Purification and Macromolecular Properties of a Sialic Acid-specific Lectin from the Slug Limax flavus

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A lectin (LFA) which is highly specific for sialic acid has been purified from the slug Limax flavus by a combination of ammonium sulfate fractionation and affinity chromatography on bovine submaxillary mucin coupled to Sepharose 4B. The affinity-purified lectin appeared homogeneous by electrophoresis in the presence of sodium dodecyl sulfate. Below 1 mg/ml at pH 7, LFA exists as a species of M, = 44,000 composed of two equal sized subunits. Above 1 mg/ml, the protein solution was observed to behave as a rapidly associating-dissociating system.

N-acetylneuraminic acid and N-glycoly neuraminic acid gave a 50% inhibition of agglutination of erythrocytes by LFA at 0.13 and 0.81 mm, respectively. Galactose, N-acetylgalactosamine, galactosamine, glucose, N-acetylglucosamine, glucosamine, mannose, N-acetylgalactosamine, rhamnose, xylose, fucose, glucaric acid, α-methyl-D-glucoside, α-methyl-D-mannoside, lactose, and sucrose were ineffective inhibitors at concentrations up to 10-25 mm. Bovine submaxillary mucin, a sialoprotein, was a potent inhibitor of hemagglutination by LFA. Upon treatment of the mucin with neuraminidase, loss of inhibitory activity was observed which was proportional to the loss of sialic acid from the mucin.

Lectins are a group of proteins that interact with glycoproteins and glycolipids by binding to specific carbohydrate residues (Lis and Sharon, 1973; Sharon and Lis, 1975; Pereira and Kabat, 1979; Goldstein and Hayes, 1978). Because of the high degree of specificity exhibited by individual lectins in their interaction with glycoproteins and glycolipids, they have been employed ever increasingly as highly discriminating agents in studies of membranes of normal and cancerous cells, in blood typing, in the purification of glycoproteins, and in studies of the mitogenesis of lymphocytes.

In spite of their ubiquitous nature, relatively few lectins which are specific for N-acetylneuraminic acid have been identified. Because of their macromolecular properties and/or because their specificity is not limited to sialic acid, the lectins which have been purified and studied to date have not proven to be fully satisfactory for studies like those enumerated above. Among the prominent examples of sialic acid binding lectins are limulin and carcioncorpin, which have been purified from the hemolymph of the American horseshoe crab Limulus polyphemus (Marchalonsis and Edelman, 1968) and the Indian horseshoe crab Carcinocorpus rotundus cauda (Bishaye and Dorai, 1980), respectively. It has been reported, however, that limulin will also bind to N-acetylglucosamine (Marchalonsis and Edelman, 1968) and glucuronic acid (Nowak and Barondes, 1975) while the carbohydrate binding properties of carcioncorpin have not been extensively studied. Additionally, the large size of limulin (M, ~ 350,000) and its tendency to dissociate (Marchalonsis and Edelman, 1968) have made it somewhat more difficult to handle. An agglutinin (LAg-1) which binds AcNeu has been isolated from the lobster, Homarus americanus (Hall and Rowlands, Jr., 1974). Hemagglutination by this lectin was inhibited by AcNeu, GlyNeu, and ManNAc. These studies also indicated that LAg-1 contains a second binding site of undefined specificity. The agglutination of human erythrocytes by wheat germ agglutinin is inhibited by AcNeu and AcNeua2 → 3-lactose; however, compounds such as GlcNacβ1 → 4GlcNAC and Galβ1 → 6GlcNacβ → C6H4NOJp were much more potent inhibitors (Bhavanandan and Katic, 1979).

It was earlier observed (Pemberton, 1970) that extracts of the slug Limax flavus contain hemagglutinins for erythrocytes. Extending Pemberton’s observation, we have recently reported (Miller, 1981) that hemagglutination of erythrocytes by L. flavus extracts is strongly inhibited by AcNeu and GlyNeu and to a lesser extent by GlCN, GlcNAc, GalN, and GalNAc. It is the purpose of this communication to describe the purification and physical characterization of a lectin (LFA) from L. flavus which exhibits a narrow specificity for sialic acid. Its narrow range of specificity together with the lectin’s macromolecular properties suggest that the L. flavus lectin has excellent potential as a discriminator for sialic acid-containing glycoproteins.

MATERIALS AND METHODS

The carbohydrates were obtained from Sigma. The N-acetylneuraminic acid was Type VI from Escherichia coli. Sepharose 4B was obtained from Pharmacia. Chemicals employed were reagent grade unless otherwise indicated. Charcoal-treated and doubly deionized water was used in all preparations. The slugs, L. flavus, were collected from the Charleston, SC area.

Coupling of Botell Submaxillary Mucin to Sepharose 4B—Twenty ml of Sepharose 4B were washed with 200 ml of deionized water by filtration, and the excess water was removed. The Sepharose was subsequently washed with 20 ml of 2 M potassium phosphate (pH 7.2) by filtration and then placed in another 20 ml of the phosphate buffer. Two and one-half ml of dioxane containing 1.0 g of cyanogen bromide were added over a 6-min period while stirring the Sepharose...
in an ice bath. The Sepharose was stirred for an additional 10 min and washed with 400 ml of deionized water. Ten ml of BSM (10 mg/ml) in 1.0 M NaCl and 10 ml of 0.5 M NaHCO₃, pH 9.0, were added to the activated Sepharose and the mixture was stirred for 2 h at 22 °C. The BSM-Sepharose was washed with 100 ml of 1.0 M NaCl and 100 ml of Tris-saline buffer. The absorbance of the washes at 280 and 260 nm indicated that approximately 85% of the BSM had been coupled to the Sepharose.

Affinity chromatography of LFA—Approximately 150 g of slugs were washed with deionized water, eviscerated, and further washed with Tris-saline buffer to remove surface mucous material. One hundred g of washed tissue were placed in 400 ml of Tris-saline buffer containing 4.0 ml of phenylmethanesulfonfyl fluoride (5 mg/ml in 2-propanol). The tissue was minced with scissors and then homogenized by use of a Polytron homogenizer at full speed for 3 min. The homogenate was centrifuged at 16,000 × g for 15 min and the supernatant fraction was saved (SF-16). Powdered ammonium sulfate was added to the SF-16 to 40% saturation and the precipitate was collected by centrifugation at 16,000 × g for 15 min. The supernatant fraction was decanted and adjusted to 80% saturation by additions of powdered ammonium sulfate. The precipitate (AS-40-80) was collected by centrifugation at 16,000 × g for 15 min. The AS-40-80 pellets were resuspended in 200 ml of Tris-saline buffer and dialyzed against three 1-liter volumes of this buffer at 5 °C for 24 h.

A column (1.5 × 10 cm) of BSM-Sepharose was equilibrated with Tris-saline buffer. One hundred ml of the dialyzed AS-40-80 fraction at approximately 10 mg of protein/ml were pumped (50 ml/h) onto the column. The column was washed with Tris-saline buffer until the nonbound proteins were eluted. The lectin was then eluted with Tris-saline buffer containing 10 mM AcNeu. The fractions containing protein which eluted with AcNeu were combined and dialyzed against Tris-saline buffer. One hundred ml of the dialyzed AS-40-80 fraction of BSM-Sepharose was equilibrated with 0.01 M Tris-saline buffer containing 0.4 mg/ml of LFA. The column was washed with Tris-saline buffer until the absorbance of the washes at 280 and 260 nm indicated that approximately 85% of the BSM had been coupled to the Sepharose. The column was then equilibrated with Tris-saline buffer which contained 0.4 mg/ml of LFA. The fractions containing protein which eluted with AcNeu were combined and dialyzed against Tris-saline buffer before storage at −70 °C.

Removal of Bound AcNeu from Purified LFA—Exhaustive dialysis of affinity-purified LFA against Tris-saline did not completely remove AcNeu. Assays of dialyzed LFA by the thiorbarbituric acid assay of Warren (1959) indicated that the LFA contained approximately 8 mol of noncovalently bound AcNeu/mol of LFA. Chromatography of LFA on a column (1 × 37 cm) of Dowex 50-X8(D) which had been equilibrated with 0.01 M ammonium acetate, pH 7.0, reduced its AcNeu content to less than 0.20 mol of AcNeu/mol of LFA with little or no change in the specific activity of the LFA preparation. Because the yield of protein from such a treatment was ~70% and because the removal of AcNeu had no effect on the lectin’s specific activity, samples of LFA which had been chromatographed on the ion-exchange column were utilized for only selected experiments.

Hemagglutination Assay—Aliquots of LFA were adjusted to 0.5 ml by addition of Tris-saline buffer which contained 0.4 mg/ml gelatin. To this was added 0.5 ml of a solution containing human erythrocytes that had been washed several times with 0.9% NaCl and whose absorbance at 540 nm was ~2. After standing at room temperature for 30 min, the cells were sedimented by centrifugation at 900 g. The cells were resuspended by shaking and allowed to stand for 5 min in order to permit aggregated cells to settle. The absorbance at 620 nm of the upper 0.5 ml of the erythrocyte suspension was measured and the data were plotted as A⁶₂₀ versus micrograms or microliters of lectin. One unit of activity is defined as the amount of LFA which gave a 50% agglutination (Miller, 1981). Per cent agglutination was calculated as follows:

\[
\text{% agglutination} = \frac{A_{620} \text{ of cell suspension} - (A_{620} \text{ of cell suspension} + \text{ lectin})}{A_{620} \text{ of cell suspension}} \times 100
\]

Inhibition of Hemagglutination—An amount of purified LFA or partially purified LFA (i.e. AS-40-80 fraction) which gave greater than 95% agglutination of erythrocytes in the hemagglutination assay was mixed with varying amounts of potential inhibitors dissolved in Tris-saline buffer, and the volume was adjusted to 0.5 ml by addition of Tris-saline buffer. A 0.5-ml aliquot of erythrocytes was added to the lectin plus inhibitor solution and the per cent agglutination determined as described above. The per cent inhibition of agglutination represents the difference between the per cent agglutination with lectin alone and that obtained with lectin plus inhibitor.

Chemical Analyses—Amino acid analyses of LFA were determined on a computerized Durrum 600 amino acid analyzer. Protein samples were hydrolyzed at 110 °C in constant boiling HCl for 24, 48, and 72 h. Corrections were made for loss of thisamine and serine by hydrolysis. Cysteine was determined after performic acid oxidation of the protein. Tryptophan and tyrosine were estimated by the spectroscopic method of Edelhoch (1967) as modified by Brederman (1974). AcNeu was measured after acid hydrolysis or neuraminidase treatment of glycoproteins by the thiorbarbituric acid assay described by Warren (1959). The acid hydrolytic release of AcNeu from glycoproteins was accomplished by incubation of the glycoprotein in 0.1 M H₂SO₄ at 80 °C for 1 h.

Physical Measurements—Sedimentation velocity and equilibrium measurements were performed in a Beckman Model E analytical ultracentrifuge equipped with schlieren/interference optics and a photoelectric scanner.

Sedimentation velocity measurements were made according to standard procedures; no corrections were made for the Johnston-Warren (1959) effect. The low recovery of agglutinin activity (Table I) resulted in a 68-fold overall purification of LFA with a 22% recovery of agglutinin activity. The use of BSM in subsequent purification by affinity chromatography of LFA on BSM-Sepharose (Fig. 1) resulted in a 68-fold overall purification of LFA with a 22% recovery of agglutinin activity. The use of BSM in subsequent purification by affinity chromatography of LFA should result in the recovery of lectin(s) which bind AcNeu. The low recovery (22%) and the apparently rather low degree of purification (68-fold) of LFA may be due to a separation, at the affinity chromatography step, from other hemagglutinins which are specific for carbohydrate
residues other than sialic acid. This suspicion is supported by the data discussed below. In spite of the low recovery of hemagglutinin activity, approximately 17 mg of purified LFA were obtained from 100 g of eviscerated slug tissue.

**Homogeneity of Purified LFA**—Electrophoresis of the affinity-purified LFA on sodium dodecyl sulfate-polyacrylamide gels (12.5%), after reduction and denaturation of the protein by sodium dodecyl sulfate and β-mercaptoethanol (Laemmli, 1970), yielded a single Coomassie blue-staining band (Fig. 2). The migration of LFA relative to the migration of myoglobin, chymotrypsinogen A, and ovalbumin suggests that reduced and denatured LFA consists of a single polypeptide species of Mr. ~22,000. Some preparations of purified LFA exhibited a faster migrating minor component when a relatively large amount of LFA (>10 μg) was applied to the gel.

**Binding Properties of LFA**—As indicated in Table II, hemagglutination by the affinity-purified LFA was inhibited by 50% at 0.13 and 0.81 mM concentrations of AcNeu and GlyNeu, respectively. No inhibition was observed with the other carbohydrates tested, even at concentrations as high as 10−25 mM. In contrast, inhibition of hemagglutination by the 40−80% ammonium sulfate fraction by GalNAc, GlcNAc, and GlcN in addition to AcNeu and GlyNeu (Table II) suggests that this fraction contains hemagglutinins with binding specificities for carbohydrate residues other than sialic acid. It is unclear why so much agglutinin activity was lost during the purification of LFA on sodium dodecyl sulfate-polyacrylamide gels (12.5%), after reduction and denaturation of the protein by sodium dodecyl sulfate and β-mercaptoethanol (Laemmli, 1970), yielded a single Coomassie blue-staining band (Fig. 2). The migration of LFA relative to the migration of myoglobin, chymotrypsinogen A, and ovalbumin suggests that reduced and denatured LFA consists of a single polypeptide species of Mr. ~22,000. Some preparations of purified LFA exhibited a faster migrating minor component when a relatively large amount of LFA (>10 μg) was applied to the gel.

**Table I**

| Fraction | Volume | Protein | Specific activity | Total activity | Recovery |
|----------|--------|---------|------------------|----------------|---------|
| AS-40-80 | 104    | 946     | 169              | 334            | 108     |
| SF-16    | 434    | 5034    | 62               | 309            | 100     |
| Purified LFA | 28    | 16.8   | 4169            | 70             | 22      |

* One unit of activity is defined as that amount of lectin which gives 50% agglutination of erythrocytes in the hemagglutination assay (see “Materials and Methods”).

**Fig. 1. Affinity purification of LFA.** One hundred ml of the AS-40-80 fraction were pumped (50 ml/h) onto a column (16 × 10 cm) of BSM-Sepharose that had been equilibrated with 50 mM Tris-Cl, pH 7.5. The column was washed with the above buffer solution until the A260 of the effluent approached zero. Elution of the column with 50 mM Tris-Cl, 100 mM NaCl, 10 mM AcNeu, pH 7.5, was begun at fraction 32. A 1.0-μl aliquot of those fractions indicated was assayed for agglutinin activity. Fractions 34 through 37 were combined, dialyzed against two 1-liter volumes of tris-saline buffer, and stored at −70°C.

**Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of LFA.** Electrophoresis was carried out on 1.5-mm slab gels by the reducing and denaturing method of Laemmli (1970). Lanes 1 and 5, 5 μg each of myoglobin (Mr. ≈17,200), chymotrypsinogen A (25,000), and ovalbumin (43,000); lane 2, 10 μg of LFA; lane 3, 5 μg of LFA; lane 4, 2.5 μg of LFA.

Activity was recovered by further elution of the BSM-Sepharose column with 50 mM AcNeu in Tris-saline or with 50 mM AcNeu in 50 mM Tris-Cl, 1.0 M NaCl, pH 7.5. Also, little additional protein was recovered by washing the column with 0.01 N NaOH. Those fractions containing proteins which did not bind to the affinity matrix were without significant hemagglutinin activity (Fig. 1). Loss of agglutinin activity may be due to inactivation of lectins by denaturation or to loss of essential cations.

As mentioned above, the data of Table II suggest that the purified LFA binds free AcNeu in a highly specific manner. This specificity was further tested by use of the AcNeu-rich glycoprotein BSM. As shown in Table III, as little as 9 μg/ml of BSM gave a 50% inhibition of hemagglutination in the standard assay containing 0.34 μg of LFA. Upon treatment of BSM with *Vibrio cholerae* neuraminidase, there was a time-dependent release of AcNeu from the BSM with a concomitant loss in agglutination inhibition. The total content of AcNeu in BSM was 5% (w/w) as measured after its release by acid hydrolysis. Release of 12 μg of AcNeu/mg of BSM, i.e. 25% of its total AcNeu, virtually abolished its inhibitory activity. Thus, it appears that only about 25% of the AcNeu residues in BSM are the functional ligands in its interaction with LFA. This suggests that BSM may contain AcNeu resi-
The standard hemagglutination assay was used in these comparative studies. Twenty µg of protein from the 40-80% ammonium sulfate fraction (AS-40-80) or 0.34 µg of the purified LFA was utilized in each assay.

A 1.0 mM solution of CaCl₂ containing 2 mg/ml BSM and 38 units of V. cholerae neuraminidase was adjusted to pH 6.2 and incubated at 37 °C. Aliquots were removed at times indicated above, diluted 10-fold with 0.05 M Tris-Cl, 0.1 M NaCl, pH 7.5, and assayed with 0.34 µg of slug lectin for inhibitory activity.

The values in parentheses for Tyr and Trp were obtained by the spectrophotometric method of Edelhoch (1967) as modified by Bredlacker (1974).

Cys (half-cystine) was determined by amino acid analysis after performic acid oxidation of the slug lectin.

**TABLE II**

Inhibition of hemagglutinin activity of slug lectin by carbohydrates

| Carbohydrate                        | Carbohydrate concentration | Inhibition | AS-40-80 fraction | Purified LFA |
|-------------------------------------|-----------------------------|------------|------------------|--------------|
|                                     | mM                          | %          |                  |              |
| N-Acetyleneuraminic acid            | 0.05                        | 6          | 1                |              |
|                                     | 0.10                        | 17         | 28               |              |
|                                     | 0.15                        | 27         | 54               |              |
|                                     | 0.20                        | 94         | 100              |              |
| N-Glycolyneuraminic acid           | 0.25                        | 3          | 0                |              |
|                                     | 0.50                        | 20         | 2                |              |
|                                     | 0.75                        | 56         | 14               |              |
|                                     | 1.0                         | 88         | 85               |              |
| N-Acetyl-d-galactosamine            | 10.0                        | 75         | 0                |              |
| N-Acetyl-d-glucosamine              | 10.0                        | 53         | 0                |              |
| Galactosamine                       | 25.0                        | 45         | 0                |              |
| Glucosamine                        | 25.0                        | 19         | 0                |              |
| d-(-)-Galactose                     | 25.0                        | 0          | 0                |              |
| d-(-)-Glucose                       | 25.0                        | 0          | 0                |              |
| D-(+)-Mannose                       | 25.0                        | 0          | 0                |              |
| a(-)-Arabinose                      | 25.0                        | 0          | 0                |              |
| d-(-)-Xylose                        | 25.0                        | 0          | 0                |              |
| a(-)-Fucose                          | 25.0                        | 0          | 0                |              |
| d-(-)-Glucuronic acid               | 25.0                        | 0          | 0                |              |
| a-Methyl-d-glucoside                | 25.0                        | 0          | 0                |              |
| a-Methyl-d-mannose                  | 25.0                        | 0          | 0                |              |
| Lactose                             | 25.0                        | 0          | 0                |              |
| Sucrose                             | 25.0                        | 0          | 0                |              |

**TABLE III**

Inhibition of hemagglutinin by neuraminidase-treated bovine submaxillary mucin

| Length of incubation | AcNeu released | BSM required for 50% inhibition |
|----------------------|----------------|---------------------------------|
|                      | µg/mg BSM      | BSM incubated with neuraminidase | BSM incubated without neuraminidase |
| min                  | µg/mg BSM      | µg/mg BSM                       | µg/mg BSM                           |
| 0                    | 0              | 9                               | 9                                  |
| 15                   | 0.8            | 22                              | 9                                  |
| 30                   | 2.5            | 33                              | 10                                 |
| 45                   | 5.8            | 40                              | 8                                  |
| 60                   | 7.4            | 70                              | 10                                 |
| 120                  | 11.9           | >100                            | 9                                  |

* These values represent the average of values obtained from 24, 48, and 72-h hydrolyses in constant boiling HCl at 110 °C.

**TABLE IV**

Amino acid composition of slug lectin

| Amino acid                        | mol/22,000 g to the nearest integer |
|-----------------------------------|-------------------------------------|
| Asparagine/aspartic acid          | 26                                  |
| Threonine                         | 13b                                 |
| Serine                            | 13a                                 |
| Glutamine/glutamic acid           | 14                                  |
| Proline                           | 4                                   |
| Glycine                           | 2                                   |
| Alanine                           | 16                                  |
| Valine                            | 7                                   |
| Methionine                        | 2                                   |
| Isoleucine                        | 7                                   |
| Leucine                           | 15                                  |
| Tyrosine                          | 10 (9.6)*                           |
| Phenylalanine                     | 10                                  |
| Histidine                         | 7                                   |
| Lysine                            | 17                                  |
| Tryptophan                        | (9)*                                |
| Arginine                          | 8                                   |
| Cysteine                          | 6*                                  |

**FIG. 3**

Sedimentation velocity behavior of slug lectin. ○, slower moving component in dilute buffer, pH 7.5; □, faster moving component in dilute buffer, pH 7.5; △, slower component in the presence of a 28:1 molar ratio of AcNeu/slug lectin; ■, faster component in the presence of a 28:1 molar ratio of AcNeu/slug lectin; ▲, slower moving component in dilute buffer which also contained 0.1 mM EDTA; ▼, faster moving component in dilute buffer which contained 0.1 mM EDTA; ○, slower moving component in dilute buffer after complete removal of AcNeu.
UV circular dichroism spectra of LFA at protein concentrations at or below which only the 3.4 S species could be observed in the analytical ultracentrifuge.

The amplitude of the ellipticity band at 282 nm increased with increasing protein concentration above ~0.6 mg/ml; this increase produced a marked qualitative change in the near UV spectrum (cf. curves 1 and 2 of Fig. 4A). Below ~0.6 mg/ml, the near UV spectra were qualitatively identical, but the amplitudes of the ellipticity bands fluctuated slightly among different preparations. In the absorbance range which could be measured at 280 nm (i.e. protein concentrations ≥1 mg/ml), LFA obeyed Beer’s Law (absorption spectrum given in Fig. 4A). Thus, the near UV spectral data suggest that the increased amplitudes of the near UV circular dichroism bands reflect an induced or enhanced chiral environment of one or more aromatic side chains upon association of lectin molecules. Slight increases in the ellipticity values were observed at all wavelengths in the far UV at protein concentrations above ~0.6 mg/ml (curves 1 and 2 of Fig. 4B), but the magnitudes of these increases, up to ~13%, can be accounted for by the induced chirality of the aforementioned aromatic amino acid residues (Beychok, 1964; Goodman et al., 1968).

A cross-linked polyacrylamide gel chromatographic support medium was employed in an attempt to utilize it as a purification step and to estimate the equivalent hydrodynamic radius of native LFA. This type of support was employed rather than a gel of polyacrylamide matrix to diminish the possibility of lectin-gel interactions. However, the elution position of LFA from the polyacrylamide gel was markedly dependent upon the ionic strength of the eluting buffer; at Γ/0.14, it eluted at a position equivalent to a protein of \( M_r = 15,000 \), and at Γ/0.04, it eluted at a position equivalent to a protein of \( M_r = 37,000 \). This was apparently the result of ionic attractions between the basic protein and the support medium since a similar behavior was observed for chymotrypsinogen A, the only standard protein utilized with a pi (Kubacki et al., 1949) similar to that of LFA.

Lines 1, 2, and 3 of Table V summarize the results of our sedimentation equilibrium measurements on native LFA. Linear plots of ln (fringe displacement) versus (radius)² were obtained from individual runs in dilute buffer at protein concentrations between 0.5 and 0.05 mg/ml. When examined collectively, however, the weight-average molecular weights

![Fig. 4. Circular dichroic and absorption spectra of slug lectin.](image)

**Table V**

| Macromolecular properties of slug lectin | Physical measurement | Magnitude* |
|-----------------------------------------|----------------------|------------|
| Molecular weight by sedimentation equilibrium, dilute buffer, pH 7.5; 4 rotor speeds | 44,000 ± 2,000 (6) |
| Molecular weight by sedimentation equilibrium, AcNeu-free protein, dilute buffer 7.5 | 42,500 |
| Molecular weight by sedimentation equilibrium, dilute buffer plus 30 mM N-acetylneuraminic acid, pH 7.5; 3 rotor speeds | 43,800 (3) |
| Molecular weight by sedimentation equilibrium, dilute buffer plus 30 mM N-acetylneuraminic acid, pH 2.05 | 18,500* |
| Molecular weight by sedimentation equilibrium, dilute buffer, pH 7.5 | 3.4 S ± 0.2 S |
| Subunit molecular weight by sedimentation equilibrium, S-S reduced in 6 M Gdm-Cl | 1.35 |
| Subunit molecular weight by sedimentation equilibrium, S-S reduced in 6 M Gdm-Cl | 22,000-26,000* (4) |
| Subunit molecular weight by gel chromatography in 6 M Gdm-Cl, S-S reduced | 21,500-23,000* |
| \( R_d \) by gel chromatography in 6 M Gdm-Cl | 41.4 Å; \( M_r = 19,100 \) |
| \( R_d \) by gel chromatography in 6 M Gdm-Cl, S-S reduced | 40.8 Å |
| Subunit molecular weight by sodium dodecyl sulfate gel electrophoresis | 23,500 |
| E from amino acid composition | 2.1 ± 0.2 cm² mg⁻¹ (3) |
| pl from isoelectric focusing | 9.5 |

*Numbers in parentheses, number of determinations.

*This includes a correction for the Donnan effect (Huston et al., 1979); a time-dependent decrease in \( M_\infty \) was observed. This latter observation suggests an acid-catalyzed chain hydrolysis.

*This range of values includes the precision of the ultracentrifuge data and the uncertainty of \( \delta \) in 6 M Gdm-Cl; values equal to, or 0.01 ml/g less than, \( \delta \) were used for \( \delta \).
from these runs exhibited a tendency to increase slightly with increasing initial protein concentration. Neither the presence of a 30-fold molar excess of AcNeu (line 3) nor the complete absence of AcNeu (line 2) had an effect on the sedimentation equilibrium behavior of the protein. The native molecular weight estimated for LFA was 44,000 ± 2,000. From this molecular weight, an $d_20$ of 3.4 S, and a $f$ of 0.724 mg/ml, the estimated $f/f_m$ of 1.35 suggests that LFA is a globular protein. A limited number of sedimentation equilibrium measurements at or below pH 3 suggest that like a number of other lectins (McKenzie et al., 1972; Nagata and Burger, 1974; Fish et al., 1978) the quaternary structure of LFA is disrupted at low pH (Table V, line 4).

The molecular weights of the constituent polypeptide chains of LFA were estimated by sedimentation equilibrium in 6 M Gdm-Cl with and without reduction of disulfide bonds. In both cases (Table V, lines 7 and 8), the data were consistent with two polypeptide chains of the same molecular weight which are held together in the native molecule by noncovalent interactions. The circular dichroic spectrum of LFA (Fig. 4B, curve 3) supports the assumption that all noncovalent interactions in the protein were broken and that the minimal subunit had been obtained. These results are also supported by empirical molecular weight estimation methods in denaturing solvents (Table V, lines 9 and 11). A comparison of the gel chromatographic behavior in 6 M Gdm-Cl between reduced and unreduced LFA suggests that there is only limited restraint imposed on the polypeptide by intrachain disulfide bonds (Table V, lines 9 and 10). This observation is consistent with the half-cystine content of the protein (Table IV) which suggests a maximum of only 1.5 disulfide bonds/100 amino acid residues (Tanford, 1968).

When LFA was exposed to a 40-fold molar excess of its receptor ligand, AcNeu, the only change observed in the near UV circular dichroic spectrum of LFA was a diminution of which are held together in the native molecule by noncovalent bonds (Table V, lines 6 and 7). This observation is consistent with the half-cystine content of the protein (Table IV) which suggests a maximum of only 1.5 disulfide bonds/100 amino acid residues (Tanford, 1968).

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discussion

This report describes the purification and characterization of a highly specific sialic acid binding lectin (LFA) from the slug L. flavus. This lectin is readily purified in milligram quantities (~17 mg/100 g of viscous slugs) by ammonium sulfate fractionation and affinity chromatography of slugs. The purified LFA is demonstrated to be homogenous by a variety of techniques. Sedimentation equilibrium studies indicate that native LFA has $M_r$ = 44,000 and $d_20$ of approximately 3.4 S. LFA consists of two equal sized subunits of $M_r$ ~ 22,000 as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and sedimentation equilibrium in 6 M Gdm-Cl. The lectin dissociates into subunits at pH 2.0 and tends to associate into larger molecular species at concentrations above ~1 mg/ml at pH 7.5, as evidenced by sedimentation velocity and near UV circular dichroism data. LFA resembles the horseshoe crab lectins carciносorcin and limulin in its high content of glutamic acid and aspartic acid residues (Dorai et al., 1981; Roche and Monsigny, 1974).

The high $p$I, approximately 9–9.5, of LFA suggests that many of its acidic amino acid residues must be amidated. Although LFA contains six residues of cysteine, disulfide bonds are not involved in subunit interactions.

Hemagglutination by affinity-purified LFA was inhibited only by AcNeu and GlyNeu of the many carbohydrates tested. Hemagglutination by partially purified slug extracts was strongly inhibited by AcNeu and GlyNeu and to a lesser extent by GlcN and GlcNAc. These data suggest that the slug contains more than one lectin, some of which do not bind sialic acid. Hemagglutination by LFA was also inhibited by the sialoprotein bovine submaxillary mucin. Treatment of the mucin with neuraminidase resulted in a loss of inhibitory activity which was proportional to the release of AcNeu from the mucin. Thus, the slug lectin LFA appears to be highly specific for sialic acid residues. Although a number of lectins have been very useful in the affinity purification of analytical quantities of glycoproteins, none with a high specificity for sialic acid has been successfully employed in this manner. As a result of its macromolecular properties and high degree of specificity for sialic acid, LFA will likely be very useful in studies on the sialoproteins of cellular membranes and in the affinity purification of sialoproteins.

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REFERENCES

Adler, A. J., Greenfield, N. J., and Fasman, G. D. (1973) Methods Enzymol. 27, 675–735

Babul, J., and Stellwagen, E. (1969) Anal. Biochem. 28, 216–221

Beychok, S., and Fasman, G. D. (1964) Biochemistry 3, 1673–1678

Bhavanandan, V. P., and Katlic, A. W. (1979) J. Biol. Chem. 254, 4000–4008

Bishaye, S., and Dorai, D. T. (1980) Biochim. Biophys. Acta 623, 89–97

Bredherman, P. J. (1974) Anal. Biochem. 51, 298–301

Chervenka, C. H. (1970) Anal. Biochem. 34, 24–29

Corfield, A. P., Veh, R. W., Wember, M., Michalski, J. C., and Schauer, R. (1981) Biochim. J. 197, 293–299

Decastel, M., Bournillon, R., and Frénoy, J.-P. (1981) J. Biol. Chem. 256, 9003–9008

Dorai, D. T., Bachhawat, B. K., Bishaye, S., Kannan, J., and Rao, D. R. (1981) Arch. Biochem. Biophys. 209, 325–333

Edelhoch, H. (1967) Biochemistry 6, 1948–1954

Fish, W. F. (1975) Methods Membr. Biol. 4, 189–276

Fish, W. F., Hamlin, L. M., and Miller, H. L. (1975) Arch. Biochem. Biophys. 190, 893–898

Gilbert, G. A. (1959) Proc. R. Soc. Lond. B Biol. 250, 377–388

Goldstein, J. J., and Hayes, C. E. (1978) Adv. Carbohydr. Chem. 35, 128–340

Goodman, M., Davis, G. W., and Benedetti, E. (1968) Acct Chem. Res. 1, 275–281

Hall, J. L., and Rowlands, D. T. Jr. (1974) Biochemistry 13, 828–832

Haupt, G. W. (1952) J. Opt. Soc. Am. 42, 441–447

Huston, J. S., Fish, W. W., Mann, K. G., and Tanford, C. (1972) Biochemistry 11, 1608–1612

Johnson, J. S., Kraus, K. A., and Scatchard, G. (1954) J. Phys. Chem. 58, 1034–1039

Kubacki, V., Brown, K. D., and Laskowski, M. (1949) J. Biol. Chem. 180, 73–73

Laemmli, U. K. (1970) Nature (Lond.) 227, 680–685

Lis, H., and Sharon, N. (1973) Annu. Rev. Biochem. 42, 541–574

Marchalonis, J. J., and Edelman, G. M. (1968) J. Mol. Biol. 24, 453–465

McKenzie, G. H., Sawyer, W. H., and Nichol, L. W. (1972) Biochim. Biophys. Acta 263, 283–293

Miller, R. L. (1982) J. Infect. Pathol. 39, 210–214

Nagata, Y., and Burger, M. M. (1974) J. Biol. Chem. 249, 3116–3122

Nowak, T. P., and Barondes, S. H. (1975) Biochim. Biophys. Acta 465, 675–735
Sialic Acid-specific Lectin from Limax flavus

Pemberton, R. T. (1970) Vox Sang. 18, 74–76
Pere, M., Bourrillon, R., and Jirgensons, B. (1975) Biochim. Biophys. Acta 393, 31–36
Pereira, M. E. A., and Kabat, E. A. (1979) Crit. Rev. Immunol. 1, 33–78
Pflumm, M. N., Wang, J. L., and Edelman, G. M. (1971) J. Biol. Chem. 246, 4369–4370
Roche, A.-C., and Monsigny, M. (1974) Biochim. Biophys. Acta 371, 242–254
Sharon, N., and Lis, H. (1975) Methods Membr. Biol. 3, 147–200
Shimazaki, K., Walborg, E. F., Neri, G., and Jirgensons, B. (1975) Arch. Biochem. Biophys. 169, 731–736
Tanford, C. (1966) Adv. Protein Chem. 23, 121–282
Thomas, M. W., Walborg, E. F., Jr., and Jirgensons, B. (1977) Arch. Biochem. Biophys. 178, 625–630
Warren, L. (1959) J. Biol. Chem. 234, 1971–1975
Yphantis, D. A. (1964) Biochemistry 3, 297–317
Purification and macromolecular properties of a sialic acid-specific lectin from the slug Limax flavus.

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