Isolation and Characterization of Lectins from *Vicia villosa*

TWO DISTINCT CARBOHYDRATE BINDING ACTIVITIES ARE PRESENT IN SEED EXTRACTS

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An uncharacterized lectin from *Vicia villosa* seeds has been reported to bind specifically to mouse cytotoxic T lymphocytes (Kimura, A., Wigzell, H., Holmquist, G., Ersson, B., and Carlsson, P., (1979) J. Exp. Med. 149, 473-484). We have found that *V. villosa* seeds contain at least three lectins which we have purified by affinity chromatography on a column of immobilized porcine blood group substances eluted with varying concentrations of *N*-acetylgalactosamine and by anion exchange chromatography. The three lectins are composed of two different subunits with M, = 35,900 (subunit B) and 33,600 (subunit A), estimated from their exchange chromatography. Sedimentation equilibrium analysis of a single preparation of these lectins was obtained in relatively small amounts from seed extracts. The predominant lectin in *V. villosa* seeds, B, does not agglutinate A, B, or O erythrocytes.

Lectins of defined specificity have been used over the last few years in probing the structure and distribution of cell membrane carbohydrates (1). In 1978, Kimura and Wigzell (2) reported that a surface glycoprotein on mouse T lymphocytes appeared after immune activation by major histocompatibility complex alloantigens or polyclonal activation with concanavalin A. Expression of this glycoprotein was shown to correlate from seed extracts. Most of the lectin in *V. villosa* seeds was designated T145. Kimura (3) subsequently reported that a lectin preparation from *Vicia villosa* seeds bound specifically to this glycoprotein. Furthermore, they were able to fractionate allogen- and mitogen-activated blasts into highly cytotoxic and noncytotoxic cell populations using a *V. villosa* affinity adsorbent. Recent reports have not entirely confirmed these findings. Kaufmann and Berke (4) examined alloimmunized lymphocyte populations induced in vivo and in vitro and found that T145 was expressed on some, but not all, of these populations. No correlation was observed between the presence of T145 and the lytic activity of the populations studied. MacDonald et al. (5) analyzed the interaction of *V. villosa* lectin with T lymphoblasts using flow cytometry and were unable to demonstrate any appreciable difference in cytolytic activity between lymphoblasts that bound large or small amounts of *V. villosa* lectin. Braciale et al. (6) reported similar conclusions. Thus, both the selective expression of T145 on cytotoxic T lymphocytes and the specific interaction of these cells with a lectin from *V. villosa* seeds have been questioned.

The carbohydrate specificity of the *V. villosa* lectin preparations used in the preceding studies has not been elucidated. Kimura et al. (3) prepared the lectin by affinity chromatography on a column of human blood group A substance coupled to Sepharose 2B eluted with *N*-acetylgalactosamine, the blood group A determinant (7). In addition, *N*-acetylgalactosamine blocked the binding of cytotoxic T lymphocytes to the *V. villosa* affinity adsorbent used to fractionate mixed lymphocyte populations. Hence, the lectin has been referred to as A-specific, although its A erythrocyte agglutinating activity has not been documented. It is interesting that a number of other lectins with specificity for terminal *N*-acetylgalactosamine residues apparently did not exhibit the high selectivity of *V. villosa* for T145 (3).

We undertook the isolation of the *V. villosa* lectin in order to characterize its carbohydrate binding specificity and to examine the oligosaccharide structure of the glycoprotein T145 to which it was reported to bind. In the present investigation, we report the presence of two distinct carbohydrate binding activities in *V. villosa* seed extracts. One is a potent A erythrocyte agglutinating activity, but lectins with this activity were obtained in relatively small amounts from seed extracts. Most of the lectin in *V. villosa* seeds does not agglutinate A, erythrocytes but agglutinates erythrocytes having exposed *N*-acetylgalactosamine residues α-linked to serine or threonine residues in their surface glycoproteins (8, 8a).

**EXPERIMENTAL PROCEDURES**

1. The "Experimental Procedures" are presented in miniprint as prepared by the authors. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 82M-2881, cite the authors, and include a check or money order for $1.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

2. The abbreviations used are: PBS, phosphate-buffered saline (0.137 M NaCl, 2.7 mM KCl, 8.1 mM NaHPO₄, 1.5 mM KH₂PO₄, pH 7.4); BSA, bovine serum albumin; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

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**RESULTS**

**Isolation of Lectins from *V. villosa* Seeds by Affinity Chromatography**—Lectins were isolated from the seed extract by adsorption to the porcine blood group substances affinity column and elution with 1 and 10 mM N-acetylgalactosamine, as shown in Fig. 1. A small amount of additional protein was also eluted with 100 mM N-acetylgalactosamine (not shown). The A<sub>1</sub> erythrocyte agglutinating specific activity in the peak fraction of 14 mM N-acetylgalactosamine eluate was 2000 hemagglutinating units/mg, 40-fold higher than that in the peak fraction of the 1 mM eluate. The electrophoretic profiles of the seed extract applied to the column and these peak fractions are shown in Fig. 2. SDS-PAGE of the N-acetylgalactosamine eluates resolves 2 protein bands with estimated *M* < sub > r </ sub > = 35,900 and 33,600. These have been given the designations B and A<sub>1</sub>.

**Preparation of Porcine Blood Group Substances Affinity Column**—25-36 ml of 14 mM-Gal1-2 or 15 were washed as described by the manufacturer and added to an equal volume of 5% dextran in 0.1 M Na<sub>2</sub>PO<sub>4</sub> buffer, pH 6.3. The elution was washed through the column with 1 M Na<sub>2</sub>PO<sub>4</sub>, pH 6.3, for 1 hr at flow rate of 10 ml/hr, and then washed with 1 M Na<sub>2</sub>PO<sub>4</sub>, pH 6.3, for 1 hr at flow rate of 10 ml/hr. N-acetylgalactosamine eluate (10 ml) were collected for each tube.

** SDS-PAGE of Lectins prepared by affinity chromatography.** Affinity chromatography of *V. villosa* seed extract was performed as illustrated in Fig. 1. Electrophoresis, in the presence of SDS, of fractions from this column was performed as described under "Experimental Procedures." The profiles of the seed extract applied to the column (50 pg), the 1 mM N-acetylgalactosamine eluate peak fraction (10 pg), and the 10 mM N-acetylgalactosamine eluate peak fraction (5 pg) are shown here. Arrows indicate the migration of the molecular weight standards.
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Discontinuous polyacrylamide gel electrophoresis was performed on the 1, 10, and 100 mM N-acetylgalactosamine eluates. The 1 mM N-acetylgalactosamine eluate contained protein which migrated as 1 broad band (Fig. 3A). In addition to this protein, the 10 mM N-acetylgalactosamine eluate contained at least two major proteins, both migrating faster than the protein in the 1 mM eluate (Fig. 3A). The 100 mM N-acetylgalactosamine eluate also contained two major proteins, although another protein which migrated just below the faster of the two was also often present (Fig. 3A).

To determine the subunit structure of the *V. villosa* lectins resolved by nondenaturing gel electrophoresis, protein was eluted from the adjacent unstained gel and subjected to SDS-PAGE, as described under “Experimental Procedures.” The gel lanes in Fig. 3B are numbered to correspond to the bands in Fig. 3A. The broad band in the 1 mM N-acetylgalactosamine eluate, labeled 1, represents a lectin composed entirely of subunit B. In the electrophoretic profile of the 10 mM N-acetylgalactosamine eluate, the upper of the two major bands, labeled 2, appears to represent a lectin composed of both subunits, with A being more prominent than B. The lower of the two major bands, labeled 3, represents a lectin also composed of both subunits, with A and B being equally prominent. In the 100 mM N-acetylgalactosamine eluate, the upper major band, labeled 4, represents a lectin composed entirely of subunit A. The lower major band, labeled 5, represents a lectin composed of both subunits, with A and B being about equally prominent. The band just below the lower major band, labeled 6, appears to represent a lectin composed of both subunits as well, with B being somewhat more prominent than A. Thus, it appeared that *V. villosa* seeds contain at least three lectins corresponding to band 1, band 4, and band 3 (and 5) composed of the two subunits, A and B, resolved on SDS-PAGE. Subsequent experiments, discussed below, have shown that the intact lectins are tetrameric, and therefore they have been designated B1, A1, and A2B2, respectively.

Table I presents a quantitative summary of the isolation of lectins by affinity chromatography. In this preparation, 200 ml of seed extract were divided into equal parts (I and II), and the two parts were applied sequentially to the affinity column. The affinity column was eluted with 5 mM (part I) and 2.5 mM (part II) N-acetylgalactosamine before elution with 10 mM N-acetylgalactosamine in an attempt to improve the A1 erythrocyte agglutinating activity of the lectins in the 10 mM N-acetylgalactosamine eluates. After the column was eluted with 5 mM N-acetylgalactosamine, only 0.4 mg of protein remained to be eluted with 10 mM N-acetylgalactosamine, although the A1 erythrocyte agglutinating activity in the 10 mM eluate increased. On the other hand, elution with 2.5 mM N-acetylgalactosamine displaced only 0.6 mg of protein from the column and failed to increase the specific activity of the lectins.

**Table I**

| Volume | Protein | A1 hemagglutinating activity |
|--------|---------|------------------------------|
| ml     | mg      | HU/mg protein               |
| HU     |         |                             |
| Seed extract | 198 | 749 | 44,500* | 59.5* |
| Affinity chromatography | | | | |
| Part I | Run through | 315 | 372 | 3,630* | 8.7* |
| 1 mM GalNAc eluate | 37.7 | 14.9 | 2,100 | 141 |
| 5 mM GalNAc eluate | 29.5 | 2.3 | 3,280 | 1,400 |
| 10 mM GalNAc eluate | 17.7 | 0.4 | 1,770 | 4,090 |
| Part II | Run through | 320 | 358 | 2,650* | 6.5* |
| 1 mM GalNAc eluate | 39.5 | 16.3 | 1,980 | 121 |
| 2.5 mM GalNAc eluate | 13.8 | 0.6 | 990 | 1,700 |
| 10 mM GalNAc eluate | 25.1 | 1.5 | 3,860 | 2,560 |
| 100 mM GalNAc eluate | 22.8 | 0.4 | 2,280 | 5,080 |

* Units of A1 erythrocyte agglutinating activity minus units of O erythrocyte agglutinating activity.

Fig. 3. Subunit structure of *V. villosa* lectins. N-Acetylgalactosamine eluates were analyzed as described under “Experimental Procedures.” A, polyacrylamide gel electrophoresis under nondenaturing conditions of the 1, 10, and 100 mM N-acetylgalactosamine eluates analyzed. The 10 and 100 mM N-acetylgalactosamine eluates were concentrated prior to analysis. The stained and destained lanes are shown here. Areas of the unstained gel adjacent to bands 1–6 were analyzed in B. B, SDS-PAGE of protein from areas of the unstained gel. The SDS-PAGE analysis of the protein remaining in minced gel pieces (not shown) was not qualitatively different from the analysis of the protein eluted into SDS-containing buffer shown here. Lanes 1–6 correspond to the bands in A.
the 10 mM N-acetylgalactosamine eluate substantially. Recovery of hemagglutinating units in this preparation was 51%, although recovery in other preparations has been somewhat higher (61-70%). The yield of lectins in this preparation was 36.4 mg. In other preparations, reaplication of the run through material to the porcine blood group substances affinity column or to a column of N-acetylgalactosamine coupled to epoxy-activated cross-linked 4% beaded agarose (Selectin 5) revealed that additional protein bound and was eluted with 1 mM N-acetylgalactosamine (data not shown). With such repeated applications, the total yield of lectins was 75-80 mg/100 g of dry seeds, a value very similar to that reported by others (3, 5, 12). The N-acetylgalactosamine eluates from this preparation have been used to further purify the B4, A4, and A2B2 lectins.

Purification of B4 Lectin—Because the 1 mM N-acetylgalactosamine eluate contained some A2 erythrocyte agglutinating activity and trace amounts of band A on SDS-PAGE, the B4 lectin was purified further by anion exchange chromatography. The 1 mM N-acetylgalactosamine eluate (part I) was dialyzed extensively against 10 mM Tris-HCl buffer, pH 8.0, and applied to a DEAE-cellulose column equilibrated in the same buffer. Bound protein was displaced from the column by stepwise elution with NaCl buffered with 10 mM Tris-HCl pH 8.0. As shown in Fig. 4, all of the protein in the 1 mM N-acetylgalactosamine eluate bound to the column and 84% of the protein applied was displaced with 85 mM NaCl. The protein displaced with 85 mM NaCl migrates as a single sharp band on SDS-PAGE with M, = 35,900, i.e. the B subunit (Fig. 4, inset). The electrophoretic mobility does not vary when samples are prepared with β-mercaptoethanol, suggesting that the protein lacks interchain disulfide bonds. Sedimentation equilibrium analysis of the lectin displaced with 85 mM NaCl gave a linear plot of log concentration versus radius, indicative of physical homogeneity. The M, calculated from this analysis is 108,300, using a calculated partial specific volume of 0.702 ml/g. The M, of the dissociated subunits, determined by sedimentation equilibrium analysis in the presence of 6 M guanidine HCl, is 25,600. From these analyses, it was apparent that the intact lectin is composed of 4 B subunits, and it has been designated B4.

The 1 mM N-acetylgalactosamine eluates from the preparation summarized in Table I contain approximately 26 mg of B4 lectin, and additional B4 lectin can be purified from the run through material. The purified B4 lectin does not agglutinate A, B, or O erythrocytes at a concentration of ≤1.15 mg/ml. The protein displaced with 170 mM NaCl accounted for 87% of the A, erythrocyte agglutinating units recovered and the SDS-PAGE of this fraction showed that both A and B subunits were present (Fig. 4, inset). The small amount of protein displaced with 500 mM NaCl was not characterized further.

Purification of A4 Lectin—The 100 mM N-acetylgalactosamine eluate was dia lyzed extensively against 10 mM Na phosphate buffer, pH 7.2, and applied to a DEAE-cellulose column equilibrated in the same buffer. Bound protein was displaced from the column by elution with a linear gradient from 0-0.5 M NaCl buffered with 10 mM Na phosphate, pH 7.2. The protein in the 100 mM N-acetylgalactosamine eluate was displaced in 2 peaks with NaCl, as shown in Fig. 5A. Fractions from the first peak were pooled as indicated and concentrated. The inset shows the SDS-PAGE profiles of the 100 mM N-acetylgalactosamine eluate and this concentrated pool, labeled peak 1. SDS-PAGE of peak 1 resolves a single band, with M, = 33,600, i.e. the A subunit, and its electrophoretic mobility does not vary when samples are prepared with β-mercaptoethanol. Sedimentation equilibrium analysis of this lectin in aqueous solution gave a linear plot of log concentration versus radius. The M, calculated from this analysis is 109,500, using a calculated partial specific volume of 0.722 ml/g. On the basis of the molecular weight of the intact lectins and the estimated molecular weight of the subunit by SDS-PAGE, it appeared likely that this lectin is also composed of 4 subunits, and it has been designated A4.

Approximately 200 µg of A4 lectin were purified from 100 g of seeds. The purified A4 lectin has an A4 erythrocyte agglutinating specific activity of 5000 hemagglutinating units/mg. Purification of A2B2 Lectin—The 10 mM N-acetylgalactosamine eluates were dia lyzed extensively against 10 mM Na phosphate buffer, pH 7.2, containing 0.05 M NaCl and applied to a DEAE-cellulose column equilibrated in 10 mM Na phosphate buffer, pH 7.2. Bound protein was displaced from the column by elution with a linear gradient from 0.05-0.5 M NaCl buffered with 10 mM Na phosphate, pH 7.2. The protein in the 10 mM N-acetylgalactosamine eluates was displaced in 2 peaks with NaCl, as shown in Fig. 5B. Fractions from the second peak were pooled as indicated and concentrated. The SDS-PAGE profile of this concentrated pool, labeled peak 2, is shown in the inset. Both A and B bands are present and of approximately equal prominence. Sedimentation equilibrium analysis of this lectin in aqueous solution gave a linear plot of log concentration versus radius. Since the amino acid and carbohydrate composition of this lectin has not been determined, the partial specific volume was estimated from those of the purified A4 and B4 lectins. The M, calculated from this analysis is approximately 94,000.

Chemical Composition of B4 and A4 Lectins—The amino acid and carbohydrate analyses of purified B4 and A4 lectins are shown in Table II. Both lectins are rich in the acidic amino

![Fig. 4](http://www.jbc.org/)

**Fig. 4.** Anion exchange chromatography of the 1 mM N-acetylgalactosamine eluate. The 1 mM N-acetylgalactosamine eluate from the *V. villosa* preparation summarized in Table I (part I) was dialyzed against 10 mM Tris-HCl buffer, pH 8.0. 17 ml (0.43 mg of protein/ml) were applied to a DEAE-cellulose column (0.7 × 14 cm) equilibrated in the same buffer and the column was washed with 5 ml of starting buffer. Column fractions were monitored by absorbance at 280 nm (O---O). The column was eluted with 85 mM NaCl in 10 mM Tris-HCl buffer, pH 8.0, at a flow rate of 40 ml/h until the A200 of the effluent fell to <0.2. The column was then eluted sequentially with 170 mM, 500 mM, and 1 M NaCl buffered with 10 mM Tris-HCl, pH 8.0, each time allowing the column effluent to return to <0.02 before starting the higher salt concentration. 2.4-ml fractions were collected through 7 tubes and 1.2-ml fractions thereafter. The inset shows the SDS-PAGE profiles of the 1 mM N-acetylgalactosamine eluate applied to the column (S), the peak fraction (#15) eluting with 85 mM NaCl, and the peak fraction (#42) eluting with 170 mM NaCl. 5-19 µg of each were applied to the gel. An adjacent lane containing the molecular weight standards is shown.
Anion exchange chromatography of 100 and 10 mm N-acetylgalactosamine eluates. A and B show the elution profiles from DEAE-cellulose of the 100 mm and combined 10 mm N-acetylgalactosamine eluates, respectively, from the V. villosa preparation summarized in Table I. Column fractions were monitored by absorbance at 280 nm and by conductivity using a Radiometer conductivity meter. Bars indicate fractions which were pooled and concentrated. A, the 100 mm N-acetylgalactosamine eluate was dialyzed against 10 mm Na phosphate buffer, pH 7.2. 21.5 ml (0.62 mg of protein/ml) were applied to a DEAE-cellulose column (0.7 X 9 cm) equilibrated in the same buffer and the column was washed with 5 ml of starting buffer. No protein ran through the column and this portion of the profile is not shown. The column was then eluted with a linear 64-ml gradient from 0-0.5 M NaCl buffered with 10 mm Na phosphate, pH 7.2, at a flow rate of 25 ml/h. 1.2-ml fractions were collected. To concentrate peak 1, pooled fractions were diluted to a conductivity of 3.7 mmho with 10 mm Na phosphate buffer, pH 7.2, and applied to a DEAE-cellulose column (0.7 X 4 cm) equilibrated in 10 mm Na phosphate buffer, pH 7.2. Bound protein was eluted with 200 mm NaCl buffered with 10 mm Na phosphate, pH 7.2. B, the 10 mm N-acetylgalactosamine eluates were combined and dialyzed against 10 mm Na phosphate buffer, pH 7.2, containing 0.05 M NaCl. 38.5 ml (0.06 mg of protein/ml) were applied to a DEAE-cellulose column (0.7 X 11.2 cm) equilibrated in 10 mm Na phosphate buffer, pH 7.2, and the column was washed with 5 ml of starting buffer. No protein ran through the column and this portion of the profile is not shown. The column was then eluted with a linear 80-ml gradient from 0.05-0.5 M NaCl buffered with 10 mm Na phosphate, pH 7.2, at a flow rate of 32 ml/h. 1.2-ml fractions were collected. To concentrate peak 2, pooled fractions were diluted to a conductivity of 3.5 mmho with 10 mm Na phosphate buffer, pH 7.2, and applied to a DEAE-cellulose column (0.7 X 5.7 cm) equilibrated in 10 mm Na phosphate buffer, pH 7.2. After eluting the column with 100 mm NaCl, bound protein was eluted with 250 mm NaCl buffered with 10 mm Na phosphate, pH 7.2. The inset shows the SDS-PAGE profiles of a concentrated 100 mm N-acetylgalactosamine eluate and peaks 1 and 2, concentrated as described above. 5-10 µg of each were applied to the gel. Arrows mark the position of the molecular weight standards.

FIG. 5. Anion exchange chromatography of 100 and 10 mm N-acetylgalactosamine eluates. A and B show the elution profiles from DEAE-cellulose of the 100 mm and combined 10 mm N-acetylgalactosamine eluates, respectively, from the V. villosa preparation summarized in Table I. Column fractions were monitored by absorbance at 280 nm and by conductivity using a Radiometer conductivity meter. Bars indicate fractions which were pooled and concentrated. A, the 100 mm N-acetylgalactosamine eluate was dialyzed against 10 mm Na phosphate buffer, pH 7.2. 21.5 ml (0.62 mg of protein/ml) were applied to a DEAE-cellulose column (0.7 X 9 cm) equilibrated in the same buffer and the column was washed with 5 ml of starting buffer. No protein ran through the column and this portion of the profile is not shown. The column was then eluted with a linear 64-ml gradient from 0-0.5 M NaCl buffered with 10 mm Na phosphate, pH 7.2, at a flow rate of 25 ml/h. 1.2-ml fractions were collected. To concentrate peak 1, pooled fractions were diluted to a conductivity of 3.5 mmho with 10 mm Na phosphate buffer, pH 7.2, and applied to a DEAE-cellulose column (0.7 X 4 cm) equilibrated in 10 mm Na phosphate buffer, pH 7.2. Bound protein was eluted with 200 mm NaCl buffered with 10 mm Na phosphate, pH 7.2. B, the 10 mm N-acetylgalactosamine eluates were combined and dialyzed against 10 mm Na phosphate buffer, pH 7.2, containing 0.05 M NaCl. 38.5 ml (0.06 mg of protein/ml) were applied to a DEAE-cellulose column (0.7 X 11.2 cm) equilibrated in 10 mm Na phosphate buffer, pH 7.2, and the column was washed with 5 ml of starting buffer. No protein ran through the column and this portion of the profile is not shown. The column was then eluted with a linear 80-ml gradient from 0.05-0.5 M NaCl buffered with 10 mm Na phosphate, pH 7.2, at a flow rate of 32 ml/h. 1.2-ml fractions were collected. To concentrate peak 2, pooled fractions were diluted to a conductivity of 3.5 mmho with 10 mm Na phosphate buffer, pH 7.2, and applied to a DEAE-cellulose column (0.7 X 5.7 cm) equilibrated in 10 mm Na phosphate buffer, pH 7.2. After eluting the column with 100 mm NaCl, bound protein was eluted with 250 mm NaCl buffered with 10 mm Na phosphate, pH 7.2. The inset shows the SDS-PAGE profiles of a concentrated 100 mm N-acetylgalactosamine eluate and peaks 1 and 2, concentrated as described above. 5-10 µg of each were applied to the gel. Arrows mark the position of the molecular weight standards.

FIG. 6. Binding of purified lectins to A1 erythrocytes. Binding assays were performed as described under "Experimental Procedures." The specific activities of the '*'I-labeled lectins used were 731,200 and 603,900 cpm/µg for B4 and A4, respectively. Binding of A4 lectin to A1 erythrocytes (○-○) and binding of B4 lectin to A1 erythrocytes (□□□□) are shown here. Each point represents the mean of duplicate assays. Nonspecific binding in the presence of 100 mm N-acetylgalactosamine was 1% of total binding and has been subtracted. The Scatchard plot of A4 lectin binding to A1 erythrocytes is shown in the inset. Binding parameters were calculated using the molecular weight of the intact A4 lectin.

**TABLE II**

Amino acid and carbohydrate composition of purified lectins

Purified B4 and A4 lectins were analyzed as described under "Experimental Procedures." The amino acid composition of the B4 lectin is based on the analysis of duplicate hydrolysates after 24, 48, and 72 h of hydrolysis. The composition of the A4 lectin is based on the analysis of a single 24-h hydrolysate. The carbohydrate compositions of the B4 and A4 lectins are based on the analysis of three separate samples and a single sample, respectively. The compositions of B4 and A4 lectins were calculated on the assumption of 27,075 g/mol of subunit and 27,357 g/mol of subunit, respectively.

| Residue | B4 lectin | A4 lectin |
|---------|-----------|-----------|
| Aspartic acid | 36.7 | 31.4 |
| Threonine | 17.5* | 21.7 |
| Serine | 30.0* | 28.6 |
| Glutamic acid | 21.1 | 19.7 |
| Proline | 12.3 | 13.7 |
| Glycine | 23.1 | 23.3 |
| Alanine | 17.1 | 9.3 |
| Valine | 7.9* | 15.7 |
| Isoleucine | 5.7* | 7.6 |
| Leucine | 15.1 | 29.0 |
| Tyrosine | 7.2* | 3.2 |
| Phenylalanine | 11.8 | 11.7 |
| Histidine | 6.7 | 7.6 |
| Lysine | 10.4 | 11.3 |
| Arginine | 5.5 | 6.0 |
| Methionine | 0 | 0 |
| Cysteine | 0 | 0 |
| Tryptophan | 1.4 | ND* |
| Mannose | 9.5 | 4.7 |
| Galactose | 0 | 2.4 |
| N-Acetylgalactosamine | 3.2 | 1.7 |
| Fucose | 3.2 | 2.3 |

* Determined by extrapolation to zero time of hydrolysis.

b Determined from the 72-h hydrolysate.

' ND, not determined.

Acids, aspartic acid and glutamic acid, and the hydroxyl amino acids, serine and threonine. In addition, neither lectin contains any cysteine or methionine. The B4 lectin contains mannose, N-acetylgalactosamine, and fucose, and these comprise 9.8% of its weight. The A4 lectin, which is composed of 6.7% carbohydrate by weight, contains galactose in addition to those sugars. Glucose was also present in the carbohydrate analyses of the purified lectins but because its content varied considerably among analyses, it was assumed to be a contaminant. A monosaccharide having a retention time identical to that of xylose was also present, but this was not analyzed further.
Binding of Purified Lectins to A1 Erythrocytes—Fig. 6 shows the binding of purified lectins to A1 erythrocytes, performed as described under “Experimental Procedures.” The curve demonstrates that binding of the A1 lectin to A1 erythrocytes at 4°C is saturable. The Scatchard plot of these data, shown in the inset, is linear. The association constant derived from this analysis is 1.8 × 10⁶ M⁻¹ (Kₐ = 5.5 × 10⁻⁸ M) and there are approximately 273,000 binding sites/cell. SDS-PAGE and autoradiography of the erythrocyte pellet confirmed that the bound radiolabeled was associated with intact A subunits (not shown). In contrast, B₁ lectin does not bind to A₁ erythrocytes. The A₂B₂ lectin also bound to A₁ erythrocytes, with a similar number of binding sites per cell and an association constant approximately half of that of the A₁ lectin (data not shown). SDS-PAGE and autoradiography of the erythrocyte pellet showed that bound radiolabeled was associated with both A and B subunits (not shown). Since the B₁ lectin does not bind to A₁ erythrocytes, finding radiolabeled associated with the B subunit in the erythrocyte pellet provides additional evidence for the presence of a lectin composed of both A and B subunits.

DISCUSSION

The data presented in this paper demonstrate that V. villosa seeds contain at least three different lectins composed of two different subunits. The relationship of these lectins to the lectin preparations in the literature is not entirely clear. Kimura et al. (3) prepared the lectin used in their initial lymphocyte binding studies by affinity chromatography on immobilized human blood group A substance. Subsequently, others have prepared lectin using an affinity adsorbent of N-acetylgalactosamine coupled to epoxy-activated Sepharose 6B (5, 12). We have used a column of immobilized porcine blood group substances. The glycopeptides found in porcine stomach mucin are heterogeneous (26), although some of the structures have A blood group reactivity.

We first attempted to prepare V. villosa lectins according to the published method (3) in which bound material was eluted from the affinity column with 10 mM N-acetylgalactosamine. Analysis of fractions in this eluate revealed 1) that the A₁ erythrocyte agglutinating specific activity increased markedly across the protein peak and 2) that 2 bands of protein were resolved on SDS-PAGE. Furthermore, the lower of the 2 bands became more prominent across the protein peak, in parallel with the increasing A₁ erythrocyte agglutinating specific activity. This observation suggested that the hemagglutinating activity was contaminated with another protein that bound less tightly to the affinity column, and we subsequently isolated lectins by elution with increasing concentrations of N-acetylgalactosamine.

The yield of lectins prepared by chromatography on various adsorbents is quite similar, i.e. 75–80 mg/100 g of seeds. Our work indicates that most of the protein that binds to the affinity column (86% in the preparation summarized in Table I) is eluted with 1 mM N-acetylgalactosamine and that the most prevalent lectin in this eluate, i.e. the B₁ lectin, has no A₁ erythrocyte agglutinating activity. The 5.2 mg (the remaining 14%) of protein eluted from the affinity column with >1 mM N-acetylgalactosamine accounted for 54% of the hemagglutinating activity recovered. Because the eluates contain mixtures of lectins and because all of the hemagglutinating activity in the seed extract was not recovered, it is difficult to estimate from these data the amount of A₁ erythrocyte agglutinating lectins present in the seed extract. On the other hand, if we assume that the A subunit-containing lectins have a specific activity of 2500–5000 hemagglutinating units/mg, 100 g of seeds could contain no more than 17.8 mg of these lectins. Although the A₁ erythrocyte agglutinating specific activity is not reported in other published lectin preparations, our data suggest that these preparations probably do not contain exclusively the A₁ erythrocyte agglutinating lectins.

The report of Grubboffer et al. (27) suggested that the A₁ erythrocyte agglutinating activity of different cultivars within the species V. villosa varied. These workers prepared lectin from V. villosa seeds using an affinity column of N-(6-aminohexanoyl)-D-galactosamine coupled to Sepharose 4B. The yield of a purified lectin prepared was 22 mg/100 g of seeds, and this lectin agglutinated human blood group A₁ erythrocytes at a minimum concentration of 15 μg/ml. Their hemagglutination inhibition studies differ significantly from those reported in the accompanying paper for the B₁ lectin, suggesting that they have in fact characterized the A₁ erythrocyte agglutinin.

The B₁ lectin was readily purified from the material eluted with 1 mM N-acetylgalactosamine from the affinity column, since it was by far the most prevalent lectin in this eluate. Purification of the A₁ lectin was less straightforward. Most (81%) of the hemagglutinating activity that remained bound to the affinity column in the presence of 1 mM N-acetylgalactosamine was eluted with ≤10 mM N-acetylgalactosamine. As described under “Results” and shown in Fig. 3, the upper of the 2 major bands (band 2) in the electrophoretic profile of the 10 mM N-acetylgalactosamine eluate contained both A and B subunits, although A was more prominent. Since gel electrophoresis of the B₁ lectin under nondenaturing conditions revealed that it migrated as a rather broad band, it is likely that band 2 in fact represents the A₁ lectin and a contaminating amount of B₁ lectin. Nevertheless, we have been unable to separate completely the A₁ lectin from the B₁ lectin in the 10 mM N-acetylgalactosamine eluate by anion exchange chromatography. SDS-PAGE analysis of column fractions from anion exchange chromatography of the 10 mM N-acetylgalactosamine eluates (Fig. 5B) revealed that both A and B subunits were present in the first peak eluted (data not shown). As shown in Fig. 3, the upper of the 2 major bands in the electrophoretic profile of the 100 mM N-acetylgalactosamine eluate contained only A subunit, and we subsequently purified the A₁ lectin from this eluate by anion exchange chromatography. As shown in Fig. 3, the lower of the 2 major bands in the electrophoretic profiles of both the 10 and 100 mM N-acetylgalactosamine eluates contained both A and B subunits, of approximately equal prominence. We purified the A₂B₂ lectin from the 10 mM eluate because it was present there in a somewhat larger amount and because the 100 mM eluate also contained a protein which migrated just below the lower major band on gel electrophoresis.

The purified B₄ and A₄ lectins have similar molecular weights, and analysis of the lectins under denaturing conditions suggests that each is composed of four apparently identical subunits. The B subunit appears to have a somewhat larger apparent molecular weight by SDS-PAGE, perhaps because it is more heavily glycosylated. The chemical compositions of the purified lectins are remarkably similar and characteristic of plant lectins generally (28). The B₁ lectin does not bind to A₁ erythrocytes and does not agglutinate A, B, or O erythrocytes. In contrast, there are approximately 273,000 binding sites for the A₁ lectin on A₁ erythrocytes. The number of binding sites per cell is similar to those reported using other blood group A, agglutinins and to the receptor number determined with the blood group B-specific lectin from Griffithia simplicifolia (29).

The similarity of the amino acid composition of the B and A subunits and the identification of at least one lectin which appears to be composed of both A and B subunits suggest that
V. villosa seeds may contain a family of isolectins, i.e. tetrameric structures composed of two unique subunits in various proportions. Such families of isolectins are not uncommon, have similar amino acid compositions, but, interestingly, differ in their carbohydrate binding specificity. The G. simplicifolia I A, isolectin agglutinates A but not B erythrocytes, whereas G. simplicifolia I AB and B lectins are highly blood group B-specific. Within the lectins derived from P. vulgaris, the L subunit is a potent leukoagglutinin and mitogen that lacks elucidated potent erythroagglutinin with little or no mitogenic activity. Their carbohydrate binding specificities have recently been elucidated (34, 35).

It is not clear which of the lectins we have described interacts with the oligosaccharide structure of T145 on cytotoxic T lymphocytes. Preliminary binding studies in our laboratory using purified B and A lectins and the cloned lines of T lymphocytes established by Braciale et al. (36) suggest that both lectins bind better to cytotoxic T lymphocytes than to lymphocytes with no cytotoxic activity, although the number of B lectin binding sites on the cytotoxic T lymphocytes is considerably higher than the number of A lectin binding sites. The presence of lectins with different carbohydrate binding specificities in V. villosa lectin preparations used in published studies may account in part for the conflicting conclusions reported. Studies with purified V. villosa lectins should clarify their interaction with membrane glycoproteins on cytotoxic T lymphocytes and other cell lines.

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