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Note

An affinity adsorbent for the rapid purification of wheat germ agglutinin*

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Sugar-specific adsorbents have become increasingly popular for use in the purification of glycoproteins. During our investigations on the use of immobilized lectins for the purification of certain glycoproteins, we discovered that commercially available wheat germ agglutinin (WGA) was not acceptable for use. As a result we developed a simplified method for the purification of this agglutinin.

Crude wheat germ lipase was first reported by Aub *et al.*[1] to possess a heat-stable component with the ability to agglutinate certain tumor cells. Subsequent work by Burger and co-workers[2–4] has led to the purification of WGA. It has been recently characterized as a 34,000-molecular-weight protein comprised of two identical subunits[5,6]. Amino acid analysis of the agglutinin has shown it to contain an unusually high mole percentage of glycine and half-cystine, approximately 24% and 17%, respectively[4,5]. A number of reports have appeared describing the use of affinity chromatography for the purification of WGA. Shaper *et al.*[5] were able to chemically synthesize an affinity ligand, 6-amino-1-hexyl-2-acetamido-2-deoxy-β-D-glucopyranoside, and couple it to Sepharose 4B with cyanogen bromide. Sepharose 4B-bound ovomucoid was used by Marchesi[7] as a specific adsorbent from which WGA could be eluted with 0.1 *N* acetic acid. Chitin has also been used as an affinity polymer in a purification procedure for the agglutinin[8]. However, this last procedure requires two other column chromatography steps in order to obtain a homogeneous protein. The present paper describes the use of aspariginyl-N-acetylglucosamine as an affinity ligand for the purification of WGA.

EXPERIMENTAL

Materials

Epichlorhydrin was obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.). 2-Acetamido-1-(L-β-aspartamido)-1,2-di-deoxy-β-D-glucose (aspariginyl-N-acetylglucosamine) was purchased from Cyclo Chemicals (Los Angeles, Calif., U.S.A.). All

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other chemicals were purchased from Mallinckrodt (St. Louis, Mo., U.S.A.) and were reagent grade or better. Raw wheat germ was purchased from a local health foods store. Sepharose 4B was obtained from Sigma (St. Louis, Mo., U.S.A.).

Preparation of adsorbent

The method of Porath *et al.*[9] was used to prepare epichlorohydrin-desulphated Sepharose 4B (ECD-Sepharose 4B) and this was then activated with cyanogen bromide using the procedure of Porath *et al.*[10]. Packed ECD-Sepharose 4B (100 ml) and 5 *M* potassium phosphate, pH 11.9 at a 10-fold dilution, were mixed with 40 ml of cyanogen bromide solution (100 mg/ml) and allowed to incubate at room temperature for 10 min with constant mixing. After thoroughly washing the activated ECD-Sepharose with 0.02 *M* potassium phosphate, pH 7.0, an equal volume of 0.1 *M* sodium borate buffer, pH 9.4, containing 50 mg of ligand, was added and the mixture was allowed to react overnight at 4° with constant stirring. The ECD-Sepharose coupled aspariginyl-N-acetylglucosamine served as the specific affinity adsorbent for WGA.

Determination of WGA purity

The purity of the WGA was assessed by sodium dodecyl sulphate gel electrophoresis using the method of Weber and Osborn[11]. Half-cystine and glycine content was determined using an automatic amino acid analyzer.

RESULTS AND DISCUSSION

Raw wheat germ was extracted 4 or 5 times with petroleum ether (b.p. 40–60°) and air dried. The defatted material (100 g) was then suspended in 500 ml of cold distilled water and stirred overnight at 4°. After filtering the suspension through cheesecloth, the remaining precipitate was removed by centrifugation for 15 min at 10,000 *g* and 4°. The resulting supernatant (*ca.* 400 ml) was heated to 55° in a water-bath and maintained at 55–60° for 15 min. After rapidly chilling the sample to 4° in an ice-bath, the precipitate was removed as before. The supernatant (*ca.* 350 ml) was collected and dialyzed overnight against two 8-l volumes of 0.02 *M* potassium phosphate, pH 7.0.

Following dialysis, the sample was applied to a 3.6 × 10 cm column of the prepared adsorbent previously equilibrated with 0.02 *M* potassium phosphate, pH 7.0, at a flow-rate of 80 ml/h. After the sample was applied, the column was washed with the same buffer until the absorbance at 280 nm was less than 0.1. It is important that the entire sample be applied to the column immediately after dialysis since a precipitate appears upon prolonged storage (2 days). Fractions collected during the sample application and wash did not contain detectable amounts of the lectin as assayed by the ability of the effluent to agglutinate rabbit red blood cells. The agglutinin was eluted from the affinity column with the same phosphate buffer containing 5 mg/ml of N-acetylglucosamine (purchased from Sigma).

Fig. 1 shows the elution profile of a preparation of heat-treated wheat germ extract when passed through the aspariginyl-N-acetylglucosamine ECD-Sepharose 4B column. As can be seen the bulk of the material absorbing at 280 nm is eluted during the sample application and subsequent wash. Elution with buffer containing 5 mg/ml N-acetylglucosamine gives rise to a broad trailing peak which was found to contain

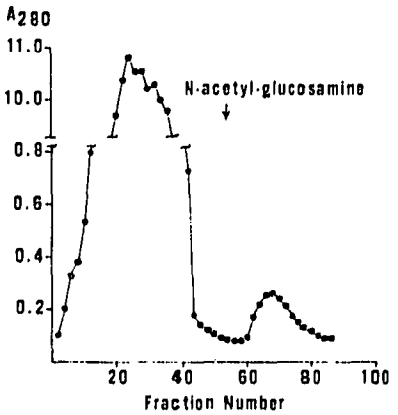

Fig. 1. Affinity chromatography of WGA. Conditions were as described in the text. No red blood cell-agglutining activity was detected in the initial absorbance peak. Elution with N-acetylglucosamine gave rise to a second absorbance peak (Fractions 60–80) which was high in red blood cell agglutinating activity. The column was eluted at a flow-rate of 60 ml/h. Fraction size, 10 ml.

red blood cell-agglutinating activity. Fractions within this peak were pooled and concentrated by pressure dialysis (Amicon pressure dialysis chamber with a PM-10 membrane).

The purity of the concentrated pool of WGA was determined as described in Experimental. An overloaded gel (*ca.* 100 μg) revealed only one major protein band.

Fig. 2. Sodium dodecyl sulphate gel electrophoresis of purified WGA. Direction of electrophoresis was from top to bottom. Gel was run at 5 mA per gel according to the method of Weber and Osborn[11].

which migrated close to the bromophenol blue marker dye (Fig. 2). During the destaining process several faint, more slowly migrating bands were discernible, but were not visible when the gel was completely destained.

A partial amino acid analysis of the WGA pool recovered from affinity chromatography was performed. An aliquot of the concentrated pool was dialyzed overnight against several changes of distilled water and then oxidized with performic acid according to the method of Hirs[12]. Following the conversion of the half-cystine to cysteic acid by this method, the sample was hydrolyzed with 6 *N* HCl for 24 h at 100°. As mentioned earlier, WGA has a characteristic high half-cystine and glycine content. On the basis of 140 amino acid residues per 17,000-molecular-weight molecule, we have calculated half-cystine and glycine contents of 17.1% and 25.7%, respectively, for our preparation of WGA. This is in excellent agreement with the values previously reported for half-cystine and glycine by two other groups (Table I).

TABLE I

GLYCINE AND HALF-CYSTINE CONTENT OF WHEAT GERM AGGLUTININ

Values are expressed as mole percent.

	Ref. 4	*Ref. 5*	*Present study*
Glycine	22.7	24.5	25.7
Half-cystine	17.8	16.9*	17.1*

* Determined as cysteic acid.

The scheme reported here for obtaining highly purified WGA requires a single affinity column chromatography step and avoids using denaturing conditions, such as 0.1 *N* acetic acid, for removal of the protein from the column. In addition, the asparaginyl-N-acetylglucosamine used for preparing the specific adsorbent is available commercially which eliminates time-consuming preparations of affinity ligands.

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