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Interactions of five D-mannose-specific lectins with a series of synthetic branched trisaccharides*,†

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ABSTRACT

The interaction of a series of synthetic, branched trisaccharides with five D-mannose-specific lectins was studied by precipitation-inhibition assay. The branched methyl α-D-mannotrioside, α-D-Man*p*-(1→3)-[α-D-Man*p*-(1→6)]-α-D-Man*p*OMe, the best inhibitor of the Con A–Dextran interaction, was 42 times more potent than α-D-Man*p*OMe, and 3–6 times more potent than the two trisaccharides substituted with D-glucosyl groups, and 8–15 times those with D-galactosyl groups. Surprisingly, methyl *O*-α-D-mannopyranosyl-(1→3)-α-D-mannopyranoside was bound to Con A 8-fold more avidly than methyl α-D-mannopyranoside. However, the related pea lectin (PSA) was singularly different from Con A in its carbohydrate-binding activity, showing no significantly enhanced binding to any of the sugars examined. The trisacchrides containing terminal, nonreducing, (1→3)-linked α-D-mannopyranosyl groups, *i.e.*, α-D-Man*p*-(1→3)-[α-D-Glc*p*-(1→6]-α-D-Man*p*OMe, α-D-Man*p*-(1→3)]-α-D-Gal*p*-(1→6)]-α-D-Man*p*OMe, and α-D-Man*p*-(1→3)-[α-D-Man*p*-(1→6)]-α-D-Man*p*OMe, were the best inhibitors of the snowdrop lectin (GNA)–D-mannan precipitation system. On the other hand, all branched trisaccharides exhibited very similar inhibitory potencies toward the daffodil lectin (NPA)–D-mannan interaction, whereas α-D-Man*p*-(1→3)-[α-D-Gal*p*-(1→6)]-α-D-Man*p*OMe and α-D-Man*p*-(1→3)-[α-D-Man*p*-(1→6)]-α-D-Man*p*OMe were somewhat better inhibitors than the other branched trisaccharides of the amaryllis lectin (HHA)–D-mannan precipitation reaction. Of the oligosaccharides studied, the linear trisaccharide α-D-Man*p*-(1→6)-α-D-Man*p*-(1→6)-D-Man appears to be the most complementary to the combining site(s) of NPA and HHA.

INTRODUCTION

Lectins from the snowdrop (*Galanthus nivalis*, GNA), daffodil (*Narcissus pseudonarcissus*, NPA), and amaryllis (*Hippeastrum hybr.*, HHA), which belong to the Amarylliaceae family of monocotyledonous plants, have been isolated from their bulbs and a detailed study of their carbohydrate-binding specificity reported[1-4]. These lectins can readily distinguish D-mannose from D-glucose units, demonstrating a marked preference for an axial OH-2. This property distinguishes these monocotyledonous lectins from other D-mannose–D-glucose-binding lectins [*e.g.*, Con A, pea, lentil, and *Vicia*

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faba (broad bean) lectin], which also recognize D-glucose and *N*-acetyl-D-glucosamine units.

These three monocotyledonous lectins differ somewhat in their fine carbohydrate-binding specificity and in their recognition of oligo- and poly-saccharides containing D-mannosyl groups[3,4]. In their immobilized form, they also show different chromatographic behavior toward glycosyl-asparagine glycopeptides. For example, terminal *O*-α-D-mannopyranosyl-(1→3)-D-mannose units are necessary for interaction with GNA[3]. On the other hand, NPA and HHA recognize internal as well as terminal D-mannose units. Additionally, NPA exhibits a preference for *O*-α-D-mannopyranosyl-(1→6)-D-mannose units whereas HHA interacts with high affinity toward both (1→3)- and (1→6)-linked α-D-mannopyranosyl groups[4].

Concanavalin A (Con A) is classified as a D-mannose–D-glucose-specific lectin, which requires the D-*manno* or D-*gluco* configuration for lectin binding. Carver *et al.*[5] and Brewer and assoc.[6–10] have studied the interaction of Con A with oligosaccharide fragments of complex, high-mannose and hybrid-type glycopeptides by X-ray crystallographic refinement techniques with coordinates for the glycopeptides obtained from ^{1}H-n.m.r. measurements[5], and by nuclear magnetic relaxation dispersion (n.m.r.d.) and precipitation assay[6–10]. Their results demonstrated an extended binding site in Con A which appears to recognize α-D-Man*p*-(1→3)-[α-D-Man*p*-(1→6)]-D-Man units and their unique spacial arrangement. In addition, the binding specificity of Con A is directed toward this component in larger complex, bisected-hybrid, and high-mannose-type glycopeptides.

Pea lectin is also a D-mannose–D-glucose-specific lectin; its carbohydrate-binding specificity has been studied by inhibition of agglutination and precipitation[11,12]. Interestingly, unlike Con A, 3-*O*-substituted monosaccharides (3-*O*-methyl-D-glucose, 3-*O*-benzyl-D-glucose, methyl 2,3-di-*O*-methyl-α-D-glucopyranoside, and methyl 2-acetamido-2-deoxy-3-*O*-methyl-α-D-glucopyranoside are at least 10 times more strongly inhibitory than their parent sugars[12].

We report herein the interaction of the aforementioned five lectins with a series of synthetic branched trisaccharides substituted, at O-3 or O-6 of the D-mannose unit with D-glucopyranosyl (C-2 epimer of mannose) or D-galactopyranosyl (which differs in configuration at C-2 and C-4 from D-mannose) groups. We also compare the carbohydrate-binding specificty of these five D-mannose-binding lectins as studied by a precipitation-inhibition assay.

EXPERIMENTAL

Lectins. — The snowdrop, daffodil, and amaryllis lectins were isolated from extracts of their bulbs by affinity chromatography on an immobilized D-mannose column as previously reported[1,2]. Concanavalin A was purified by the procedure of Agrawal and Goldstein[13]. Pea lectin (PSA) was the gift of Dr. J. P. Carver of the University of Toronto.

Sugars. — Methyl α-D-mannopyranoside (αManMe), and methyl *O*-α-D-mannopyra-

nosyl-(1→6)- and -(1→3)-α-D-mannopyranosyl (αMan→6 ManMe and αMan→3ManMe) were purchased from Pfanstiehl Laboratories, Inc. (Waukegan, IL) and Sigma Chemical Co. (St. Louis, MO), respectively. *O*-α-D-Mannopyranosyl-(1→3)-D-mannose (αMan→3Man) and *O*-α-D-mannopyranosyl-(1→6)-*O*-α-D-mannopyranosyl-(1→6)-D-mannose (αMan→6αMan→6Man) were the gifts of Dr. K. L. Matta of Roswell Park Memorial Institute, Buffalo, NY. The synthesis of the trisaccharides **1**, **2**, **3**, and **4** has been published (see Scheme 1)[14]. Trisaccharide **5** was the gift of Dr. J. P. Carver of the University of Toronto. The D-mannans and Dextran B-1355-S used were available from a previous study[4].

Precipitation-inhibition assay. — Sugar inhibition of the precipitation reaction was carried out by adding increasing amounts of the branched oligosaccharides to the Con A–Dextran B-1355-S (14.7 μg of Con A and 5 μg of Dextran), PSA–*Saccharomyces cerevisiae* D-mannan (120 μg PSA and 20 μg D-mannan), GNA–*H. capsulata* D-mannan (15 μg GNA and 15 μg Mannan), and NPA (15 μg)– and HHA (10 μg)–*P. pastoris* D-mannan (10 μg) precipitation systems in a total volume of 100 μL as previously described[3,4,15]. After incubation at 37° for 1 h, followed by storage for 2 days at 4°, the precipitates were collected in a microcentrifuge (11 500 r.p.m., 10 min) and washed twice with cold 10mM phosphate buffer (pH 7.2) containing 0.1mM $CaCl_2$, 0.04% NaN_3 and 150mM NaCl. The protein content of the precipitates was determined by the method of

α-D-Gal*p*
1
↓
6
α-D-Man*p*-(1→3)-α-D-Man*p*OMe

1 [αMan→3(αGal→6)αManMe]

α-D-Man*p*
1
↓
6
α-D-Gal*p*-(1→3)-α-D-Man*p*OMe

2 [αGal→3(αMan→6)αManMe]

α-D-Glc*p*
1
↓
6
α-D-Man*p*-(1→3)-α-D-Man*p*OMe

3 [αMan→3(αGlc→6)αManMe]

α-D-Man*p*
1
↓
6
α-D-Glc*p*-(1→3)-α-D-Man*p*OMe

4 [αGlc→3(αMan→6)αManMe]

α-D-Man*p*
1
↓
6
α-D-Man*p*-(1→3)-α-D-Man*p*OMe

5 [αMan→3(αMan→6)αManMe]

Scheme 1. Structures and abbreviations of synthetic, branched trisaccharides.

Lowry *et al.*[16] using bovine serum albumin as standard. Sugar concentrations were determined by the phenol–H_2SO_4 procedure[17].

RESULTS AND DISCUSSION

It was a great surprise to discover that the disaccharide glycoside α-D-Man*p*-(1→3)-α-D-Man*p*OMe was almost 8 times more potent an inhibitor than α-D-Man*p*OMe of the Con A–Dextran precipitation system. The fact that α-D-Man*p*-(1→3)-D-Man was only twice as good an inhibitor as α-D-Man*p*OMe (also observed by Bhattacharyya *et al.*[9] using a hemagglutination–inhibition of rabbit erythrocyte assay) indicated that the methyl α-D-glycosidic linkage of α-D-Man*p*-(1→3)-α-D-Man*p*OMe probably makes an important contribution to the binding energy. Until this finding, we had observed a substantial increase in inhibitory potency with an increase in the number of glycosyl units of an homologous series only in the case of the (1→2)-α-D-mannopyranosyl-oligosaccharide series. In that case, α-D-Manp-(1→2)-D-Man and α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-D-Man were 4- and 24-fold more active, respectively, than α-D-Man*p*OMe[15], a phenomenon attributed initially to an extended binding site; later, it was argued to represent a statistical effect[18] due to the possible interaction of the Con A binding sites with OH-3, OH-4, and OH-6 of the (1→2)-linked α-D-mannosyl residues[15] (see however William *et al.*[19]). But this argument cannot be advanced for α-D-Man*p*-(1→3)-D-Man inasmuch as OH-3 of the reducing D-mannose residue is involved in a glycosidic bond. Therefore, it would appear reasonable to attribute this enhanced inhibitory activity to an extended binding site. On the basis of modeling studies and thermodynamic calculations, a similar conclusion was reached by Carver and Mackenzie[20].

Of the synthetic, branched trisaccharides (**1**–**5**) studied by inhibition of precipitation analysis, α-Man→3(Man→6)αManMe (**5**) was the best inhibitor of the Con A–Dextran interaction (Fig. 1), being 42 times more potent than α-D-Man*p*OMe, and 6 and 16 times more potent than the α-D-(1→3)- and α-D-(1→6)-linked methyl mannobiosides, respectively. Compound **5** was also 8 to 15 times better an inhibitor than the trisaccharides containing D-galactosyl groups (**1** and **2**), and 3 to 6 times more active than **3** and **4**, in which D-glucosyl groups replaced D-mannosyl groups in the methyl (3→6)-linked D-mannotrioside (Tables I and II). Furthermore, substitution of a D-glucosyl group at O-3 of the penultimate D-mannosyl residue gave a trisaccharide **4** that was only 50% more active than the trisaccharide (**2**) containing a D-galactosyl group at this position; however trisaccharide **1** was 15 and 6 times less active than **5** and **3**, respectively. These observations suggested that equatorial OH-4 of the α-D-mannosyl residue linked at O-6 may be more important a requirement for binding than that of the configuration at C-2.

It is well established that Con A requires the D-*manno* configuration for optimal lectin binding[21]; the D-*manno* configuration, with an axial OH-2, binds to Con A with a 4.5-fold higher affinity than that of the D-*gluco* configuration. αMan→6αMan→6Man was similar in inhibitory potency to α-D-Man*p*OMe and, interestingly,

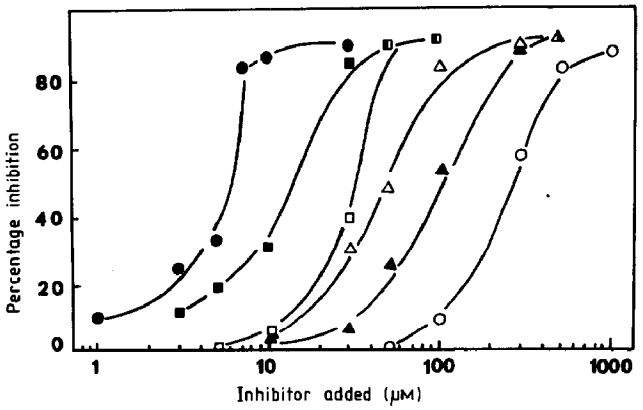

Fig. 1. Inhibition by branched trisaccharides **1**–**5** and methyl α-D-mannopyranoside (α-D-Man*p*OMe) of Con A–Dextran B-1355 interaction. Each tube contained Con A (15 μg), Dextran (5 μg), and inhibitor as noted, in a total volume of 100 μL; (●) **5**, (■) **3**, (□) **4**, (△) **2**, (▲) **1**, and (○) α-D-Man*p*OMe.

TABLE I

Inhibition of D-mannose-specific lectins by synthetic, branched trisaccharides

Sugar	*Concentration* (mM) *required for 50% inhibition*				
	Con A[a]	*PSA*[b]	*GNA*[c]	*NPA*[d]	*HHA*[d]
α-D-Man*p*OMe	0.26	0.71	5.6	6.4	13.0
αMan→3(αMan→6)αManMe (**5**)	0.0062	0.55	0.39	1.9	1.5
αGlc→3(αMan→6)αManMe (**4**)	0.034	0.45	4.0	2.4	4.6
αMan→3(αGlc→6)αManMe (**3**)	0.016	0.43	0.16	2.2	3.5
αGal→3(αMan→6)αManMe (**2**)	0.052	1.18	2.0	2.2	4.8
αMan→3(αGal→6)αManMe (**1**)	0.094	0.58	0.21	1.9	1.6

[a] In the Con A–Dextran B-1355 precipitation system. [b] In the PSA–*S. cerevisiae* D-mannan precipitation system. [c] In the GNA–*H. capsulata* D-mannan precipitation system. [d] In the *P. pastoris* D-mannan precipitation system.

TABLE II

Inhibition of D-mannose-specific lectins by branched trisaccharides

Sugar	*Relative inhibitory potency*				
	Con A	*PSA*	*GNA*	*NPA*	*HHA*
α-D-Man*p*OMe	1.0	1.0	1.0	1.0	1.0
αMan→3αManMe	7.6	1.0	8.9	2.6	7.0
αMan→6αManMe	2.7	0.5	2.7	4.3	5.5
αMan→6αMan→6Man	1.6	0.9	3.7[a]	10.3	13.0
αMan→3(αMan→6)αManMe (**5**)	41.9	1.4	14.3	3.2	9.0
αGlc→3(αMan→6)αManMe (**4**)	7.6	1.6	1.4	2.5	2.8
αMan→3(αGlc→6)αManMe (**3**)	16.3	1.7	35.0	2.7	3.7
αGal→3(αMan→6)αManMe (**2**)	5.0	0.6	2.8	2.7	2.7
αMan→3(αGal→6)αManMe (**1**)	2.8	1.3	26.7	3.2	7.9

[a] From Shibuya *et al.*[3]

αMan→6αManMe was almost 3-fold more potent than α-D-Man*p*OMe, perhaps because it contains a methyl group in α configuration in place of the "potentially reducing" (1→6)-α-D-mannosyl residue which could hinder its binding to Con A.

Commenting on their molecular-modeling studies, Carver *et al.*[5] stated that the OH-3, OH-4, and OH-6 of the (1→6)-linked α-D-mannopyranosyl group in αMan→3(αMan→6)Man were directed toward the protein surface of the lectin, occupying the methyl α-D-mannoside site. This supports our observation that **3** has an affinity for Con A higher than that of **1**; however, **2** and **4**, both of which have a (1→6)-linked α-D-mannopyranosyl group in their structure, are less active than **3**. These observations suggested that the (1→3)-linked α-D-mannopyranosyl group in the mannotrioside **5** also may play an important role in the Con A interaction. In this regard, Carver *et al.*[5] also reported that the two α-D-mannopyranosyl groups must be linked (1→3) and (1→6) to a common D-mannose unit [αMan→3(αMan→6)Man] in order to achieve the greatest binding affinity to Con A, and that the specificity of Con A appears directed toward the trimannosyl core of *N*-linked oligosaccharides and glycopeptides. These investigators also commented that "no hydrogen bonding was found for the (1→3)-linked α-D-mannosyl group; however a considerable number of favorable van der Waals contacts are made"[5]. Employing the technique of nuclear magnetic relaxation dispersion, Brewer *et al.*[7] also reported that Con A contains an extended binding site which recognizes the two nonreducing α-D-mannosyl groups in αMan→3(αMan→6)Man. It should be noted that the free energy differences associated with the differences in inhibitory potency observed are on the order of 4 kJ or less (except for the 42-fold difference noted for the binding of **5** to Con A), and hence may correspond to very subtle differences in the mode of binding or the flexibility of the ligands.

The pea lectin (PSA) consisting of 2α and 2β subunits is related to Con A by a circular permutation of amino acid sequences, such that the α-chain corresponds to residues 70–119 of Con A, and the β-chain begins at residue 120 of Con A and passes through the carboxyl terminus of the chain to residue 69 of the lectin molecule[22,23]. As seen from the inhibition data in Tables I and II, PSA (and almost certainly the related lentil and *V. faba* lectins) is singularly different from Con A in its carbohydrate-binding specificity, showing no enhanced binding to αMan→3αManMe and little difference in the binding of the branched trisaccharides with the exception of a diminished reactivity toward **2**. It is especially noteworthly that **5** binds to the pea lectin with only slightly greater affinity than methyl α-D-mannopyranoside, an observation also made by Stubbs *et al.*[24]

Turning to the monocotyledonous lectins, compound **5** was 2 to 5 times better an inhibitor of the GNA–*H. capsulata* D-mannan precipitation system than the (1→3)- and (1→6)-linked methyl α-mannobiosides. Interestingly, **1** and **3** containing a nonreducing (1→3)-linked α-D-mannosyl end group had 5 to 25 times greater inhibitory activity than the other branched trisaccharides **2** and **4** (Tables I and II). These results confirmed the previous report by Shibuya *et al.*[3] that GNA has a high affinity for (1→3)-linked terminal D-mannosyl groups. At this time, we cannot explain why **5** is less inhibitory than **1** and **6**.

On the other hand, all the branched trisaccharides exhibited very similar inhibitory activity of the NPA–D-mannan precipitation system. However, NPA did recognize the linear mannotriose more strongly than the branched trisaccharides; αMan→6αMan→6Man exhibited a 3- to 4-fold greater inhibitory activity than the branched D-*manno*-trisaccharides (Table II).

Whereas **2**, **3**, and **4** appear to display very similar inhibitory activity of the HHA interaction, **1** and **5** showed somewhat greater activity than the other branched trisaccharides. Similar to NPA, HHA also binds preferentially to the α-D-(1→6)-linked linear mannotriose (Table II). Interestingly, the inhibitory activity of **1** is of the same order as that of **5**. However, when O-6 was substituted with an α-D-glucosyl group, the resulting trisaccharide **3** was only one half as potent as **1**. At the present time, we cannot explain these results.

In summary, we have investigated the binding specificity of five D-mannose-specific lectins with a series of unique synthetic, branched trisaccharides. These lectins showed differential specificity for these synthetic oligosaccharides. Notably, Con A showed high affinity for **5**; PSA showed no significant enhancement for any sugars; GNA recognized the terminal, nonreducing (1→3)-linked α-D-mannosyl groups; and NPA and HHA exhibited a specificity for the linear (1→6)-linked α-D-mannosyl residue (and group). The investigation of the detailed sugar-binding specificity of these lectins should make these proteins useful reagents for structural studies of D-mannose-containing glycoproteins, and oligo- and poly-saccharides.

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