isolated by paper chromatography (R_{MP} = 1.37, R_{MTet} = 0.89). This tetrasaccharide was then reduced with $NaBH_4$ and the product alditol (**8**) was purified by passing the borate-free mixture through a small column of Dowex AG1 (OH^-), and then by paper chromatography (R_{MP} = 1.52, R_{MTet} = 0.98).

The origins of Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)[U-^{14}C]Galβ1-4GlcNAc (**15**) [12], [U-^{14}C]Galβ1-4GlcNAcβ1-6Galβ1-4Glc-ol (**7**) [5], [U-^{14}C]Galβ1-4GlcNAcβ1-6[U-^{14}C]Galβ1-4GlcNAc (**16**) [2], [U-^{14}C]Galβ1-4GlcNAcβ1-3[U-^{14}C]Galβ1-4GlcNAc (**17**) [2] and Galβ1-4[^{3}H] GlcNAcβ1-3Gal (**19**) [13] have been described in the cited papers.

Galβ1 - 4(Fucα1 - 3)GlcNAcβ1 - 3[Galβ1 - 4(Fucα1 - 3)GlcNAcβ1-6]Galβ1-4[1-^{3}H]Glc-ol (**1**) was prepared by reducing Galβ1-4(Fucα1-3)GlcNAcβ1-3[Galβ1-4(Fucα1-3)GlcNAcβ1-6]Galβ1-4Glc (BioCarb, Lund, Sweden) with NaB[^{3}H]$_4$. The alditol was purified with a small column of Dowex AG1 (OH^-) [14] and subsequently by paper chromatography (R_{MH} = 0.55, R_{MP} = 0.27). The octasaccharide **1** was defucosylated partially by a 10 min hydrolysis with 0.02 M H_2SO_4 at 100°C. A mixture of the monodefucosylated heptasaccharides (**2** and **3**) (R_{MH} = 0.80, R_{MP} = 0.39) was isolated by paper chromatography in a 10 day run with solvent A. Although inseparable by paper chromatography, the two heptasaccharides were well separated by WGA-chromatography: heptasaccharide **2** was not retained (Δ = 0), but heptasaccharide **3** was retained substantially (Δ = 10). In order to determine the position of the Fucα1-3 group in the two heptasaccharide fractions, they were separately digested with β-galactosidase and β-*N*-acetylhexosaminidase. Paper chromatography revealed that pentasaccharides had been formed from both peaks; as expected [15], the fucosylated branches had resisted the enzyme treatment. The two pentasaccharides obtained revealed slightly different migration rates in solvent A. The pentasaccharide obtained from the nonretained heptasaccharide **2** revealed a slower migration rate (R_{MH} = 1.70, R_{MP} = 0.86) than that obtained from the retained heptasaccharide **3** (R_{MH} = 1.83, R_{MP} = 0.97). The slower moving pentasaccharide proved to be Galβ1-4(Fucα1-3)GlcNAcβ1-6Galβ1-4Glc-ol (**5**), and the faster one represented Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-ol (**6**). This became evident when the fucose residues of the pentasaccharides were removed using 45 min hydrolysis with 0.02 M H_2SO_4; the tetrasaccharide obtained from the slow pentasaccharide (**5**) migrated on paper like authentic Galβ1-4GlcNAcβ1-6Galβ1-4Glc-ol (**7**) (R_{MP} = 1.37, R_{MTet} = 0.84), and the tetrasaccharide obtained from the fast pentasaccharide (**6**) migrated like authentic Galβ1-4GlcNAcβ1-3Galβ1-4Glc-ol (**8**) (R_{MP} = 1.51, R_{MTet} = 0.95). These experiments establish the structure of heptasaccharide **2** that was

Table 1. The structures of key saccharides in the present experiments.[a)]

1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	

[a)] The construction and structural characterization of the saccharides are described in the Materials and methods section.

not retained in the WGA-agarose column as Galβ1-4GlcNAcβ1-3[Galβ1-4(Fucα1-3)GlcNAcβ1-6]Galβ1-4Glc-ol, and the structure of heptasaccharide **3** that was retained by WGA as Galβ1-4(Fucα1-3)GlcNAcβ1-3[Galβ1-4GlcNAcβ1-6]Galβ1-4Glc-ol.

[6-^{3}H]Galβ1-4GlcNAcβ1-3[Galβ1-4(Fucα1-3)GlcNAcβ1-6]Galβ1-4Glc (**9**) was prepared from Galβ1-3GlcNAcβ1-3[Galβ1-4(Fucα1-3)GlcNAcβ1-6]Galβ1-4Glc (**10**) (monofucosyllacto-*N*-hexaose, BioCarb) by a prolonged incubation with jack bean β-galactosidase, and subsequent galactosylation using UDP-[^{3}H]Gal and bovine milk β(1-4)-galactosyltransferase. The resulting radiolabelled heptasaccharide (**9**) was purified using paper chromatography ($R_{MH} = 0.82$, $R_{MP} = 0.41$); it was obtained in a good yield. The conditions used for prolonged β-galactosidase treatment of **10** (incubation for 72 h; addition of fresh enzyme at 24 h and 48 h) degalactosylated Galβ1-3GlcNAcβ1-3Gal completely (O. Renkonen *et al.*, unpublished results); hence, it was not surprising that also the type 1 linked galactose unit from the monofucosyllacto-*N*-hexaose appeared to be cleaved under these conditions. In contrast, the Galβ1-4(Fucα1-3)GlcNAc-R sequence of **10** probably resisted the β-galactosidase treatment [15]. *D. pneumoniae* β-galactosidase treatment cleaved [^{3}H]Gal from **9**, establishing that the newly inserted [^{3}H]Gal units were β(1-4) linked [16].

Fucα1-2Galβ1-4GlcNAcβ1-3(Fucα1-2Galβ1-4GlcNAcβ1-6)[U-^{14}C]Galβ1-4GlcNAc (**12**) was prepared by fucosylating the hexasaccharide Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)[U-^{14}C]Galβ1-4GlcNAc (**15**) with GDP-fucose and β-galactoside α(1-2)-fucosyltransferase. The fucosylated octasaccharide **12** was purified by paper chromatography in an 8 day run with solvent A ($R_{MH} = 0.92$, $R_{MP} = 0.45$). The chromatogram also revealed a peak with the mobility of a heptasaccharide ($R_{MH} = 1.16$, $R_{MP} = 0.57$). The heptasaccharide fraction, supposed to contain a mixture of Fucα1-2Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)[U-^{14}C]Galβ1-4GlcNAc and Galβ1-4GlcNAcβ1-3(Fucα1-2Galβ1-4GlcNAcβ1-6)[U-^{14}C]Galβ1-4GlcNAc was characterized in an analogous manner as the heptasaccharides **2** and **3** (see above). It was digested with β-galactosidase and β-*N*-acetylhexosaminidase, and the digest was analysed by paper chromatography, which revealed two well resolved peaks. These proved to represent the isomeric pentasaccharides Fucα1-2Galβ1-4GlcNAcβ1-6[U-^{14}C]Galβ1-4GlcNAc (**13**) ($R_{MP} = 1.24$, $R_{MTet} = 0.80$) and Fucα1-2Galβ1-4GlcNAcβ1-3[U-^{14}C]Galβ1-4GlcNAc (**14**) ($R_{MP} = 1.56$, $R_{MTet} = 0.98$): defucosylation of the pentasaccharides with 30 min hydrolysis with 0.02 M H_2SO_4 converted the saccharides **13** and **14** into products migrating on paper chromatography like authentic radiolabelled markers of Galβ1-4GlcNAcβ1-6Galβ1-4GlcNAc (**16**) and Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc (**17**) [2], respectively.

[^{3}H]Fucose and [^{3}H]GlcNAc-, as well as [U-^{14}C]Gal-labelled versions of the tetrasaccharide Galβ1-4(Fucα1-3)GlcNAcβ1-3Gal (**18**) were isolated from endo-β-galactosidase digests of metabolically labelled poly-*N*-acetyllactosaminoglycans of embryonal carcinoma cells (line PC 13) [13]. They were obtained as an α-galactosidase resistant neutral tetrasaccharide fraction by paper chromatography with solvent A ($R_{Lact} = 0.465$). The [^{3}H]fucose-labelled version of **18** resisted β-galactosidase and α-L-fucosidase of type 2 [17] from almond emulsin. However, it was cleaved completely by α-L-fucosidase of type 1 [18] from almond emulsin. The [^{3}H]GlcNAc-labelled version of **18** was converted by the α-L-fucosidase of type 1 into Galβ1-4[^{3}H]GlcNAcβ1-3Gal (**19**), which was identified by paper chromatography. The [U-^{14}C]Gal-labelled version of **18** was used in the WGA chromatography experiment.

Results

WGA affinity chromatography of α(1-3)-fucosylated saccharides and their nonfucosylated counterparts

Figure 1 shows a series of WGA-affinity chromatograms for biantennary reduced saccharides. The difucosylated octasaccharide **1** showed no affinity for WGA (Fig. 1A), nor did the heptasaccharide **2** (Fig. 1B). The heptasaccharide **3**, however, was distinctly retarded in the column ($\Delta = 9.7$; Fig. 1C). The nonfucosylated hexasaccharide **4** was the most retarded among the four biantennary saccharides ($\Delta = 11.4$; Fig. 1D), implying that α(1-3)-fucosylation of the penultimate *N*-acetylglucosamine unit(s) decreased the WGA-affinity. Specifically, the α(1-3)-fucosylation of the (1-6) branch completely abolished the WGA-affinity; the fucose unit in the (1-3) branch had a much lower effect.

These findings were extended by studying the reducing saccharides [6-^{3}H]Galβ1-4GlcNAcβ1-3[Galβ1-4(Fucα1-3)GlcNAcβ1-6]Galβ1-4Glc (**9**) and [6-^{3}H]Galβ1-4GlcNAcβ1-3([6-^{3}H]Galβ1-4GlcNAcβ1-6)Galβ1-4Glc (**11**). The fucosylated heptasaccharide **9** revealed very weak affinity towards WGA ($\Delta = 0.25$), while the nonfucosylated hexasaccharide **11** possessed considerable affinity, eluting with $\Delta = 11.1$ (Fig. 2).

The high affinity tetrasaccharide alditol Galβ1-4GlcNAcβ1-6Galβ1-4Glc-ol (**7**) ($\Delta = 30$; Fig. 3B) also experienced a dramatic decrease in WGA-affinity when it was α(1-3)-fucosylated in the penultimate *N*-acetylglucosamine unit. The fucosylated pentasaccharide alditol Galβ1-4(Fucα1-3)GlcNAcβ1-6Galβ1-4Glc-ol (**5**) was completely devoid of affinity (Fig. 3A).

The tetrasaccharide Galβ1-4(Fucα1-3)GlcNAcβ1-3Gal (**18**) eluted from the WGA column without showing significant affinity (Fig. 4A). The trisaccharide Galβ1-4GlcNAcβ1-3Gal (**19**) gave on WGA chromatography an elution pattern, where most of the material eluted very soon after the void volume ($\Delta = 0.5$), but a distinct tail was also seen (Fig. 4B). Rechromatography of the tail (tubes 13–29 in Fig. 4B) reproduced the original profile (not shown). This kind of behaviour has been suggested to be due to different affinities of the anomeric forms of saccharides bearing galactose in the reducing end [3, 5].

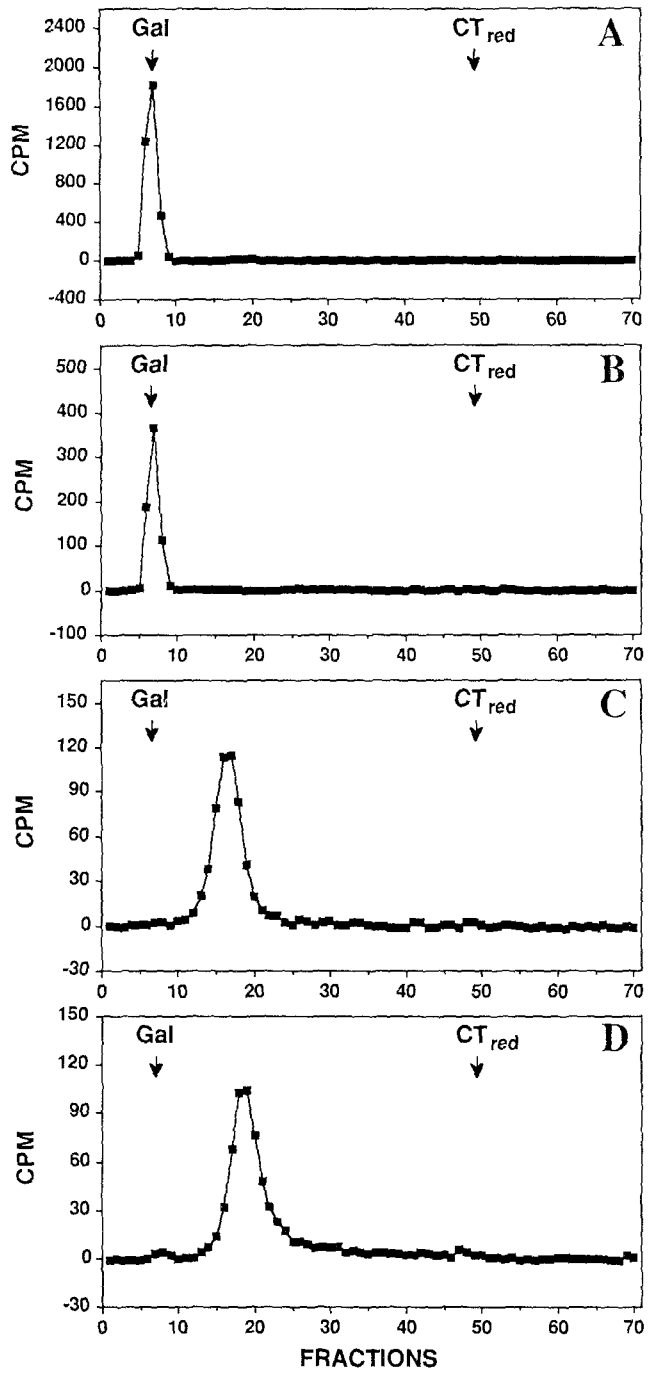


Figure 1. WGA agarose chromatography of the biantennary oligosaccharide alditols **1** (A), **2** (B), **3** (C) and **4** (D). The arrows labelled 'Gal' and 'CT_{red}' show the elution positions of galactose and reduced *N,N',N''*-triacetylchitotriose, respectively.

WGA affinity chromatography of α(1-2)-fucosylated saccharides and their nonfucosylated counterparts

WGA-chromatography revealed rather similar affinities for the difucosylated octasaccharide Fucα1-2Galβ1-4GlcNAcβ1-3(Fucα1-2Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAc (**12**) ($\Delta = 4.2$) and for the backbone hexasaccharide Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAc (**15**) ($\Delta = 6.25$), as shown in Fig. 5. Also the linear α(1-2)-fucosylated pentasaccharides Fucα1-2Galβ1-4GlcNAcβ1-6Galβ1-4GlcNAc (**13**) and Fucα1-2Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc (**14**) were retarded in the WGA-agarose column approximately as much as their nonfucosylated counterparts **16** and **17**, respectively (Figs 6, 7).

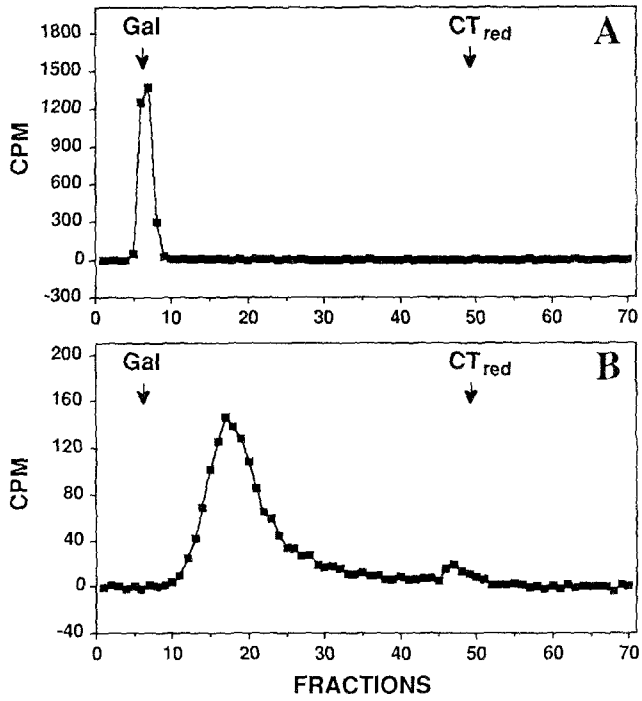


Figure 2. WGA agarose chromatography of the biantennary reducing saccharides **9** (A) and **11** (B).

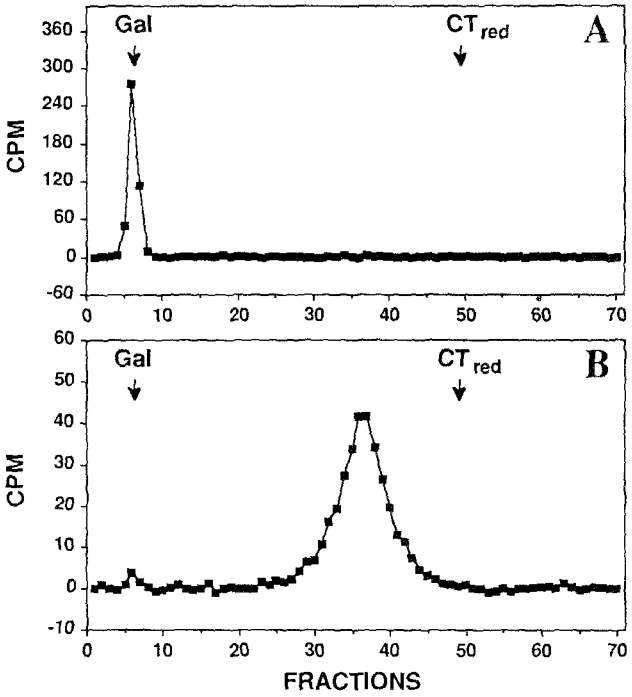


Figure 3. WGA agarose chromatography of the linear oligosaccharide alditols **5** (A) and **7** (B).

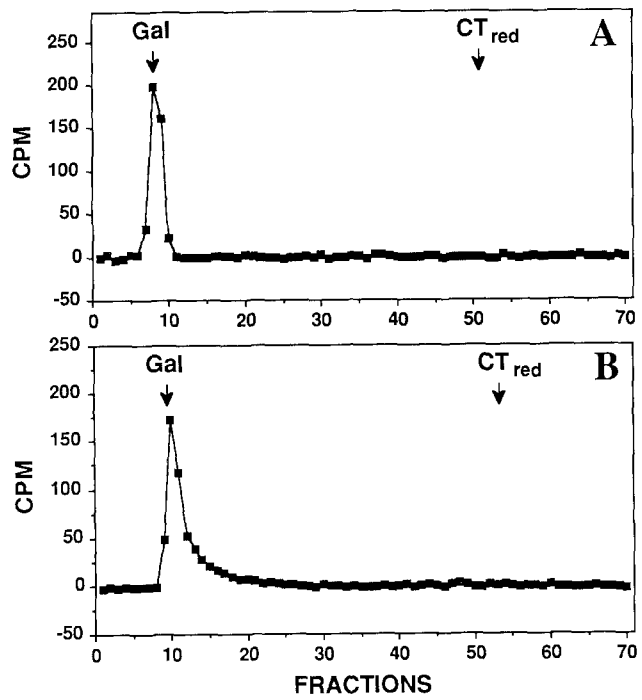


Figure 4. WGA agarose chromatography of the oligosaccharides **18** (A) and **19** (B).

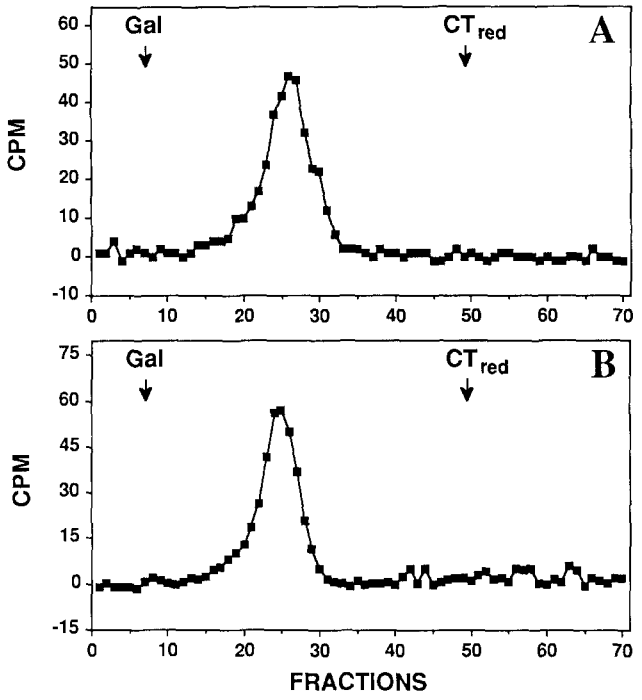


Figure 6. WGA agarose chromatography of the saccharides **13** (A) and **16** (B).

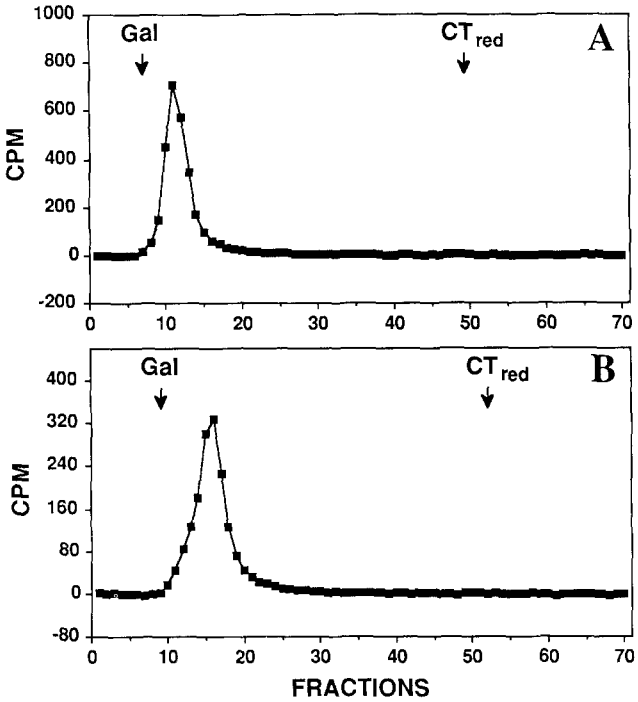


Figure 5. WGA agarose chromatography of the biantennary oligosaccharides **12** (A) and **15** (B); the experiment with **12** was carried out nearly two years later than the run with **15**.

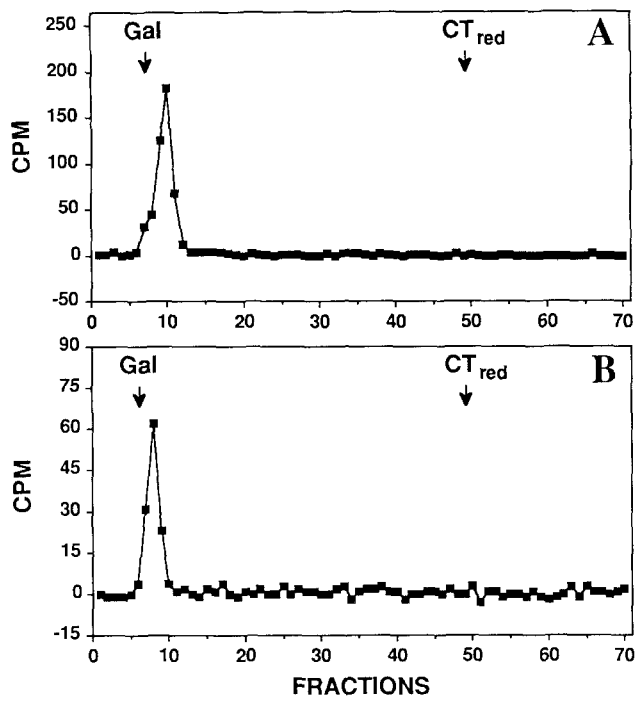


Figure 7. WGA agarose chromatography of the saccharides **14** (A) and **17** (B).

Discussion

The present experiments show that α(1-3)-fucosylation of the *N*-acetylglucosamine unit in Galβ1-4GlcNAcβ1-6Galβ1-4Glc-ol (**7**) results in a dramatic loss of the high WGA-affinity of the tetrasaccharide alditol. Similar changes are observed when the high-affinity hexasaccharide alditol Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4Glc-ol (**4**), or the hexasaccharide Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAc

β1-6)Galβ1-4Glc (**11**), were compared with derivatives which were α(1-3)-fucosylated in the *N*-acetylglucosamine unit of the (1-6) branches. However, α(1-3)-fucosylation of the *N*-acetylglucosamine unit of the (1-3) branch of the hexasaccharide alditol **4** had a rather small effect, showing that the (1-3) branch is of minor importance in the WGA-binding. If WGA chromatography can differentiate also between other α(1-3)-fucosylated *N*-acetylglucosamine units in oligo-*N*-acetylactosaminoglycans, it may actually help to define the binding epitopes of the nonfucosylated saccharides.

Even the saccharides of low WGA-affinity, like Galβ1-4GlcNAcβ1-3Gal experienced, upon α(1-3)-fucosylation a distinct reduction in their WGA-affinities, implying that the hydroxyl group at position 3 of the *N*-acetylglucosamine unit is probably important for the weakly bound as well as for the strongly bound oligo-*N*-acetyllactosaminoglycans.

Our present findings are compatible with the early work of Allen *et al.* [6], in which they showed that methylation of the hydroxyl group at C-3 of *N*-acetylglucosamine methyl glycosides destroys its WGA-affinity. These findings lead us to postulate that lacto-*N*-tetraose and its derivatives should have distinctly smaller affinities than lacto-*N*-neotetraose and its derivatives, and that it should be possible to separate type 1 from type 2 backbones with WGA chromatography.

The present findings show that α(1-2)-fucosylation of the nonreducing end galactose has little influence on the WGA-affinities of oligo-*N*-acetyllactosaminoglycans. This implies that WGA chromatography can be used to differentiate between α(1-2)- and α(1-3)-fucosylated backbones in a nondestructive manner. Rather little information is available concerning the effect of other "capping units" on the WGA-affinities of oligo-*N*-acetyllactosaminoglycans. Tarrago *et al.* [19] have reported that NeuAcc(2-6)Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4Glc-ol has a lower affinity than the nonsialylated hexasaccharide alditol. We have found in preliminary experiments that Galα1-3Galβ1-4GlcNAcβ1-6Galβ1-4GlcNAc has a higher WGA-affinity than Galβ1-4GlcNAcβ1-6Galβ1-4GlcNAc (O. Renkonen *et al.*, unpublished observations).

Kawashima *et al* [4] have recently shown that several poly-*N*-acetyllactosamine-binding lectins possess well-defined affinities towards small backbones of oligo-*N*-acetyllactosaminoglycans. It remains to be seen how α(1-2)- and α(1-3)-fucosylation of the backbones affect the binding with these lectins.

Our present data show that the α(1-2)-fucosylated derivatives of Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc and Galβ1-4GlcNAcβ1-6Galβ1-4GlcNAc are separated by paper chromatography as easily as the nonfucosylated tetrasaccharides. Also, the (1-3)-fucosylated derivatives of Galβ1-4GlcNAcβ1-3Galβ1-4Glc-ol and Galβ1-4GlcNAcβ1-6Galβ1-4Glc-ol are separated like the nonfucosylated tetrasaccharide alditols. Among the fucosylated saccharides, as well as among the parent, nonfucosylated tetrasaccharides, the isomers containing the mid-chain GlcNAcβ1-3Gal sequence migrate faster than their isomeric counterparts containing the mid-chain GlcNAcβ1-6Gal unit. As exemplified in the present experiments, these separations are quite useful in the structural analysis of picomolar samples of oligo-*N*-acetyllactosaminoglycans.

Acknowledgements

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