SUBUNIT STRUCTURE OF WHEAT GERM AGGLUTININ

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SUMMARY

Wheat germ agglutinin was found to have a molecular weight of 36,200 ± 5% at pH 7.2 by high-speed sedimentation equilibrium. At this pH, the protein had a sedimentation coefficient of 3.5 S. These data indicate that the native agglutinin consists of dimers of the 18,000-dalton subunits observed upon sodium dodecyl sulfate polyacrylamide gel electrophoresis. Agglutinin treated with 0.05 N HCl, in which it sedimented at 2.1 S, and then dialyzed back to pH 7.2 sedimented as the native protein, suggesting reversible denaturation of the protein or dissociation of subunits.

Plant-derived agglutinins (lectins) have found wide use in studies of cell surface structure due to their property of specific binding of simple sugars and complex carbohydrates (1). Chemical characterization of some commonly used lectins has indicated that their multivalent binding ability is conferred by their subunit structure (1). While WGA[1] has received much attention because it binds to many types of cells, its subunit structure and hence valence have remained unclear. Several laboratories have reported a molecular weight for the protein in the range of 20,000 as determined by gel electrophoresis in the presence of the detergent sodium dodecyl sulfate (2,3,4) and by sedimentation equilibrium centrifugation in 0.05 N HCl (2). Sephadex gel filtration under nondenaturing conditions has provided estimates of molecular weight in the same range (2,3,4), suggesting that the native protein may be monomeric. In view of indications of one saccharide binding site per 23,000-dalton monomer (3), however, it has been suggested that agglutination of cells, if requiring a multivalent character, occurs as a result of aggregation of WGA

[1]Abbreviation used: WGA, wheat germ agglutinin.

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monomers (1). To test this suggestion, we have investigated the subunit structure of the native protein using ultracentrifugation.

MATERIALS AND METHODS

WGA was purified from raw wheat germ (*Triticum vulgaris*), kindly donated by Dr. P.K. Stumpf (University of California, Davis), essentially as described by Marchesi (5) following extraction of lipids with petroleum ether. The three isolectins were then isolated and further purified by ion-exchange column chromatography on SP- and QAE-Sephadex (4). WGA fractions I and II of Allen *et al.* (4), were used in this work since WGA fraction III was obtained only in small amounts from the available strain of wheat germ.

For determination of extinction coefficients, WGA fractions were dialyzed against 0.1 M NaCl and then dialyzed exhaustively against water. The protein was lyophilized and dried to constant weight by dessication *in vacuo* over phosphorus pentoxide at room temperature. (A sample of WGA II showed no further weight loss upon drying *in vacuo* at 105°.) No difference in extinction coefficient was noted between solutions in water, in which the dried protein dissolved readily, or phosphate buffered saline (0.15 M NaCl in 0.01 M phosphate buffer, pH 7.2). The protein exhibited an absorption maximum at 277 nm where extinction coefficients of 1.70 (WGA I) and 1.60 (WGA II) cm^2/mg were obtained. Samples for measurement of partial specific volumes were dialyzed 24 hr against phosphate buffered saline and diluted gravimetrically with dialysate for density measurements at 25°C on a Paar mechanical oscillator densimeter as described by Kratky *et al.* (6). Due to difficulties in maintaining protein solubility in buffer, densities of solutions of WGA I were measured at the relatively low concentrations of 1.5, 1.0 and 0.5 mg/ml. The accuracy of measurement under these conditions was estimated at 1%. The value of 0.680 ml/g obtained for partial specific volume, slightly smaller than the value of 0.69 ml/g estimated by Nagata and Burger (2) from amino acid composition data, is not unreasonable in view of the unusually high content of glycine and cystine in WGA I as reported by Allen *et al.* (4). One sample of WGA II (0.5 mg/ml) also gave a value of 0.680 ml/g for its partial specific volume.

Sedimentation equilibrium runs at 20.0°C in phosphate buffered saline were performed in a Beckman Model E ultracentrifuge equipped with electronic speed control and Rayleigh optics using the conventional six-well cell and the long column modification of Chervenka (7) under conditions of meniscus depletion (8). Fringe patterns obtained were measured on a Nikon comparator at 50X magnification. The average displacement of three fringes was used to calculate weight average molecular weights (using the experimentally determined value for WGA partial specific volume) by means of a least squares fit computer program (courtesy of D. Bylund, University of California, Davis). Sedimentation velocity runs at 20.0°C were done at 60,000 rpm in a conventional cell of 1.2 cm pathlength at a 0.5 mg per ml protein concentration. Photographs obtained using uv optics were scanned on a Shoeffel recording densitometer for measurement of radial position of the protein boundary. Apparent sedimentation coefficients calculated from repeated runs agreed to within 0.1 S.

Gel electrophoresis in the presence of sodium dodecyl sulfate was performed in 10% gels of 6 cm length essentially as described by Weber and Osborn (9). Samples containing 5 to 10 μg of each protein in 40 μl of a solution which was 1% in sodium dodecyl sulfate, 10 mM in sodium phosphate, pH 7.2, 20 mM in dithiothreitol, and 10% in glycerol were heated in a boiling water bath for 3 min before application to the gels. Ovalbumin was used as an internal standard for comparison of gels electrophoresed in parallel containing two to four protein bands.

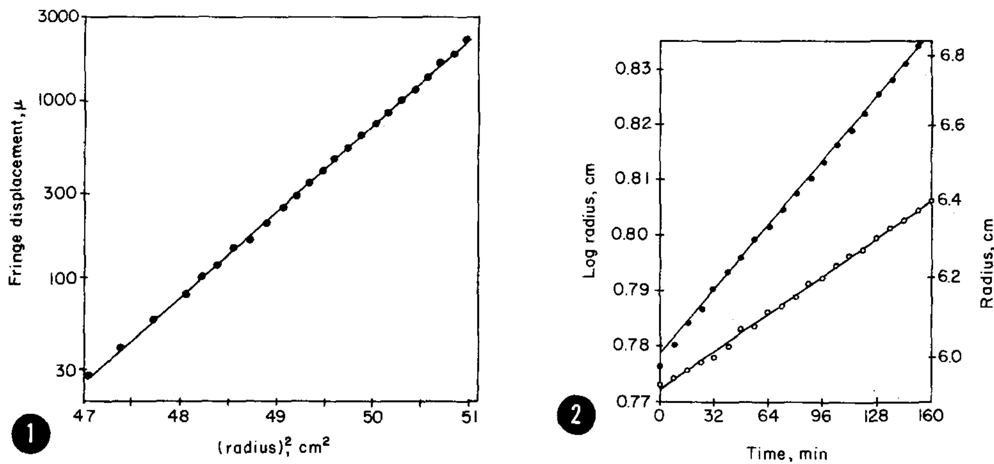

Fig. 1. Sedimentation equilibrium of WGA I in phosphate buffered saline. A 0.05 ml aliquot of dialyzed protein solution of 1.9 mg/ml concentration was placed in the sample sector of a 12 mm double sector capillary synthetic boundary cell and the reference sector was filled with 0.45 ml dialysate. The rotor was accelerated to 24,000 rpm and the interference pattern was photographed after 22 hr. The speed was lowered to 22,000 and the interference pattern, photographed 6 hr later, was used to plot the above graph.

Fig. 2. Sedimentation velocity of WGA I. Samples in phosphate buffered saline (●-●) were compared with those in 0.05 N HCl (o-o).

RESULTS

The molecular weight of native WGA I in phosphate buffered saline was investigated by high speed sedimentation equilibrium. Using the long column method of Chervenka (7), the data presented in Fig. 1 were obtained. The logarithm of protein concentration was essentially linearly dependent upon the square of radius over nearly a 100-fold range of concentration. In addition to estimates obtained at 22,000 rpm and 24,000 rpm in the long column method, estimates were obtained by conventional sedimentation equilibrium (32,000 rpm) at average cell concentrations of 0.12, 0.24 and 0.47 mg/ml. The calculated molecular weights were quite similar in the five cases and were averaged to yield the overall value of 36,200 (estimated uncertainty 5%). No indications of larger molecular weight aggregates were obtained in these experiments. Calculated molecular weights at 0.12 mg/ml were noted to be several percent higher than at 0.47 mg/ml, however, possibly indicating some nonideality; this effect will require further work for substantiation.

WGA was subjected to sedimentation velocity in phosphate buffered saline, pH 7.2, and in 0.05 N HCl. The observed sedimentation behavior, shown in Fig. 2, indicated a gross conformational difference under the two conditions. The value of 3.5 S obtained at the neutral pH for both WGA I and II is not unreasonable for a protein of 36,000 daltons. The observed value of 2.1 S in 0.05 N HCl, as obtained by Nagata and Burger (2), presumably does not reflect the native conformation. In view of the molecular weight of 23,500 estimated by equilibrium sedimentation in 0.05 N HCl (2), it would appear that WGA may be largely dissociated into subunits under these conditions. Treatment of the protein with 0.05 N HCl appears not to cause irreversible denaturation, since the 2.1 S form sedimented at 3.5 S following overnight dialysis against phosphate buffered saline. Further work on the possible reversible denaturation or disaggregation of WGA into subunits is in progress.

The molecular weight of WGA was investigated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. WGA I and II migrated identically in the gels with mobility corresponding to about 18,000 daltons. Though this estimate may be uncertain by 5 to 10% due to the scatter of standard protein mobilities in the range of 10,000 to 20,000 daltons our data favor the estimate of Allen _et al_. (4), of 18,000 daltons in comparison to the 23,000-24,000-dalton estimate of Nagata and Burger (2) and LeVine _et al_. (3). The small discrepancy in these results may be due to the different electrophoresis systems used by the different investigators even though they all employed sodium dodecyl sulfate to swamp out intrinsic protein charge.

DISCUSSION

The present results indicate that at neutral pH, where this protein is routinely employed in cell binding and agglutination tests, WGA is a dimer of approximately 18,000-dalton subunits. This finding is in conflict only with the reported behavior of WGA upon Sephadex gel filtration (2,3,4). Since this agglutinin has been noted to interact with such diverse substances as polyacrylamide beads (4) and cellulose filters (10), it seems likely that its elu-

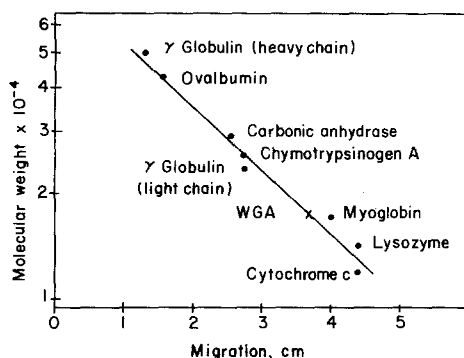

Fig. 3. Gel electrophoresis of WGA I in the presence of sodium dodecyl sulfate. Logarithm of molecular weight is plotted against migration for a typical experiment.

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tion from Sephadex is affected by weak interaction with the polysaccharide support. This phenomenon has also been encountered with the RCA II agglutinin from *Ricinus communis* (11).

The present indications that WGA is dimeric help rationalize the ability of this protein to cause crosslinking of cells, as suggested by Lis and Sharon (1). Though no indications have been obtained of dissociation into monomers at neutral pH, we have not attempted to investigate this possibility at the very low concentrations often used in some cell binding experiments. Further details of subunit structure in regard to pH and concentration dependence remain to be investigated so that association of dimers under some conditions is not yet ruled out.

ACKNOWLEDGMENTS

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