Glycoconjugate Journal (1992) **9**:141–147

Fine sugar specificity of the *Butea frondosa* seed lectin

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Received 31 October 1991, revised 11 February 1992

Various monosaccharides and oligosaccharides were used to define the specificity of the *Butea frondosa* lectin using the hapten inhibition technique of human erythrocyte agglutination. Although *B. frondosa* lectin exhibited higher affinity for *N*-acetylgalactosamine, lactose and *N*-acetyllactosamine appeared to be relatively good inhibitors of haemagglutination. The behaviour of *N*-acetyllactosamine-type oligosaccharides and glycopeptides on a column of *B. frondosa* lectin immobilized on Sepharose 4B showed that the sugar-binding specificity of the lectin is directed towards unmasked *N*-acetyllactosamine sequences. Substitution of these *N*-acetyllactosamine sequences by sialic acid residues completely abolished the affinity of the lectin for the saccharides. The presence of one or several αFuc(1-3)GlcNAc groups completely inhibited the interaction between the glycopeptides and the lectin. Substitution of the core β-mannose residue by an additional bisecting β(1-4)GlcNAc residue decreases the affinity of the lectin for these structures as compared with the unsubstituted ones.

Keywords: *Butea frondosa* lectin; sugar specificity; *N*-acetyllactosamine-type oligosaccharides

Seeds of *Butea frondosa* were shown to contain a Gal/GalNAc-specific lectin, previously isolated by Horejsi *et al.* [1]. The monosaccharide specificity of the lectin is very similar to other well-known Gal/GalNAc-specific lectins [2]. However, we have now compelling evidence that lectins of similar monosacharide specificity can in fact exhibit quite different fine specificities towards more complex glycans or glycopeptides [3, 4]. In this study, the fine sugar specificity of the *B. fondosa* lectin was examined by two complementary approaches.

Materials and methods

Isolation of lectin

The lectin from seeds of *Butea frondosa* Roxb. (Erythrineae), was purified by affinity chromatography on a column of lactose immobilized on 4% crosslinked agarose (Pierce), as previously described [5]. The lectin was coupled to Sepharose 4B (Pharmacia) that had been CNBr-activated according to the procedure of March *et al.* [6]. The amount of lectin bound to Sepharose was estimated to be 3 mg per ml of gel, by subtracting the amount of unbound protein found in the supernatant and washing solutions after coupling. Protein was determined by the method of Lowry *et al.* [7], using bovine serum albumin (Sigma) as standard.

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Origin of glycopeptides and oligosaccharides

All the simple monosaccharides and their derivatives, and the unbranched oligosaccharides were purchased from Sigma. Oligosaccharides **1**–**3**, as shown in Table 2, with ^{14}C-labelled terminal nonreducing D-galactosyl groups, were gifts from Dr. F. Piller (Centre National de Transfusion Sanguine, Paris). Oligosaccharides **5**–**12** and glycopeptide **19**, were isolated by Dr. G. Strecker (Lille, France) from the urines of patients with various lysosomal diseases [8]. Glycopeptides isolated from human serotransferrin (structure **13**), from human lactotransferrin (structures **14**–**16**), from hen ovotransferrin (structure **21**), from Cohn's fraction IV (structure **22**), and from turtle-dove ovomucoid (structure **20**), were gifts from Prof. G. Spik (Lille, France). Glycopeptides **17** and **18**, isolated from human α1-acid glycoprotein, were obtained from Prof. B. Fournet (Lille, France). The purity of all oligosaccharides and glycopeptides was checked by ^{1}H-NMR spectroscopy. Oligosaccharide **4** is described in the next section.

Labelling of glycopeptides and oligosaccharides

Glycopeptides were labelled with [^{14}C]acetic anhydride (10–30 mCi mmol^{-1}, Amersham) as described by Koide *et al.* [9], and purified by gel filtration on a column of Bio-Gel P-2 (Bio-Rad) equilibrated with water. Oligosaccharides **5**–**12** were labelled at the reducing-terminal *N*-

acetylglucosamine residues by reduction with tritiated sodium borohydride (5–20 Ci mmol^{-1}, Amersham) as described by Takasaki and Kobata [10]. Oligosaccharide **4** was released from human serotransferrin by hydrazinolysis and labelled by *N*-reacetylation with [^{14}C]acetic anhydride (10–30 mCi mmol^{-1}, Amersham).

Affinity chromatography of oligosaccharides or glycopeptides on the immobilized B. frondosa lectin-Sepharose 4B column

Labelled glycopeptides or oligosaccharides (2–30 × 10^3 disintegrations min^{-1}; 0.1–10 nmol), were applied to the column of lectin-Sepharose 4B (1 × 10 cm) equilibrated at room temperature in 10 mM sodium phosphate buffer (pH 7.2) containing 0.15 M NaCl (PBS). Elution was performed first with PBS at a flow rate of 9 ml h^{-1}, and then with PBS containing 0.15 M lactose. Fractions (1.5 ml) were collected and aliquots counted in a Beckman LS-1800 scintillation counter. Recovery of radioactivity from the column was always >95%.

Hapten-inhibition of haemagglutination

Agglutination of human ORh+ erythrocytes by *B. frondosa* lectin and inhibition of haemagglutination by various mono- and oligosaccharides and glycoproteins, was carried out in PBS in U-bottomed micro-plates (Flow Laboratories) [3, 11]. Results were expressed as the minimum concentration (mM) of mono- or oligosaccharides or glycoproteins required to inhibit completely four haemagglutination doses. Account was taken of the threefold dilution caused by the addition of lectin and erythrocytes.

Results and discussion

As examined by the hapten inhibition technique, the most potent inhibitory mono- and oligosaccharides against the *B. frondosa* lectin range were: *N*-acetylgalactosamine, 2′-fucosyllactose [α-L-Fuc(1-2)-βGal(1-4)Glc], *p*-nitrophenyl *N*-acetyl-α- and β-galactopyranosaminide, lactose, lactulose, methyl galactopyranoside, *p*-nitrophenyl α- and β-galactopyranoside, MurNAc, β-Gal(1-3)GlcNAc, β-Gal(1-4)GlcNAc, β-Gal(1-6)-β-Gal(1-*O*)-*p*$NO_2C_6H_4$ (Table 1).

Although *N*-acetylgalactosamine is the most potent inhibitor of the lectin, other galactosides behave as good inhibitors, especially those having free hydroxyl groups at positions 3 and 4 of both terminal or core β-D-galactopyranose moieties. Accordingly, 2′-fucosyllactose [α-L-Fuc(1-2)-β-Gal(1-4)Glc] is as potent an inhibitor as *N*-acetylgalactosamine, while 3′-fucosyllactose is much less inhibitory.

The high inhibitory potency of galactosides containing aromatic aglycones, e.g., the *p*-nitrophenyl group, suggests

Table 1. Inhibition of *Butea frondosa* lectin by monosaccharides, oligosaccharides and glycoproteins, ranged according to their inhibitory potencies.[a]

Inhibitor	*Minimum concentration completely inhibiting four haemagglutinating doses*
N-Acetylgalactosamine	0.21 mM
Fucα1-2Galβ1-4Glc	0.21
p-Nitrophenyl *N*-acetyl-α-galactosaminide	0.31
p-Nitrophenyl *N*-acetyl-β-galactosaminide	0.31
Lactose	0.41
Lactulose	0.41
Methyl α-galactopyranoside	0.82
Methyl β-galactopyranoside	0.82
p-Nitrophenyl α-galactoside	0.82
p-Nitrophenyl β-galactoside	0.82
N-Acetylmuramic acid	0.82
Galβ1-3GlcNAc	0.82
Galβ1-4GlcNAc	0.82
Galβ1-6Galβ(1-*O*)-*p*-$NO_2C_6H_4$	0.82
Galβ1-6GlcNAc	1.25
GlcNAcβ1-6Gal	1.25
GlcNAcβ1-6Galβ1-4Glc	1.25
Raffinose	1.25
Galactose	1.65
Galactosamine	1.65
p-Nitrophenyl β-thiogalactoside	1.65
N-Acetylneuraminic acid	1.65
Muramic acid	1.65
Galβ1-6Gal	1.65
Galβ1-4Man	1.65
Galα1-4Gal	1.65
Galβ1-3GlcNAc β(1-*O*)-*p*-$NO_2C_6H_4$	1.65
Galβ1-4Galβ1-4GlcNAc	1.65
NeuAc2-3Galβ1-4Glc	1.65
Galβ1-3GlcNAcβ1-3Galβ1-4Glc	1.65
Melibiose	1.65
Human lactotransferrin	1.65
Human serotransferrin	1.65
Galβ1-3GalNAc	3.3
Galβ1-4Galβ1-4Glc	>3.3
Galβ1-4[Fucα1-3]Glc	>3.3
Galacturonic acid	5.0
Glucuronic acid	10.0

[a] The following sugars and glycoproteins gave values >10 mM: arabinose, ribose, glucose, mannose, fructose, L-rhamnose, glucosamine, *N*-acetylglucosamine, methyl α-glucopyranoside, methyl β-glucopyranoside, methyl α-mannopyranoside, saccharose, human orosomucoid, and chicken ovalbumin.

that a hydrophobic region could occur in the vicinity of the galactosyl-binding site of the lectin. Such a finding has been observed already with various legume lectins [2]. In this respect, the available x-ray solved monosaccharide:lectin complexes obtained between Con A [12] or the *Lathyrus ochrus* isolectin I (LoLI) [13] and D-glucose, D-mannose and their methyl derivatives, show that hydrophobic residues adjacent to the monosaccharide-binding site, e.g., Phe 123 in LoLI, participate in the binding. Similarly, the x-ray analysis of the lactose complex of the *Erythrina corallodendron* lectin showed that four hydrophobic residues Ala_{88}, Tyr_{106}, Phe_{131} and Ala_{218}, participate in the binding of the galactose moiety to the combining site of the lectin [14]. The carbohydrate-binding site of the *Griffonia simplicifolia* GS4 lectin was shown to be surrounded by several hydrophobic residues involved in the binding of the terminal tetrasaccharide unit of the Lewis^b human blood group determinant [15]. Other hydrophobic interactions were observed allowing the recognition of tri- and octasaccharides of the *N*-acetyllactosamine group by LoLI [16, 17]. Along this line, chemical modifications have shown that both Tyr and Trp residues are important for carbohydrate-binding properties of lectins from potato [18], winged-bean [19] and artocarpin isolated from *Artocarpus lakoocha* seeds [20].

Methyl derivatives of both α- and β-galactopyranoside are also good inhibitors of the lectin.

As shown with some other Leguminosae lectins [21], MurNAc, a major component of the bacterial cell-wall glycopeptide, readily reacts with the *B. frondosa* lectin. Although *B. frondosa* lectin exhibits higher affinity for *N*-acetylgalactosamine; 2′-fucosyllactose and, to a lesser extent, lactose and *N*-acetyllactosamine, also behave as good inhibitors of haemagglutination. Therefore, the behaviour of *N*-acetyllactosamine type oligosaccharides and glycopeptides related to the *N*-glycosylamine linkage was studied using a column of *B. frondosa* lectin immobilized on Sepharose 4B.

Four elution profiles were obtained when *N*-acetyllactosamine type oligosaccharides and glycopeptides were applied to the immobilized *B. frondosa* lectin–Sepharose column. Some oligosaccharides or glycopeptides were eluted at the void volume of the column (fraction FNR) because of a lack of interaction between the immobilized lectin and the saccharides. All the other saccharides were eluted from the column by PBS as retarded fractions (fractions FR) according to their affinity for the lectin: under the experimental conditions used, FR1 was eluted between fractions 10–13, FR2 between fractions 12–17, and FR3 between fractions 14–18; the maximum elution being at fractions 12, 14 and 16, respectively (Fig. 1). There is a considerable overlap between fractions FR1, FR2 and FR3, when using a 1 cm × 10 cm column, which could be a limiting factor in the fractionation of mixtures of oligosaccharides and glycopeptides of diverse origins.

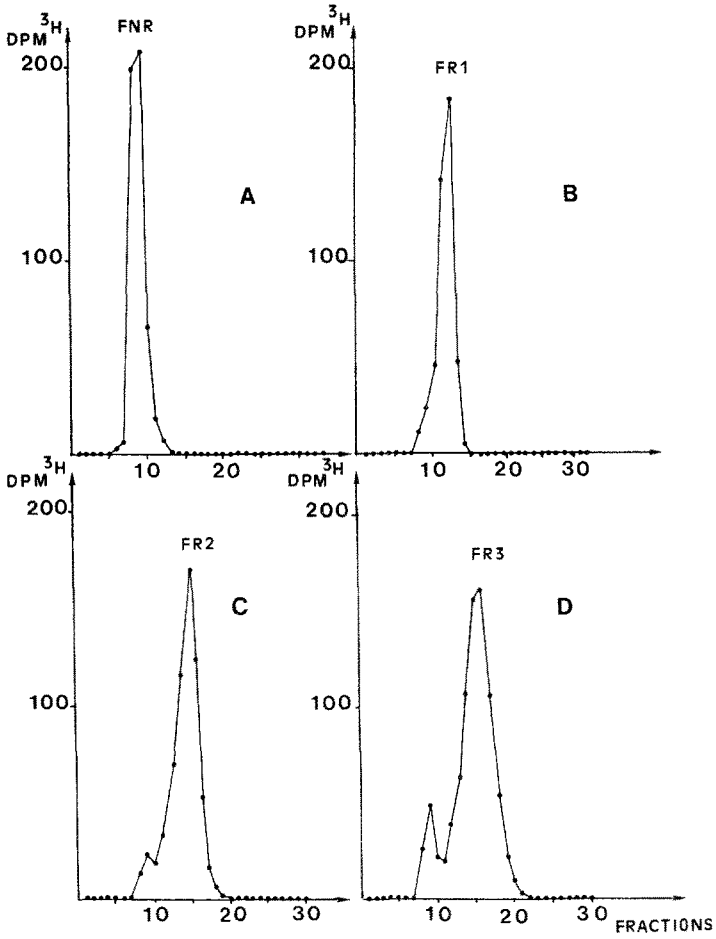

Figure 1. Elution profiles of *N*-glycosylpeptides or related oligosaccharides on immobilized *B. frondosa* lectin–Sepharose column. Labelled glycopeptides or oligosaccharides (20–30 × 10^3 disintegrations min^{-1}; 0.1 to 10 mmol) were applied to the lectin–Sepharose column (1 × 10 cm) equilibrated at room temperature in 10 mM PBS, pH 7.2. Elution was performed with buffer at a flow rate of 9 ml h^{-1}. Fractions of 1.5 ml were collected and aliquots counted by liquid scintillation in a Beckman LS-1800 counter. A, B, C and D correspond, respectively, to profiles obtained (8000 disintegrations min^{-1} ^{3}H each) with compounds **5** (non-retained fraction FNR), **6** (retarded fraction FR1), **7** (retarded fraction FR2) and **9** (retarded fraction FR3).

However, improvement in the separation of these fractions might be achieved by using a long and thin column (0.5 cm × 20 cm) which would be more efficient than a wider column containing the same volume of immobilized lectin.

Under the experimental conditions used, no strong interaction occurred between immobilized lectin and *N*-acetyllactosamine type structures, as suggested by the retarded profiles (FR) obtained by elution with the starting buffer. However, the strength of some interactions could be increased by a higher amount of lectin coupled to Sepharose 4B. The behaviour of 22 *N*-acetyllactosamine-type glycopeptides or related oligosaccharides on the immobilized lectin is described in Table 2.

Table 2. Behaviour of *N*-acetyllactosamine-type oligosaccharides and glycopeptides on an immobilized *Butea frondosa* lectin–Sepharose column.

Compound	Glycan structure	Elution profile			
		FNR	FR1	FR2	FR3
1	β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-GlcOMe	+			
2	β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-GlcOMe	+			
3	β-Gal-(1→4)-β-GlcNAc-(1→3) β-Gal-(1→4)-β-GlcNAc-(1→6) ⟩ β-Gal-(1→4)-GlcOMe		+		
4	α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6) ⟩ β-Man-(1→4)-β-GlcNAc-(1→4)-GlcNAc	+			
5	β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6) ⟩ β-GlcNAc-(1→4)-β-Man-(1→4)-GlcNAc	+			
6	β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6) ⟩ β-Man-(1→4)-GlcNAc		+		
7	β-Gal-(1→4)-β-GlcNAc-(1→4) β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6) ⟩ β-Man-(1→4)-GlcNAc			+	
8	β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6) β-Gal-(1→4)-β-GlcNAc-(1→6) ⟩ β-Man-(1→4)-GlcNAc			+	
9	β-Gal-(1→4)-β-GlcNAc-(1→4) β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6) β-Gal-(1→4)-β-GlcNAc-(1→6) ⟩ β-Man-(1→4)-GlcNAc				+
10a	β-Gal-(1→4)-β-GlcNAc-(1→3)$_1$— [β-Gal-(1→4)-β-GlcNAc-(1→4) β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6) β-Gal-(1→4)-β-GlcNAc-(1→6)] ⟩ β-Man-(1→4)-GlcNAc			+	
11a	β-Gal-(1→4)-β-GlcNAc-(1→3)$_2$— [β-Gal-(1→4)-β-GlcNAc-(1→4) β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6) β-Gal-(1→4)-β-GlcNAc-(1→6)] ⟩ β-Man-(1→4)-GlcNAc		+		
12a	β-Gal-(1→4)-β-GlcNAc-(1→3)$_3$— [β-Gal-(1→4)-β-GlcNAc-(1→4) β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6) β-Gal-(1→4)-β-GlcNAc-(1→6)] ⟩ β-Man-(1→4)-GlcNAc			+	
13	α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6) ⟩ β-Man-(1→4)-β-GlcNAc-(1→4)-β-GlcNAc-(1→N)-Asn	+			
14	β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6) ⟩ β-Man-(1→4)-β-GlcNAc-(1→4)-β-GlcNAc-(1→N)-Asn 6 ↑ 1 α-Fuc		+		

(continued)

Table 2 (*continued*)

Compound	Glycan structure	Elution profile			
		FNR	FR1	FR2	FR3
15	α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6) β-Man-(1→4)-β-GlcNAc-(1→4)-β-GlcNAc-(1→N)-Asn 6 ↑ 1 α-Fuc	+			
16	α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6) β-Man-(1→4)-β-GlcNAc-(1→4)-β-GlcNAc-(1→N)-Asn 3 ↑ 1 α-Fuc 6 ↑ 1 α-Fuc	+			
17	β-Gal-(1→4)-β-GlcNAc-(1→4) β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6) β-Man-(1→4)-β-GlcNAc-(1→4)-β-GlcNAc-(1→N)-Asn			+	
18	β-Gal-(1→4)-β-GlcNAc-(1→4) β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6) β-Gal-(1→4)-β-GlcNAc-(1→6) β-Man-(1→4)-β-GlcNAc-(1→4)-β-GlcNAc-(1→N)-Asn			+	
19a	[β-Gal-(1→4)-β-GlcNAc- 3 ↑ 1 α-Fuc] (1→2) (1→4) (1→6) [α-Man-(1→3) α-Man-(1→6) β-Man-(1→4)-β-GlcNAc-(1→4)-β-GlcNAc-(1→N)-Asn 6 ↑ 1 α-Fuc]	+			
20a	α-Gal-(1→4)$_2$– α-NeuAc-(2→6)$_1$– β-Gal-(1→4)-β-GlcNAc-(1→4) β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) β-Gal-(1→4)-β-GlcNAc-(1→2) β-Gal-(1→4)-β-GlcNAc-(1→6)-α-Man-(1→6) β-Gal-(1→4)-β-GlcNAc-(1→4) β-Man-(1→4)-β-GlcNAc-(1→4)-β-GlcNAc-(1→N)-Asn	+			
21	β-GlcNAc-(1→4) β-GlcNAc-(1→2)-α-Man-(1→3) β-GlcNAc-(1→2)-α-Man-(1→6) β-GlcNAc-(1→4)-β-Man-(1→4)-β-GlcNAc-(1→4)-β-GlcNAc-(1→N)-Asn	+			
22	α-NeuAc-(2→3)-β-Gal-(1→4)-β-GlcNAc-(1→4) α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3) α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6) β-Man-(1→4)-β-GlcNAc-(1→4)-β-GlcNAc-(1→N)-Asn	+			

[a] For oligosaccharides 10–12 and 19–20, brackets around the antennae mean that the exact position of the mono- or oligosaccharide residues substituting the basic structures is not known.

The data in Table 2 demonstrate that the lectin interacts with all of the glycopeptides or oligosaccharides on condition that they possess at least two unmasked β-Gal(1-4)-β-GlcNAc 1-sequences (structures **3**, **6–12**, **14**, **17**, **18**). However, the *B. frondosa* lectin does not show any affinity for a penta-antennary glycopeptide from turtle-dove ovomucoid (structure **20**), which possesses two unmasked *N*-acetyllactosamine sequences, two *N*-acetyllactosamine sequences each substituted by an α-D-galactose residue and a fifth *N*-acetyllactosamine sequence substituted by an α-NeuAc residue, perhaps because of steric hindrance introduced by these substituting residues. In this respect, *B. frondosa* lectin is similar to *Erythrina* lectins [22].

Substitution of the *N*-acetyllactosamine sequence by sialic acid residues (structures **4**, **13**, **15**, **16**, **22**), completely abolishes the affinity of the lectin for any saccharide. Substitution of the β-linked mannose residue by an additional β(1-4)GlcNAc residue (structure **5**) abolishes the affinity of *B. frondosa* lectin for this kind of structure, as compared to the unsubstituted one (structure **6**). In this respect, *B. frondosa* lectin resembles the *Ricinus communis* agglutinins RCAI and RCAII [23], *Erythrina* lectins [22] and, to some extent, Con A [23, 24].

The presence of one or several fucosyl residues linked α(1-3) to the peripheral *N*-acetylglucosamine residues of *N*-acetyllactosamine-type glycopeptides (structures **16** and **19**), completely abolishes the interaction between the glycopeptides and the immobilized lectin. Such influence of α-Fuc(1-3)GlcNAc on binding to Con A, to erythro- and leucoagglutinating lectins from *Phaseolus vulgaris*, and to *Erythrina* lectins, has also been reported previously [22, 25, 26].

The affinity between *B. frondosa* lectin and oligosaccharides increases with the number of unmasked *N*-acetyllactosamine sequences present in the carbohydrate structures. This clearly occurs in the interaction between the immobilized lectin and structures **6–9**. In this respect, *B. frondosa* lectin also behaves like *Erythrina* lectins [22] and *Ricinus* lectins [23].

The behaviour of *N*-acetyllactosamine-type oligosaccharides and glycopeptides on a column of *B. frondosa* lectin immobilized on Sepharose 4B confirms and extends the results obtained by hapten inhibition of haemagglutination. These results show that the sugar-binding specificity of this Gal/GalNAc-specific lectin is directed towards unmasked *N*-acetyllactosamine sequences. They also indicate that, after careful calibration with well-defined oligosaccharides and glycopeptides, the immobilized *B. frondosa* lectin could provide a valuable tool for both the fractionation and structural analysis of *N*-acetyllactosamine-containing oligosaccharides and glycopeptides of diverse origins. Further, due to the high affinity of *B. frondosa* lectin for *N*-acetylgalactosamine, we plan to study the behaviour of asparagine-linked oligosaccharides containing terminal nonreducing β-linked *N*-acetylgalactosamine residues found in bovine lutropin [27] or in Tamm-Horsfall glycoprotein [28] on a column of *B. frondosa* lectin. The affinity of the immobilized lectin for Asn-linked oligosaccharides with α-linked *N*-acetylgalactosamine residues, present in human small intestinal epithelial cells from blood group A individuals [29], should also be examined.

Acknowledgements

This work was supported in part by the Centre National de la Recherche Scientifique (Unité Mixte n° 111, Relations structure-fonction des constituants membranaires; Dir. Prof. A. Verbert), by the Université des Sciences et Techniques de Lille-Flandre-Artois and by the Ministère de l'Education Nationale. The authors are very grateful to Drs. B. Fournet, F. Piller, G. Spik and G. Strecker for providing the glycopeptides and oligosaccharides used in this study.

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