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A COMPARATIVE STUDY OF MANNOSE-BINDING LECTINS FROM THE AMARYLLIDACEAE AND ALLIACEAE

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Key Word Index—*Allium*; Alliaceae; *Galanthus*; Amaryllidaceae, bulbs; lectin; mannose-binding; chemotaxonomy.

Abstract—A comparative study of the lectins from the families Amaryllidaceae and Alliaceae reveals many common features: all bind D-mannose exclusively and have similar molecular structures and amino acid compositions. All these lectins contain subunits of M_r 11 500–14 000 which are not linked by disulphide bonds and occur as dimers (in *Allium sativum, A. vineale, A. ursinum, A. moly, Narcissus pseudonarcissus* and *Clivia miniata*) or tetramers (in *Galanthus nivalis, Hippeastrum* hybrid, *Allium cepa* and *A. porrum*. Most of these lectins were shown to occur as complex mixtures of isolectins. In general, the lectin concentration in Amaryllidaceae bulbs is higher than in Alliaceae bulbs. Antisera raised in rabbits against the *Galanthus nivalis* and the *Narcissus* cv Carlton lectins gave, upon double immunodiffusion, single precipitation bands and lines of identity with purified lectins from all Amaryllidaceae species, *A. cepa* and *A. porrum*. Similar single lines of identity were obtained between purified lectins from other *Allium* species and Amaryllidaceae lectins when challenged with rabbit anti-daffodil antiserum. However, two immunoprecipitin bands were obtained when the same assay was carried out with antiserum against *Galanthus nivalis* lectin, one line cross-reacting with the Amaryllidaceae lectins with the formation of a spur. Cross-reactions were also observed between *Allium cepa* and *A. porrum* lectins and the lectins from *A. moly, A. vineale, A. ursinum* and *A. sativum*. Although all lectins are serologically related, there are differences in their reaction with various antisera.

INTRODUCTION

With the exception of the large group of Gramineae lectins, most interest in plant lectins during the past decades has focused on lectins in dicotyledonous plant species. However, evidence has accumulated that lectins are not confined to a single family of monocotyledonous species but rather are widespread within this group. Indeed, lectins have been found in tulip (*Tulipa gesneriana*) [3, 9], meadow saffron (*Colchicum autumnale*) [10]. twayblade (*Listera ovata*) [15], snowdrop (*Galanthus nivalis*) [14], daffodil (*Narcissus pseudonarcissus*) [6, 16] and snowflake (*Leucojum aestivum and Leucojum vernum*) [16]. Moreover the isolation and characterization of these lectins has revealed that some of them exhibit several interesting properties. For instance, the lectins from *Galanthus, Narcissus* and *Leucojum* exclusively recognize mannose [11, 14, 16] which differ from all previously reported mannose-binding lectins from legume species (which also bind glucose and *N*-acetylglucosamine). Their absolute specificity towards mannose makes the Amaryllidaceae lectins very useful tools in glycoconjugate research and has already been exploited successfully for the one-step purification of IgM monoclonal antibodies from murine serum and α_2-macroglobulin from human serum by affinity chromatography on a column of immobilized *Galanthus nivalis* lectin [12] and the isolation from human sera of α_2-macroglobulin, haptoglobin and β-lipoprotein on immobilized *Narcissus pseudonarcissus* and *Hippeastrum hybr.* lectin [Kaku, H. and Goldstein, I. J., unpublsihed results]. Furthermore, Amaryllidaceae lectins have been shown to be potent and selective inhibitors of retroviruses (e.g. HIV) and cytomegalovirus [1].

Since detailed studies of various characteristics of all these mannose-specific lectins have shown that they very definitely differ in some properties (e.g. different molecular strucutres), it seemed worthwhile to seek new representatives of this class of lectins. Similarly, to understand the possible physiological role of these lectins additional information is needed regarding their occurrence within this family or within other taxonomic groups. This paper deals with a comparative study of different Amaryllidaceae and Alliaceae lectins. We present evidence that representatives of the family Alliaceae also contain mannose-binding lectins which strongly resemble the Amarylidaceae lectins with respect to their molecular structure, carbohydrate-binding specificity, amino acid composition and serological properties.

RESULTS

Occurrence of mannose-specific lectins in Amaryllidaceae and Alliaceae species

Previous studies have shown that within the Amaryllidaceae, mannose-specific lectins occur in the bulbs of species belonging to three genera indigenous to Mediterranean Europe: *Galanthus, Narcissus* and *Leucojum* [16]. Recently species of two other genera, *Hippeastrum* and *Clivia*, which have their centre of variation in America and southern Africa, respectively, but are often cultivated in Western Europe, were also shown to contain

reasonable amounts of the mannose-specific lectins. Unlike all other species, *Clivia* does not have a bulb but a rhizome. For practical reasons, however, the lectin was isolated from the leaves.

Because of the general occurrence of mannose-binding lectins within the Amaryllidaceae we looked for the presence of similar lectins in species of the Alliaceae for two reasons. First, there are indications that both families are closely related taxonomically and second, the occurrence of an unspecific agglutinating activity in bulbs and leaves of several *Allium* species had been reported by Sun and Yu [13]. Very soon it became evident that mannose-binding lectins similar to the Amaryllidaceae lectins also occur within the tribe Allieae of the Alliaceae which, like the Amaryllidaceae, are perennial herbs with a bulb or, more rarely, a rhizome. Because the genus *Allium* is widely distributed and contains both wild species and a large number of economically important species we were interested to see whether the lectins of Alliaceae and Amaryllidaceae are related. Therefore, we isolated the lectins from four cultivated Alliaceae species (*Allium cepa* (onion), *Allium porrum* (leek), *Allium sativum* (garlic) and *Allium moly* (dwarf flowering onions)) and two wild species *Allium ursinum* (ramsons) and *Allium vineale* (crow garlic). Because the agglutination activity of crude extracts with rabbit erythrocytes could only be inhibited by mannose, all Alliaceae lectins were purified following the purification scheme described for the Amaryllidaceae lectins [16]. Subsequently the lectins were characterized in some detail.

Since crude extracts from bulbs of different Alliaceae species showed a lower agglutination titre than extracts from Amaryllidaceae species it could be expected that Alliaceae bulbs contain less lectin than Amaryllidaceae bulbs. Whereas the yield of affinity purified lectin is about 2–4 mg per g fresh weight tissue for *Narcissus* cv Carlton, *Galanthus nivalis* and *Hippeastrum* hybr. bulbs, most Alliaceae species tested contained *ca* 1 mg of lectin per g fresh weight tissue except for the edible plants (*Allium cepa* and *A. porrum*) which contain even less lectin (0.5–10 μg per g fresh weight).

Molecular structure

The molecular strucutre of the mannose-specific lectins was determined using SDS-PAGE and gel filtration. Whereas upon SDS-PAGE (Fig. 1) the lectins isolated from *Galanthus nivalis* (lane 1) and *Narcissus* cv Carlton (lane 2) yielded a single polypeptide band of M_r 13 000, as previously reported [16], the *Hippeastrum* lectin (lane 3) yielded a polypeptide with a slightly higher M_r (*ca* 14 000). The *Clivia miniata* lectin (lane 4) migrated as two polypeptides of M_r 13 000 to 14 000, respectively. Whereas the *A. porrum* (lane 5) and *A. cepa* (lane 6) lectins yielded polypeptides of M_r 13 000 and 12 500, respectively, the lectins isolated from *A. moly* (lane 10), *A. sativum* (lane 7), *A. vineale* (lane 9) and *A. ursinum* (lane 8) migrated as two polypeptide bands of M_r 12 500 and 11 500, respectively. These results were identical whether electrophoresis was conducted in the presence or absence of β-mercaptoethanol indicating that subunits are not joined by disulphide bonds (Fig. 1).

Gel filtration experiments on a Superose 12 column demonstrated that the lectins isolated from *A. sativum*, *A. vineale*, *A. ursinum*, *A. moly* and *Clivia miniata* elute with an apparent M_r of 25 000 indicating that they are probably dimers. Although the lectins from *A. moly*. *A. sativum*, *A. vineale*, *A. ursinum* and *Clivia miniata* contain two different polypeptides all these lectins coelute with the *Narcissus* cv Carlton lectin when chromatographed on a Superose column. The lectins isolated from *Hippeastrum* hybr., *A. cepa* and *A. porrum* coelute with the *Galanthus nivalis* lectin (M_r 50 000) indicating that these proteins are composed of four subunits (Table 1).

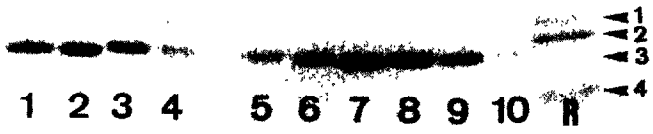

Fig. 1. SDS-PAGE of various Amaryllidaceae and Alliaceae lectins after reduction and alkylation of the proteins. About 30 μg of each lectin was loaded on the gel in the presence of 2% β-mercaptoethanol. Lectins were loaded as follows: lane 1, *Galanthus nivalis*; lane 2, *Narcissus* cv Carlton; lane 3, *Hippeastrum* hybr.; lane 4, *Clivia miniata*; lane 5, *Allium porrum*; lane 6, *A. cepa*; lane 7, *A. sativum*; lane 8, *A. ursinum*; lane 9, *A. vineale*; lane 10, *A. moly*. M_r reference proteins are shown in lane R: (1) myoglobin (M_r 17 200), (2) myoglobin I and II (M_r 14 600), (3) myoglobin I (M_r 8240) and (4) myoglobin II (M_r 6380).

Table 1. Molecular structure of Amaryllidaceae and Alliaceae lectins

Species	M_r on SDS-PAGE	M_r on Gel filtration	Molecular structure
Amaryllidaceae			
Galanthus nivalis	13 000	50 000	Tetramer
Narcissus cvs	13 000	25 000	Dimer
*Leucojum aestivum**	13 000	25 000	Dimer
*Leucojum vernum**	13 000	25 000	Dimer
Clivia miniata	13 000 and 14 000	25 000	Dimer
Hippeastrum hybr.	14 000	50 000	Tetramer
Alliaceae			
Allium sativum	11 500 and 12 500	25 000	Dimer
A. vineale	11 500 and 12 500	25 000	Dimer
A. moly	11 500 and 12 500	25 000	Dimer
A. ursinum	11 500 and 12 500	25 000	Dimer
A. cepa	12 500	50 000	Tetramer
A. porrum	13 000	50 000	Tetramer

*The results for *Leucojum* are taken from Van Damme *et al.* [15].

Ion exchange chromatography

When the purified lectins were analysed by high resolution ion exchange chromatography, it became evident that they all yield a very complex mixture of isolectins (Fig. 2). Although all lectins, except the *A. ursinum* agglutinin, eluted from the anion exchanger column with approximately the same NaCl concentration, different isolectin patterns were obtained. Using the same experimental conditions as for the other lectins, the *A. ursinum* agglutinin was only partially retained on the Mono-Q column. When the pH of the running buffer was increased from pH 7.8 to 8.7 all *A. ursinum* isolectins were bound to the column. However, they eluted at very low salt concentrations.

The occurrence of multiple isolectins was confirmed by isoelectric focusing. As shown in Fig. 3 the purified lectins yield a complex pattern of polypeptides of differing intensity indicating that many isolectins are present, some in higher concentrations than others. The *A. ursinum* agglutinin apparently contains only a few isolectins with a p*I* near 4.5 whereas all the other lectins contain polypeptides with p*I* ranging from 4.5 to 2.8. All isolectins appear to possess the same molecular structure and exhibit similar agglutination properties and a binding specificity for mannose (results not shown).

Carbohydrate-binding and agglutination properties

Amaryllidaceae and Alliaceae lectins readily agglutinate rabbit erythrocytes but are completely inactive towards human erythrocytes, irrespective of the blood group. The specific agglutination activity of all lectins was approximately the same, being 3.75 $\mu g\,ml^{-1}$ for achieving complete agglutination, except that the lectins from *A. sativum* and in particular *A. moly* exhibited a lower agglutination activity (10 and 60 $\mu g\,ml^{-1}$ for achieving complete agglutination, respectively). It was previously reported that rabbit erythrocytes of different individuals exhibit considerable variation in sensitivity in agglutination assays [16]. Therefore these results should not be compared quantitatively with the figures from previous reports.

The carbohydrate-binding specificity of the lectin was assessed by hapten inhibition assays using a whole series of simple sugars. Of all monosaccharides tested only D-mannose was inhibitory. However, all Alliaceae lectins and the *Clivia miniata* lectin require a two-fold higher mannose concentration for inhibition than the *Galanthus*, *Narcissus* and *Hippeastrum* lectins.

Amino acid and carbohydrate analysis

The amino acid composition of three Alliaceae lectins was determined and compared to those of the Amaryllidaceae lectins. All lectins have a very similar but not identical amino acid composition typified by high contents of aspartic acid, threonine, glycine, serine and leucine (Table 2). Sugar determinations of purified lectins indicated no covalently bound carbohydrate, suggesting that the lectins are not glycosylated.

Serological relationship between Amaryllidaceae and Alliaceae lectins

Because the Amaryllidaceae and Alliaceae lectins strongly resemble each other in their molecular structure and carbohydrate-binding properties it seemed worthwhile to examine the serological relationship among the lectins. When challenged with antisera raised against the *Galanthus nivalis* and the *Narcissus* cv Carlton lectins in double immunodiffusion assays, purified lectins from all Amaryllidaceae species, *A. cepa* and *A. porrum* produced single immunoprecipitin bands forming lines of identity with the different lectins (Fig. 4). Similarly, when purified lectins from *A. moly*. *A. sativum*, *A. vineale* and *A. ursinum* were challenged with an antiserum against the *Narcissus* cv Carlton lectin, single immunoprecipitin lines of identity were also obtained between these Alliaceae lectins and Amaryllidaceae lectins. However, when the same assay was carried out with antiserum against the *Galanthus nivalis* lectin two immunoprecipitin lines were obtained, one cross-reacting with the Amaryllidaceae lectins and forming a spur at the end of the arc of precipitation. This cross-reaction was also observed between the *A. cepa* and

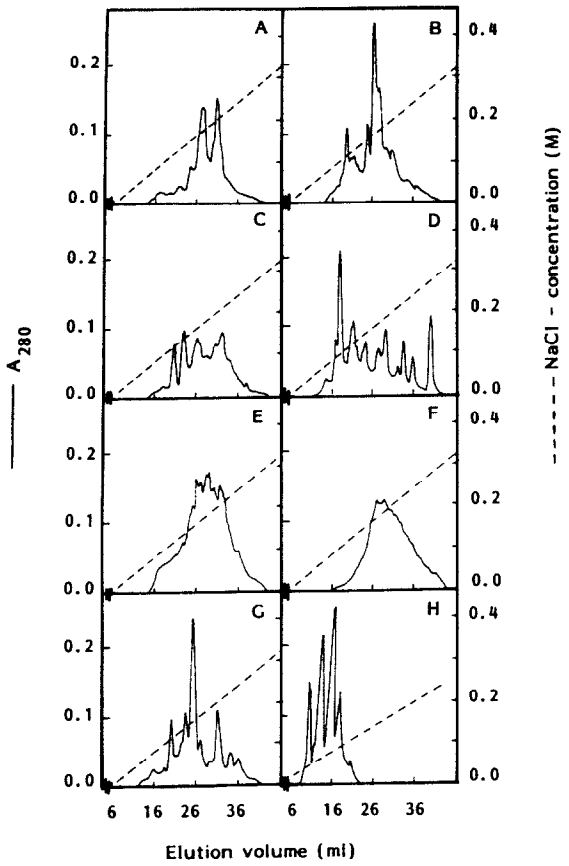

Fig. 2. Ion exchange chromatography of Amaryllidaceae and Alliaceae lectins. Purified lectin (*ca* 250 μg) was chromatographed on a Mono-Q column. The running buffer was 20 mM Tris–HCl pH 7.8 for all lectins except the *Allium ursinum* lectin which was chromatographed in 20 mM Tris–HCl pH 8.7. Lectins were eluted using a linear NaCl gradient from 0 to 0.4 M except for the *A. ursinum* lectin where we used a gradient from 0 to 0.25 M NaCl. The flow rate was 2 ml min^{-1}. Lectins are shown as follows: *Galanthus nivalis* (A); *Narcissus* cv Carlton (B); *Hippeastrum* hybr. (C); *Clivia miniata* (D); *A. porrum* (E); *A. cepa* (F); *A. sativum* (G); *A. ursinum* (H).

A. porrum lectins and the lectins from *A. moly*, *A. vineale*, *A. ursinum* and *A. sativum*. From these results we can conclude that the lectins isolated from *A. porrum* and *A. cepa* are more closely related to the Amaryllidaceae lectins than the other Alliaceae lectins. Whereas the *A. cepa* and the *A. porrum* lectin yield immunoprecipitin lines of identity with the Amaryllidaceae lectins, the other Alliaceae lectins appear to possess some epitopes that are different from those of Amaryllidaceae lectins.

All lectins reacted with both antisera used in the immunodiffusion assays. However, as double immunodiffusion experiments cannot be interpreted quantitatively, both antisera were challenged with known concentrations of the lectins in an ELISA. All Amaryllidaceae lectins and both the *A. cepa* and *A. porrum* lectin reacted strongly with the antisera prepared against the *Galanthus nivalis* and the *Narcissus* cv Carlton lectins (Fig. 5). The lectins isolated from *A. sativum*, *A. vineale*, *A. moly* and *A. ursinum* also bind to the antiserum but this reaction is

Fig. 3. IEF of Amaryllidaceae and Alliaceae lectins. A highly purified lectin sample (*ca* 100 μg) was loaded on to the gel. Focusing was carried out in the presence of 8 M urea. Lectins were loaded as follows: lane 1, *Allium moly*; lane 2, *A. vineale*; lane 3, *A. ursinum*; lane 4, *A. sativum*; lane 5, *Galanthus nivalis*; lane 6, *Narcissus* cv. Carlton; lane 7, *Hippeastrum hybr.*; lane 8, *Clivia miniata*; lane 9, *A. porrum*; lane 10, *A. cepa*. The positions of the p*I* marker proteins (1) glucose oxidase (p*I* 4.15), (2) amyloglucosidase (p*I* 3.50) and (3) pepsinogen (p*I* 2.80) are indicated.

weaker. We can conclude that although all lectins are serologically related there are certainly quantitative differences in their reaction with the various antisera.

DISCUSSION

In this paper we have presented evidence that in addition to members of the Amaryllidaceae, representatives of the Alliaceae also contain mannose-specific lectins, albeit in lower concentrations than bulbs from the former family. Furthermore, the Alliaceae and Amaryllidaceae lectins have similar molecular structures, agglutination properties and amino acid compositions. When analysed by ion exchange chromatography or isoelectric focusing all lectins yielded complex mixtures of isolectins. However, as none of the lectins appears to be glycosylated, the occurrence of multiple isoforms cannot be explained by differences in glycosylation. Moreover, the isolectin patterns are highly reproducible [18] so that artefacts due to *in vitro* modifications are not likely to occur. A study of the serological relationship among these lectins indicates that the lectins from *A. cepa* and *A. porrum* are serologically identical to the Amaryllidaceae lectins. On the other hand, the lectins isolated from *A. moly*, *A. sativum*, *A. vineale* and *A. ursinum* exhibit only a few epitopes in common with the Amaryllidaceae lectins as they produce pronounced spurs in their cross-reaction with these lectins in a double immunodiffusion assay.

At present the taxonomic classification of the Alliaceae is still a matter of dispute. Some botanists include the Alliaceae in the (heterogeneous) Liliaceae. Others consider the Alliaceae as part of the Amaryllidaceae, or regard them as a separate family which is morphologically related to the Amaryllidaceae. However, the latter is not unequivocally supported by some chemical characteristics in as much as Amaryllidaceae species probably do not contain saponins and possess unique alkaloids. According to Dahlgren *et al.* [4] an analysis of the

Table 2. Amino acid composition of Amaryllidaceae and Alliaceae lectins

Amino acid	(1)*	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Asp	17.0	16.8	16.5	14.3	17.9	14.5	14.0	11.1	12.6
Thr	9.0	7.6	9.1	7.7	5.4	7.0	4.2	9.0	7.6
Ser	7.4	7.1	9.3	7.4	6.8	6.4	12.0	9.0	9.0
Glu	6.8	8.0	8.1	9.7	9.1	8.6	11.7	7.4	9.4
Pro	3.7	3.7	3.1	4.3	1.6	2.8	2.7	1.8	3.0
Gly	11.2	12.9	12.6	11.5	11.4	13.1	11.6	10.8	12.6
Ala	3.4	5.0	5.8	5.8	4.5	6.0	6.2	3.5	5.2
Val	5.6	3.9	5.1	4.8	9.9	5.2	6.4	3.1	5.1
Met	1.7	1.8	0.6	1.9	1.6	2.2	0.9	1.7	2.0
Ile	5.5	5.9	4.8	4.5	3.5	4.0	3.3	6.4	4.8
Leu	8.8	8.0	8.3	7.8	6.0	6.9	7.0	9.5	7.3
Tyr	5.8	5.1	5.6	5.8	5.8	6.5	4.7	8.1	6.3
Phe	3.0	1.7	2.6	2.2	3.7	3.6	1.9	4.8	2.9
Lys	2.8	2.9	2.5	3.6	2.5	3.3	3.6	4.0	2.3
His	0.9	0.9	2.2	1.3	0.0	1.6	2.9	1.9	2.2
Arg	2.9	3.8	3.1	4.8	6.4	5.2	3.8	2.8	3.5
Cys	2.9	3.0	0.6	0.9	2.2	1.4	1.8	2.8	1.4
Trp	1.7	2.0	2.0	1.8	1.6	1.8	1.5	1.9	2.1

* The amino acid composition of the lectins isolated from *Galanthus nivalis* (1), *Narcissus* cv Carlton (2), *Clivia miniata* (3), *Hippeastrum* hybr. (4), *Allium vineale* (5), *A. ursinum* (6), *A. sativum* (7), *A. cepa* (8) and *A. porrum* (9) is calculated in mol%.

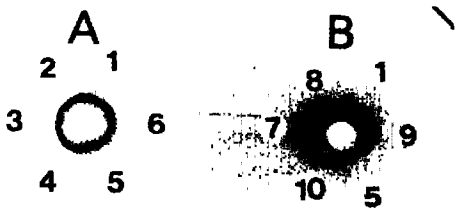

Fig. 4. Double immunodiffusion of purified Amaryllidaceae and Alliaceae lectins against anti-*Narcissus* cv Carlton lectin and anti-*Galanthus nivalis* lectin antisera. Antisera against *N.* cv Carlton (A, central well) and *G. nivalis* lectin (B, central well) were challenged with 10 μg of purified lectin isolated from *Galanthus nivalis* (1), *Narcissus* cv Carlton (2), *Hippeastrum hybr.* (3), *Clivia miniata* (4), *Allium porrum* (5), *A. cepa* (6), *A. sativum* (7), *A. vineale* (8), *A. ursinum* (9) and *A. moly* (10).

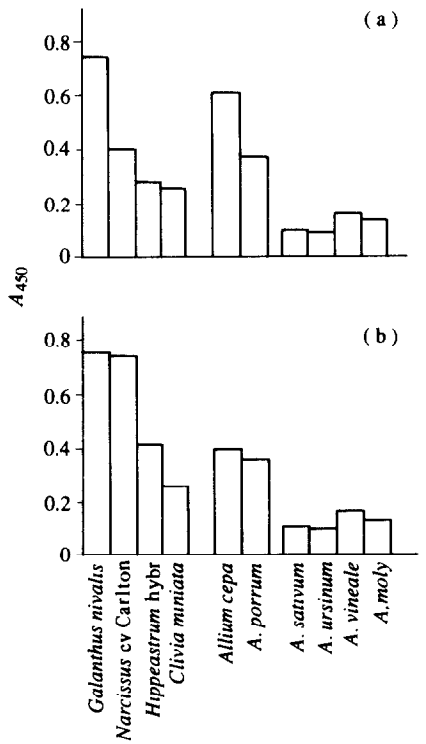

Fig. 5. ELISA of anti-*Galanthus nivalis* lectin (A) and anti-*Narcissus* cv Carlton lectin (B) antiserum challenged with purified Amaryllidaceae and Alliaceae lectins. This figure shows A_{405} when 0.5 μg of a purified lectin was assayed.

variation within the Alliaceae is needed as this family is not as homogenous as is generally assumed.

A certain degree of heterogeneity within the tribe Allieae can be inferred from our results. When we compare the lectins from different Alliaceae species it is surprising that the *A. ursinum*, *A. vineale*, *A. sativum* and the *A. moly* lectins differ from the *A. cepa* and *A. porrum* lectins in that they are proteins comprised of two different subunits. Furthermore, it is striking that *A. cepa* and *A. porrum* contain lectins which are serologically more related to Amaryllidaceae lectins than the other Alliaceae lectins tested.

Although the lectin concentration in Alliaceae bulbs is lower than in Amaryllidaceae bulbs, all Amaryllidaceae and Alliaceae lectins except the *A. cepa* and the *A. porrum*

lectin represent one of the most prominent proteins, quantitatively, in a crude bulb extract. The question arises as to why the edible onion and leek plants contain about a hundred-fold less lectin compared to the other Alliaceae species. Unfortunately we cannot resolve this problem based on our results.

EXPERIMENTAL

Plant material. Bulbs of *Galanthus nivalis* L. (snowdrop), *Allium ursinum* L. (ramsons) and *A. vineale* L. (crow garlic) were collected locally at the beginning of the growing season. Dry bulbs of *Narcissus* cv Carlton (daffodil), *Hippeastrum* hybr. Herb. (amaryllis), *A. cepa* L. (onion), *A. sativum* L. (garlic) and *A. moly* L. (dwarf flowering onions), *A. porrum* L. (leek) plants and *Clivia miniata* Regel plants were purchased from a local store.

Extraction and lectin isolation. For all Amaryllidaceae and Alliaceae species the same procedure was followed to extract and isolate the lectin from the plant material [16]. The bulbs or leaves were homogenized with a blender using 50 ml 1 M $(NH_4)_2SO_4$ per g fr. tissue. The extract was filtered through cheese cloth and centrifuged (4000 *g* for 10 min). The resulting supernatant was frozen overnight at −20°. After thawing, the ppt. was removed by a second centrifugation. The clarified supernatant was then applied to a column of mannose-Sepharose (50 ml bed vol.) equilibrated with 1 M $(NH_4)_2SO_4$. Unbound proteins were eluted and the lectin desorbed using unbuffered 20 mM 1,3-diaminopropane. To remove all phenolic compounds, the affinity purified lectin was brought to 1 M $(NH_4)_2SO_4$ by adding the solid salt and applied to a column of phenyl Sepharose (15 × 3 cm) equilibrated with 1 M $(NH_4)_2SO_4$. After washing the column, lectins were eluted using H_2O or 1,3-diaminopropane (20 mM, unbuffered solution).

Ion exchange chromatography. The purified lectins were further analysed by ion exchange chromatography using a Chromatography system equipped with a Mono-Q column (type HR 5/5) equilibrated with Tris buffer. Lectin samples were dialysed against the same buffer and loaded on the Mono-Q column. After washing the column with 4 ml of buffer the lectin was eluted using a linear gradient of increasing NaCl concn in this buffer. The experimental conditions used are described in the legends to the figures. The A_{280} of the eluate was monitored with a UV detection monitor.

Isoelectric focusing (IEF). IEF on 5% (w/v) acrylamide gels was performed in the presence or absence of 8 M urea using the LKB Midget System. The ampholytes used ranged from pH 2.5 to 5.0. Focusing was carried out as described in ref. [18].

Enzyme-linked immunosorbent assay (ELISA). ELISA was carried out as described previously [17]. Purified lectins of *G. nivalis* and *N. pseudonarcissus* in concns ranging from 0.1–12 ng ml^{-1} were used to prepare standard curves. Phosphate-buffered saline was used as a blank. Alkaline phosphatase conjugated rabbit anti-*Galanthus nivalis* lectin and anti-*Narcissus* cv Carlton lectin antibodies were prepared following the procedure of ref. [19] using glutaraldehyde to couple the enzyme to the antibody.

Amino acid analysis. Amino acid analyses [7] were performed by the University of Michigan Protein Sequencing Facility.

Agglutination assays. Agglutination assays were conducted as reported previously [16]. The sugars tested were: glucose, galactose, galactosamine, *N*-acetylglucosamine, *N*-acetylgalactosamine, mannose, lactose, melibiose, fucose, arabinose, amylose, ribose, fructose, trehalose, sorbose, xylose, sucrose, maltose and sorbitol (all sugars are of the D-configuration with the exception of L-fucose and L-sorbose).

Production of antisera. Antibodies against the *G. nivalis* and the *N.* cv Carlton lectins were raised in rabbits immunized with the purified lectins. The immunization procedure has been described previously [16]. The antisera were partially purified as in ref. [17].

Double immunodiffusion assay. Double immunodiffusion assays were done in small Petri dishes filled with a layer of 1% agarose containing 4% polyethylene glycol (M_r 6000) and 0.1 M mannose (to prevent aspecific binding of the lectin). After 15 hr at 37° unreacted proteins were washed from the plates with Pi buffered saline and the gel stained with Coomassie Brilliant Blue.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Lectin preparations were analysed by SDS-PAGE using a discontinuous system on 12.5–25% (w/v) acrylamide gradient gels as described in ref. [8].

Protein assay. Protein concentration was estimated by the method of ref. [2] using BSA and lectins as standards.

Sugar determination. Total neutral sugar content was determined by the phenol–H_2SO_4 method [5].

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