

Chromatography of Proteins from Wheat Gluten on Polyacrylamide Gel¹

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ABSTRACT

Glutenin and gliadin fractions from wheat flour and cyanoethylglutenin (CN-glutenin) were chromatographed on polyacrylamide gel in 8*M* urea. Glutenin emerged continuously over a range of R_f values from 1.0 to 0.5, consistent with the concept that glutenin is a mixture of materials which have different molecular weights. Examination of reduced and cyanoethylated fractions from glutenin by starch-gel electrophoresis disclosed a deficiency of slow-moving electrophoretic components in fractions containing materials of low molecular weight. CN-glutenin was separated into three fractions, each of which contained a different mixture of proteins. Comparison of elution patterns for CN-glutenin and gliadin showed that portions of CN-glutenin migrated as particles with apparent molecular weights near 100,000 and 40,000. The heavier fraction contained the major electrophoretic components in CN-glutenin; the lighter, fewer components that migrated slowly upon electrophoresis. Chromatography of the viscous solutions of wheat gluten proteins was facilitated by use of glass beads as support for the polyacrylamide gel.

The glutenin fraction from wheat flour was shown by Jones *et al.* (1) to consist of different-sized particles with molecular weights (MW) ranging from around 30,000 to approximately 3,000,000, even in dissociating solvents. Subsequently, Nielsen *et al.* (2) demonstrated that cleavage of the disulfide bonds in glutenin causes its physical MW to drop to about 20,000. These two observations suggest that the polypeptide subunits in glutenin are linked by intermolecular disulfide bonds into molecular complexes of varying size (2). It is uncertain, however, whether large glutenin particles arise by random association of the same basic subunits or, instead, are composed of subunits that are structurally different from those in small glutenin particles. The glutenin fraction from wheat flour has not been adequately resolved to test these possibilities.

Whereas the glutenin fraction appears to contain different-sized particles in the ultracentrifuge, it migrates as a relatively homogeneous peak (3) during moving-boundary electrophoresis. Apparently glutenin molecules differ more in molecular dimensions than in mass-charge ratio. Accordingly, gel permeation chromatography (GPC), which combines simplicity and high resolving power on the basis of molecular size (4), seemed the most suitable means by which to fractionate glutenin or derivatives of glutenin. Although the fractionation of small molecules and soluble proteins by GPC is well documented, applications of this technique to mixtures of sparingly soluble or highly aggregated polymers, like the proteins in wheat gluten, have been published only recently. Meredith and Wren (5) resolved whole gluten into at least four components on Sephadex G-200, and Inamine *et al.* (6) used GPC on agarose in a preliminary fractionation of wheat

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flour proteins. We report here our experiences with chromatography on polyacrylamide gel in the fractionation of components in glutenin, cyanoethylglutenin (CN-glutenin), and gliadin.

MATERIALS AND METHODS

Glutenin and gliadin were prepared from wheat flour (variety, Ponca) by the method of Jones *et al.* (3). CN-glutenin, in which only cysteine residues were derivatized, was prepared as follows: Glutenin (10 g.) was dissolved in 500 ml. of 0.05M phosphate-urea (6M) buffer, pH 8.3, reduced with 6.7 ml. of mercaptoethanol (Eastman Kodak Co., Rochester, N. Y.) under nitrogen when stirring for 1 hr. at room temperature, and then alkylated with 10.5 ml. of acrylonitrile (Eastman) for 15 min. at room temperature. The reaction was stopped by acidification with glacial acetic acid, and the solution was dialyzed exhaustively against 0.01M acetic acid and then lyophilized.

Solutions of urea (Analytical Reagent, Mallinckrodt Chemical Works, St. Louis, Mo.) were prepared and filtered through Whatman No. 1 filter paper just before use.

Column Chromatography

Bio-Gel P-300 (Bio-Rad Laboratories, Richmond, Calif.) was expanded in water, and freed of fines by decantation of any material still suspended after 15 min. of standing. This sizing procedure continued until the supernatant was clear, and then it was repeated in 8M urea.

The chromatographic column (3.8 × 160 cm.) was prepared from a 2,000-ml. slurry composed of 8M urea, 12.2 g. of Bio-Gel P-300, and 2,670 g. of 4-mm. glass beads coated with dimethyldichlorosilane. Smaller columns (2 × 65 cm.) of Bio-Gel P-300 were similarly prepared. Column uniformity and hold-up volumes were determined with Sephadex Blue Dextran 2000 (Pharmacia, Uppsala, Sweden).

Samples of glutenin for chromatography were dissolved in 8M urea and filtered through medium sintered glass to eliminate traces of a globular solid, which otherwise plugged the columns. Solutions of gliadin and CN-glutenin did not require filtration. Chromatography was carried out in 8M urea at room temperature. Column effluents were collected in 6- to 14-ml. fractions and their absorbances were measured at 280 m μ with a Beckman DU spectrophotometer. Effluent corresponding to each absorbance peak was combined, dialyzed exhaustively against 0.01M acetic acid, and lyophilized before further use. Sample recoveries were calculated on a dry-weight basis.

Starch-gel electrophoresis of the fractions was conducted in 0.025M aluminum lactate-3M urea buffer, pH 3.1, according to the method of Woychik *et al.* (7) as modified by Beckwith *et al.* (8)

Amino acid analyses were performed by the method of Spackman *et al.* (9) with a Phoenix model K-8000 automatic analyzer. Samples taken for analysis were hydrolyzed by the method of Beckwith *et al.* (8).

RESULTS

To carry out GPC with viscous samples in 8M urea on columns of low-

density polyacrylamide at reasonable flow rates, it was necessary to devise a means to prevent compaction of the gel. Upward flow did not prove satisfactory. Similarly, columns constructed with bundles of small-bore glass tubing were useless. Glass beads, however, provided sufficient support to allow the columns of Bio-Gel P-300 to be operated with flow rates comparable to those of columns of more highly cross-linked polyacrylamide gels or Sephadex. One large column (3.8×160 cm.) had a useful life of approximately 5 months, during which the flow rate slowly decreased from about 20 ml./hr. to 1–2 ml./hr. Generally, columns were run at about 6 ml./hr.

Glutenin

The elution pattern from gel filtration of glutenin on Bio-Gel P-300 is shown in Fig. 1, A. Although the sample emerged over a range from

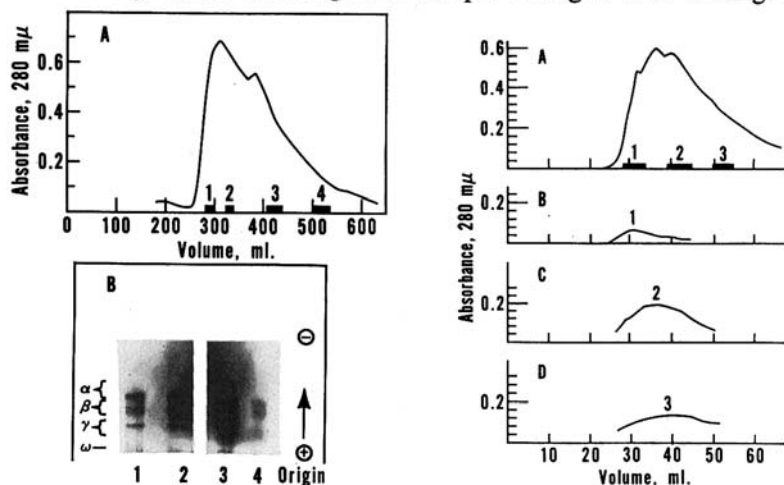


Fig. 1 (left). A, gel permeation chromatography of glutenins (5 ml. of 1% solution) on Bio-Gel P-300 (column, 3.8×155 cm.) in 8M urea. Heavy lines on abscissa indicate combined fractions. B, starch gel electrophoresis of glutenin fractions (after reduction and alkylation).

Fig. 2 (right). A, GPC of glutenin (0.5 ml. of 1% solution) on Bio-Gel P-300 (column, 2×65 cm.) in 8M urea. B–D, rechromatography of glutenin fractions.

R_f 1.0 to R_f 0.5, irregularities in absorbance suggested that some fractionation had occurred. Nevertheless, when samples taken at various points across the peak were reduced and alkylated, they gave essentially the same starch-gel-electrophoretic pattern (Fig. 1B) as that published by Woychik *et al.* (10) for reduced and alkylated glutenin.

Nomenclature used in describing the electrophoretic mobility of derivatives of glutenin follows the system suggested for gliadin by Woychik and co-workers (10) in that three relatively distinct groups of components in CN-glutenin have been called alpha-, beta-, or gamma-CN-glutenin in order of decreasing electrophoretic mobility.

The distribution of glutenin was essentially the same on repoured columns or columns with different dimensions. In Fig. 2, A shows the elution pattern obtained on a column 2×65 cm. Upon rechromatography of selected fractions from this distribution (Fig. 2, B-D), the initial fraction again emerged at the column volume (Fig. 2, B). The other fractions, however, were distributed over nearly the same range as the original sample (Fig. 2, C and D).

CN-Glutenin

In contrast to glutenin, CN-glutenin was resolved into three relatively distinct fractions by GPC on Bio-Gel P-300 (Fig. 3, A). Fraction I, which

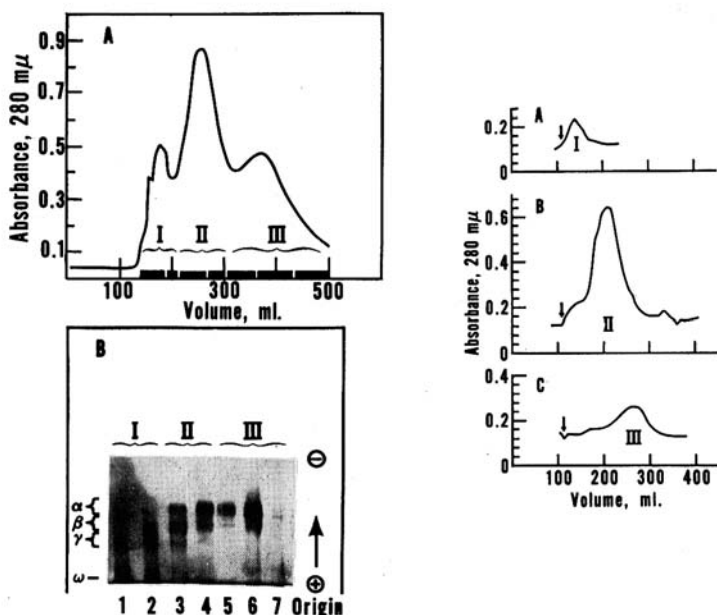


Fig. 3 (left). A, GPC of cyanoethylglutenin (CN-glutenin) (5 ml. of 4% solution) on Bio-Gel P-300 (column, 3.8×155 cm.) in $8M$ urea. Heavy lines on abscissa indicate combined fractions. B, starch-gel electrophoresis of CN-glutenin fractions.

Fig. 4 (right). Rechromatography of CN-glutenin fractions on Bio-Gel P-300 (column, 3.8×155 cm.) in $8M$ urea. Arrows indicate void volume.

generally accounted for about 11% of the material recovered (Table I), consisted of a mixture of poorly defined electrophoretic components with low mobilities (Fig. 3, B). On the other hand, fraction II contained nearly all the components commonly recognized in CN-glutenin. This fraction accounted for about 50% of the material recovered. Fraction III (Fig. 3, B), the last material eluted, was noticeably deficient in gamma-components and comparatively rich in alpha-CN-glutenins. Upon rechromatography (Fig. 4), each of the separated fractions I-III migrated at nearly the same R_f 's as those calculated from the original elution pattern (Table I).

TABLE I
CHROMATOGRAPHIC MOBILITIES AND WEIGHT DISTRIBUTION OF GLIADIN AND
CYANOETHYLGLUTENIN COMPONENTS ON BIO-GEL P-300

SAMPLE	CONCENTRATION	R _f OF FRACTIONS ^a			DISTRIBUTION OF SAMPLE ^b		
		I	II	III	I	II	III
	% w./v.						
Gliadin	2	0.72	0.38		9	52	
CN-Glutenin	1	0.86	0.69	0.54			
	2	0.76	0.57	0.45	18	36	22
	4	0.80	0.54	0.37	8	27	41
	5	0.81	0.62	0.51	14	51	15
	10	0.81	0.56	0.41	3	31	32
	Average	0.81	0.58	0.46	11	36	28
Rechromatographed:							
Fraction I		0.79					
Fraction II			0.52				
Fraction III				0.42			

^aR_f = void volume/elution volume.

^bPercentage of sample applied to column (dry weight).

The effect of concentration on the distribution of CN-glutenin was tested by chromatographing samples at five different concentrations from 1 to 10%. Figure 5 shows the results for one intermediate and two extreme

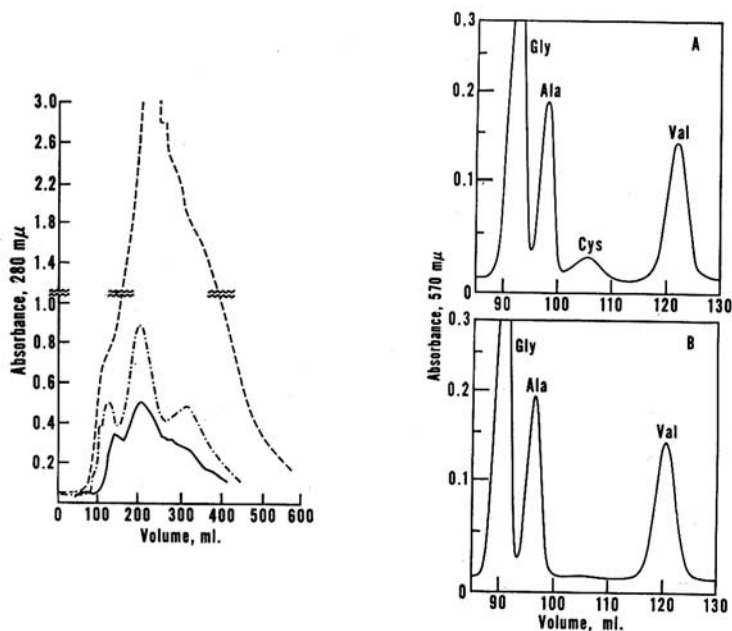


Fig. 5 (left). Gel permeation chromatography of CN-glutenin at 1% (—), 2% (—•—), and 10% (— — —) on Bio-Gel P-300 (column, 3.8 × 155 cm.) in 8*M* urea.

Fig. 6 (right). Portions of elution patterns from amino acid analyses of: A, glutenin, and B, CN-glutenin.

concentrations. In each, the general shape of the distribution was the same, although resolution was poorer at higher concentrations. Generally, the best separations were at a protein concentration of 2% (w./v.) (Fig. 5).

To check the possibility that this distribution of CN-glutenin might have been due to incomplete reduction, two different samples of the CN-glutenin used in the GPC were subjected to amino acid analysis along with a sample of glutenin. Figure 6 compares portions of the elution patterns from amino acid analyses of CN-glutenin and glutenin. Whereas cystine was present in glutenin, the reduced and alkylated proteins were essentially devoid of cystine or cysteic acid. As an additional check, fractions I-III were again reduced and alkylated. This second cyanoethylation did not appear to alter their electrophoretic patterns.

Gliadin

Beckwith and co-workers (8) showed that the gliadin fraction from wheat contains a small amount of material that has a MW of about 100,000. Since most components in the gliadin fraction have much smaller MW's (about 25,000), this high MW impurity could be removed readily by GPC on Sephadex G-100 (8). Thus characterized as a mixture of different molecular species, crude gliadin afforded an estimate of the MW range over which Bio-Gel P-300 is effective in fractionating glutenin and CN-glutenin. Figure 7, C shows the elution pattern obtained when gliadin was chromato-

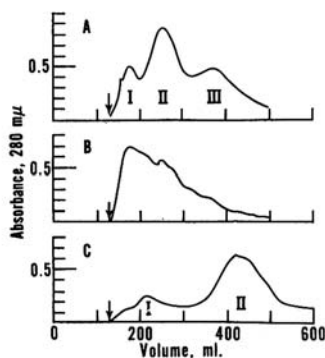


Fig. 7. Comparison of elution patterns from gel permeation chromatography of: A, CN-glutenin, B, glutenin, and C, gliadin on Bio-Gel P-300 (column, 3.8×155 cm.) in 8M urea. CN-glutenin and glutenin, 50 mg. in 1% solution; gliadin, 200 mg. in 2% solution. Arrows indicate void volume.

graphed on Bio-Gel P-300. Nearly all the gliadin emerged at R_f near 0.3, as expected for molecules around 25,000 MW. The high-MW components emerged at R_f near 0.8, but the shape of the elution pattern suggests that small amounts of even larger materials might be present. Interestingly, the elution volume of the high-MW component in gliadin was between the elution volumes of fractions I and II from CN-glutenin.

DISCUSSION

From the results of GPC of glutenin (Fig. 1, A), it is evident that a

large portion of the glutenin components in wheat flour have apparent MW's near or greater than the exclusion limit of Bio-Gel P-300 in 8M urea. True MW's of the glutenin proteins are uncertain, since distributions on rechromatography suggested that association was occurring under the conditions used. Most of the glutenin retarded on the gel appeared to contain the same electrophoretic components (Fig. 1, B). This similarity suggests that glutenin molecules might differ only in the degree to which the same subunits are repeated in their composition. The fact that a fraction (Fig. 1, A, fraction 4) taken near the end of the elution pattern was deficient in slow-moving electrophoretic components (Fig. 1, B) could indicate that there are structural differences in smaller glutenin particles. However, in view of the apparent MW of material in this fraction (about 25,000), the data seem more consistent with the possibility that some proteins are held in the glutenin complex by associative forces or, at most, by very labile covalent bonds.

To some extent, multiple bands in the gel-electrophoretic patterns might be due to modification of amino groups in the proteins by carbamylation during chromatography in 8M urea. It is doubtful, however, that carbamylation alone was responsible for the similarity between most of the fractions from glutenin. If carbamylation were extensive it would also have obscured the separation obtained with CN-glutenin.

GPC produced substantially better resolution of CN-glutenin (Fig. 3, A), and each fraction obtained appeared different from the other two on rechromatography (Fig. 4, A-C). Differences between the three fractions from CN-glutenin in terms of gel-electrophoretic patterns, apparent MW's, and weight distributions suggest there may be substantial compositional or structural variation between individual constituents of glutenin.

The presence of three distinct fractions in CN-glutenin emphasizes the dissimilarity of alkylated-reduced glutenin and gliadin. By comparison to gliadin (Fig. 7), fractions II and III from CN-glutenin appear to contain materials that have MW's near 100,000 and 40,000, respectively. The presence of high-MW components is not surprising in view of the conclusion of Nielsen (11) that disulfide-cleaved glutenin can form aggregates even in dissociating solvents. If the three fractions arise by aggregation, however, it is surprising that the aggregates appear comparatively stable and specific, and that changes in concentration produce little, if any, alteration in the distribution of CN-glutenin components. Alternatively, differences in molecular shape might be responsible for the distributions obtained in our studies. Further experiments will determine to what extent the unique properties of glutenin and gliadin result from fundamental chemical differences.

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