Lectin Purification on Affinity Columns Containing Reductively Aminated Disaccharides*

(Received for publication, July 6, 1976)

REINHOLD J. BAUES AND GARY R. GRAY†
From the Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455

A method is described for isolating lectins in pure form and quantitative yield in a single step by affinity chromatography on aminoethyl polyacrylamide gels containing reductively aminated disaccharide residues. The affinity columns were prepared in two steps: (a) direct reductive amination of the disaccharide and aminoethyl gel with sodium cyanoborohydride in aqueous solution at pH 9; (b) N-acetylation of excess amino groups. Affinity columns prepared by reductive amination of lactose, melibiose, maltose, and di-N-acetylchitobiose were used to purify the following lectins: lactose, peanut, castor bean; melibiose, Bandeiraea simplicifolia; maltose, jack bean, common lentil; di-N-acetylchitobiose, wheat germ. These columns are extremely stable, have good flow rates, and high binding capacities.

The increasing use of lectins in chemical and biological research has prompted the development of many methods for their purification. Because of its specificity, affinity chromatography has been used most widely, and several types of ligands and supports have been employed. In some cases, polysaccharide gels are themselves used as the ligand (2-8), while in others, carbohydrate-containing ligands are covalently attached to a supporting gel, or are copolymerized with the gel as it is formed (9). The ligands utilized have either been glycoproteins (10-14) or synthetically prepared glycosides (15-21), and the supports most commonly used have been the polysaccharide gels.

The affinity columns prepared by these procedures suffer a number of disadvantages, including low binding capacity, instability (especially those prepared by cyanogen bromide coupling), the use of polysaccharide gels as supports, or the need to synthesize a carbohydrate hapten of known anomeric configuration. Ideally, a polyacrylamide gel should be used as a support because of the absence of unwanted specificity, and the coupling procedure should utilize readily available starting materials and give a stable linkage and a high degree of substitution. Recently, a procedure was described by Gray (22) for coupling reducing disaccharides to proteins and derivatized gels in a single step in aqueous solution. This method relies on the ability of cyanoborohydride anion to selectively reduce the Schiff base formed between the aldehyde groups of reducing sugars and the amino groups of proteins or aminoethyl polyacrylamide gels. In the work reported here, affinity columns prepared from aminoethyl Bio-Gel P-150 and several readily available disaccharides are used to isolate in high yields, lectins which bind to terminal α- and β-D-galactopyranosyl, α-D-glucopyranosyl, and β-D-2-acetamido-2-deoxyglucopyranosyl residues.

EXPERIMENTAL PROCEDURES

Materials

Seeds—Jack bean meal and raw wheat germ were from Sigma Chemical Co.; Bandeiraea simplicifolia, Calbiochem; castor beans, Northrop King and Co., Minneapolis; unroasted Spanish peanuts and dried lentils, local market.

Chemicals—Sodium cyanoborohydride (Alfa Inorganics) was purified as described by Boreh et al. (23). Aminoethyl Bio-Gel P-150 (32 μmol of NH₂/mg of gel) was obtained from Bio-Rad. Chitosan, lactose, maltose, and melibiose were obtained from Pfaltz and Bauer. D-N-acetylchitobiose was prepared from chitosan as described by Raftery et al. (24). Yeast mannan and neuraminidase (type V) were from Sigma Chemical Co. Recently out-dated blood was kindly supplied by the Blood Bank, University of Minnesota Hospitals.

Methods

Hemagglutinating Assay—Human erythrocytes were washed three times with phosphate-buffered saline and used as a 3% (v/v) suspension in the same buffer. Each 50 μl of a 2-fold serial dilution of lectin solution and an equal volume of the erythrocyte suspension was incubated 1 h at room temperature, and the highest dilution that gave agglutination (titer) was determined by visual inspection. A hemagglutination unit is defined as the titer/mg of protein.

Lectins from wheat germ, common lentil, and B. simplicifolia were titered with type A erythrocytes, and the castor bean lectins with type B erythrocytes. The peanut lectin was titered with type B erythrocytes which had been incubated with neuraminidase (0.4 unit/ml, 15 min, 37°C), and then washed three times with NaCl/Pi (25). All assays were carried out in duplicate.

Precipitin Reaction for Concanavalin—Concanavalin A was titrated against yeast mannan by a modification of the procedure of So and Goldstein (26). The amount of protein precipitable by yeast mannan was determined by Lowry assay (27) after dissolution of the precipitates with 0.25 M methyl a-D-mannopyranoside.

* This work was supported by Grant CA15325 from the National Institutes of Health and by a Young Faculty Grant from the Du Pont Corporation. A preliminary report of this work has appeared (1).
† Recipient of Faculty Research Award 143 from the American Cancer Society. To whom requests for reprints should be addressed.

57
**Lectin Purification**

Extraction of Seeds - Raw peanuts (85 g) were ground to a fine powder and extracted three times with ether (1 ml/g) at 4°C, filtered, and air-dried (42 g). Untreated wheat germ and B. simplicifolia seeds were defatted by the method of Nagata et al. (28). Extracts were prepared by grinding the seeds with an equal volume of NaCl/Pi in an Omni-Mixer at high speed for 2 min (external cooling), followed by dilution with NaCl/Pi to 10 volumes the original weight of seeds. The extracts were stirred 24 h at 4°C and clarified by centrifugation at 14,000 x g.

Preparation of Affinity Gels - Affinity gels were prepared as described in the text (Fig. 2), followed by N-acetylation of the excess amino groups. A slurry of the gel (20 ml) in 0.2 M NaOAc was treated with 4 ml of acetate anhydride at 0°C for 30 min, followed by 4 ml of acetate anhydride at 30 min at room temperature. The gel was washed by filtration sequentially with water, 0.1 M sodium hydroxide, water, and NaCl/Pi. Titration by the method of Inman and Dintzis (29) indicated that all derivatized gels contained <3 μmol of NH₃/ml of gel.

**RESULTS**

Preparation of Affinity Columns - The effect of pH on the rate of reductive amination of lactose to aminoethyl Bio-Gel P-150 is shown in Fig. 1. Coupling is fastest at pH 9, but significantly slower at pH 6, 7, and 8. For preparative work, the reductive amination was conducted at pH 9. The rate of coupling of lactose, maltose, and melibiose to aminoethyl Bio-Gel P-150 (Fig. 2) shows some variation with disaccharide, but in all cases, high degrees of substitution are readily obtained. A smaller amount of di-N-acetylchitobiose under similar conditions (250 mg of GlcNAc, 110 mg of NaBH₄CN, and 10 ml of aminoethyl Bio-Gel P-150 in 0.2 M potassium phosphate, pH 9.0, 15 ml volume) gave a degree of coupling of 1.5 μmol of GlcNAc/ml of gel after 45 days.

Purification of Lectins - Affinity chromatography of lectins from the jack bean, common lentil, peanut, castor bean, R. deirea simplicifolia, and wheat germ is shown in Figs. 3 to 8. An NaCl/Pi extract from each source was applied without N-acetylation to the affinity column, and effluents were assayed for protein (A₄₀₀) and hemagglutinating activity. After removal of unbound proteins by elution with NaCl/Pi, each column was eluted with the appropriate inhibitor in NaCl/Pi. Fractions were assayed for absorbance at 280 nm, and the hemagglutinating activity of each fraction was determined after exhaustive dialysis against water to remove inhibitor.

With the exception of the common lentil, all hemagglutinating activity was bound to the affinity column in each case examined, and was removed specifically upon elution with inhibitor. The lentil lectin was not expected to be strongly retained by the maltose affinity column, as its specificity for α-D-glucopyranosyl residues is known to be poor (30). The other lectins, however, were quantitatively retained, and within experimental error, all hemagglutinating activity applied to the column was recovered upon elution with inhibitor (Table I). Inspection of Table I also reveals the high binding capacities of these columns. Concanavalin A (559 mg) was purified in a single run on a 6-ml column containing 15.1 μmol of glucose/ml of gel, and the capacity of the column was still not exceeded.

The purity of the lectins was examined by electrophoresis on polyacrylamide gels (31), applying 20 to 200 μg of protein. With the exception of the castor bean, all lectins gave a single band with the same mobility observed by other workers. As expected, the castor bean lectin gave two bands, corresponding in their mobilities to RCA₁ and RCA₂ (32).

**DISCUSSION**

Affinity columns containing carbohydrate residues are readily prepared by reductive amination of reducing sugars and aminoethyl polyacrylamide gels in aqueous solution. Since the ring form of the reducing sugar is destroyed, disaccharides must be used to obtain a monosaccharide determinant in the gel. Considering the number of disaccharides commercially available, or readily obtained by degradation of polysaccharides, the method is potentially useful for the isolation of a wide variety of lectins. Although only a limited number of lectins...
Lectin Purification

FIG. 4. Purification of the castor bean lectins by affinity chromatography on N-1-(1-deoxylactitol)aminoethyl Bio-Gel P-150. An NaCl/Pi extract from 4.9 g of delipidated castor bean was applied to the column (8 ml, 10 μmol of galactose/ml of gel), and after removal of unbound proteins by elution with NaCl/Pi, the lectins were eluted with 0.2 M lactose in NaCl/Pi. Fractions (3 ml) were collected at a flow rate of 15 ml/h at 4°C.

FIG. 5. Purification of Bandeiraea simplicifolia lectin by affinity chromatography on N-1-(1-deoxymelibitol)aminoethyl Bio-Gel P-150. An NaCl/Pi extract from 33 g of seeds was applied to the column (6 ml, 17.2 μmol of galactose/ml of gel), and after elution with NaCl/Pi to remove unbound proteins, the lectin was eluted with 0.1 M melibiose in NaCl/Pi. Fractions (2.2 ml) were collected at a flow rate of 21 ml/h at 4°C.

FIG. 6. Purification of concanavalin A by affinity chromatography on N-1-(1-deoxymaltitol)aminoethyl Bio-Gel P-150. An NaCl/Pi extract from 25 g of jack bean meal was applied to the column (6 ml, 15.1 μmol of glucose/ml of gel), and after elution of unbound proteins with NaCl/Pi, the lectin was eluted with 0.1 M methyl o-n-mannopyranoside (α-Me-MAN) in NaCl/Pi. Fractions (2.7 ml) were collected at a flow rate of 16 ml/h at 4°C.

FIG. 7. Purification of the common lentil lectin by affinity chromatography on N-1-(1-deoxymaltitol)aminoethyl Bio-Gel P-150. An NaCl/Pi extract from 20 g of seeds was applied to the column (9.8 ml, 15.1 μmol of glucose/ml of gel), and after elution of unbound proteins with NaCl/Pi, the lectin was eluted with 0.1 M methyl o-n-mannopyranoside (α-Me-MAN) in NaCl/Pi. Fractions (3.8 ml) were collected at a flow rate of 14 ml/h at 4°C.

FIG. 8. Purification of wheat germ agglutinin by affinity chromatography on N-1-(1-deoxydi-N-acetylchitobiitol)aminoethyl Bio-Gel P-150. An NaCl/Pi extract from 12.8 g of wheat germ was applied to the column (4.8 ml, 2.5 μmol of N-acetylglucosaminie/ml of gel), and after elution of unbound proteins with NaCl/Pi, the lectin was eluted with 0.1 M N-acetylglucosamine in NaCl/Pi. Fractions (3.8 ml) were collected at a flow rate of 10 ml/h at 4°C.

have been isolated so far by this procedure, the usefulness of this approach has clearly been demonstrated.

Besides its generality, the cyanoborohydride method has other advantages over commonly used procedures. Polyacryl-
### Lectin Purification

**Table I**

Lectin purification on acrylamide gels containing reductively aminated disaccharides

<table>
<thead>
<tr>
<th>Source</th>
<th>Column</th>
<th>Total protein*</th>
<th>Total activity*</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Applied</td>
<td>Bound</td>
<td>Eluted with inhibitor</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut</td>
<td>Lactosaminyl AE-P-150 (8 ml)</td>
<td>4,060</td>
<td>53</td>
<td>576,000</td>
</tr>
<tr>
<td>Castor bean</td>
<td>Lactosaminyl AE-P-150 (8 ml)</td>
<td>499</td>
<td>27.7</td>
<td>532,400</td>
</tr>
<tr>
<td><em>Bandeiraea simplicifolia</em></td>
<td>Melibiosaminyl AE-P-150 (6 ml)</td>
<td>878</td>
<td>43.3</td>
<td>72,000</td>
</tr>
<tr>
<td>Jack bean</td>
<td>Maltosaminyl AE-P-150 (6 ml)</td>
<td>3,060</td>
<td>559</td>
<td>6W</td>
</tr>
<tr>
<td>Common lentil</td>
<td>Maltosaminyl AE-P-150 (10 ml)</td>
<td>1,870</td>
<td>3.9</td>
<td>32,000</td>
</tr>
<tr>
<td>Wheat germ</td>
<td>Chitobiosaminyl AE-P-150 (5 ml)</td>
<td>556</td>
<td>4</td>
<td>8,160</td>
</tr>
</tbody>
</table>

* Lowry assay; bovine serum albumin as standard.


t Hemagglutination units (titer/mg of protein × total protein).


c Protein precipitated by yeast mannan.

Amine are present in equimolar proportions.

The affinity gels described here were prepared with approximately a 2-fold excess of carbohydrate, so it is possible that tertiary amines were formed via reductive amination of two disaccharides per amino group. The mechanism of this reaction is under further investigation.

**Acknowledgments** — We wish to thank Mr. Robert C. Wade, Ventron Corporation, for a generous gift of sodium cyanoborohydride.

**REFERENCES**

2. Liener, I. E., and Olson, M. O. J. (1967) Biochemistry 6, 105-111

* S. Danielson and G. R. Gray, unpublished data.
Lectin purification on affinity columns containing reductively aminated disaccharides.
R J Baues and G R Gray


Access the most updated version of this article at [http://www.jbc.org/content/252/1/57](http://www.jbc.org/content/252/1/57)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/252/1/57.full.html#ref-list-1](http://www.jbc.org/content/252/1/57.full.html#ref-list-1)