The Purification and Characterization of Wheat-Germ Agglutinin

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The purification of wheat-germ agglutinin by precipitation with ammonium sulphate and by chromatography on Sephadex G-75, Sepharose-ovomucoid and CM-cellulose is described. This procedure gave agglutinin preparations which were homogeneous on polyacrylamide gels under a variety of conditions. Purified wheat-germ agglutinin formed colourless solutions and was relatively insoluble at neutral pH; maximum solubility in 1 mM-tris-HCl buffer, pH 7.4, was approx. 1 mg/ml. The agglutinin was a glycoprotein containing a single polypeptide chain with an approximate molecular weight of 23000. The *N*-terminus of the oxidized agglutinin was cysteic acid and the *C*-terminus was glycine. The amino acid composition showed that the protein was extremely rich in cysteine and cystine; there were 15-17 free SH groups/mol. The absorption maximum for the protein was at 272 nm and the molar extinction coefficient at 280 nm was 1.09×10^5 litre $\cdot$ mol⁻¹ $\cdot$ cm⁻¹. Equilibrium dialysis indicated that there was only one binding site per molecule for *N*-acetylglucosamine.

A major topic of current research is the study of the fundamental differences between normal and transformed cells. One such difference is the ability of transformed cells to be agglutinated by plant lectins (Aub *et al.*, 1963; Inbar & Sachs, 1969; Burger, 1969; Ben-Bassat *et al.*, 1970); this property reflects a major change in the surface membranes of cells after transformation (Burger & Noonan, 1970; Eckhart *et al.*, 1971; Ben-Bassat *et al.*, 1971). Plant lectins are therefore being used to probe the differences between the membranes of normal cells and transformed cells at the molecular level.

Lectins have been detected in extracts of many plant seeds (Boyd, 1963) but few have been purified and fully characterized. The function of lectins has not been clearly established but it seems unlikely that they play any important role in the vital processes of the plant (Brücher *et al.*, 1969). Lectins all appear to possess one common property, that of binding specific carbohydrates and carbohydrate-containing macromolecules (Boyd, 1963). For example, wheat-germ agglutinin binds to *N*-acetylglucosamine, to di-*N*-acetylchitobiose and to ovomucoid (Burger & Goldberg, 1967) but concanavalin A, the lectin from jack beans, binds only to oligosaccharides and glycoproteins possessing terminal α -D-glucopyranosyl and α -D-mannopyranosyl residues (Goldstein *et al.*, 1965; So & Goldstein, 1967). This property of lectins is almost certainly related to the mechanism of cell agglutination, which is possibly analogous to the precipitation of antigens by antibodies (Goldstein *et al.*, 1965).

Wheat-germ agglutinin is one of the plant lectins that has been extensively used during investigations on the interactions between this group of proteins

and cell membranes. There is no quantitative difference between the binding of wheat-germ agglutinin to transformed cells and to equivalent normal cells (Ozanne & Sambrook, 1971); however, with few exceptions, agglutination occurs only with transformed cells (Aub *et al.*, 1965; Liske & Franks, 1968; Gantt *et al.*, 1969). A satisfactory procedure for purifying wheat-germ agglutinin has not been described; the purification procedures described by Burger & Goldberg (1967) and by Ozanne & Sambrook (1971) both give agglutinin preparations which yield two protein bands on polyacrylamide gel electrophoresis. In the present paper we describe a procedure for the purification of wheat-germ agglutinin to homogeneity and also describe some of its properties.

Experimental

Materials

Wheat-germ lipase and wheat-germ acid phosphatase were isolated from a mixture of *Triticum* species and were obtained from Miles-Pentex Inc., Kankakee, Ill., U.S.A. KB cells were obtained from Dr. A. Shatkin, Hoffmann-La Roche, Nutley, N.J., U.S.A.; Joklik's modified Eagle's spinner medium (W. K. Joklik, personal communication), horse serum and calcium-free magnesium-free phosphate-buffered saline (8 g of NaCl, 0.2 g of KCl, 1.5 g of Na₂HPO₄ in 1 litre of glass-distilled water) were obtained from Gibco, Grand Island, N.Y., U.S.A. Sephadex G-75 and Sepharose 4B were obtained from Pharmacia Fine Chemicals Inc., Piscataway, N.J., U.S.A., and CM-cellulose (CM52) was obtained

from H. Reeve Angel Inc., Clifton, N.J., U.S.A. Aquasol liquid-scintillation fluid and *N*-[1-¹⁴C]-acetyl-D-glucosamine were obtained from New England Nuclear, Boston, Mass., U.S.A. Trypsin (twice crystallized) was obtained from Worthington Biochemical Corp., Freehold, N.J., U.S.A. Equilibrium dialysis cells (1 ml capacity) were purchased from Metaloglass Inc., Boston, Mass., U.S.A. Aquacide II (CM-cellulose, mol. wt. approx. 250000, for concentrating solutions) was obtained from Calbiochem, Los Angeles, Calif., U.S.A. Plastic trays used in agglutination assays were obtained from Linbro, New Haven, Conn., U.S.A. The elementary analysis was performed by Kross-Reference Laboratories, Bellmore, N.Y., U.S.A.

Methods

Agglutination assay. KB cells were grown in suspension at 37°C in Joklik's modified Eagle's spinner medium containing 5% horse serum (W. Sugden, personal communication). Cells were removed from this suspension by centrifugation (10000g for 10 min) and were washed three times with calcium-free magnesium-free phosphate-buffered saline. Cells were finally suspended in this medium at a concentration of 4×10^7 cells/ml (determined by counting in a haemocytometer); 0.3 ml of this suspension was used for each agglutination assay. Normally 0.1 ml of agglutinin solution was used for each assay and agglutination was scored on a rough scale of 0 to +++++.

Preparation of the Sepharose-ovomucoid affinity adsorbent. Ovomucoid was coupled to Sepharose 4B after activation of the resin by cyanogen bromide (Axen *et al.*, 1967; Cuatrecasas, 1970). Sepharose 4B was swollen in water and cyanogen bromide (300 mg/ml of packed Sepharose) was then added with gentle stirring. The suspension was maintained at pH 11.5 by the addition of 5M-NaOH and the temperature was maintained at 20°C by the addition of ice. After 12 min the temperature of the suspension was decreased to 0°C by the addition of large quantities of ice and the suspension was then quickly filtered and washed with cold 0.5M-Na₂CO₃. The activated Sepharose was immediately suspended in 0.1M-Na₂CO₃ and an equal volume of cold ovomucoid [1% (w/v) in 0.1M-NaHCO₃] quickly added. The suspension was gently stirred at 4°C for 20 h and then washed with water and finally with 0.1M-NaCl.

Electrophoresis. Disc gel electrophoresis was performed by the method of Ornstein & Davis (1961) in 5%, 7.5% and 10% cross-linked polyacrylamide gels polymerized in glass tubes (10 cm × 5 mm diam.). Gels were prepared in 0.38M-tris adjusted to pH 8.7 with 2M-HCl, with and without 8M-urea, in 0.38M-acetic acid adjusted to pH 4.5 with 1M-KOH, with and without 8M-urea, and in 0.1M-sodium phosphate

adjusted to pH 7.2 with 2M-NaOH, containing 0.1% sodium dodecyl sulphate. The current was 2.5–5.0 mA/tube. The molecular weight of purified wheat-germ agglutinin was determined by the method of Shapiro *et al.* (1967) by electrophoresis in 15% cross-linked polyacrylamide slab gels (F. W. Studier, personal communication) prepared in 0.38M-tris adjusted to pH 8.8 with 2M-HCl, containing 0.1% sodium dodecyl sulphate.

Gels were stained with 0.025% (w/v) Coomassie Brilliant Blue in methanol–acetic acid–water (9:2:9, by vol.). Gels were destained either electrophoretically at 200 mA for 24 h or by repeated washing in methanol–acetic acid–water (2:3:35, by vol.). In some experiments gels were stained for glycoproteins by the method of Zacharius *et al.* (1969).

Amino acid analysis. Amino acid compositions were determined with a Technicon TSM amino acid analyser. Samples were hydrolysed in 6M-HCl at 105°C for 24 h in evacuated, sealed tubes. Hydrolysates were dried *in vacuo* and stored dry at 4°C until analysis. In some analyses cysteine and cystine were determined either as cysteic acid, after performic acid oxidation of the protein (Hirs, 1956), or as *S*-carboxymethylcysteine, after total reduction and *S*-carboxymethylation (Hirs, 1967). Tryptophan was determined by spectrophotometric titration with *N*-bromosuccinimide (Funatsu *et al.*, 1964).

Determination of SH groups. The number of free SH groups in purified agglutinin preparations was determined by titration with *p*-chloromercuribenzoate (Boyer, 1954). The protein was dissolved in 0.01M-sodium phosphate, adjusted to pH 7.0 with 2M-NaOH, and the titration of free SH groups with 76 μM-*p*-chloromercuribenzoate was measured spectrophotometrically at 255 nm. The end-point of the reaction was determined graphically.

***N*- and *C*-Terminal analysis.** The *N*-terminus of performic acid-oxidized protein was determined by reaction with dansyl chloride (Gray, 1967) and the *C*-terminus was determined by digestion with carboxypeptidase A (Ambler, 1967).

Digestion with trypsin. Trypsin was treated with diphenylcarbamoyl chloride to inhibit chymotryptic activity (Erlanger & Cohen, 1963). The purified agglutinin (approx. 2 mg) was oxidized with performic acid (Hirs, 1956) and then dried *in vacuo*. The dried material was dissolved in 0.1M-acetic acid adjusted to pH 8.5 with 6M-ammonia, trypsin (enzyme/substrate ratio 1:10, w/w) was added and the solution was incubated at 37°C for 18 h. The tryptic peptides were separated on Whatman no. 1 paper by ionophoresis at pH 3.5 at 57 V/cm for 1 h (Ryle *et al.*, 1955) in the first dimension and by descending chromatography in butan-1-ol–acetic acid–water–pyridine (15:3:12:10, by vol.) (Waley & Watson, 1953) in the second dimension. Peptides were detected by staining with 0.2% (w/v) ninhydrin in acetone

(Toennies & Kolb, 1951); glycopeptides were detected by staining with aqueous 1% (w/v) sodium metaperiodate and ammoniacal 5% (w/v) silver nitrate (Clamp & Putnam, 1964).

Equilibrium dialysis. Membranes used for equilibrium dialysis were acetylated by the procedure of Myer & Schellman (1962) and stored at 4°C under ethanol. Before use the membranes were hydrated by soaking in 1 mM-tris adjusted to pH 7.4 with 2 M-HCl, which was the buffer used during equilibrium dialysis. Dialysis cells were prepared by placing the agglutinin (5 nmol) and various amounts of *N*-[1-¹⁴C]-acetyl-D-glucosamine (specific radioactivity 10 μ Ci/ μ mol) on opposite sides of the membrane. The cells were agitated on a rotary table at 22°C and duplicate samples (50 μ l) were withdrawn from each compartment every 24 h until equilibrium was reached (168 h). The samples were dried on glass-fibre discs, Aquasol (2.5 ml) was added and the samples were counted for ¹⁴C radioactivity in an Intertechnique liquid-scintillation spectrometer. The amount of protein on each side of the membrane at equilibrium was determined by amino acid analysis and final calculations were corrected for the small amount of protein penetration that had occurred.

Results and Discussion

Purification procedure

Commercial preparations of wheat-germ lipase and wheat-germ acid phosphatase were used throughout as the source of the lectin. The lot numbers and the previous histories of the preparations are shown in Table 1. The buffer used during the majority of these procedures was 1 mM-tris adjusted to pH 7.4 with 2 M-HCl.

(1) **Heat extraction.** Wheat-germ lipase or acid phosphatase (10 g) was suspended in 1 mM-tris-HCl buffer, pH 7.4 (1:20, w/v), and was incubated at 63°C for 15 min. The resulting precipitate was removed by centrifugation (12000g for 20 min) at 4°C.

(2) **(NH₄)₂SO₄ fractionation.** The supernatant, obtained as above, was fractionated by sequential precipitation with (NH₄)₂SO₄ at 19, 45 and 60% saturation. The supernatant was stirred at 0°C and sufficient (NH₄)₂SO₄ (saturated at room temperature) was added to yield a suspension at the required degree of saturation. The suspension was stirred for 2 h at 0°C and the precipitate then recovered by centrifugation (12000g for 20 min) at 4°C. The resulting pellets, which were suspended in 1 mM-tris-HCl buffer, pH 7.4 (approx. 10 mg of protein/ml), and the final supernatant were dialysed at 4°C for 16 h against 1 mM-tris-HCl buffer, pH 7.4 (four changes). The resulting solutions were assayed for agglutinating activity; the agglutinin was precipitated almost entirely by (NH₄)₂SO₄ at 45% saturation. A small precipitate, representing approx. 1% of the total protein, usually remained after dialysis of this (NH₄)₂SO₄ fraction; this precipitate contained no agglutinating activity and was removed by centrifugation before the chromatographic separations were done.

(3) **Exclusion chromatography on Sephadex G-75.** The fraction obtained with 45% (NH₄)₂SO₄ was concentrated by dialysis against Aquacide II and then applied to a column of Sephadex G-75. Elution was with 1 mM-tris-HCl buffer, pH 7.4, and eluent fractions were monitored by measuring their *E*₂₈₀ and by determining their agglutinating activity (Fig. 1). The column was calibrated by the elution of proteins of known molecular weight under identical conditions (Fig. 1); the agglutinin was eluted from Sephadex G-75 with an elution volume that was compatible with a molecular weight of 22000. In later preparations this step was omitted.

(4) **Affinity chromatography on Sepharose-ovomucoid.** Either the 45% (NH₄)₂SO₄ fraction or the agglutinin from Sephadex G-75 was applied, without prior concentration, to the Sepharose-ovomucoid affinity column. The column was washed with 0.1 M-NaCl until the *E*₂₈₀ was at background and the agglutinin was subsequently eluted with 0.1 M-acetic

Table 1. Sources used for the preparation of wheat-germ agglutinin

Lipase and acid phosphatase preparations were obtained from wheat-germ by (NH₄)₂SO₄ and ethanol precipitation.

Source	Manufacturer's lot no.	Previous history	Yield of lectin/g of starting material (mg)
Wheat-germ lipase	5A-2	Prepared from defatted wheat germ	3.0
Wheat-germ lipase	6	Prepared from non-defatted wheat germ	0.2
Wheat-germ lipase	15B	Crude acid phosphatase preparation, containing 50% (w/v) (NH ₄) ₂ SO ₄ , from defatted wheat germ	0.1
Wheat-germ acid phosphatase	BE2	Freeze-dried salt-free preparation from defatted wheat germ	2.9

Fig. 1. Elution of the 45% $(\text{NH}_4)_2\text{SO}_4$ fraction from Sephadex G-75

Approx. 250mg of the 45% $(\text{NH}_4)_2\text{SO}_4$ fraction and 50mg of a mixture of ovalbumin (mol.wt. 40000), trypsin (mol.wt. 24000) and α -chymotrypsin (mol.wt. 21000) were applied separately to a column (85cm $\times$ 2.5cm diam.) of Sephadex G-75. Elution was with 1 mM-tris-HCl buffer, pH 7.4, the flow rate was 50ml/h and 20ml fractions were collected. ●, 45% $(\text{NH}_4)_2\text{SO}_4$ fraction; ▲, molecular weight markers. The height of the vertical bars represents the agglutinating activity of the fractions.

acid (Fig. 2). The agglutinin was concentrated by dialysis against Aquacide II and then dialysed against 1 mM-tris-HCl buffer, pH 7.4, at 4°C for 16h (three changes). A white protein precipitate, which contained approx. 70% of the agglutinating activity, was obtained during dialysis and was recovered by centrifugation.

(5) *Chromatography on CM-cellulose.* The supernatant from the previous step was applied to a column of CM-cellulose and was eluted with 1 mM-tris-HCl buffer, pH 7.4. Effluent fractions were monitored as before (Fig. 3). Two protein peaks were recovered after this chromatographic separation. The first

peak was brown in colour and contained little or no agglutinating activity; the second peak, which contained the agglutinin, was colourless. Effluent fractions containing the agglutinin were pooled and concentrated by dialysis against Aquacide II; these fractions were sometimes turbid.

Gel electrophoresis

Some of the gel patterns obtained with the agglutinin which precipitated after affinity chromatography and with the agglutinin obtained after CM-

EXPLANATION OF PLATE I

Polyacrylamide gel electrophoresis of purified agglutinin

(1) 10% cross-linked polyacrylamide in 0.1 M-sodium phosphate buffer, pH 7.2, containing 0.1% sodium dodecyl sulphate. (2) 7.5% cross-linked polyacrylamide in 8M-urea, pH 8.7. (3) 5% cross-linked polyacrylamide in 8M-urea, pH 8.7. (4) 5% cross-linked polyacrylamide, pH 8.7. (5) 7.5% cross-linked polyacrylamide in 8M-urea, pH 4.5. (6) 5% cross-linked polyacrylamide, pH 4.5. Electrophoresis was performed as described in the Experimental section; 20–50 μ g of pure agglutinin was used.

Fig. 2. Elution of the agglutinin from Sepharose-ovomucoid

Approx. 50mg of protein, obtained after chromatography on Sephadex G-75, was applied to a column (14cm × 1cm diam.) of Sepharose-ovomucoid. Elution was with 0.1 M-NaCl (600ml) followed by the stepwise addition of 0.1 M-acetic acid (200ml) (marked by the arrow); the flow rate was 100ml/h and 25 ml fractions were collected. The height of the vertical bars represents the agglutinating activity.

cellulose chromatography are shown in Plate 1. In all cases only a single protein band was detected; the protein was therefore considered homogeneous. The agglutinin gave a positive reaction when stained for glycoprotein.

On sodium dodecyl sulphate-polyacrylamide gels the protein migrated as a homogeneous molecular species with a molecular weight of 23000 (Fig. 4). In some experiments protein bands of higher molecular weight were detected. These were considered to be aggregation artifacts which formed during preparation of the samples for electrophoresis. Aggregation was enhanced by high concentrations of 2-mercaptoethanol.

Solubility

Maximum solubility of the agglutinin in 1 mM-tris-HCl buffer, pH7.4, was approx. 1 mg/ml. It is sug-

gested that the low solubility at neutral pH resulted in the precipitation of the agglutinin after affinity chromatography and subsequent concentration and in the formation of turbid solutions after CM-cellulose chromatography. The solubility of wheat-germ agglutinin was greatly increased at low pH; maximum solubility in 0.1 M-acetic acid was greater than 10mg/ml. Mixing experiments with the proteins eluted in the first peak from CM-cellulose (there were at least three as determined by electrophoresis in polyacrylamide gels with buffers containing 8 M-urea) indicated that these proteins increased the solubility of wheat-germ agglutinin at neutral pH. These proteins were responsible for the brownish colour of impure solutions of the agglutinin. It was assumed that at least one of these proteins was the contaminant that had interfered with previous purification attempts (Burger & Goldberg, 1967; Ozanne & Sambrook, 1971).

Fig. 3. Elution of the agglutinin supernatant from CM-cellulose

Approx. 5 mg of protein, obtained after dialysis of the agglutinin-containing fractions from Sepharose-ovomucoid, was applied to a column (26 cm × 1.5 cm diam.) of CM-cellulose. Elution was with 1 mM-tris-HCl buffer, pH 7.4; the flow rate was 12 ml/h and 3 ml fractions were collected. The height of the vertical bars represents the agglutinating activity.

Amino acid composition

The amino acid composition of different agglutinin preparations is shown in Table 2. There was no significant difference between the composition of the agglutinin from different batches of wheat-germ lipase or acid phosphatase. The protein contained large quantities of half-cystine and glycine but only small amounts of the hydrophobic amino acids. Tryptophan was not detected in significant quantities; however, the titration of this amino acid with *N*-bromosuccinimide may have been masked by the large amounts of half-cystine.

The unusually high half-cystine content of the protein was confirmed in three ways. First, the elementary analysis of the protein (C, 43.4; H, 6.3; N, 15.7; O, 27.9; S, 6.7%) was comparable with that predicted from the amino acid composition (C, 47.5; H, 6.1; N, 15.9; O, 23.6; S, 6.9%). Elementary analysis of proteins is normally an insensitive indicator of amino acid composition but such an analysis on wheat-germ agglutinin unambiguously confirmed the abnormally high sulphur content. Secondly, there was quantitative recovery of half-cystine as cysteic acid after performic acid

Fig. 4. Determination of the molecular weight of wheat-germ agglutinin on sodium dodecyl sulphate-polyacrylamide gels

Electrophoresis was performed as described in the Experimental section. Approx. $10\mu\text{g}$ of the pure agglutinin and $10\mu\text{g}$ of the molecular weight markers were used. The mobility is measured relative to Bromophenol Blue.

oxidation of the protein. Finally, there was quantitative recovery of half-cystine as *S*-carboxymethyl-cysteine after reduction and *S*-carboxymethylation of the protein. Titration of the protein with *p*-chloromercuribenzoate indicated the presence of 15–17 cysteine residues/mol.

Because of the exceptionally high sulphur content it is tempting to speculate that the protein fulfils a unique function within the plant. The lectin may therefore function as a form of food storage or, as suggested by Ensgraber (1958), may serve as a means of fixing, transporting and storing carbohydrate material.

Absorption spectrum

The absorption spectrum of the agglutinin in 0.01 M-sodium phosphate buffer, pH7.0, and in 8M-

urea, pH4.0, demonstrated that the protein had an unusually low absorption maximum (272nm). The molar extinction coefficient at 280nm was $1.09 \times 10^5 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ and was $1.2 \times 10^5 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ at 272nm.

Structural characterization

The *N*-terminus of oxidized agglutinin was cysteic acid; the *N*-terminus was not detected if oxidation of the protein was omitted. Digestion of the protein with carboxypeptidase A released 2mol of glycine/mol of protein; the protein therefore had a *C*-terminal sequence of -Gly-Gly-OH. Digestion of the oxidized agglutinin with trypsin gave no core material and the peptide 'map' of this digest is shown in Fig. 5. Two glycopeptides were detected; the carbohydrate moieties of these peptides have not been identified. Free cysteic acid was not detected and hence the high content of half-cystine observed in agglutinin preparations was not due to the fortuitous binding of cysteine or cystine.

A single protein band was observed during polyacrylamide-gel electrophoresis of purified wheat-germ agglutinin under denaturing conditions. The protein had an approx. molecular weight of 23000 and it was assumed that the molecule was composed of a single polypeptide chain. The number of peptides detected on the tryptic peptide 'map', which was compatible with the number predicted from the lysine and arginine content of the protein, confirms this assumption.

Equilibrium dialysis

Equilibrium dialysis of the protein was done against *N*-acetylglucosamine at different concentrations (Fig. 6). Virtually no binding was observed with low concentrations of *N*-acetylglucosamine; at equilibrium and with high concentrations of this sugar only 1 mol of *N*-acetylglucosamine was bound per mol of protein. The agglutinin therefore appears to be univalent with respect to *N*-acetylglucosamine binding.

This result sharply contrasts with the results of binding studies on other lectins. Both concanavalin A (Yariv *et al.*, 1968) and the phytohaemagglutinin from *Lens culinaris* (Stein *et al.*, 1971) contain two carbohydrate-binding sites. This may imply that wheat-germ agglutinin functions as a dimer during agglutination. This seems unlikely as the lectin is still active in agglutination when isolated as the monomer with a molecular weight of 23000. There are two other alternatives. Either agglutination by wheat-germ agglutinin is by a mechanism other than bridge formation between cells or the lectin is in fact bivalent with respect to carbohydrate binding but the

Fig. 5. Tryptic peptide 'map' of wheat-germ agglutinin

The tryptic digest of approx. 2mg of oxidized agglutinin was fractionated on Whatman no. 1 paper by ionophoresis at pH 3.5 (56 V/cm) for 1 h in the first dimension and by descending chromatography in butan-1-ol-acetic acid-water-pyridine (15:3:12:10, by vol.) for 20 h in the second dimension. Peptides were detected by ninhydrin staining and glycopeptides (cross-hatched areas) were detected by staining with sodium meta-periodate and ammoniacal silver nitrate. XCFE represents the blue dye marker Xylene Cyanole FF.

N-acetylglucosamine binding site is only part of the receptor site. The presence of sialic acid on cell membranes has been implicated as a requirement for agglutination and a receptor membrane protein for wheat-germ agglutinin, containing sialic acid, has been isolated (Smith & Walborg, 1972). Hence the complete receptor site involved in cell agglutination may contain both *N*-acetylglucosamine and sialic

acid-binding properties and it will be of interest to investigate the cooperative binding of *N*-acetylneuraminic acid (one of the sialic acids) and *N*-acetylglucosamine to wheat-germ agglutinin.

Note added in proof. While this paper was in preparation Nagata & Burger (1972) published a similar characterization of wheat-germ agglutinin.

Table 2. Amino acid composition of wheat-germ agglutinin preparations

Results are expressed as mol/100 mol of recovered amino acids.

Lot no. ...	15B	6	5A-2*	5A-2†	BE2*	BE2†	Residues/23 000 g‡ (nearest integer)
Lys	3.6	4.2	4.1	4.7	3.6	4.5	9
His	1.0	0.7	0.8	0.7	1.0	0.8	2
Arg	2.5	2.5	2.1	2.1	2.5	2.5	6
Asp	9.0	8.9	8.2	8.8	8.8	8.2	20
Thr	2.8	2.6	2.4	2.8	2.9	2.4	6
Ser	4.6	5.3	5.5	6.6	5.4	5.6	13
Glu	9.8	9.3	8.7	9.0	9.3	8.5	21
Pro	3.7	3.4	4.9	3.2	3.9	3.2	9
Gly	20.6	22.5	21.3	21.9	21.9	20.8	49
Ala	7.3	6.9	7.4	7.2	7.6	6.6	17
Cys	19.9	20.7	21.1	21.1	18.7	19.6	46
Val	1.8	1.0	1.0	0.8	2.2	1.2	3
Met	2.3	1.5	1.4	1.6	1.2	1.1	3
Ile	1.5	1.3	1.2	1.3	1.7	1.2	3
Leu	2.8	2.4	2.2	2.0	2.9	2.2	6
Tyr	4.3	4.7	5.2	4.7	4.3	4.2	11
Phe	1.9	1.6	2.0	1.6	2.1	1.9	4
Trp	0.06	0.0	0.05	0.2	—	—	

* Precipitated after affinity chromatography.

† From CM-cellulose chromatography.

‡ Calculated from the mean of the data presented.

Fig. 6. Equilibrium dialysis of wheat-germ agglutinin against N-acetylglucosamine

Dialysis was with 5 nmol of agglutinin/ml at 22°C for 168 h in 1 mM-tris-HCl buffer, pH 7.4. For further details see the text.

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