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Interactions of *Bowringia mildbraedii* agglutinin with complex- and hybrid-type glycans

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Affinity chromatography on *Bowringia mildbraedii* agglutinin (BMA) Sepharose of glycopeptides confirmed a previous report using oligosaccharides (Animashaun, T. and Hughes, R.C. (1989) J. Biol. Chem. 264,4657–4663) that high affinity binding requires the sequence Manα1→2 Manα1→6 Manα1→6 Manβ1→4. However, moderate binding was still exhibited by structures lacking this sequence provided the oligosaccharide core sequence Manα1→3[Manα1→6]Manβ1→4GlcNAc was present. This moderate binding was not affected by substitution with *N*-acetylglucosamine at C2 and C4, respectively, of the Manα1→3 and Manβ1→4 residues and BMA Sepharose should prove to be a useful tool for the isolation of bisected or non-bisected hybrid-type glycans.

Bowringia mildbraedii agglutinin; Lectin; Carbohydrate binding specificity

1. INTRODUCTION

Previously we described a novel lectin isolated from a Nigerian legume *Bowringia mildbraedii* Harms [1]. The lectin, *Bowringia mildbraedii* agglutinin or BMA, was shown to differ significantly in carbohydrate-binding specificity from other well-known lectins of leguminous plants such as Concanavalin A (Con A). Oligosaccharides bearing the sequence Manα1→2 Manα1→6 Manα1→6 Man, for example $Man_9GlcNAc$ and certain structural isomers of $Man_8GlcNAc$ and $Man_7GlcNAc$ released from glycoproteins with endoglycosidase H, interacted with BMA more avidly than other $Man_8GlcNAc$ and $Man_7GlcNAc$ isomers. More highly processed structures such as $Man_6GlcNAc$ and $Man_5GlcNAc$ were shown to bind relatively weakly. Thus, BMA binds preferentially to glycans present in glycoproteins subjected to early steps of oligosaccharide processing in the endoplasmic reticulum and less so to those exposed to more extensive processing by Golgi mannosidases [2], making BMA a useful histochemical marker for the endoplasmic reticulum [1].

We have now examined further the carbohydrate-binding specificity of BMA using glycopeptide and oligosaccharide standards. The results confirm and extend the previously reported [1] specificity for oligomannosidic structures and show that affinity chromatography on BMA Sepharose is a useful tool for the separation of oligomannosidic glycopeptides as well as endo H-released oligosaccharides. The binding of complex- and hybrid-type glycans to BMA Sepharose has also been studied.

Abbreviations: BMA, *Bowringia mildbraedii* agglutinin; Con A, Concanavalin A; endo H, endoglycosidase H; endo D, endoglycosidase D; $GlcNAc_{OT}$, *N*-acetylglucosaminitol.

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2. EXPERIMENTAL

2.1. *Materials*

BMA was purified from *Bowringia mildbraedii* seeds by affinity chromatography on *p*-aminophenyl-α-D-mannopyranoside-agarose [1]. BMA Sepharose was prepared as described [1] and Con A Sepharose was from Pharmacia/LKB, Milton Keynes, UK. 2-[^{3}H]Mannose (13.4 Ci/mmol), [L-5,6-^{3}H]fucose (45 Ci/mmol) and [1-^{14}C]acetic anhydride (32 Ci/mmol) were from Amersham International, UK. Sodium boro [^{3}H]hydride (600 Ci/mmol) was from NEN Dupont. Endoglycosidases-H (endo H) and -D (endo D) were from Boehringer-Mannheim. *Arthrobacter ureafaciens* sialidase was from Nacalai Tesque, Kyoto, Japan. Other reagents were from Sigma Corp.

2.2. *Oligosaccharides and glycopeptides*

Oligosaccharide 1 (Table 1) was prepared from BHK cells, metabolically labelled for 2 days with [^{3}H]mannose, as described [3]. Briefly, the glycopeptides obtained from labelled cells by pronase digestion were fractionated on Con A Sepharose, the oligomannosidic glycopeptides eluted with 200 mM methyl-α-mannoside were treated with endo H and the released oligosaccharides were separated by paper chromatography. Oligosaccharide 2 was prepared from the biantennary glycan released from human serum transferrin [4] by hydrazinolysis followed by exhaustive digestion with *Arthrobacter ureafaciens* sialidase followed by jack bean β galactosidase and β *N*-acetylglucosaminidase. This and other oligosaccharides, as indicated, were labelled by reduction with NaB^3H_4 [1]. Glycopeptides 3 and 4 were obtained from [^{3}H]mannose- or [^{3}H]fucose-labelled ricin-resistant Ric^R21 cells as describe d [5]. Oligosaccharides 5, 6 and 7 were obtained from swainsonine-treated BHK cells, metabolically labelled with [^{3}H]mannose for 2 days, by sequential chromatography of glycopeptides on Con A Sepharose, lentil lectin Sepharose and DEAE Sephacel columns followed by endo H digestion, exo-glycosidase treatments and Bio-Gel P-4 chromatography as described [6,7]. Glycopeptides 8 and 9 and oligosaccharides 16 and 17 were gifts from G. Strecker (Universite des Sciences et Techniques, Lille, France). The

glycopeptides were labelled by treatment with [1-^{14}C]acetic anhydride [5]. The remaining compounds were gifts from S. Sato (NIMR, London) and were prepared and labelled as described [8].

2.3. *Pulse-chase labelling*

Ricin-resistant Ric^{R}14 cells [9,10] were grown at 37°C in 6 cm diameter dishes as monolayers in Eagle's medium supplemented with 10% foetal calf serum. Nearly confluent monolayers were rinsed twice with Eagle's medium containing 2 mM (one tenth) of the usual concentration of glucose and 10% dialysed serum and incubated for 30 min with the same medium (2 ml) containing 1 mCi [^{3}H]mannose. After labelling, the monolayers were rinsed twice with Eagle's medium containing the normal amount of glucose plus 5 mM non-radioactive mannose and chased at 37°C for 4 h. The cells were harvested, lipid extracted and a glycopeptide fraction was obtained by pronase digestion of cellular glycoproteins as described [11]. Briefly, defatted cell residues were suspended in 50 mM Tris–HCl, pH 7.5, 20 mM $CaCl_2$, heated at 90°C for 5 min and then treated with 100 μl of pronase (0.4% in the above buffer) at 37°C for 2 days. The digests were re-heated and the glycopeptides were isolated by Bio-Gel P-2 chromatography.

2.4. *Endo glycosidase digests*

Glycopeptides dissolved in 0.2 M sodium acetate buffer, pH 6, (20 μl) were treated with endo H (2.5 μl, 2.5 mU) plus endo D (2.5 μl, 2.5 mU) at 37°C for 1–2 days. After incubation the digests were heated at 90°C for 5 min.

2.5. *Chromatography*

Radioactively labelled glycopeptide or oligosaccharide samples (approximately 1–2 × 10^3 counts/min) were applied in 1 ml of elution buffer 10 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$ to columns (1.5 ml) of BMA Sepharose or Con A Sepharose equilibrated with elution buffer at room temperature. After incubation for 1 h at room temperature the columns were washed sequentially with elution buffer (9 ml) and 10 ml each of 10 mM methyl-α-glucoside, 10 mM methyl-α-mannoside and 200 mM methyl-α-mannoside in elution buffer. Fractions (1 ml) were collected and counted for radioactivity. Paper chromatography of oligosaccharides was performed on Whatman 1 Chr paper using ethyl acetate/pyridine/acetic acid/water (5:5:1:3, by volume) as solvent. Following development for 3–5 days the dried paper was cut into 1 cm segments: each segment was eluted with 1 ml of water and eluates were counted for radioactivity.

3. RESULTS AND DISCUSSION

3.1. *Interactions of BMA with oligomannosidic glycopeptides*

The fact that BMA appears to be a marker for the endoplasmic reticulum [1], expected to be rich in poorly processed oligomannosidic structures [2], suggested that the unique binding specificity of BMA established using endo H-released oligosaccharides [1] could be extended to peptide-linked glycans. Results (Fig. 1) of affinity chromatography on BMA Sepharose of a glycopeptide mixture obtained from the ricin-resistant Ric^{R}14 cell line, deficient in GlcNAc transferase I and unable to convert oligomannosidic structures into complex- or hybrid-type glycans [9,10], supported this supposition. Glycopeptides generated from [^{3}H]mannose-labelled cells were treated with endo H and endo D and the released oligosaccharides were analyzed by paper chromatography. The major products were $Man_5GlcNAc$ and $Man_9GlcNAc$ with smaller, about

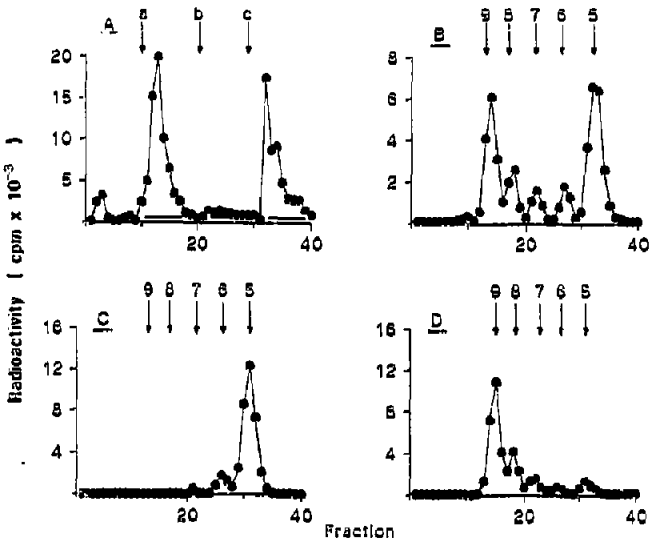


Fig. 1. Fractionation of oligomannosidic glycopeptides on BMA Sepharose. (A) A glycopeptide mixture obtained from [^{3}H]mannose-labelled Ric^{R}14 cells by pronase digestion (15 × 10^4 counts/min) was applied to BMA Sepharose. Fractions (1 ml) were eluted with buffer, 10 mM methyl-α-glucoside (a), 10 mM methyl-α-mannoside (b) and 200 mM methyl-α-mannoside (c). (B–D) Paper chromatography of endo H digests of total glycopeptides (B) and glycopeptides eluted from BMA Sepharose with 10 mM methyl-α-glucoside (C) or 200 mM methyl-α-mannoside (D). After chromatography using approximately 5 × 10^3 counts/min, the paper strips were cut into 1 cm segments, eluted with water and counted for radioactivity. The migration of standard oligosaccharides is indicated: (9) $Man_9GlcNAc$; (8) $Man_8GlcNAc$; (7) $Man_7GlcNAc$; (6) $Man_6GlcNAc$; (5) $Man_5GlcNAc$.

equal amounts of $Man_6GlcNAc$, $Man_7GlcNAc$ and $Man_8GlcNAc$ (Fig. 1B). When the glycopeptide mixture was subject ed to affinity chromatography on BMA Sepharose, major fractions were eluted with 10 mM methyl-α-glucoside and with 200 mM methyl-α-mannoside (Fig. 1A). Each glycopeptide fraction was treated with endo H and endo D and analyzed by paper chromatography. The material eluted with 10 mM methyl-α-glucoside contained over 90% of the $Man_5GlcNAc$ and $Man_6GlcNAc$ with a minor peak of $Man_7GlcNAc$ and no detectable $Man_8GlcNAc$ or $Man_9GlcNAc$ (Fig. 1C). This material eluted with 200 mM methyl-α-mannoside contained all of the $Man_9GlcNAc$ and $Man_8GlcNAc$, about 70% of the $Man_7GlcNAc$ and only trace amounts of $Man_6GlcNAc$ and $Man_5GlcNAc$ (Fig. 1D).

3.2. *Interactions with complex- and hybrid-type glycans*

Eighteen oligosaccharides or glycopeptides of known structures (Table 1) were analysed by BMA Sepharose affinity chromatography under standard conditions given in section 2. Representative elution profiles are shown in Fig. 2 and all of the results are collected in Table 1. In the following discussion we refer to moderate- and high-affinity binding in relation to oligosac-

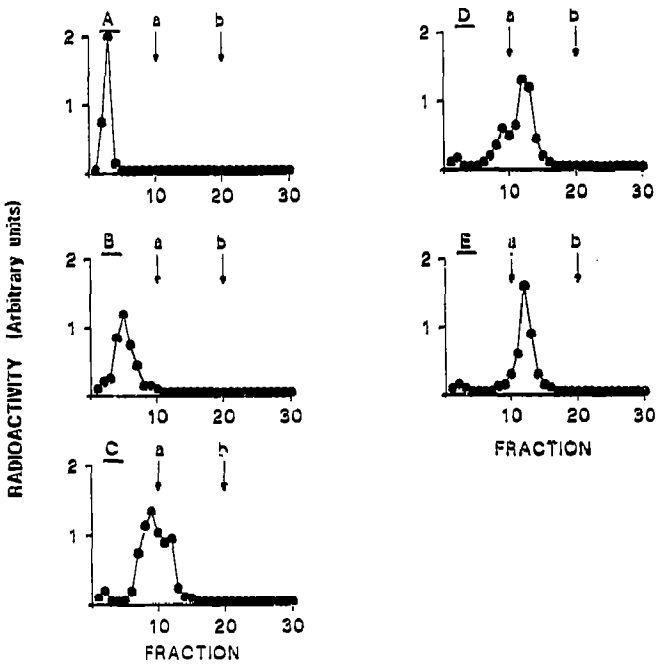


Fig. 2. Affinity chromatography of complex- and hybrid-type oligosaccharides on BMA Sepharose. Samples (approximately 10^3 counts/min) were applied in 1 ml of elution buffer to columns (1 ml) of BMA Sepharose and fractions (1 ml) were eluted with elution buffer followed by 10 mM methyl-α-glucoside (a) and 10 mM methyl-α-mannoside (b) in elution buffer. No additional radioactivity was eluted with 200 mM methyl-α-mannoside. (A) Compound 18; (B) compound 14; (C) compound 9; (D) compound 8; (E) compound 2. See Table 1 for structures.

charides or glycopeptides eluted from lectin columns with 10 mM and 200 mM sugar, respectively. Oligosaccharides showing no affinity are eluted in the void volume with buffer and weak-affinity binding is indicated by a retarded elution with buffer.

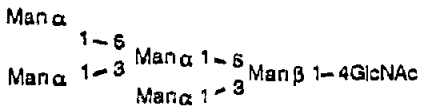
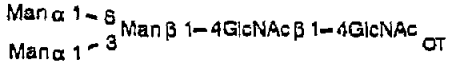
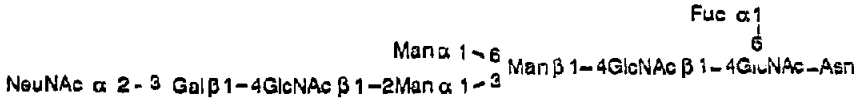
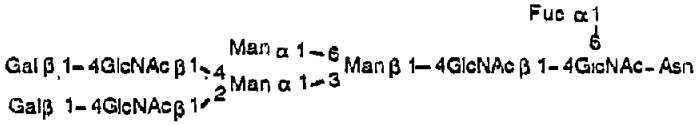
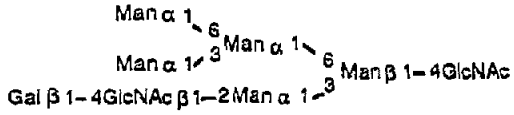
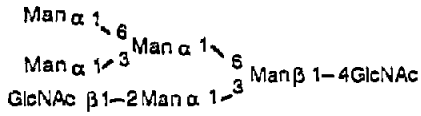
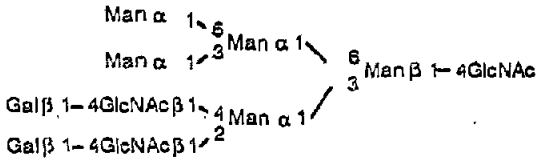
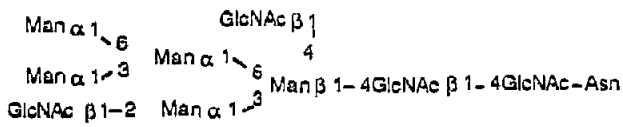
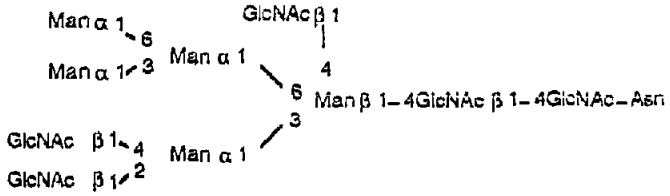
High affinity binding to Con A requires only two appropriately placed, non-reducing mannose residues [12,13], as are present on the outer branches of compounds 1 and 2 (Table 1), and the Manα1→2 Manα1→6 Manα1→6 sequence recognised by BMA (Fig. 1 and [1]) is not an essential requirement. Conversely, two non-reducing mannose termini are not by themselves sufficient for high affinity binding to BMA, since compounds 1 and 2 were eluted from BMA Sepharose with 10 mM sugar (Table 1). However, we showed previously [1] that a straight-chain compound Manα1→3 Manβ1→4GlcNAc bound weakly to BMA Sepharose and was eluted with buffer alone, showing that two interacting mannose units do increase the affinity of glycans for BMA as they do for Con A [12]. In the case of Con A, substitution of the interacting mannose residues at C2 may weaken but does not abolish binding [12,13] and compound 3 (Table 1) binds with high affinity. Similarly, with BMA, substitution of the Manα1→3 unit at C2 in compound 3 (Table 1) had only a small weakening effect on recognition of the core pentasaccharide, compound 2 (Table 1). However, substitution at C2 and C4, as in compound 4 (Table 1), completely abolished binding to BMA, as it does for high affinity Con A binding [5]. By contrast, the Man_5-hybrid structures 5 and 7 (Table 1) bound to similar extents to BMA Sepharose presumably due to the presence in both compounds of two non-reducing mannose residues in the outer branches. Similarly, Con A binds with high affinity to both these compounds [6,14]. Interestingly, shortening of the antennae on the Manα1→3 residue of the core, and exposure of a non-reducing GlcNAc residue (compound 6, Table 1) appeared to increase interactions with BMA Sepharose.

BMA retains interactions with bisected hybrid-type compounds during affinity chromatography as shown by similar chromatographic behaviours of compounds 6 and 8, and compounds 7 and 9, respectively (Table 1). Attachment of a bisecting GlcNAcβ1→4 residue to the β-linked mannose is believed to restrict the rotational flexibility of the α1→6 linked mannose unit [15] and appears to hinder adoption of a conformation suitable for high affinity binding to Con A [12,13,16]. Evidently the orientation of the α1→6 linked mannose relative to the C5–C6 bond of the core β-linked mannose, especially in monoantennary hybrid-type compounds, plays a less important role in binding to BMA than it does to Con A, or the two lectins recognize different conformational variants of this structure.

As is the case for affinity chromatography on Con A Sepharose [12], substitution of both α-linked mannose residues of the core pentasaccharide at C2, as in biantennary complex-type structures 10–12 (Table 1), weakened binding to BMA Sepharose but did not abolish it and the compounds were eluted by buffer in a retarded position (Table 1, Fig. 2). In agreement with the results obtained for hybrid-type compounds discussed above, exposure of a terminal GlcNAc residue in compound 13 increased significantly the binding of a biantennary glycan as shown by the more retarded elution position of compound 13 compared with the fully galactosylated derivatives 10–12. A similar behaviour has been demonstrated for Con A binding [17]. The biantennary compound 14, containing Galβ1→3GlcNAc termini, behaved similarly on BMA Sepharose as compounds 10–12 with a Galβ1→4GlcNAc terminal sequence. The behaviour on BMA Sepharose of tri- and tetra-antennary glycans, compounds 15–18 showed (Table 1, Fig. 2) that all of these structures are unretarded as they are on Con A Sepharose [12].

In conclusion, the present data extend knowledge of the carbohydrate binding specificity of BMA. The overall similarities in binding specificities suggest that BMA shares a common evolutionary origin with Con A and probably other legume lectins. However, BMA clearly shows some unique binding properties and represents a

Table 1 Structure of oligosaccharides and glycopeptides and their elution profiles on BMA Sepharose

Sample	Structure	Elution Profile [1]
1		E
2		E
3		D
4		A
5		C
6		D
7		C
8		D
9		C

10	Galβ1-4GlcNAcβ1-2Manα1-6 Galβ1-4GlcNAcβ1-2Manα1-3 Manβ1-4GlcNAcβ1-4GlcNAc-Asn	B
11	Galβ1-4GlcNAcβ1-2Manα1-6 Galβ1-4GlcNAcβ1-2Manα1-3 Manβ1-4GlcNAcβ1-4GlcNAc OT	B
12	Galβ1-4GlcNAcβ1-2Manα1-6 Galβ1-4GlcNAcβ1-2Manα1-3 Manβ1-4GlcNAc OT	B
13	GlcNAcβ1-2Manα1-6 Galβ1-4GlcNAcβ1-2Manα1-3 Manβ1-4GlcNAc OT	C
14	Galβ1-3GlcNAcβ1-2Manα1-6 Galβ1-3GlcNAcβ1-2Manα1-3 Manβ1-4GlcNAcβ1-4GlcNAc OT	B
15	Galβ1-4GlcNAcβ1-2Manα1-6 Galβ1-4GlcNAcβ1-4Manα1-3 Manβ1-4GlcNAcβ1-4GlcNAc OT Galβ1-4GlcNAcβ1-2	A
16	Galβ1-4GlcNAcβ1-2Manα1-6 Galβ1-4GlcNAcβ1-4Manα1-3 Manβ1-4GlcNAc OT Galβ1-4GlcNAcβ1-2	A
17	Galβ1-4GlcNAcβ1-6 Galβ1-4GlcNAcβ1-2Manα1-6 Galβ1-4GlcNAcβ1-2Manα1-3 Manβ1-4GlcNAc OT	A
18	Galβ1-4GlcNAcβ1-6 Galβ1-4GlcNAcβ1-2Manα1-6 Galβ1-4GlcNAcβ1-4Manα1-3 Manβ1-4GlcNAcβ1-4GlcNAc OT Galβ1-4GlcNAcβ1-2	A

1 See fig. 2

new and useful member of this family for the separation and analysis of glycan structures.

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