The Purification, Composition, and Specificity of the Anti-T Lectin from Peanut (Arachis hypogaea)*

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Peanut agglutinin was purified by affinity chromatography on Sepharose-ε-aminocaproyl-β-D-galactopyranosylamine. The purified lectin obtained in a yield of 150 mg/100 g of defatted peanut was homogeneous on polyacrylamide gel electrophoresis, ultracentrifugation, and gel filtration. The intrinsic sedimentation coefficient ($s_{20,w}$) and the intrinsic diffusion coefficient ($D_{20,w}$) were estimated at pH 7.4 as $5.7 \pm 0.1$ S and $5.0 \times 10^{-7}$ cm$^2$ s$^{-1}$, respectively. The molecular weight of the agglutinin, determined by sedimentation and diffusion and by gel filtration, was found to be 110,000. Disc gel electrophoresis and gel filtration, both in the presence of sodium dodecyl sulfate, gave a single component of $M_r = 27,500$ suggesting that the lectin is a tetramer composed of four subunits. Four alanine residues per 110,000 g were found by NH$_2$-terminal analysis and the sequence of the five NH$_2$-terminal amino acids was: Ala-Glu-Ser-Val-Thr. Each cycle in a sequenator gave a single amino acid, suggesting that the four subunits are identical.

Peanut agglutinin does not contain covalently bound sugar; it is devoid of cysteine and cystine, low in methionine, histidine, and tryptophan, but rich in acidic and hydroxyamino acids. The lectin agglutinated erythrocytes of human ABO blood types equally well, but only after they have been treated with neuraminidase. Of the monosaccharides tested for inhibition of hemagglutination only $\beta$-D-galactose and (β- and α-D-galactosides were active. High inhibitory activity was found with the disaccharide $\beta$Gal/3(1+3)GalNAc and with the desialylated glycoproteins: α$_1$-acid glycoprotein, fetuin, glycophorin, and human blood group NN or MM antigen. These desialylated glycoproteins also reacted with the lectin to form precipitin bands in Ouchterlony double diffusion in agar.

Extracts of peanut (Arachis hypogaea) have been known for some time to agglutinate neuraminidase-treated human red blood cells (1, 2). The agglutinin was designated "anti-T agglutinin" since it gave the same immunological reaction as the anti-T antibody of mammalian sera which is responsible for T-polyagglutination occurring in several bacterial and viral infections (3, 4). In fact peanut extracts have been used for clinical determination of T-polyagglutinability (5), but no attempt has been made to obtain the lectin in pure, homogeneous form. Since the agglutinating activity of peanut extracts could be inhibited by galactose and by lactose (1, 2), we tried to purify the lectin by affinity chromatography on a column of Sepharose-ε-aminocaproyl-β-galactopyranosylamine previously prepared by us for the isolation of soybean agglutinin (6). Peanut agglutinin thus purified was obtained in a homogeneous form. In this paper we describe the procedure for the purification of peanut agglutinin as well as some of the physicochemical and biological properties of the purified lectin.

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EXPERIMENTAL PROCEDURE

Materials

Source of Lectin—Peanuts, variety Shulamit (1974 crop), were purchased from Hazera Co., Haifa, Israel.

Glycoproteins and Sugars—Lacto-N-tetraose, lacto-N-neotetraose, and T-antigen (prepared from isolated O,MM and O,NN antigens) were the gifts of Dr. G. F. Springer, Department of Immunology Research, Evantion Hospital, Northwestern University; α$_1$-acid glycoprotein was a gift of Dr. K. Schmid, Boston University School of Medicine, and glycophorin of Dr. I. Kahane, Department of Clinical Microbiology, The Hebrew University Hadassah Medical School, Jerusalem. Fetuin was purchased from Gibco. Galβ(1→3)GalNAc was a gift of Dr. H. M. Flowers, Department of Biophysics, of this institute. N-Acetyllactosamine (Galβ(1→4)GlcNAc) was a gift from the late Dr. R. L. Katzman. All other sugars were of the highest quality commercially available.

Enzymes—Neuraminidase was obtained from Behringwerke AG, Marburg/Lahn, Germany, and trypsin from Worthington.

Chemicals—Sepharose 4B and Sephadex G-150 (superfine) were from Pharmacia, Uppsala, Sweden. Cyanogen bromide and all chemicals used for electrophoresis were from Eastman Chemicals. Sodium

The structures of the oligosaccharides mentioned in this paper are as follows: Lacto-N-tetraose: Galβ(1→3)GlcNAcβ(1→3)Galβ(1→4)Glc; lacto-N-neotetraose: Galβ(1→4)GlcNAcβ(1→3)Galβ(1→4)Glc. All sugars used in this study are in the pyranose form and of the β configuration unless otherwise noted.
dialyzed extensively, first against water and then against 0.9% NaCl. The precipitate was dissolved in water (150 ml) and cold, and then collected by centrifugation at 15,000 × g for 30 min. The precipitate which formed was allowed to settle overnight in the combined supernatants (730 ml) to 60%~saturation at 4°C, the precipitate was resuspended in 0.9% NaCl (500 ml) and the above procedure was repeated. Ammonium sulfate (285 g) was added to the combined supernatants (730 ml) to 60% saturation at 4°C, the precipitate which formed was allowed to settle overnight in the cold and then collected by centrifugation at 15,000 × g for 30 min. The precipitate was dissolved in water (150 ml) and dialyzed extensively, first against water and then against 0.9% NaCl. Insoluble material in the dialysis bag was removed by centrifugation (25,000 × g for 20 min) and the clear supernatant (225 ml) was applied at 4°C to a column (2.7 × 17 cm) of Sepharose-ε-aminocaproyl-β-galactopyranosylamine (61). The column, kept at 4°C, was eluted at a rate of 100 ml/hour, first with 0.9% NaCl (800 ml) until no significant amount of material absorbing at 280 nm was detected in the effluent (A280 < 0.05) and then with a solution of galactose (0.05 M in 0.9% NaCl). The fractions eluted with galactose and containing material absorbing at 280 nm were pooled, dialyzed extensively against water, centrifuged (25,000 × g, 20 min), and lyophilized. The elution pattern of the ammonium sulfate fraction on the affinity column is shown in Fig. 1. Usually 130 to 150 mg of purified peanut agglutinin with a specific hemagglutinating activity of 2,000 units/mg toward neuraminidase-treated human erythrocytes were obtained (for the definition of a hemagglutinating unit see Refs. 7 and 8). The recovery of the activity applied to the affinity column was higher than 98%, with an over-all yield of 87% of the activity present in the original extract and a 66-fold purification (Table 1).

**RESULTS**

**Purification of Peanut Agglutinin**—Peanuts (200 g) were ground with a Waring Blender, defatted with petroleum ether in a Soxhlet extractor, and dried in an air stream. The product (100 g) was suspended in 0.9% NaCl (500 ml), stirred for 3 hours at room temperature, centrifuged at 10,000 × g for 20 min, and the supernatant was retained. The precipitate was resuspended in 0.9% NaCl (500 ml) and the above procedure was repeated. Ammonium sulfate (285 g) was added to the combined supernatants (730 ml) to 60% saturation at 4°C, the precipitate which formed was allowed to settle overnight in the cold and then collected by centrifugation at 15,000 × g for 30 min. The precipitate was dissolved in water (150 ml) and dialyzed extensively, first against water and then against 0.9% NaCl. Insoluble material in the dialysis bag was removed by centrifugation (25,000 × g for 20 min) and the clear supernatant (225 ml) was applied at 4°C to a column (2.7 × 17 cm) of Sepharose-ε-aminocaproyl-β-galactopyranosylamine (61). The column, kept at 4°C, was eluted at a rate of 100 ml/hour, first with 0.9% NaCl (800 ml) until no significant amount of material absorbing at 280 nm was detected in the effluent (A280 < 0.05) and then with a solution of galactose (0.05 M in 0.9% NaCl). The fractions eluted with galactose and containing material absorbing at 280 nm were pooled, dialyzed extensively against water, centrifuged (25,000 × g, 20 min), and lyophilized. The elution pattern of the ammonium sulfate fraction on the affinity column is shown in Fig. 1. Usually 130 to 150 mg of purified peanut agglutinin with a specific hemagglutinating activity of 2,000 units/mg toward neuraminidase-treated human erythrocytes were obtained (for the definition of a hemagglutinating unit see Refs. 7 and 8). The recovery of the activity applied to the affinity column was higher than 98%, with an over-all yield of 87% of the activity present in the original extract and a 66-fold purification (Table 1).

**Homogeneity of Peanut Agglutinin**—Disc electrophoresis at pH 8.9 (14), at pH 4.3 (15), and at pH 7.2 (16) (the latter in the presence of 0.1%(w/v) sodium dodecyl sulfate) of the purified agglutinin revealed a single protein band (Fig. 2). Gel filtration on Sephadex G-150 (supernate) gave a single symmetrical protein peak with constant specific activity throughout (2,000 hemagglutinating units/mg). In sedimentation velocity studies in the ultracentrifuge, peanut agglutinin (14 mg/ml in buffer centrifuged at 56,000 rpm) afforded a single symmetrical peak.

**Molecular Weight**—Gel filtration of peanut agglutinin on a column of Sephadex G-150 (supernate) which had been calibrated with proteins of known molecular weight, gave a value of 110,000 ± 10,000 relative to bovine γ-globulin (M, = 150,000), soybean agglutinin (M, = 120,000), bovine serum albumin (M, = 68,000), ovalbumin (M, = 45,000), and chymotrypsinogen (M, = 25,000) (Fig. 3).

**Dissociation into Subunits**—In disc gel electrophoresis, in the presence of 0.1% sodium dodecyl sulfate (16), peanut agglutinin migrated to a distance corresponding to a molecular weight of 27,000 ± 1,500 relative to bovine serum albumin (M, = 68,000), ovalbumin (M, = 45,000), glyceraldehyde phosphate dehydrogenase (M, = 36,000), soybean agglutinin (M, = 30,000), chymotrypsinogen (M, = 25,000), and lysozyme (M, = 14,400) (Fig. 4). Gel filtration on Sephadex G-150 (supernate) in the presence of 0.1% sodium dodecyl sulfate afforded a single peak of protein which emerged from the column at a position corresponding to a molecular weight of 28,000 ± 2,500 relative to catalase (M, = 68,000), ovalbumin (M, = 45,000), soybean agglutinin (M, = 30,000), chymotrypsinogen (M, = 25,000), and cytochrome c (M, = 12,400) (Fig. 3).

These results indicate that peanut agglutinin is a tetramer composed of four apparently identical subunits.

**Amino Acid Composition and Carbohydrate Analysis**—Amino acid analysis of the agglutinin (Table II) revealed a particularly high content of acidic and hydroxyamino acids, a low content of methionine, tryptophan, and histidine, and the
FIG. 1 (left). Affinity chromatography of peanut agglutinin on a column of Sepharose-ε-aminocaproyl-β-D-galactopyranosylamine. The ammonium sulfate (60%) fraction (225 ml) obtained from an extract of 100 g of defatted peanut was applied to a column (2.7 x 17 cm) and after washing out the unbound proteins with 800 ml of 0.9% NaCl (20-ml fractions), the lectin was eluted (10-ml fractions) with 0.05 M β-galactose in 0.9% NaCl. The arrow denotes the point of application of the galactose solution. The flow rate was 100 ml/hour and chromatography was carried out at 4°. For the determination of hemagglutinating activity (H.A.) samples were dialyzed extensively against 0.9% NaCl.

FIG. 2 (right). Disc electrophoresis of agglutinin on polyacrylamide gels. The direction of migration was from the top. Electrophoresis was performed in 7.5% acrylamide gels with 100 µg of peanut agglutinin at pH 8.9 at 1 ma for 5 hours (a); at pH 4.3 at 3 ma for 4 hours (b); and in the presence of 0.1% sodium dodecyl sulfate at 8 ma for 4 hours (c). The gels were stained for protein with Coomassie brilliant blue R-250.

TABLE I

<table>
<thead>
<tr>
<th>Fraction*</th>
<th>Volume</th>
<th>Protein concentration*</th>
<th>Total protein (mg)</th>
<th>Hemagglutinating activity*</th>
<th>Total activity (HU)</th>
<th>Specific activity (HU/mg)</th>
<th>Yield of activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract from 100 g defatted peanut</td>
<td>730</td>
<td>14.7</td>
<td>10,700</td>
<td>480</td>
<td>350,400</td>
<td>33</td>
<td>100</td>
</tr>
<tr>
<td>Ammonium sulfate (60%) precipitate</td>
<td>225</td>
<td>13.8</td>
<td>3,100</td>
<td>1,380</td>
<td>310,500</td>
<td>100</td>
<td>88</td>
</tr>
<tr>
<td>Galactose eluate from affinity column</td>
<td>107</td>
<td>1.37</td>
<td>147</td>
<td>2,860</td>
<td>306,020</td>
<td>2,080</td>
<td>87</td>
</tr>
</tbody>
</table>

*Each fraction was dialyzed extensively against 0.9% NaCl before dialysis against water and lyophilization.

Protein in the first two fractions was determined by the method of Lowry et al. (10) and in the last fraction by weighing the lectin after complete absence of cysteine and cystine. No traces of amino sugars could be observed in the amino acid analyzer. Likewise, no neutral sugar (less than 0.5% w/w) could be observed (in a 5-mg sample) by the colorimetric phenolsulfuric acid method using mannose as standard (20).

NH4-terminal Sequence—Quantitative determination of NH4-terminal amino acids gave only dinitrophenylalanine in an amount corresponding to 0.8 mol/27,500 g of peanut agglutinin. The sequence of the five NH4-terminal amino acids was found to be: alanine, glutamic acid, serine, valine, and threonine. Each cycle in the sequenator gave a single amino acid suggesting that the four subunits of the lectin are identical.

Hemagglutinating Activity—The lectin did not agglutinate untreated or trypsin-treated human erythrocytes whether type A, B, or O. However, after treatment of the erythrocyte with neuraminidase all the above erythrocytes were highly susceptible to agglutination by peanut agglutinin (2,000 ± 300 HU/mg).

Inhibition Studies—The affinity of peanut agglutinin for various mono-, di-, and oligosaccharides as well as for the circulating glycoproteins fetuin and α1-acid glycoprotein and the human erythrocyte membrane glycoproteins, glycoporphin, and T-antigen, was probed by measuring their ability to inhibit hemagglutination of human type B, neuraminidase-treated human type B erythrocytes. When plotted as percentage of inhibition versus the inhibitor concentration on a logarithmic scale (data not shown), the inhibition curves showed a characteristic

*The abbreviation used is: HU, hemagglutinating units.
The disaccharide Galf(1+3)GalNac was a much better inhibitor than any of the above saccharides (Table IIIC). The most desialylated glycoproteins tested were more potent inhibitors (30-fold) than the above di- and tetrasaccharides. All the tetrasaccharides, lacto-l+r-tetraose and lacto-l+r-neotetraose were slightly more potent inhibitors (1.66-fold) than galactose. The unmodified glycoproteins did not exhibit any inhibition activity at 0.25 M concentration: galactose 6-sulfate; 2-deoxygalactose, galactitol, γ-galactosylactone, calcium galactonate, UDP-galactose, galacturonic acid, N-acetylgalactosamine, D-nitrophosphoryl α- or β-N-acetylgalactosamine, glucose, methyl-α-glucoside, glucosamine, N-acetylglucosamine, N-nitrophosphoryl α- or β-N-acetylgalactosaminidase, mannose, methyl-α-mannoside, N-acetylmannosamine, L-arabinose, xylose, D- or L-fucose, and rhamnose. Native (not desialylated) α, α-acid glycoprotein, fetuin, and glycoporphin were not inhibitory.

**Ouchterlony Double Diffusion in Agar**—Peanut agglutinin formed precipitin bands with the desialylated glycoproteins: α, α-acid glycoprotein, fetuin, and glycophorin, and with T-antigen. The unmodified glycoproteins did not form precipitin bands with peanut agglutinin under the same experimental conditions (Fig. 5).

**DISCUSSION**

This report is the first to describe the isolation and characterization of the lectin from peanut (*Arachis hypogaea*). For the purification of peanut agglutinin we used the affinity adsorbent Sepharose-ε-aminocaproyl-α-galactopyranosylamine (6), which is routinely employed in our laboratory for the purification of soybean agglutinin, another galactose-specific lectin. The purification procedure described herein afforded a lectin which was homogeneous by a variety of techniques. Peanut agglutinin is similar in its molecular weight (110,000) and subunit structure (four subunits) to a number of other purified lectins such as soybean agglutinin and wax bean agglutinin (26). Furthermore, the abundance of acidic and basic amino acids is similar in the two lectins.
TABLE III

Inhibitory effect of various sugars and glycoproteins on hemagglutinating activity of peanut agglutinin

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Concentration needed for 50% inhibition</th>
<th>Relative inhibitory activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Monosaccharides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Methyl-α-galactopyranoside</td>
<td>40</td>
<td>2.5</td>
</tr>
<tr>
<td>Methyl-β-galactopyranoside</td>
<td>80</td>
<td>1.25</td>
</tr>
<tr>
<td>β-Nitrophenyl-α-galactopyranoside</td>
<td>60</td>
<td>1.86</td>
</tr>
<tr>
<td>α-Nitrophenyl-β-galactopyranoside</td>
<td>80</td>
<td>1.25</td>
</tr>
<tr>
<td>Galactosamine</td>
<td>70</td>
<td>1.43</td>
</tr>
<tr>
<td>B. Di- and tetrasaccharides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melibiose</td>
<td>125</td>
<td>0.8</td>
</tr>
<tr>
<td>Lactose</td>
<td>60</td>
<td>1.66</td>
</tr>
<tr>
<td>N-Acetyllactosamine</td>
<td>60</td>
<td>1.66</td>
</tr>
<tr>
<td>Lacto-N-tetraose</td>
<td>60</td>
<td>1.66</td>
</tr>
<tr>
<td>Lacto-N-neotetraose</td>
<td>60</td>
<td>1.66</td>
</tr>
<tr>
<td>Galβ(1→3)GalNAc</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>C. Glycoproteins (desialylated)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α1-Acid glycoprotein</td>
<td>0.4</td>
<td>20</td>
</tr>
<tr>
<td>Fetuin</td>
<td>0.1</td>
<td>1,000</td>
</tr>
<tr>
<td>Glycophorin</td>
<td>0.01</td>
<td>10,000</td>
</tr>
<tr>
<td>T-Antigen (desialylated O,N,N blood group substance)</td>
<td>0.008</td>
<td>12,500</td>
</tr>
</tbody>
</table>

*The concentration of inhibitors before the addition of 1 ml of neuraminidase-treated human type B erythrocyte suspension.

†The hemagglutination inhibitory activity of galactose was taken as 1.

‡Due to scarcity of material the inhibition was assayed in microtiter plates (21) and the results were calculated in relation to the inhibitory activity of galactose in the same assay system.

§For comparison, the concentration of inhibitors has been normalized to their molar galactose content as given in the literature for α1-acid glycoprotein (22), fetuin (23), glycophorin (24), and NN blood group substance (25).

hydroxyamino acids and the paucity of sulfur-containing amino acids found in peanut agglutinin have also been reported for other lectins (26). However, unlike most of the lectins which are glycoproteins, peanut agglutinin does not contain any covalently bound carbohydrate.

Our inhibition experiments (Table III) confirm the original observations of Bird (1) and Uhlenbruck et al. (2), made with crude extracts of peanut, that galactose and lactose are poor inhibitors of peanut agglutinin and that the disaccharide Galβ(1→3)GalNAc and the desialylated human erythrocyte N-substance (T-antigen) are very potent inhibitors of this lectin.

We have extended the study to many other saccharides and in contrast to the suggestion of Uhlenbruck et al. (2) that only β-galactosides are inhibitors, we find that both α- and β-galactosides possess inhibitory activity.

Assuming that the hemagglutination inhibition activity of saccharides reflects the affinity of peanut agglutinin for the saccharides, it is possible to specify the structural requirements of the lectin combining site. Among galactosides differing only in the configuration about anomeric C-1, there seems to be a slight preference for the α anomers, except for melibiose which is less active than lactose or galactose. The aglycon of the galactoside (α- or β-N-nitrophenyl and α- or β-methyl groups) does not influence markedly binding of the sugar by peanut agglutinin. Furthermore, aromatic aglycons of galacto-
Extended combining site (Fig. 6). The presence of extended sugar binding sites has also been proposed for concanavalin A (31) for wheat germ agglutinin (32), and for the lectin from potato (33).

Peanut agglutinin unlike most of the lectins described thus far does not agglutinate untreated or trypsin-treated human erythrocytes. Preliminary experiments indicate that untreated cells bind very little $^{125}$I-labeled agglutinin; however, after treatment of the cells with neuraminidase there is a great enhancement in the binding of the lectin and the erythrocytes are agglutinated. Similar results were obtained for the binding of peanut agglutinin to lymphocytes from a variety of sources and it was observed that the lectin was mitogenic to human peripheral blood lymphocytes and to rat lymphocytes only after the cells have been treated with neuraminidase.  

The availability of a newly purified galactose-specific lectin with a sugar specificity somewhat different from that of soybean agglutinin makes it possible to gain further insight into the role of galactosyl residues on lymphocyte surface membrane in triggering blastogenesis. The purified peanut agglutinin may be used for the clinical determination of T-polyagglutinability of erythrocytes (5) and for probing cell membranes from cells of healthy mammary glands and benign lesions. The purified peanut agglutinin may thus be of great use in the characterization of the tumor-specific antigens on the surface of malignant cells.

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