Purification and Characterization of Two Lectins from Caragana arborescens Seeds*

ROBERT BLOCH, JENNIFER JENKINS, JEFFREY ROTH,§ AND MAX M. BURGER

From the Department of Biochemistry, Biocenter of the University of Basel, Klingelbergstrasse 70, Basel 4056 Switzerland

A glycoprotein fraction with hemagglutinating activity was purified by affinity chromatography from seeds of the pea tree, Caragana arborescens. Subsequent fractionation resolved two components, which could be separated on a preparative scale using different affinity matrices. The major component binds to N-acetylgalactosamine coupled to Sepharose 4B. It is a glycoprotein with high hemagglutinating activity. It is composed of two types of polypeptides, present in nonstoichiometric amounts, with apparent molecular weights near 30,000. In the native molecule, the subunits are cross-linked by disulfide bonds to form dimers, which in turn appear to be in rapid equilibrium with tetramers. The minor component binds to underivatized Sepharose 4B. It, too, is a glycoprotein but has low hemagglutinating activity. It is composed of three types of polypeptides which, although they have apparent molecular weights near 30,000, are distinguished from the subunits of the major hemagglutinin by a number of physical and chemical properties. The native molecule is dimeric, with a mass of 60,000 daltons.

The major component has high affinity (K = 0.1 mm) for the haptenic sugar, N-acetylgalactosamine, but will also bind α-galactose. Neither lectin has ABO blood group specificity, nor are they toxic to cultured mouse fibroblasts. Both agglutinate normal and transformed mouse fibroblasts to the same extent.

Although carbohydrates on the outer surfaces of cells have been intensively studied, their function has not yet been elucidated. They may exist as structural components, possibly defining the asymmetry of the membrane, or they may play an active role in determining the responses of cells to environmental changes. The presence of sugar-specific proteins on cell surfaces (1–5) has led some authors (4–7) to suggest that cellular interactions might be mediated by specific complex formation by these proteins with surface oligosaccharides of neighboring cells. This hypothesis may be tested by studying cells in culture with the presence of artificial carbohydrates and carbohydrate-specific proteins.

Ideally the sugar binding proteins in such experiments should be those actually found on cell surfaces. It is difficult, however, to prepare sufficient quantities of highly purified and solubilized membrane components. We have, therefore, chosen to work instead with lectins, which are easily accessible, soluble proteins that agglutinate animal cells by simultaneously binding to specific sugar residues on two or more cells (8).

This approach assumes that in vitro binding of lectin to surface carbohydrates simulates the effects of the natural sugar binding moiety, and it requires the use of nontoxic lectins (9). As many lectins have been shown to kill cultured cells (9), we have screened for and purified lectins which appear to be nontoxic.

We describe here the purification of two proteins with agglutinating activity from seeds of the pea tree, Caragana arborescens. Such activity was first identified in crude extracts of whole seeds by Mäkelä (10). The purified preparations are composed of two distinct proteins. One of these has high hemagglutinating activity, whereas the activity of the second is low. Neither appears to be toxic to cultured mouse fibroblasts.

EXPERIMENTAL PROCEDURES

Materials—Seeds from the pea tree, Caragana arborescens, were obtained from a local source (Habersak Sons. A.-G., Bottmingen, Switzerland). β-Galactosidase was purchased from Nutritional Biochemicals or from Koch-Light. Monosaccharides and lactose were from Merck (Germany). The p-nitrophenyl derivatives of α-D-galactose, β-D-galactose, and α- and β-N-acetyl-D-galactosamine were from Sigma, Serva, and Koch-Light, respectively. Methyl-α-D-galactopyranoside was also from Koch-Light. Methyl-α-D-galactopyranoside and raffinose were from Sigma. Serva, and Koch-Light, respectively. Methyl-β-D-galactopyranoside was also from Koch-Light. Methyl-α-D-galactopyranosyl-N-acetyl-D-galactosamine, was the generous gift of Dr. W. Gielen (University of Cologne). Standard proteins were...
Two Lectins from Caragana arborescens Seeds

Two lectins from Caragana arborescens seeds were obtained from Sigma, Morok, and Boehringer Mannheim. The catalytic subunit of aspartate transcarbamylase was the gift of Mr. Peter Suter (Biocenter).

Whole blood was obtained from the local blood bank, L1, Ehrlich ascites, 3T3, and Simian virus 40-transformed 3T3 (SV101-3T3) cells have been carried in this laboratory (9, 11). All cells were washed before use to remove plasma and buffy coat or ascites fluid. Medium and calf serum for tissue culture were purchased from Gibco-Biocult (Glasgow, Scotland).

Affinity chromatography—Sepharose 4B (Pharmacia) was activated with cyanogen bromide (12) for 30 to 45 min. Synthesis of the affinity matrix, Sepharose-2-acetamido-0-(p-aminophenyl)-2-deoxy-β-D-galactopyranoside, was as reported (13). The coupled resin contained 0.4 to 0.7 μmol of 2-acetamido-0-(p-aminophenyl)-2-deoxy-β-D-galactoside/ml of packed resin and was diluted with 4 volumes of unmodified Sepharose 4B before use, to a total volume of 125 ml. When an alternative method for activating Sepharose with cyanogen bromide was used (14), the ability of the substituted resin to retain agglutinating activity was greatly reduced.

Assays—Agglutination of 3T3 and SV101-3T3 cells was as described (15). Agglutination of other cells was performed with minor modifications of previous procedures (15) to permit measurements after 40 min. Agglutination of human erythrocytes was scored using a scale of 0, (+), +, ++, +++, and +++ to represent approximate levels of agglutination of 0, 25, 50, 75, 90, and 100%, respectively. Quantitation of agglutination of other cells was as reported (15). We define 1 unit of activity as that amount of lectin which gives + agglutination of 1 ml of a 1% suspension of type O erythrocytes.

Glycosidase activities were measured by standard procedures (16) with slight modifications to permit determinations at neutral pH. Protein concentrations during purification and of the homogenized material were determined according to the method of Lowry et al. (17), using bovine serum albumin as a standard. The concentration of mixtures of the two proteins was determined using an absorption coefficient (A280) of 12, which we obtained from amino acid analyses.

Chemical Analysis—Amino acid analyses were done according to the method of Spackman et al. (18) using a Beckman multichromatograph, using p-aminobenzoic acid as an internal standard. The aromatic amino acids were determined according to the method of Hirs (19). Tryptophan was determined spectrophotometrically (20).

The carbohydrate contents of the purified lectins were determined by the anthrone method (21) using glucose as standard. Component sugars were identified and quantitated as their alditol acetate derivatives (22) on a Hewlett-Packard model 7620A gas-liquid chromatograph, using t-arabinose as an internal standard. L-Arabinose is not a component of either lectin. The column (0.26 x 125 cm) was packed with 3% ECNSS-M on Gas-chrom Q (Applied Science). Peak integration was performed automatically by a model 3370B integrator.

Electrophoresis—Polyacrylamide gel electrophoresis in the presence of dodecyl sulfate was performed as described for discontinuous slab gels (23) using the buffer system of Lai et al. (24). Electrophoresis on cellulose acetate strips (4 x 17 cm, Chemetron, Milan) was as reported (25).

Purification—Meal from C. arborescens (19 g), obtained by grinding seed frozen in liquid nitrogen to a fine powder, was suspended in 100 ml of cold buffered saline (consisting of 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na2HPO4, 0.2 g of KH2PO4, 0.62 g of CaCl2·H2O, and 0.1 g of MgCl2·6H2O in 1 liter of water, pH 7.4) and stirred overnight at 4°. The suspension was strained through cheesecloth and centrifuged at 6000 x g for 1 h. The supernatant was heated for 30 min in a constant temperature water bath maintained at 52°, with frequent mixing. The precipitate was removed by centrifugation (6000 x g, 1 h, 4°). All subsequent operations were carried out at 4°. β-Galactosidase was added to the supernatant to a final concentration of 0.25 mm. The solution was then applied to a column of Sepharose 4B (Pharmacia) derivatized with N-acetylglucosamine which had previously been equilibrated with buffered saline containing 0.25 mm galactose. The column was first washed with four column volumes of buffered saline and then with an identical volume of this solution containing 0.25 mm galactose and then with an identical volume of this solution containing 0.25 mm galactose and 200 mm lactose (Fig. 1). All fractions eluting with lactose were pooled if their absorption at 280 nm was greater than 0.01. Solutions were concentrated by vacuum dialysis or by ultrafiltration in an Amicon apparatus with PM 10 filters. Lactose was removed by repeated diluting and dialyzing solutions with buffered saline or by dialysis. Resulting solutions containing between 1 and 3 mg of protein/ml were stored at -20°. Storage at 4° resulted in the formation of inactive precipitate. Small amounts of precipitate were always found in concentrated solutions of either of the two lectins.

RESULTS

Purification and Demonstration of Two Components—Purification of hemagglutinating activity is summarized in Table I, as is the purification away from contaminating glycosidases. To inhibit binding of β-galactosidase to the affinity column, p-galactai, a competitive inhibitor of Escherichia coli β-galactosidase (26), was present in the column buffers. Galactose at 0.25 mm inhibits galactosidase activity by 50% at 22°, but it has no effect on the agglutination reaction (see below). Its presence, therefore, allows removal of this enzyme from the column while lectin remains bound to the matrix (Fig. 1).

Because the peak of agglutinating activity in Fig. 1 was asymmetric and did not coincide with the peak of optical density, we suspected that we had purified more than one protein. We, therefore, examined our purified fractions by electrophoresis on cellulose acetate strips (27) and analyzing the isolated fractions by dodecyl sulfate gel electrophoresis. The results, presented in Fig. 3, show that the two fractions are distinct in several ways: (a) species I consists of one major and one minor band (Fig. 3, b and e), while species II is composed of one major and two minor bands (Fig. 3, c and f); (b) the mobilities of individual bands after reduction with 2-mercaptoethanol (Fig. 3, e and f) are different; and (c) unreduced samples of Fraction I migrate as dimers, whereas the mobilities of the polypeptide chains of Fraction II are not affected by reduction. The apparent molecular weights of the various bands are presented in Table II.

Preparation of Two Lectins—Pure preparations of the two

![Fig. 1. Purification of Caragana arborescens lectins on Sepharose 4B derivatized with N-acetylglucosamine. Agglutination of type O erythrocytes was assayed using 10 μl of the protein solution to be tested. Samples eluting with lactose were dialyzed for 2 h before assay. β-Galactosidase activity is represented by the optical density at 400 nm (16). The column (2.5 x 25 cm) was eluted at a flow rate of 60 ml/h, and 20-ml fractions were collected.](http://www.jbc.org/)

Downloaded from http://www.jbc.org/ by guest on March 24, 2020

http://www.jbc.org/
Two Lectins from Caragana arborescens Seeds

Purification of lectins from Caragana arborescens

10 g of ground meal were processed as described.

| Step                  | Protein | Total activity | Specific activity | Total activity | Glycosidases | Specific activity | Total activity | Glycosidases | Specific activity |
|-----------------------|---------|----------------|-------------------|----------------|--------------|-------------------|----------------|--------------|-------------------|
|                       | mg      | units × 10^-2  | units/mg          | (μmol/min)     | μmol/min/mg protein | (μmol/min/mg protein) |
| Crude extract         | 920     | 6.9            | 7.5               | 14             | 1.4          | 1.5 × 10^-2       | 1.6 × 10^-2       |
| Heat treatment        | 710     | 16             | 23                | 3.3            | 0.78         | 4.7 × 10^-3       | 1.1 × 10^-3       |
| Sepharose-GalNAc      | 12      | 12             | 1000              | 9.5 × 10^-4    | 9.9 × 10^-4  | 7.9 × 10^-4       | 8.3 × 10^-4       |

* Incubations for the sample obtained after affinity chromatography on Sepharose derivatized with N-acetylgalactosamine were for 10% h, for other samples, 2 h, in buffered saline.

Characterization of Two Lectins—The amino acid compositions of the two proteins, reported in Table III, show significant differences, particularly in the basic amino acids, proline, leucine, and half-cystine. As expected from the amino acid composition and the presence of interchain disulfide bonds (Fig. 3), lectin I has no free cysteine groups (< 0.1/polypeptide chain) when assayed by the method of Ellman (28) in the presence of 6 M guanidine hydrochloride.

Both lectins I and II are glycoproteins, as found using antrone reagent (21). Determination of their sugar compositions using gas-liquid chromatography (22) showed them to contain the following sugars in approximately equal amounts per polypeptide chain: mannose (3); xylose (1); glucosamine (1 to 2); and galactosamine (0). Both contained galactose and glucose, but we could not exclude the possibility that their presence was due to small amounts of lactose. The only difference we could detect in their sugar compositions was the virtual absence of fucose in lectin I, but not in II, which

---

**Fig. 2.** Separation of two species of Caragana lectins by electrophoresis on cellulose acetate. Protein fractions obtained from affinity chromatography were dialyzed against 10 mM sodium phosphate and 10 mM MgCl₂, pH 6.5. This pH was chosen after preliminary experiments showed the lectins to have isoelectric points in the range of 5.9 to 6.5. Approximately 100 μg were applied to the cellulose acetate strip, and electrophoresis was for 40 min at 13 mA, 4°C. The electrophoresis buffer was 20 mM sodium phosphate and 10 mM MgCl₂, pH 6.5. Staining was with amido black.

**Fig. 3.** Analysis of the Caragana lectins by dodecyl sulfate polyacrylamide slab gel electrophoresis in the presence and absence of 2-mercaptoethanol. The samples and the amounts applied to the gel were: a and d, (I + II) 9 μg; b and c, Fraction I from electrophoresis on cellulose acetate (see text and Fig. 2), 7.5 μg; e and f, Fraction II from electrophoresis on cellulose acetate, 7 μg; and g, a standard protein mixture containing, in order of decreasing molecular weight, bovine serum albumin (68,000), pyruvate kinase (57,000), ovalbumin (43,000), glyceraldehyde-3-P-dehydrogenase (36,000), carbonic anhydrase (29,000), and lysozyme (14,000) which runs with the tracker dye. Samples were boiled for 3 min in dissociation buffer (25): d to g, in the presence of 1% 2-mercaptoethanol, and a to c, in the absence of 2-mercaptoethanol. The acrylamide concentration in the gel was 10%.
proteins to have single sedimentation constants performed using both the mixture (I + II) and the purified proteins. Sedimentation velocity analysis showed the pure proteins to have single sedimentation constants \( s_{20, w} \) of 6.4 and 4.6 s, respectively. Similar values (6.2 ± 0.4 s and 4.3 ± 0.5 s) were obtained with the mixture. Sedimentation equilibrium studies of lectin II gave a plot of log concentration versus the square of the radial distance which was linear (Fig. 5). The average molecular weight obtained from such experiments between 0.25 and 0.5 mg of protein/ml is 64,000 ± 3,000. Similar plots for lectin I were nonlinear (Fig. 5). However, a tangential line can be drawn through most of the points at protein concentrations greater than 0.2 mg/ml yielding an average molecular weight of 103,000 ± 3,000. The curvature seen at lower protein concentrations may be due to dissociation of this form. This would be consistent with results obtained in gel filtration experiments using the mixture (I + II), in which Fractions I and II eluted with distribution coefficients \( K_v \) corresponding to those of globular proteins with masses of 85,000 and 55,000 daltons, respectively (data not shown).

**Interactions with Cells**—The lectin mixture (I + II) does not show ABO blood group specificity in hemagglutination tests. The final concentration of lectin needed to agglutinate about 75% of the erythrocytes is approximately 1.5 μg/ml, but much higher concentrations are needed to agglutinate L,

**TABLE II**

| Component | Plus 2-mercaptoethanol | Minus 2-mercaptoethanol |
|-----------|------------------------|-------------------------|
| Ia        | 30,400                 | 54,300                  |
| Ib        | 29,400                 | 51,600                  |
| IIA       | 32,000                 | 31,700                  |
| IIB       | 30,000                 | 29,700                  |
| IIC       | 28,000                 | 28,500                  |

**FIG. 4.** Preparation of pure lectins by chromatography on different affinity matrices. Aliquots of crude extract obtained after heat treatment (see "Experimental Procedures" and Table I) were applied at 4° either to a column containing (A) Sepharose 4B (2.5 x 20 cm) or (B) Sepharose derivatized as described under "Experimental Procedures" with p-aminophenyl-β-GalNAc (1.5 x 14 cm). Columns were washed first with several column volumes of buffered saline containing 0.25 M galactose (vertical arrows). Material eluting with lactose was pooled, concentrated, and analyzed by dodecyl sulfate gel electrophoresis in the absence of 2-mercaptoethanol, as described in Fig. 3. Photographs of stained gels are presented next to their representative elution profiles. The horizontal arrows indicate the places in the gels where Bands Ia and IIb should appear. The high A\text{280} seen in Fractions 85 to 90 in A is due to high background absorption by the lactose solution used in this experiment; that of Fractions 85 to 110 in B is due to the long trailing of lectin I on undiluted Sepharose-GalNAc. Fractions of 10 ml each were collected in both experiments. Volumes of extract applied were: A, 300 ml; B, 100 ml.

**TABLE III**

Amino acid composition of Caragana lectins

| Residue | Mol/30,000 g |
|---------|--------------|
|         | I            | II           |
| Lys     | 17.9         | 10.1         |
| His     | 5.4          | 8.3          |
| Arg     | 4.3          | 9.5          |
| Asx     | 29.2         | 27.2         |
| Thr\*   | 18.9         | 24.4         |
| Ser\*   | 30.2         | 33.1         |
| Glx     | 19.2         | 19.8         |
| Pro     | 13.3         | 9.7          |
| Gly     | 22.3         | 21.4         |
| Ala     | 19.4         | 15.1         |
| 1/2-Cys\* | 1.1       | 0.2          |
| Val     | 18.0         | 18.5         |
| Met     | 1.0          | 1.2          |
| Ile     | 12.1         | 15.1         |
| Leu     | 27.5         | 19.1         |
| Tyr     | 5.5          | 3.9          |
| Phe     | 15.3         | 20.1         |
| Trp\*   | 5.2          | 6.5          |

\* Value extrapolated to zero time, from single 24- and 72-h hydrolysates.

\* Determined as cysteic acid, assuming a recovery of 80% (19). Both lectins contain less than 0.1 free cysteine group/30,000 g (28).

\* Determined spectrophotometrically (27).
Two Lectins from Caragana arborescens Seeds

The values given are the sugar concentrations (K-1 μg/ml) which inhibit hemagglutination by 25% (from +++ to +). All values are subject to a 50% S.D. inherent in the assay procedure. The following sugars gave no inhibition of agglutination at concentrations up to 80 mM: d-glucose, d-mannose, L-fucose, d-arabinose, N-acetyl-d-mannosamine, N-acetyl-d-glucosamine, and maltose.

**Table IV**

| Sugar                       | K (μg/ml) |
|-----------------------------|-----------|
| N-Acetyl-d-galactosamine    | 0.09      |
| d-Galactose                 | 0.7       |
| d-Galactal                  | 8         |
| l-Arabinose                 | 9         |
| l-Rhamnose                  | 15        |
| Lactose                     | 0.9       |
| Melibiose                   | 2         |
| Raffinose                   | 2         |
| Stachyose                   | 4         |
| 3-O-β-d-galactosyl-N-acetyl-d-galactosamine | 1.5 |
| Methy1-a-d-galactopyranoside | 0.7       |
| Methy1-β-d-galactopyranoside | 1.4       |
| p-Nitrophenyl-α-d-galactopyranoside | 0.8       |
| p-Nitrophenyl-β-d-galactopyranoside | 0.5       |
| p-Nitrophenyl-2-acetamido-2-deoxy-β-d-galactopyranoside | 0.14       |
| p-Nitrophenyl-2-acetamido-2-deoxy-α-d-galactopyranoside | 0.05       |

**DISCUSSION**

We have described the preparation and properties of two lectins from *C. arborescens* seeds. Both are heterogeneous with

![Graph](https://example.com/graph1.png)

Fig. 7. Agglutination of normal and transformed mouse fibroblasts by the *Caragana* lectins. Agglutination by four different preparations was determined after 30 min. □ and ■, Lectin I; O and ●, mixture (I + II); △ and ▲, a mixture containing 3 parts of II to 1 part of I. ▽ and ▼, lectin II. Lectin I was prepared by overnight dialysis of I + II against 1 mM sodium phosphate and 1 mM MgCl2, pH 6.5, as described in the text. Lectin II was prepared as described in the legend to Fig. 5. Open symbols represent agglutination of 3T3 cells; closed symbols, agglutination of SV101-3T3 cells.

![Graph](https://example.com/graph2.png)

Fig. 6 (right). Hemagglutination by the two *Caragana* lectins. Lectins were prepared as described in the legend to Fig. 5. Serial dilutions of both were prepared and mixed with appropriate volumes of a dilute human erythrocyte suspension (type A) to give final volumes of 0.10 to 0.11 ml. Agglutination was measured after 45 min (see "Experimental Procedures"). The symbols are: O, lectin I; △, lectin II; ●, agglutination by lectin I in the presence of 100 μg/ml of lectin II.

![Graph](https://example.com/graph3.png)

Fig. 5 (left). Sedimentation equilibrium analysis of the two *Caragana* lectins. Lectins were purified as described in the text and the legend to Fig. 5. They were then concentrated by ultrafiltration and dialyzed against buffered saline to free them of lactose. Sedimentation was performed in this buffer at 20° using an AN-F rotor. The equilibrium distribution of protein in the cell was measured using the photoelectric scanning absorption system of the analytical ultracentrifuge. Logarithms of the optical density at 280 nm are plotted against the square of the radial distance, R, from the axis of rotation. Initial protein concentrations and centrifugation speeds were: lectin I (O), 0.5 mg/ml, 10,000 rpm; lectin II (●), 0.5 mg/ml, 10,000 rpm. The arrows indicate the upper and lower menisci found in the analysis of lectin II.

**Experimental Procedures**

Treatment of erythrocytes with neuraminidase (29) or with trypsin (30) increases the agglutination by both lectins approximately 5-fold, but it does not render agglutination by either blood group-specific.

When assayed with fibroblasts, the lectins, either separately or together, agglutinate transformed and normal cells with the same efficiency (Fig. 7). While lectin I can agglutinate both blood group-specific.

The sugar specificity of lectin I has been investigated using inhibition of hemagglutination as a measure of interaction. The concentration of sugar which decreases the agglutination reaction from nearly complete agglutination (+++++) to a point where approximately 75% (or +++) of the cells are clumped can be taken as a measure of the affinity of the lectin for the sugar (15). The results obtained with several monosaccharides and glycosides are reported in Table IV. Lectin I has highest affinity for N-acetylgalactosamine but is also inhibited by galactose. The lectin seems to prefer monosaccharide to disaccharide haptens, suggesting that the sugar binding site of lectin I accommodates only 1 monosaccharide unit. We found no consistent preferential interaction with α or β anomers.

Similar measurements with lectin II are not yet possible. As the hemagglutinating activity of this lectin may be due to contaminating amounts of lectin I, we are now beginning to study hapten binding using methods which do not rely on agglutination.

Because our ultimate aim is to use the lectins from *Caragana arborescens* with growing and differentiating cells, we tested their effects on the mouse fibroblast line, 3T3, in culture. The data in Table V show that 3T3 cells cultured in the presence of 100 or 200 μg/ml of the mixture (I + II) grow as well as controls. Hemagglutinating activity after incubations with cells was undiminished. These lectins are, therefore, nontoxic to 3T3 cells under conditions in which they retain full activity. Similar results were found both with the purified lectins and with SV101-3T3 cells in log phase growth using the mixture.

![Graph](https://example.com/graph4.png)

Fig. 3. Hemagglutination of mouse erythrocytes. A, lectin I; B, lectin II; C, mixture (I + II).
old preparations of lectin I, we found an additional faint band, that partial proteolysis, either
raphies, we consider both proteins to be essentially pure, with not the protease or another contaminant. Its presence suggests the Caragana lectins.

barely visible in Fig. 3, migrating faster than Band Ib. As the mobility of this band is also increased by treatment with sulfate gel electrophoresis. Since, however, these component polypeptides always co-purified upon electrophoresis, iso-
respect to their polypeptide content, as revealed by dodecyl sulfate gel electrophoresis. Since, however, these component polypeptides always co-purified upon electrophoresis, iso-
electric precipitation, and gel filtration and affinity chromatogra-
phies, we consider both proteins to be essentially pure, with microheterogeneity. We took special precautions to avoid co-purifying degradative enzymes, particularly glycosidases of similar sugar specificity, and only trace amounts of these or other enzymes are likely to be present in our preparations. A proteinase may be a trace contaminant. When we examined old preparations of lectin I, we found an additional faint band, barely visible in Fig. 3, migrating faster than Band Ib. As the mobility of this band is also increased by treatment with 2 mercaptoethanol, we believe it is a derivative of lectin I and not the protease or another contaminant. Its presence suggests that partial proteolysis, either in vivo or during and perhaps after purification, may be responsible for the heterogeneity of the Caragana lectins. Although they are both glycoproteins consisting of several polypeptide chains of similar molecular weights, the two Caragana lectins are dissimilar in most other respects. Lectin II is a dimer under nondenaturing conditions; lec
tion I is most probably a tetramer, which, judging from sedimentation equi-
librium analyses (Fig. 5), can dissociate to dimers in dilute solution. The mobilities of the subunits of lectin I in dodecyl sulfate gel electrophoresis depend on whether the sample is first reduced. As the only half-cystine of lectin I is present as the disulfide, I is likely to be composed of dimers which are covalently linked by disulfide bonds. Lectin II contains no interchain disulfide bonds and indeed contains no half-cystine. It further differs from lectin I in its content of a number of other amino acids and also in its very low agglutinating activity. Perhaps the most striking distinction between the two proteins is their different specificities for the two components of the affinity matrix on which they were purified. This almost certainly means that the sugar hapten specificities of the two proteins are also different, but we have not yet been able to confirm this inference in quantitative experiments using lectin II.

The two Caragana lectins have a number of properties which distinguish them from other lectins with specificity for N-
acetylgalactosamine (31-38) and which also make them potentially useful for studying growing and differentiating cells. Foremost in this respect is their lack of toxicity to cultured mouse fibroblast cells. They also have rather high affinity for sugar haptons. As cells in culture are often severely affected by sugars added to the culture medium, this is a further advantage. Finally, the fact that these lectins agglutinate normal and transformed mouse cells to the same extent offers a good opportunity to compare their properties to those of galactose and N-acetylgalactosamine-specific lectins which preferentially agglutinate transformed cells (31, 37).

Acknowledgments—We wish to acknowledge the early contribu-
tions of Ms. Claudia Metz, whose partial purification of the Caragana arborescens lectins served as the basis for the purification scheme presented here. We are also grateful to the following colleagues for their assistance in the experiments noted: Ms. Marianne Grob, for the agglutination tests with 3T3 and SV101-3T3 cells; Dr. Kenneth Talmadge, for advice on gas-liquid chromatography; Ariel Lustig, for the ultracen-
trifugation studies; Dr. Rolf Schaefer, for instructions on cellulose acetate electrophoresis; Dr. Akira Tsugita and his colleagues, for the amino acid analyses. We also thank our colleagues in the Biocenter who critically read the manuscript, and Dr. Jürg Rosenbusch, who made very useful suggestions throughout the course of the work.

REFERENCES

1. Shur, B. D., and Roth, S. (1975) Biochim. Biophys. Acta 415, 473-475
2. Prentki, A., Lombardo, A., Cestaro, B., Zambotti, S., and Tettamanti, G. (1974) Biochim. Biophys. Acta 350, 406-414
3. Fleischer, R., and Fleischer, S. (1969) Biochim. Biophys. Acta 183, 265-275
4. Rosen, S. D., Kafka, J. A., Simpson, D. L., and Barondes, S. H. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 9554-9557
5. Gartner, T. K., and Podleski, T. R. (1975) Biochem. Biophys. Res. Commun. 67, 972-978
6. Roseman, S. (1970) Chem. Phys. Lipids 5, 279-297
7. Novak, T. P., Haywood, P. L., and Barondes, S. H. (1976) Biochim. Biophys. Res. Commun. 68, 650-657
8. Liu, H., and Sharon, N. (1970) Annu. Rev. Biochem. 40, 541-574
9. Burger, M. M., and Noonan, K. D. (1970) Nature 228, 512-515
10. Mäkelä, O. (1957) Ann. Med. Exp. Biol. Fenn. 35, Suppl. 11
11. Burger, M. M., and Goldberg, A. R. (1967) Proc. Natl. Acad. Sci. U. S. A. 57, 399-396
12. Cuatrecasas, P. (1970) J. Biol. Chem. 245, 3069-3066
13. Bloch, R., and Burger, M. M. (1974) FEBS Lett. 44, 206-229
14. March, S. C., Parikh, I., and Cuatrecasas, P. (1974) Anal. Biochem. 60, 149-152
15. Burger, M. M. (1974) Methods Enzymol. 32, 615-621
16. Li, Y. T., and Li, S. C. (1972) Methods Enzymol. 28, 702-713
17. Bailey, J. L. (1967) Techniques in Protein Chemistry, 2nd ed, p. 340, Elsevier, New York
18. Spackman, D. H., Stein, W. H., and Moore, S. (1958) Anal. Chem. 30, 1190-1206
19. Hirs, C. H. W. (1967) Methods Enzymol. 11, 59-63

resin by a faster, gentler procedure (14) does not destroy the ability of the resin to bind lectin II.

1 R. Bloch, unpublished observations.
Two Lectins from Caragana arborescens Seeds

20. Edelhoch, H. (1967) Biochemistry 6, 1948-1954
21. Spiro, R. G. (1966) Methods Enzymol. 8, 3-26
22. Albersheim, P., Nevins, D. J., English, P. D., and Karr, A. (1967) Carbohydr. Res. 5, 340-345
23. Studier, F. W. (1975) J. Mol. Biol. 79, 237-248
24. Laemmli, U. K. (1970) Nature 227, 680-685
25. Heil, Α., and Zillig, W. (1970) FEBS Lett. 11, 165-168
26. Lee, Y. C. (1969) Biochem. Biophys. Res. Commun. 35, 161-167
27. Schaefer, R., Hinnen, R., and Franklin, R. M. (1974) Eur. J. Biochem. 50, 15-27
28. Ellman, G. L. (1959) Arch. Biochem. Biophys. 82, 70-77
29. Gahmberg, C. G., and Hakomori, S. (1973) J. Biol. Chem. 248, 4311-4317
30. Gordon, J. A., Sharon, N., and Lis, H. (1972) Biochim. Biophys. Acta 264, 387-391
31. Nicolson, G. L., Blaustein, J., and Etzler, M. E. (1974) Biochemistry 13, 196-204
32. Galbraith, W., and Goldstein, I. J. (1972) Biochemistry 11, 2976-2981
33. PusztaI, A., and Watt, W. B. (1974) Biochim. Biophys. Acta 365, 57-71
34. Novakova, N., and Kocourek, J. (1974) Biochim. Biophys. Acta 369, 320-333
35. Etzler, M. E., and Kabat, E. A. (1970) Biochemistry 9, 869-877
36. Hammerschmidt, S., and Kabat, E. A. (1971) Biochemistry 10, 1684-1692
37. Lis, H., Sela, B.-A., Sachs, L., and Sharon, N. (1970) Biochim. Biophys. Acta 211, 582-585
38. Poretz, R. D., Riss, H., Timberlake, J. W., and Chien, S. (1974) Biochemistry 13, 250-256
Purification and characterization of two lectins from Caragana arborescens seeds.
R Bloch, J Jenkins, J Roth and M M Burger

*J. Biol. Chem. 1976, 251:5929-5935.*