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BINDING-SITE SPECIFICITY OF LECTINS FROM *Bauhinia purpurea alba*, *Sophora japonica*, AND *Wistaria floribunda**

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ABSTRACT

The binding-site specificities of lectins isolated from the seeds of *Bauhinia purpurea alba*, *Sophora japonica*, and *Wistaria floribunda* were studied by hemagglutination-inhibition assays utilizing a variety of saccharides as inhibitors. For *Bauhinia* lectin, 2-acetamido-2-deoxy-D-galactose was found to be the best monosaccharide inhibitor and the free monosaccharide inhibitor was as active as its glycosides. D-Galactose was a weak inhibitor and so were some of its glycosides. Some of the oligosaccharides having a D-galactose nonreducing terminus were good inhibitors, but substitution on the D-galactose or 2-acetamido-2-deoxy-D-galactose residues with other saccharides abolished the inhibitory activity. No specificity for anomeric configuration or linkage position could be demonstrated. The presence of aromatic aglycon groups did not enhance inhibitory activity of the saccharides tested and, in some cases, the inhibitory activity was decreased. In contrast to the results for the *Bauhinia* lectin, compounds having aromatic aglycon groups were markedly better inhibitors for *Sophora* and *Wistaria* lectins than the corresponding compounds without aromatic aglycons. D-Galactose was a weak inhibitor for *Sophora* and *Wistaria* lectins, whereas 2-acetamido-D-galactose was a poor inhibitor of *Sophora* lectin but a good inhibitor of *Wistaria* lectin. *Sophora* and *Wistaria* lectins were somewhat similar in their activity as some of the saccharides having a D-galactose in penultimate position to an L-fucose residue were weak inhibitors. However, *Sophora* lectin has a binding preference for β anomers, whereas *Wistaria* lectin did not demonstrate a clear preference for α or β anomers. For some pairs of compounds, the α was a better inhibitor than the β anomer; in other cases, the reverse was true.

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INTRODUCTION

The use of lectins has gained considerable popularity in recent years as a tool for the study of the carbohydrate constituents of cell surfaces[1-5] and glycoproteins[6-11]. The properties and uses of lectins have been the subject of several reviews[12-17]. We have engaged in the development of simple, affinity-chromatographic procedures for the isolation of lectins[18-20], and in the determination of their binding-site specificities by hemagglutination-inhibition assays[21]. We previously reported the development of procedures for the isolation of D-galactose- and 2-acetamido-2-deoxy-D-galactose-binding lectins on columns of acid-treated agarose[19]. Three of these lectins were from *Bauhinia purpurea alba*, *Sophora japonica*, and *Wistaria floribunda* seeds. These lectins were found to differ with respect to pH optima of ligand binding, with respect to temperature dependence of binding to affinity columns, and with respect to the degree of acid-treatment that agarose beads were required to undergo in order to prepare satisfactory affinity adsorbents.

Although several of the D-galactose- and 2-acetamido-2-deoxy-D-galactose-binding lectins have been studied in detail with respect to their carbohydrate specificities[22-26], the three lectins just mentioned require further study to more clearly determine their binding-site specificities. In this report, we describe the carbohydrate-binding specificities of these lectins as determined by hemagglutination-inhibition assay using a variety of saccharides as inhibitors.

EXPERIMENTAL

Materials and methods. — Lectins from the seeds of *Bauhinia purpurea alba*, *Sophora japonica*, and *Wistaria floribunda* were isolated as previously described[19]. *N*-Acetylneuraminic acid, raffinose, stachyose, 2-amino-2-deoxy-D-galactose, lactose, D- and L-fucose, D-glucose, D-galactose, melibiose, 2-acetamido-2-deoxy-D-galactose, methyl α- and β-D-galactopyranoside (Me-α- and -β-Gal)*, *o*-nitrophenyl α- and β-D-galactopyranoside (ONP-α- and -β-Gal), *p*-nitrophenyl α- and β-D-galactopyranoside PNP-α- and -β-Gal), *o*-nitrophenyl 2-acetamido-2-deoxy-α-D-galactopyranoside (ONP-α-GalNAc), and methyl α-D-mannopyranoside (Me-α-Man) were obtained from commercial sources, and most were purchased from Sigma Chemical Co., St. Louis, MO 63178. All other saccharides, except chitin oligosaccharides [$(GlcNAc)_n$], were synthesized in Dr. Matta's laboratory. The synthesis of some of these saccharides has been reported[27-31]. In addition, ONP-α- and -β-Gal, PNP-α- and -β-Gal, Me-α- and -β-Gal, and ONP-α-GalNAc were also synthesized in this laboratory and used in addition to the commercial compounds. $(GlcNAc)_n$ was

*In addition to the usual abbreviations for carbohydrate residues (without indication of the D or L configurations), the following abbreviations are used: All (allyl), Bzl (benzyl), Me (methyl), ONP (*o*-nitrophenyl), PAP (*p*-aminophenyl), PNP (*p*-nitrophenyl) and S (thio). Gal, GalNAc, GlcNAc, and Man are in the D, Fuc in the L configuration (except where indicated), and all in the pyranose form.

prepared by hydrolysis of chitin in conc. hydrochloric acid for 2 h at room temperature, followed by 1 h at 40°. The suspension was then chilled, neutralized with 50% sodium hydroxide, and then filtered. Aliquots of the filtrate were chromatographed on a Bio-Gel P-2 column (92 × 2.5 cm), equilibrated with 0.1M acetic acid. Fractions were analyzed by t.l.c., and appropriate fractions were pooled and freeze-dried to give the oligosaccharides indicated. Sepharose 2B and clam chitin were obtained from Sigma. Bio-Gel P-2 was obtained from Bio-Rad Laboratories, Rockville Centre, NY 11571.

Hemagglutination assays were carried out as previously described[21], except that "V" plates were used. For the inhibition assays, the final concentrations in the wells were 5.2 μg/mL for *Bauhinia*, 20.8 μg/mL for *Sophora*, and 10.4 μg/mL for *Wistaria* lectin. *Bauhinia* and *Wistaria* lectins were tested at pH 7.0 with rabbit erythrocytes as indicator cells. *Sophora* lectin was tested at pH 8.3 with human type-B erythrocytes as indicator cells. The data are reported as the minimum concentration of inhibitor that gives complete hemagglutination inhibition. For some of the compounds tested, complete inhibition of hemagglutination was not achieved; the hemagglutination score is also given for these compounds.

RESULTS AND DISCUSSION

The purified lectins were titered against human and rabbit erythrocytes. The results are presented in Table I. *Bauhinia* lectin showed no difference in reactivity towards the different human blood groups, but it was much more active against rabbit erythrocytes. These results indicate that the red-blood-cell receptor for the *Bauhinia* lectin is comprised of sites other than those expressing blood-group A, B, or O (H) activity, in accord with the findings of other investigators[7,32,33]. Uhlenbruck and Dahr[34] also found the *Bauhinia* lectin to be more active against rabbit erythrocytes, as compared to human cells. In contrast to *Bauhinia* lectin, *Sophora* lectin was more active against human type-B erythrocytes than against types A and O

TABLE I

MINIMUM CONCENTRATION[a] OF LECTIN GIVING A POSITIVE HEMAGGLUTINATION REACTION

Red-blood-cell type	*Lectin*		
	Bauhinia purpurea alba	Sophora japonica	Wistaria floribunda
A	2.6	42	5.2
B	2.6	5.2	7.8
O(H)	2.6	>333	6.5
Rabbit	0.3	18.9	0.6

[a]In μg/mL.

TABLE II

MINIMUM CONCENTRATION OF INHIBITOR GIVING COMPLETE INHIBITION OF AGGLUTINATION OF ERYTHROCYTES

Compound	*Lectin*					
	Bauhinia purpurea		Sophora japonica		Wistaria floribunda	
	Conc.[a]	*Score*[b]	*Conc.*[a]	*Score*[b]	*Conc.*[a]	*Score*[b]
α-Fuc-(1→2)-Gal	>10	3.0	>10	3.0	>10	2.0
β-Fuc-(1→2)-Gal	>10	3.0	>10	3.0	>10	1.0
Bzl-α-Fuc-(1→2)-β-Gal	>10	3.0	>10	1.0	>10	1.0
Bzl-β-Fuc-(1→2)-β-Gal	>10	3.0	>10	1.0	1.88	
PNP-α-Fuc-(1→3)-β-Gal	>10	3.0	>10	3.0	>10	3.0
PNP-α-Fuc-(1→2)-β-Gal	>10	3.0	5.0		5.0	
Fuc	>10	3.0	>10	3.0	>10	3.0
Glc	>10	3.0	>10	3.0	>10	3.0
NeuAc	>10	3.0	>10	3.0	>10	3.0
Me-α-Man	>10	3.0	>10	3.0	>10	3.0
Raffinose	>10	2.5	>10	3.0	>10	3.0
Stachyose	>10	2.0	>10	3.0	>10	3.0
ONP-α-Gal	>10	1.5	>10	1.0	1.88	
GalN	>10	1.0	>10	3.0	>10	3.0
Lactose	>10	1.0	5.0		7.50	
PAP-β-SGal	>10	1.0	>10.0		>10	0.5
Bzl-β-Gal	>10	0.5	10.0		3.75	
PNP-β-Gal	10.0		1.25		2.50	
ONP-β-Gal	10.0		1.25		1.25	
Me-β-Gal	10.0		>10	2.0	>10	0.5
Me-α-Gal	10.0		>10	1.5	5.0	
All-α-Gal	10.0		>10	2.0	2.50	
Gal	10.0		>10	2.5	>10	2.0
β-D-Fucopyranosyl azide	10.0		>10	1.5	5.0	
N-Acetyllactosamine	10.0		5.00		0.63	
Melibiose	10.0		>10	3.0	>10.00	
D-Fuc	10.0		>10	3.0	>10	2.0
PNP-β-D-Fuc	>6.66	1.0	5.00		1.67	
ONP-α-GalNAc	>5.0	1.0	2.50		<0.04	
β-D-Galactopyranosyl azide	7.50		10.00		10.00	
PNP-α-Gal	5.00		10.00		5.00	
6-*O*-Methyl-D-galactose	5.00		>10	1.0	5.00	
PNP-β-GlcNAc-(1→6)-β-Gal	>3.33	3.0	>3.33	3.0	>3.33	3.0
Ph-α-GalNAc	2.50		5.00		0.16	
Me-β-Gal-(1→3)-α-GlcNAc	2.50		1.88		0.31	
PNP-β-Gal-(1→3)-β-GlcNAc	1.67		1.67		1.67	
Me-α-GalNAc	1.25		>3.33	1.0	0.10	
PNP-β-Gal-(1→6)-β-GlcNAc	1.25		[c]		[c]	
GalNAc	1.25		10.00		0.31	
PNP-β-Gal-(1→3)-β-GlcNAc-(1→6)-β-Gal	1.08		[c]		[c]	
PNP-β-Gal-(1→3)-β-GalNAc	0.94		>2.50	2.5	>2.50	1.0
PNP-β-GalNAc	>0.83	1.5	0.42		0.08	
Me-β-Gal-(1→3)-α-GalNAc	0.63		>10	2.0	>5.0	1.0
β-GalNAc-(1→6)-Gal	0.63		0.63		<0.08	

TABLE II *(continued)*

Compound	*Lectin*					
	Bauhinia purpurea		Sophora japonica		Wistaria floribunda	
	Conc.	*Score*	*Conc.*[a]	*Score*[b]	*Conc.*[a]	*Score*[b]
$(GlcNAc)_2$	[c]		>3.33	3.0	>3.33	3.0
$(GlcNAc)_{2-4}$	[c]		>3.33	3.0	>3.33	3.0
$(GlcNAc)_{3-7}$	[c]		>3.33	3.0	>3.33	3.0
$(GlcNAc)_4$	[c]		>3.33	3.0	>3.33	3.0
$(GlcNAc)_{5-7}$	[c]		>3.33	3.0	>3.33	3.0
$(GlcNAc)_{6,7}$	[c]		>3.33	3.0	>3.33	3.0
$(GlcNAc)_{7,8}$	[c]		>3.33	3.0	>3.33	3.0

[a]Conc. in mM. [b]Hemagglutination score: 3, maximal agglutination; blank, no agglutination. [c]Not determined. [d]Conc. in mg/mL.

(H), and rabbit rbc. Erythrocytes were not typed for Ii activity. *Wistaria* lectin was found to be more active against rabbit erythrocytes than against human rbc, with no preference for type A, B, or O (H) erythrocytes.

The results of the hemagglutination-inhibition assays are given in Table II. The low-molecular-weight compounds are listed with the weakest inhibitors, with respect to *Bauhinia* lectin, at the top of the list. Of the three lectins reported here, *Bauhinia* lectin is the most distinctively different with respect to its binding-site specificity, and it will be discussed first. In contrast to many lectins[21,24], the presence of an aromatic aglycon group did not enhance the inhibitory activity of the low-molecular-weight compounds tested. For example, the results for ONP-α- and -β-Gal, PNP-β-Gal, benzyl β-D-galactopyranoside (Bzl-β-Gal), and *p*-aminophenyl β-D-thiogalactopyranoside (PAP-β-SGal) were compared with those for Me-α- and -β-Gal, allyl α-D-galactopyranoside (All-α-Gal), and Gal. In some cases, the presence of an aromatic aglycon group clearly diminished the inhibitory activity of the saccharide; for example, GalNAc gave complete inhibition at a 1.25mM concentration, whereas ONP-α-GalNAc did not give complete inhibition at a 5.0mM concentration. This lack of reactivity toward aromatic aglycon groups is similar to that observed for peanut lectin[25]. *Bauhinia* lectin is also similar to peanut lectin in that substitution of the Gal or GalNAc with glycosidically-linked Fuc or GlcNAc residues abolished inhibitory activity of the former saccharides. This is evidenced by comparing the results for α-Fuc-(1→2)-Gal, β-Fuc-(1→2)-Gal, benzyl 2-*O*-α- and -β-L-fucopyranosyl-β-D-galactopyranoside [Bzl-α- and -β-Fuc-(1→2)-β-Gal], and *p*-nitrophenyl 3-*O*-α-L-fucopyranosyl-β-D-galactopyranoside [PNP-α-Fuc-(1→3)-β-Gal] with those for Me-, ONP-, and PNP-β-Gal; All-α-Gal; and Gal. In contrast to peanut lectin, 6-*O*-methyl-D-galactose is a slightly better inhibitor of *Bauhinia* lectin than is Gal.

We could not demonstrate clearly a binding specificity for anomeric configuration. No difference was observed between Me-α- and -β-Gal. However, the position

of the nitro group on the phenolic aglycon group may influence the inhibitory activity when the glycoside is in the α-D configuration, for example, the results for ONP- and PNP-α-Gal differ, whereas ONP- and PNP-β-Gal gave identical results. It is also evident that phenyl 2-acetamido-2-deoxy-D-galactopyranose (Ph-α-GalNAc) is a better inhibitor than ONP-α-GalNAc.

Of the free monosaccharides tested, Fuc and Gal were equivalent inhibitors. GalNAc was the most potent, however, being 8 times more effective. Several disaccharides were tested for inhibitory activity. When Gal was the nonreducing residue, as for Me-β-Gal-(1→3)-α-GlcNAc, PNP-β-Gal-(1→3)- and -(1→6)-β-GlcNAc, PNP-β-Gal-(1→3)-*p*-GalNAc, and Me-β-Gal-(1→3)-α-GalNAc, the inhibitory activity increased several-fold relative to free Gal, and was near that of free GalNAc. Kaifu and Osawa[35] reported that *Bauhinia* lectin had a greater specificity for the Gal→GalNAc than for the Gal→GlcNAc sequence. The results reported here confirm their observations. Me-β-Gal-(1→3)-α-GalNAc was a better inhibitor than Me-β-Gal-(1→3)-α-GlcNAc, and PNP-β-Gal-(1→3)-β-GalNAc was a better inhibitor than PNP-β-Gal-(1→3)-β-GalNAc. This binding preference for the Gal→GalNAc sequence is in contrast to that found for *Sophora* and *Wistaria* lectins. The insertion of a GlcNAc*p* residue between two Gal residues to form the trisaccharide PNP-β-Gal-(1→3)-β-GlcNAc-(1→6)-β-Gal gave a compound having an activity similar to that of the disaccharides tested. β-GalNAc-(1→6)-Gal was similar in activity to free GalNAc; this is in contrast to the results with Gal, as discussed above.

Apparently, *Bauhinia* lectin has a binding specificity for atoms involved in the glycosidic bond of the reducing residue of disaccharides, as lactose, *N*-acetyllactosamine, and melibiose are weaker inhibitors than the disaccharides that have their reducing residues glycosidically linked to methyl or nitrophenyl groups. The potential size and complexity of the *Bauhinia* lectin binding-site may be appreciated by noting that raffinose and stachyose are relatively weak inhibitors, even though the residues penultimate to the nonreducing Gal residue do not have free anomeric hydroxyl groups.

In support of the proposal that the *Bauhinia* lectin binding-site for D-galactosyl-containing saccharides recognizes internal residues, we found that, even after removal of sialic acid, fetuin and α_1-acid glycoprotein are not potent inhibitors[36] (unpublished observation). Irimura *et al.*[37] have reported that *O*-desialosylated porcine thyroglobulin glycopeptide B is also a poor inhibitor. After *O*-desialosylation, these three glycoproteins have in common the general oligosaccharide structure Gal→GlcNAc→Man. As Me-β-Gal-(1→3)-α-GlcNAc, and PNP-β-Gal-(1→3)- and -(1→6)-β-GlcNAc have GlcNAc as potential reducing residues and are also inhibitors, and as PNP-β-Gal-(1→3)-β-GlcNAc-(1→6)-β-Gal is a good inhibitor, it may be that the presence of D-mannopyranosyl residues is responsible for the low inhibitory activity of the *O*-desialosylated glycoproteins mentioned. This requires further study, however, since the demonstration by Kaifu and Osawa[38] that β-Gal-(1→4)-β-GlcNAc-(1→2)-Man was a slightly better inhibitor than β-Gal-(1→4)-GlcNAc and Gal.

Our results for *Bauhinia* lectin are in general accord with those reported in the

literature[7,34,37-39]. The lectin is more active against rabbit rbc than against human rbc; the lectin binds primarily to red-blood-cell receptors other than A, B, O (H) blood-group-active components; GalNAc is the best monosaccharide inhibitor; there is no apparent strong preference for the configuration at C-1; some saccharides containing D-galactosyl residues are better inhibitors than free Gal whereas others are not; and the presence of an aromatic aglycon group did not enhance the inhibitory activity of saccharides. Irimura *et al.*[37] concluded that the *Bauhinia* lectin could not bind to the Gal→GlcNAc sequence on the basis of results obtained with porcine thyroglobulin glycopeptide B. As shown here, however, and in later work by Kaifu and Osawa[38], the lectin can bind effectively to the sequence Gal→GlcNAc→R, where R is not sucrose and, possibly in the case of glycoproteins, not Man.

The apparent large-size of the binding site of *Bauhinia* lectin is in contrast to that proposed for other Gal–GalNAc-binding lectins[26]. Since nonreducing terminal Fuc, GlcNAc, and sialyl residues block the binding of penultimate Gal and GalNAc residues, and since the presence of aromatic aglycon groups can diminish the inhibitory activity of saccharides, the binding site may be a narrow, deep cleft with proximal, hydrophilic amino acid residues. The presence of a small subsite is possible in view of the different inhibitory characteristics of Gal and GalNAc and their conjugates. Recent studies on the *Bauhinia* lectin by quantitative precipitin and precipitin-inhibition assays[40] extend and, in general, confirm the results presented here.

The binding-site specificity of *Sophora* lectin has been partially elucidated by others[37,41-45]. These earlier studies elucidated the anti-I activity of this lectin and demonstrated a binding preference for the β-D anomers of nonreducing terminal residues of Gal and GalNAc. It was also shown that the presence of aromatic aglycon groups increases the inhibitory activity of saccharides toward the *Sophora* lectin. Our results confirm and extend these observations.

Gal residues in penultimate position to Fuc residues are not always blocked from interacting with *Sophora* lectin in contrast to their effect on *Bauhinia* lectin. For example, PNP- and Bzl-α-Fuc-(1→2)-β-Gal, and Bzl-β-Fuc-(1→2)-β-Gal show inhibitory activity. However, both the presence of aromatic aglycon groups and the linkage position of Fuc→Gal are influential since α- and β-Fuc-(1→2)-Gal, and Bzl-α-Fuc-(1→3)-β-Gal are not inhibitory at the concentration tested. The specificity for β-D-glycosides is demonstrated by comparing the results for lactose and melibiose, PNP-α- and -β-Gal, PNP-β-GalNAc, and ONP-α-GalNAc.

Of interest is the observation that whereas Me- and PNP-β-Gal-(1→3)-β-GlcNAc, and *N*-acetyllactosamine are good inhibitors of *Sophora* lectin, the Gal→GalNAc sequence is not, since PNP-β-Gal-(1→3)-β-GalNAc and Me-β-Gal-(1→3)-α-GalNAc showed little inhibitory activity. This is in agreement with the observation of anti-I activity of *Sophora* lectin[43], since the anti-I determinant has been shown to be comprised, at least in part, by the Gal→GlcNAc sequence[46,47]. However, GalNAc-(1→6)-Gal was one of the most potent inhibitors tested.

In view of the inhibitory activity of the Gal→GlcNAc sequence, several oligomers of GlcNAc were tested for inhibitory activity. None of the compounds

tested were active, in agreement with the results obtained for PNP-β-GlcNAc-(1→6)-β-Gal, which also has a terminal nonreducing GlcNAc residue.

The isolation of *Wistaria floribunda* lectin has been reported by several investigators[19,48–51]. However, detailed studies on its binding-site specificity have not been previously reported. Some data have been reported by Toyoshima *et al.*[51], Irimura *et al.*[37], and Kaifu and Osawa[35,38], which showed that GalNAc was the most potent monosaccharide-inhibitor, and that α- and β-D-glycosides were equally good inhibitors.

In contrast to *Bauhinia* lectin, *Wistaria* lectin can recognize penultimate Gal and GalNAc residues. Bzl-β-Fuc-(1→2)-β-Gal was as good an inhibitor as ONP-α-Gal. It is of interest that Kaifu and Osawa[35] concluded that *Wistaria* lectin may have a partial specificity for serine based upon inhibition assays with synthetic compounds. It should be noted that PNP-β-GlcNAc-(1→6)-β-Gal was not inhibitory at a 3.33 mM concentration.

Although *Wistaria* lectin seemed to be the least specific of the lectins studied here, some binding restrictions are evident. Whereas lactose (a β-D-galactoside) and melibiose (an α-D-galactoside) were essentially equivalent in inhibitory activity, raffinose and stachyose were inactive. *N*-Acetyllactosamine was about 12 times more active than lactose, indicating that *Wistaria* lectin must recognized penultimate residues.

Wistaria lectin was similar to *Sophora* lectin in that PNP-β-Gal-(1→3)-β-GalNAc and Me-β-Gal-(1→3)-α-GalNAc were relatively poor inhibitors, whereas β-GalNAc-(1→6)-Gal was a very potent inhibitor. In agreement with Kaifu and Osawa[35], the Gal→GlcNAc sequence was found to be a better inhibitor than the Gal→GalNAc sequence. However, as for *Sophora* lectin, all of the GlcNAc oligomers tested were inactive. *L. terrestris* and honey-locust mucins were inhibitors of *Wistaria* but they had little or no inhibitory activity towards *Sophora*[36] lectin.

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