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The glycosylation of glycoprotein lectins. Intra- and inter-genus variation in *N*-linked oligosaccharide expression*

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ABSTRACT

Glycosylated lectins represent a series of glycoproteins with related activities and, in the case of the *Leguminosae*, related amino acid sequences. Therefore, they offer a model system in which to study the diversity of *N*-linked oligosaccharide structures of plant glycoproteins. The influence of the polypeptide on the type of oligosaccharide substitution and the problem of inter- and intra-genus variation in glycosylation can also be addressed. Analysis of the glycosylation of 18 lectins has shown that they can be classified into four qualitatively similar groups on the basis of the Bio-Gel P-4 elution profiles of the oligosaccharides released by hydrazinolysis: (a) The *Erythrina cristagalli* profile, with a major component at 8.8 glucose units (gu) and minor components at 8.0, 7.2, and 5.8 gu. The major component is the heptasaccharide, α-D-Man*p*-(1→3)-[α-D-Man*p*-(1→6)]-[β-D-Xyl*p*-(1→2)]-β-D-Man*p*-β-D-Glc*p*NAc-(1→4)-[α-L-Fuc*p*-(1→3)]-D-GlcNAc. (b) The *Phaseolus vulgaris* profile, which was characterized by peaks at 12.5, 11.7, 10.8, and 9.9 gu, in addition to the peaks at 8.8, 8.0, 7.2, and 5.8 gu mentioned above. These higher-mol.-wt. components were oligo-D-mannose oligosaccharides containing 9, 8, 7, and 6 D-mannose residues, respectively. (c) The *Lonchocarpas capassa* profile, which had a major peak at ~8 gu. (d) The soybean agglutinin profile, which has a single peak at 12.5 gu. This peak consisted solely of an oligomannose undecasaccharide containing 9 D-mannose residues. This lectin is unique in that it shows no microheterogeneity.

INTRODUCTION

Many plant lectins are glycoproteins, but little information is available on either the structure of the oligosaccharide units or the factors that determine the glycosylation pattern of glycoprotein lectins. It is now clear that *N*-linked oligosaccharides in plants fall into two major classes and these are both found in lectins. Oligosaccharides of the "oligomannose" type are the only structures present on soybean agglutinin (SBA, *Glycine max*)[1], and plant specific "xylose–fucose" or "modified" type oligosaccharides only are found on *Erythrina cristagalli*[2] and *Sophora japonica* lectins[3]. Phytohaemagglutinin from *Phaseolus vulgaris* (PHA) carries both oligosaccharide types on a single polypeptide chain but at different glycosylation sites[4,5].

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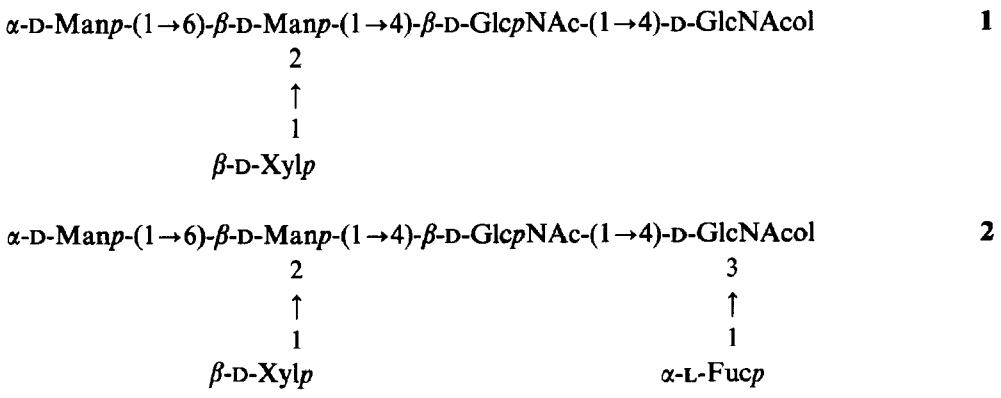
Glycosylated lectins represent a series of glycoproteins with related activities, similar structure, and in the case of the *Leguminosae* extensive amino acid sequence homology[6]. These lectins can be used as a model system in which to study the diversity of *N*-linked oligosaccharide structures present on plant glycoproteins, the influence of the polypeptide on the type of oligosaccharide substitution, and also the inter- and intra-genus variation in glycosylation. In this paper, we report the glycosylation patterns of 18 lectins and suggest a grouping based on these patterns.

EXPERIMENTAL

Materials. — Lectins from *Caragana arborescens, Dolichos biflorus, Griffonia simplicifolia* (lectins I and II), *Lens culinaris* (lentil), *Phaseolus vulgaris* (PHA), *Phaseolus coccineus, Sophora japonica,* and *Wisteria floribunda* were purchased from Sigma Chemical Co. Ltd. (Poole, Dorset, UK). *Phaseolus lunatus* (lima bean) lectin was a product of E.Y. Laboratories Inc., and was purchased from Bradsure Biologicals Ltd. (Market Harborough, Leicestershire, UK). *Glycine max* (SBA), *Erythrina* species (*E. caffra, E. corallodendron, E. cristagalli,* and *E. lysistomon*), *Lonchocarpas capassa,* and *Vicia faba* lectins were isolated as previously described[7–10]. All lectins were affinity purified. 1,4-Diaminobutane was purchased from Aldrich Chemical Co. Ltd. (Gillingham, Dorset, UK).

The following oligosaccharides were released by hydrazinolysis, isolated, reduced, and purified as previously described[2]. Compounds **1** and **2** were obtained from pineapple stem bromelain[11], **3** and **4** were obtained from *E. cristagalli* lectin[2], **5** was obtained from hen ovomucoid[12], **6** and **7** were obtained from bovine pancreatic ribonuclease B[13], and **8** was obtained from bovine thyroglobulin[14]. The sources of all other enzymes, chemicals, and radiochemicals were as previously described[2].



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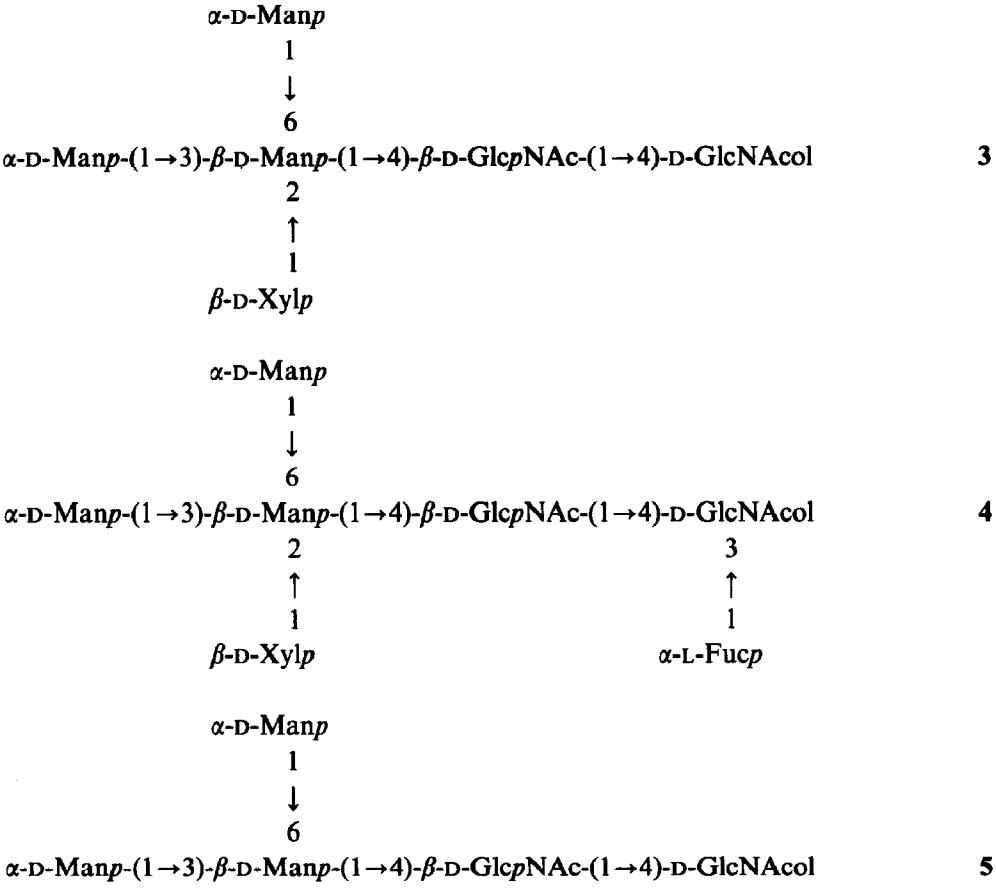
α-D-Manp
1
↓
6
α-D-Manp-(1→3)-β-D-Manp-(1→4)-β-D-GlcpNAc-(1→4)-D-GlcNAcol
2
↑
1
β-D-Xylp
3
α-D-Manp
1
↓
6
α-D-Manp-(1→3)-β-D-Manp-(1→4)-β-D-GlcpNAc-(1→4)-D-GlcNAcol
2
↑
1
β-D-Xylp
3
↑
1
α-L-Fucp
4
α-D-Manp
1
↓
6
α-D-Manp-(1→3)-β-D-Manp-(1→4)-β-D-GlcpNAc-(1→4)-D-GlcNAcol
5
α-D-Manp
1
↓
6
α-D-Manp-(1→3)-α-D-Manp-(1→6)-β-D-Manp-(1→4)-β-D-GlcpNAc-(1→4)-D-GlcNAcol
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α-D-Manp
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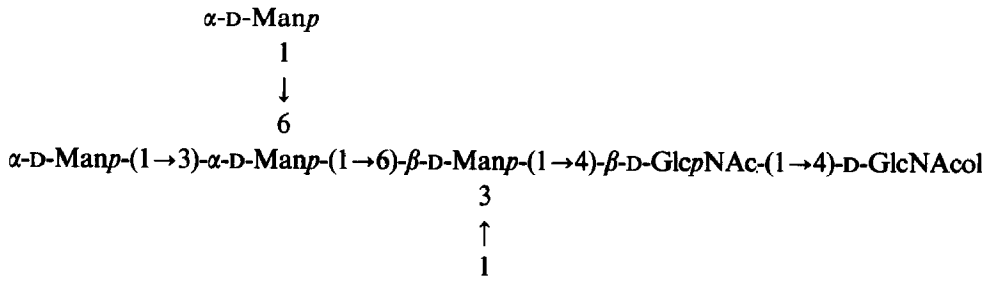

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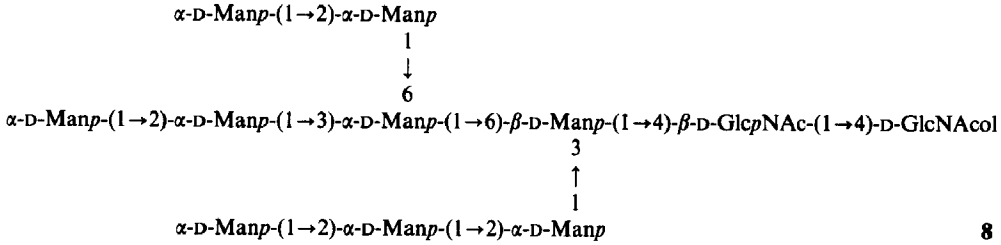

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Methods. — ^{1}H-N.m.r. spectroscopy was performed as previously described[15]. H.p.l.c. of oligosaccharides was carried out on 4.9 × 250-mm columns, packed either with LiChrosorb-NH_2 (5 μm, Hichrom Ltd., Reading, Berkshire, UK) or with 3 μm silica (Spherisorb S3W, Hichrom Ltd.) modified with 1,4-diaminobutane. Separation conditions were as described by Turco[16], using a Waters dual-pump gradient system with computer control, data acquisition, and analysis. Columns were standardised with the xylose–fucose-type oligosaccharides **1–4** and the oligomannose-type oligosaccharides **5–8**. Radioactivity in the column effluent was detected by a Berthold LB503 HPLC flow monitor (Lab-Impex Ltd., Twickenham, Middlesex, UK). Hydrazinolysis, isolation of oligosaccharides and their subsequent reduction with NaB^3H_4, high-voltage paper electrophoresis, gel filtration on Bio-Gel P-4 columns, measurement and detection of radioactivity, and exoglycosidase digestion were performed as previously described[2]. The proportions of radioactivity associated with each pooled peak from the Bio-Gel P-4 column were determined either directly, if sufficient resolution was obtained, or after repassage through the gel-filtration column, and are expressed as the molar percentage of recovered radioactivity. The elution positions and relative proportions of each peak were reproducible in repeat experiments.

RESULTS

High-voltage paper electrophoresis of the oligosaccharides released from each lectin by treatment with hydrazine, followed by acetylation and reduction with NaB^3H_4, demonstrated that in each case >90% comigrated with lactose and were, therefore, uncharged. This neutral fraction of each lectin was then subjected to gel filtration on Bio-Gel P-4, and the fractions corresponding to peaks of radioactivity were collected and pooled.

Table I shows the elution position on Bio-Gel P-4, expressed as glucose units (gu), and the molar percentage of each peak fraction for all the lectins analyzed. Figures 1–3 show typical Bio-Gel P-4 elution profiles from which these peaks were pooled. Three clear groupings of glycosylation patterns can be seen, in addition to the unique glycosylation of SBA. The first pattern (Table I, group I; and Fig. 1) is characterised by a major peak at 8.8 gu, identical to the elution position of **4**. The molar proportion of this

TABLE I

The elution position and molar percentages of neutral oligosaccharides released from lectins by hydrazinolysis and separated by gel filtration on Bio-Gel P-4

Lectin	*Molar percentage of recovered radioactivity in each peak*[a]								
	12.5	*11.7*	*10.8*	*9.9*	*9.5*	*8.8*	*8.0*	*7.2*	*5.8*
Group I									
Caragana arborescens	[b]	[b]	[b]	[b]	[b]	63.8	25.2	6.4	4.6
Dolichos biflorus	[b]	[b]	[b]	[b]	4.3	62.2	22.8	5.9	4.9
Erythrina caffra	[b]	[b]	[b]	[b]	[b]	76.8	13.9	[b]	9.4
Erythrina corallodendron	[b]	[b]	[b]	[b]	[b]	72.2	17.3	[b]	10.4
Erythrina cristagalli	[b]	[b]	[b]	[b]	[b]	78.9	13.1	[b]	8.0
Erythrina latissima	[b]	[b]	[b]	[b]	[b]	62.7	29.2	[b]	8.1
Erythrina lysistemon	[b]	[b]	[b]	[b]	[b]	77.0	10.6	[b]	12.4
Griffonia simplicifolia I	[b]	[b]	[b]	[b]	5.3	64.3	22.4	5.3	2.8
Sophora japonica	[b]	[b]	[b]	[b]	[b]	52.2	29.0	9.1	9.7
Wisteria floribunda	[b]	[b]	[b]	[b]	[b]	64.0	22.1	10.0	3.8
Group II									
Lens culinaris	6.0	2.5	15.1	[b]	11.4	26.3	24.1	7.6	6.8
Phaseolus coccineus	12.2	14.4	8.9	[b]	9.9	33.6	13.6	3.1	4.2
Phaseolus lunatus	6.9	9.7	9.9	[b]	15.7	37.3	16.7	2.7	1.0[c]
Phaseolus vulgaris	13.5	13.4	8.8	[b]	18.9	18.4	18.4	6.5	2.1
Group III									
Lonchocarpas capassa	[b]	[b]	[b]	[b]	[b]	26.5	62.7	8.8	1.9
Vicia faba	[b]	[b]	[b]	[b]	[b]	23.3	58.3	18.3	[b]
Griffonia simplicifolia II	[b]	[b]	[b]	[b]	[b]	30.3	60.0	6.0	3.2
Group IV									
Glycine max	100.0	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]

[a] The elution positions are given in glucose units relative to an internal isomaltooligosaccharide standard mixture. [b] Less than 1% of the recovered radioactivity. [c] The elution position peak was 6.7 gu.

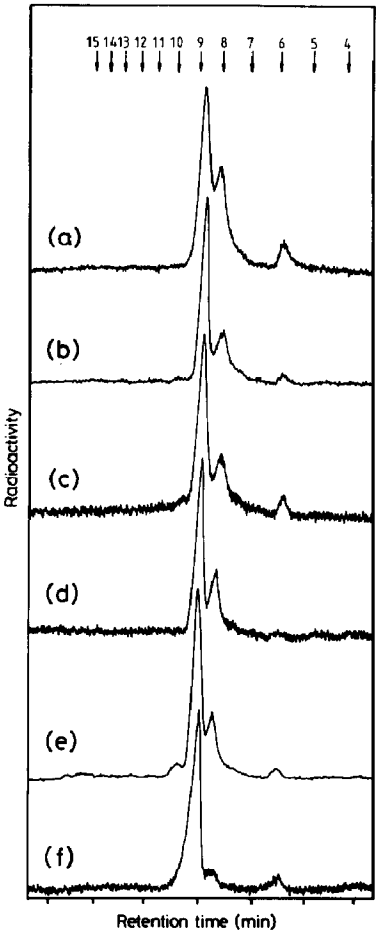


Fig. 1. Gel filtration of the neutral oligosaccharides from lectins of glycosylation group I on Bio-Gel P-4. The lectins from (a) *Sophora japonica*, (b) *Wisteria floribunda*, (c) *Dolichos biflorus*, (d) *Caragana arborescens*, (e) *Griffonia simplicifolia* (lectin I), and (f) *Erythrina cristagalli* were subjected to hydrazinolysis and the isolated oligosaccharides were reduced, purified, and separated on Bio-Gel P-4. The arrows indicate the elution position of isomaltooligosaccharides containing the corresponding number of glucose units. The time axis is marked at 100-min intervals.

component varied from 52 to 79% in the lectins from *S. japonica* and *E. cristagalli*, respectively. Lectins that possessed this peak always had components that were eluted at 8.0 and 5.8 gu, and sometimes also at 7.2 gu. The proportion of these components had a wide range; the 8.0-gu peak varied from 29 in *S. japonica* to 10.5% in *E. lysistomon*, the 7.2-gu peak from 10 in *W. floribunda* to $<1\%$ in the *Erythrina* species, and the 5.8-gu peak from 12.5 in *E. lysistomon* to 2.8% in *G. simplicifolia* I. Two lectins of this group also showed an additional peak, which was eluted before the main component, at 9.5 gu. This comprised 4.3 in *Dolichos biflorus* and 5.3% in *G. simplicifolia* I. H.p.l.c. of the Bio-Gel P-4 pool corresponding to the 8.8-gu peak of each lectin showed that it consisted of a single component and had the same retention time as that of **4**. The

sequential exoglycosidase digestion of this component from *S. japonica*, *D. biflorus*, and *Erythrina* species lectins with jack bean α-D-mannosidase, *C. lampas* β-D-xylosidase, and α-L-fucosidase showed the same susceptibility to these enzymes as did **4**, and both the component and **4** gave identical products. Material eluted at 8.0 gu was not homogeneous on h.p.l.c., as previously observed for *E. cristagalli* lectin by use of high-voltage electrophoresis of oligosaccharide–borate complexes[2]. Both **2** and **3** were eluted at this position on Bio-Gel P-4. A similar situation applies for the 7.2-gu peak fraction, where **1** and **5** were coeluted. These ambiguities can be resolved either by h.p.l.c. (see below) or by exoglycosidase sequencing.

The second glycosylation pattern that is evident (Table I, group II; and Fig. 2) has peaks >9.5-gu present in addition to the components of the first group. Again, the molar proportions varied markedly from lectin to lectin. The level of incorporation of radioactivity into oligosaccharides, on a molar basis, was very low for lentil lectin and represented <0.5 mol of oligosaccharide per mol of protein. This indicated that there was <50% occupancy of the potential glycosylation site at Asn-66 of the β-chain of lentil lectin[17]. Much more variation was seen between lectins from species of the genus *Phaseolus* than was seen between lectins from *Erythrina* species in either this in-

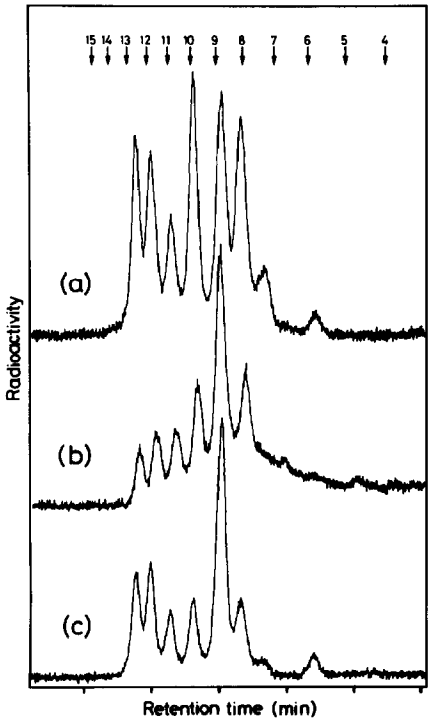
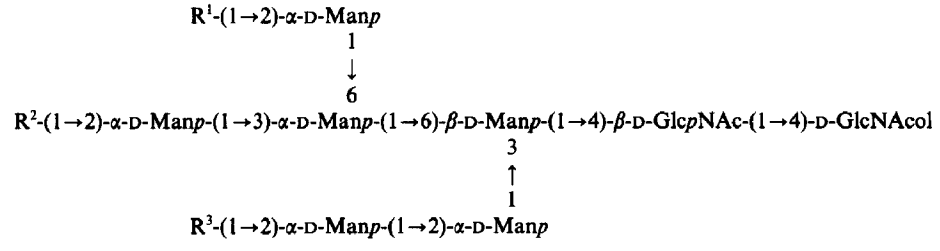


Fig. 2. Gel filtration of the neutral oligosaccharides from lectins of three *Phaseolus* species on Bio-Gel P-4. The lectins from (a) *P. vulgaris*, (b) *P. lunatus*, and (c) *P. coccineus* were subjected to hydrazinolysis and the isolated oligosaccharides were reduced, purified, and separated on Bio-Gel P-4. For details of the annotation, see legend to Fig. 1.

vestigation or as previously[2] reported. H.p.l.c. of pooled Bio-Gel P-4 fractions from PHA and *Phaseolus coccineus* lectin indicated that the 12.5- and 9.9-gu peak fractions were single components and had the same retention times as those of **8** and **7**, respectively. Pools corresponding to the 11.7- and 10.8-gu peaks showed some splitting of h.p.l.c. peaks, suggesting that they contained a number of closely related structures. H.p.l.c. of the 8.8-gu peak indicated that >95% of this peak had the same retention time as that of **4**, and the remainder had the same retention time as that of **6**. Sequential exoglycosidase treatment revealed that the peaks >9.5 gu contained only mannosyl groups at their nonreducing termini. Peak fractions at 12.5, 11.7, 10.8, and 9.9 gu lost 4, 3, 2, and 1 mannose units, respectively, with (1→2)-α-D-mannosidase, and each lost 4 further mannose units with jack bean α-D-mannosidase. Therefore, the structures of these fractions correponded to oligosaccharides containing 9, 8, 7, and 6 mannose units. ^{1}H-N.m.r. spectra were obtained for the main Bio-Gel P-4 pooled products of PHA. The spectra of the 12.5-, 9.9-, and 8.8-gu peak fractions were identical to those of **8**, **7**, and **4**, respectively. The spectra of the peak fractions containing the 7 mannose (10.8 gu) and 8 mannose (11.7 gu) structures indicated that they were composed of three isomers of $Man_7GlcNAcGlcNAcol$ and $Man_8GlcNAcGlcNAcol$, respectively. The proportions of these isomers, determined as described by Zamze *et al.*[15], are shown in Scheme 1. The distribution of Man_8-A, Man_8-B, and Man_8-C is the same as has been reported for bovine thyroglobulin[18,19], but differs from that found for the variant surface glycoproteins (VSG) of *Trypanosma brucei* MITat 1.4 and MITat 1.6, where Man_8-A and Man_8-C are present in approximately equimolar proportions[14,19]. The occurrence of a mixture of Man_7-A, Man_7-B, and Man_7-C was not seen in either of these systems. Bovine thyroglobulin carries only Man_7-A[19] and the *T. brucei* VSG carry only Man_7-B[14,19]. Man_7-C has been reported on ricin-D where it is found with Man_7-B[20].

Three lectins, *L. capassa, G. simplicifolia* II, and *V. faba*, had similar Bio-Gel P-4 elution profiles (Table I, group III; and Fig. 3 a–c) with a major component at ~8 gu and minor peaks at 8.8, 7.2, and 5.8 gu (Fig. 3 a, b, and c). H.p.l.c. of the total neutral oligosaccharide pool of *L. capassa* and *V. faba* lectins are shown in Fig. 4. The major



$Man_7GlcNAcGlcNAcol$	*$Man_8GlcNAcGlcNAcol$*
Man_7-A 25%; R^1 = α-D-Man*p*, $R^2=R^3=H$	Man_8-A 60%; $R^1=R^3$ = α-D-Man*p*, $R^2=H$
Man_7-B 50%; $R^1=R^2=H$, R^3 = α-D-Man*p*	Man_8-B 30%; $R^1=H$, $R^2=R^3$ = α-D-Man*p*
Man_7-C 25%; $R^1=R^3=H$, R^2 = α-D-Man*p*	Man_8-C 10%; $R^1=R^2$ = α-D-Man*p*, $R^3=H$

Scheme 1. The structure and molar proportions of the isomers of $Man_7GlcNAcGlcNAcol$ and $Man_8GlcNAcGlcNAcol$ from *Phaseolus vulgaris* lectin.

component of *L. capassa* had the same retention time as that of **3**, and the next most abundant component had the same retention time as that of **4**. This agrees with the relative proportions of the 8.0- and 8.8-gu peak fractions on Bio-Gel P-4 chromatography. Only a small amount of radioactivity was eluted from the h.p.l.c. column at the same retention time as that of **2**. This was in direct contrast to the h.p.l.c. distribution of radiolabeled oligosaccharides from *V. faba* where the major component had the same retention time as that of **2**. Minor peaks were eluted at the same retention times as those of **1**, **3**, and **4**. Therefore, although the Bio-Gel P-4 elution profiles were similar, these lectins had a completely different oligosaccharide distribution.

SBA is unlike all the other glycoprotein lectins tested (Table I, group IV; and Fig. 3 d). It had only a single peak on Bio-Gel P-4, which was eluted at 12.5 gu. This elution position is identical to that of **8**. H.p.l.c. also gave a single peak which had the same

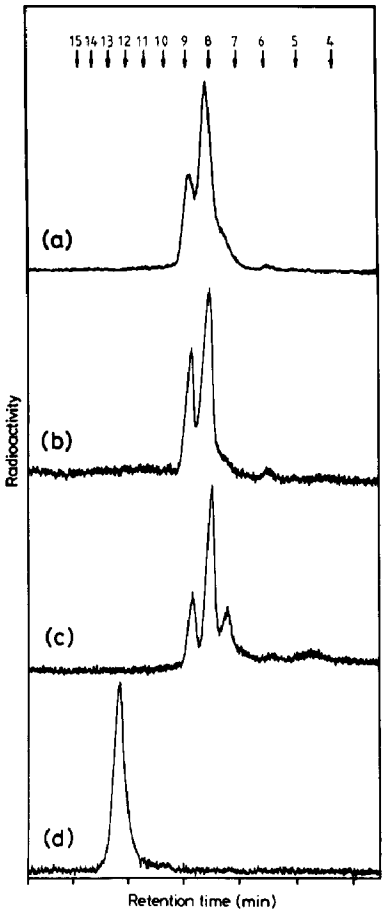


Fig. 3. Gel filtration of the neutral oligosaccharides from lectins of glycosylation group III and soybean agglutinin on Bio-Gel P-4. The lectins from (a) *Lonchocarpas capassa*, (b) *Griffonia simplicifolia* (lectin II), (c) *Vicia faba*, and (d) soybean (*Glycine max*) were subjected to hydrazinolysis and the isolated oligosaccharides were reduced, purified, and separated on Bio-Gel P-4. For details of the annotations, see legend to Fig. 1.

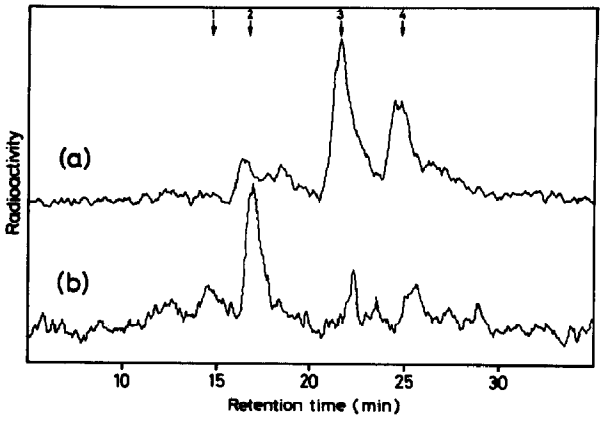


Fig. 4. H.p.l.c. separation of the neutral oligosaccharides from the lectins of *Lonchocarpas capassa* and *Vicia faba* on LiChrosorb-NH_2. The lectins from (a) *L. capassa* and (b) *V. faba* were subjected to hydrazinolysis and the isolated oligosaccharides were reduced, purified, and separated on a column of LiChrosorb-NH_2 as described in the Experimental section. The arrows indicate the retention time of **1**(1), **2**(2), **3**(3), and **4**(4), respectively.

retention time as that of **8**. Treatment with (1→2)-α-D-mannosidase released 4 mannose units and a further 4 were removed by jack bean α-D-mannosidase. The ^{1}H-n.m.r. spectrum of this component was identical to that of **8**. These findings are conformation of the results of a previous study on a glycopeptide isolated from SBA[1].

DISCUSSION

We report herein the glycosylation profiles of 18 lectins and present data on the nature of many of the oligosaccharide components that contribute to these profiles. Previous reports of the glycosylation of plant lectins have almost invariably described only the major sugar structures, rather than the full molar distribution of oligosaccharides which can be seen to be present from their profiles. The findings reported herein are consistent with or complementary to previous studies of glycoprotein lectins with only a few exceptions.

On the basis of their characteristic Bio-Gel P-4 elution profile, the glycoprotein lectins were classified into four qualitatively similar groups. Firstly, the profile of *E. cristagalli* lectin was also found for four other *Erythrina* species and five unrelated legumes. This profile has a major component at 8.8 gu and minor components at 8.0, 7.2, and 5.8 gu. The major component in each case was confirmed to be the heptasaccharide **4**.

A second group of lectins had a distinctive glycosylation pattern that resembled that of PHA. Two of these were from other *Phaseolus* species, and the third was lentil lectin. This profile was characterised by peaks at 12.5, 11.7, 10.8, and 9.9 gu, in addition to the peaks at 8.8, 8.0, 7.2, and 5.8 gu mentioned above. The higher-mol.-wt. components were oligomannose oligosaccharides containing 9, 8, 7, and 6 mannose units, respectively.

A third group of lectins had Bio-Gel P-4 elution profiles in which the major component was eluted at ~8 gu. However, the major oligosaccharides of two of these lectins, *V. faba* and *L. capassa*, were different and were shown to have the same retention times, on h.p.l.c., as that of **2** and **3**, respectively.

Finally soybean agglutinin was unique in that it showed no microheterogeneity. This is the only example in animal or plant species where this has been observed. The single peak in the Bio-Gel P-4 profile at 12.5 gu represents the oligomannose undecasaccharide structure containing 9 mannose units.

The number of different oligosaccharide structures in a single lectin can, thus, vary quite markedly, from one in SBA to > 12 in PHA. In 13 out of 18 lectins, the major component was eluted at 8.8 gu on Bio-Gel P-4 chromatography. In 12 of these, the 8.8-gu peak had the same retention time, on h.p.l.c., as that of **4**, and in 8 out of the 12, further evidence was obtained to support the identity of this component with **4**. Moreover, with the exception of SBA, this component was the second most abundant oligosaccharide in the remaining lectins. Thus, it is clear that the unreduced form of **4** is by far the most common oligosaccharide substituent of the lectins in this study. These results suggested that this oligosaccharide represents the normal end result of the biosynthetic pathway for glycosylation in plants. In the case of the *Phaseolus* lectins where significant levels of oligomannose-type oligosaccharides are found, in addition to xylose–fucose-type oligosaccharides, there may be polypeptide interactions or structural features that restrict access to certain glycosylation sites in these lectins. This hypothesis can be verified in the case of PHA. The lectin has two glycosylation sites, one which carries oligomannose-type oligosaccharides and the other xylose–fucose-type oligosaccharides[4]. In an undenatured glycoprotein precursor isolated from the endoplasmic reticulum, the site that carries the oligomannose-type structure in the mature glycoprotein was almost completely inaccessible to exo- or endo-glycosidase digestion[5]. Although similar accessibility studies have not been carried out on SBA, it might be expected that one of the reasons that SBA shows no trimming of mannose units and hence no heterogeneity is that the oligosaccharide is not available to the processing enzymes in either the endoplasmic reticulum or Golgi apparatus.

The present data serve to clarify some ambiguities that have been present in the lectin glycosylation literature for some time. The first of these is the proposed structure of the carbohydrate component of lima bean lectin[21]. The reported analysis did not take into account the contribution of oligomannose oligosaccharides that may have been present in the glycopeptide isolated after protease digestion. The presence of oligosaccharides of this type would lead to an overestimation of the proportion of 2-*O*-linked mannosyl residues in methylation analysis and also would reduce the relative molar proportion of xylose in composition analysis. The major component observed for lima bean lectin in the present study has the same properties as those of **4**. A similar situation exists, even more clearly, in the case of the lectin from tora bean (a strain of *Phaseolus vulgaris*)[22]. The present findings, together with those of other workers[4,5], suggest that the proposed structure is a composite of the oligomannose- and xylose–fucose-type oligosaccharides present in the lectin. The last rationalisation is of the omission of xylose and

fucose from the composition of *V. faba* lectin[23,24]. The results of the examination of the glycosylation of *V. faba* lectin in this paper indicate that the major oligosaccharide is identical to hexasaccharide **2**. The other oligosaccharides also contain either xylose, or xylose and fucose.

The question of the role of the glycosylation in plant lectins is tied into the ultimate question of the role of lectins in plant seeds, but it is clear that these components have to be taken into account if only in terms of their structural contribution. As more information on the three-dimensional structure of lectins accumulates, it may become possible to relate directly some of the properties of the lectin to its glycosylation. An instance of this is seen in the case of *V. faba* lectin, where the carbohydrate component is in such a position on the lectin subunits that it would prevent their interaction to form a tetramer from two dimers[25], in an analogous manner to concanavalin A. The extent of heterogeneity seen in this sample of plant glycoproteins seem less than for mammalian glycoproteins. This may reflect a smaller number of processing enzymes present in plants, or it may suggest a highly conserved interaction involving the carbohydrate parts of these lectins. If the latter were the case, the single structure found for the carbohydrate part of SBA is an intriguing and unexpected finding.

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