

Wheat Gluten Subunits: Molecular Weights Determined by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis¹

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ABSTRACT

Polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) has revealed the number and molecular weight (MW) of the different-sized subunits obtained from gliadin and glutenin after reduction of disulfide bonds. Most proteins in gliadin are single-chained, and have MW's near 36,500. The whole gliadin fraction also contains 11,400-MW polypeptides which may be albumins, a major polypeptide of MW 44,200, and Ω -gliadins of MW's 69,300 and 78,100; trace amounts of other polypeptides are also present. The 44,200-MW polypeptides, along with some of MW 36,500, are joined through disulfide bonds into higher-MW proteins. Glutenin consists of polypeptides of at least 15 unique MW's ranging from 11,600 to 133,000; two glutenin subunits correspond in mobility to the major gliadin polypeptides. Since disulfide cleavage and denaturation are complete and since no other labile cross-links can be detected, these polypeptides may represent the fundamental structural units of glutenin. Equilibrium-dialysis data establish that the amounts of SDS bound to gluten proteins and standards are similar, supporting the MW values obtained for gliadin and glutenin. In addition to determining MW distribution and number of gluten subunits, SDS-polyacrylamide gel electrophoresis can monitor column separations, serve as a criterion of purity, and detect differences in subunit composition of glutenins of different varieties.

Gliadin and glutenin, the main components of wheat gluten, are each composed of many different molecular species. The viscoelastic properties of dough are thought to arise from both the structure and interactions of these proteins. Most gliadin proteins have molecular weights (MW's) of 16,000 to 50,000, and contain single polypeptide chains whose conformations are stabilized by intramolecular disulfide bonds (1-9). Glutenin, however, consists of subunits of MW's 20,000 to 100,000 linked through intermolecular disulfide bonds into proteins with MW's of 50,000 to 2,000,000 or more (1,4,10-13).

Considerable similarity in amino acid sequence (14) and MW's (3,5,7) exists among gliadin proteins. Reduced gliadin components also resemble reduced glutenin subunits in electrophoretic mobilities (1) and, to some extent, in sequence (15,16). Because the similar chemical nature of these proteins and their large numbers have prevented complete separation and characterization, conflicting data have been reported.

Polyacrylamide-gel electrophoresis in the presence of the anionic detergent sodium dodecyl sulfate (SDS) can be used to separate and estimate MW's of reduced proteins and their subunits (17); the method's validity has been demonstrated for many proteins (18,19). Electrophoretic mobilities in this system are related to MW's alone and are proportional to lengths of the fully unfolded,

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reduced, protein-SDS complexes, since charge differences are masked with SDS (20). In standard gel electrophoresis, mobilities depend on both size and charge. SDS electrophoresis is rapid, is accurate (5 to 10% usual maximum deviation), and can simultaneously determine MW's of all subunits in mixtures (18,19).

A better understanding of the subunits of gliadin and glutenin would be beneficial in understanding their structures and properties. The heterogeneity of these subunits, difficulties in their separation, and the lack of definitive MW's, led us to examine the number and MW's of gluten protein subunits by SDS-polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

Protein Samples

Ponca hard red winter wheat flour was defatted by several extractions with dry *n*-butanol and was then washed with petroleum ether and air-dried. Gliadin and glutenin were prepared from the defatted flour by washing a doughball with 0.1% NaCl, dispersing the gluten in 70% ethanol which is 0.01N in acetic acid, and precipitating glutenin at pH 6.5 (21); the soluble protein was considered whole gliadin. Glutenin was redispersed and reprecipitated to remove traces of gliadin. "Purified gliadin" was separated from higher-MW components in whole gliadin on Sephadex G-100 columns (Pharmacia, Inc., Uppsala, Sweden) in 0.1N acetic acid (4). Fractions from this column were also examined by SDS electrophoresis. Albumins and globulins were extracted from defatted Ponca flour with water and 0.1% NaCl, respectively. Gluten proteins were converted to aminoethyl derivatives and the aminoethylglutenin was fractionated by salt precipitation as described by Rothfus and Crow (12). Glutenin was oxidized with performic acid, and *S*-sulfo-glutenin was prepared as described by Nielsen et al. (10). Attempts were made to cleave aminoethylglutenin with hydrazine and hydroxylamine (22), with sodium borohydride (23), or with 0.0001 to 0.5N NaOH for 0.5 to 24 hr.

Fractions 2, 4 to 9, and 11 of Comanche hard red winter wheat gliadin were isolated by ion-exchange chromatography on sulfoethyl cellulose (see Fig. 2 in ref. 9). Ponca γ_1 - and γ_3 -gliadins were purified by chromatography on sulfoethyl cellulose and Sephadex G-50 columns (3). Ponca α - and β -gliadins were isolated in like manner: β -, α -7-, and α -9- are the major proteins in fractions 6, 7, and 9, respectively (see Figs. 1 and 2 in ref. 3); the numerals differentiating the α -gliadins denote only their parent fractions and not any system of nomenclature. Brevor (soft white winter) Ω -gliadin eluted from Sephadex G-100 (4.2 X 93 cm.) between glutenin and gliadin, and contained three or more components. Glutenins from Red Chief and Comanche (hard red winter wheats), Selkirk (hard red spring), Wells (durum), Omar (club), Brevor (soft white winter), and Seneca (soft red winter) precipitated upon neutralization of acidic 70% ethanol dispersions of gluten and were reduced and cyanoethylated (see Fig. 1 in ref. 24). Glutenin from Red River 68 (semidwarf hard red spring) was isolated on Sephadex G-100 as described previously (see Fig. 2 in ref. 24).

SDS-Polyacrylamide Gel Electrophoresis

Electrophoresis was performed in horizontal slabs (6 mm. thick; 7.7 or 12.8 cm. wide; and 10 to 13 cm. in length) as described by Koenig et al. (25). The pH 8.9, 0.125M Tris-borate buffer containing 0.1% SDS was routinely used; 0.1M sodium

phosphate (pH 7.1, with 0.1% SDS) served as an alternate buffer. Acrylamide, *N,N*-methylenebisacrylamide, and dimethylaminopropionitril were products of Eastman; ammonium persulfate was from E-C Apparatus Corp. (Philadelphia, Pa.); SDS (Fisher) was recrystallized from boiling 95% ethanol before use. All other chemicals were reagent grade and used without further purification. Deionized water was used throughout.

Proteins (0.1 to 2.5 mg.) were dissolved in 0.1 ml. buffer containing 1% 2-mercaptoethanol and SDS equal to two to four times the weight of protein, and incubated at 40°C. for 16 hr. or 95° to 100°C. for 5 min. Samples of the reduced SDS-protein (about 5 μ l.) were then absorbed into 5-mm. squares of Whatman No. 1 paper and inserted into a slit in the gel with forceps. Safranin O (previously incubated with SDS) was added as a tracking dye. Electrophoresis was performed at 100 v. (20–40 ma.) for 150 to 180 min. with tap water circulating through cooling plates on both sides of the gel; all protein-SDS complexes migrated toward the anode. Gels were stained with Coomassie blue (Schwarz/Mann) and destained as described by Koenig et al. (25). They were photographed on Polaroid Type 55 P/N film with a Kodak No. 25 gelatin filter.

The following proteins (with source and MW in parentheses) were used as MW markers (18): cytochrome c (Mann, 11,700, exclusive of heme); ribonuclease (Schwarz/Mann, 13,700); chymotrypsinogen A (Schwarz/Mann, 25,700); ovalbumin (Schwarz/Mann, 43,000); and serum albumin (Schwarz/Mann, 68,000). High-MW markers were prepared from ovalbumin with diethyl oxydiformate (Eastman) (25); polymers up to at least the pentamer could be distinguished. Mobilities of these known proteins are proportional to log MW in the SDS media after reduction. (Fig. 1); MW's from 10,000 to 200,000 may be determined. The

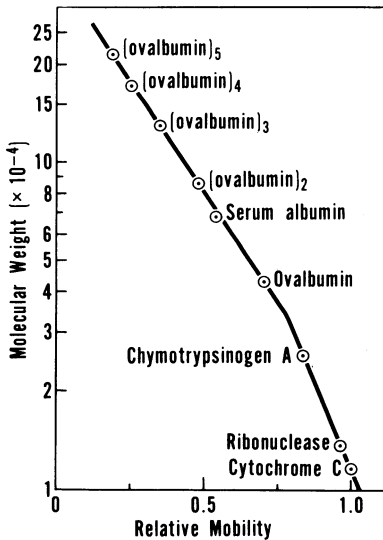


Fig. 1. Plot of logarithm of molecular weight (MW) of marker proteins vs. mobility upon SDS-polyacrylamide gel electrophoresis. Proteins giving each experimental point are indicated; MW's are listed in text.

slight downward curvature of the plot is due to the low (5%) gel concentration (19,26). Mixtures of MW markers were routinely submitted to electrophoresis next to proteins of unknown MW. MW's determined for all the sample proteins are averages obtained by comparing mobilities in several runs to those of standard proteins; no more than 1 to 2% deviation in calculated MW's was observed between runs. MW's determined for standard proteins by this method differ from accepted MW's by no more than 5 to 10%.

Equilibrium Dialysis

The method was essentially that of Reynolds and Tanford (27). Protein (about 25 mg.) was dissolved in 5.0 ml. 6M guanidine hydrochloride containing 0.1% 2-mercaptoethanol and placed in 8/32 dialysis bags, which had been pretreated with 1.5% SDS, hot tap water, and deionized water. Guanidine hydrochloride was removed by dialysis vs. 0.1% 2-mercaptoethanol; and the reduced proteins were then dialyzed at room temperature vs. 2,500 ml. of 0.02M sodium phosphate, pH 7.2, containing 0.1% SDS and 0.1% 2-mercaptoethanol. This buffer contained SDS in excess of the critical micelle concentration (27) to ensure complete reaction with the proteins. Dialysate was changed daily, and after 6 days the experiment was terminated. SDS was determined photometrically after extracting SDS-methylene blue complex into chloroform (27), protein was determined with Folin-Ciocalteu reagent by the method of Lowry, and protein-SDS complex concentrations were determined from dry weights of dialysate and protein solutions.

RESULTS AND DISCUSSION

Subunits of Gliadin

Number and MW's of Subunits. An SDS-polyacrylamide gel electrophoretic separation of reduced gliadin proteins is shown in Fig. 2. The most intense band in whole gliadin (Fig. 2b) corresponds to a polypeptide of MW 36,500; a less abundant subunit of MW 44,200 is also present. Another major band, migrating slightly faster than cytochrome *c*, indicates that the gliadin fraction contains a MW-11,400 subunit. This subunit will be considered in a following section. Whole gliadin also contains Ω -components of MW's 69,300 and 78,100 and trace amounts of subunits of MW's 25,600, 48,800, and 57,300.

The SDS-gel patterns of native gliadin and aminoethylgliadin (Fig. 2c), in which disulfides were reduced with 2-mercaptoethanol and the resulting sulfhydryls blocked by alkylation before complexing with SDS, have the same bands. Gliadin-SDS complexes prepared in the presence of 8M urea (Fig. 2D) or 6M guanidine hydrochloride (not shown) also give electrophoretic patterns equivalent to that of whole gliadin (Fig. 2b). These observations establish that gliadin was completely reduced, denatured, and complexed with SDS during preparation for electrophoresis.

Fractionation of Gliadin on Sephadex G-100. A G-100 chromatogram of whole gliadin (2.15 g.) is shown in Fig. 3. The three major areas in the chromatogram correspond to "low-MW glutenin" (fractions a to c), " Ω -gliadin" (fractions d to f), and purified gliadin (fractions g to j); SDS electrophoresis (Fig. 3) reveals the nature of the reduced polypeptides in each peak.

After reduction, the gliadin proteins excluded from G-100 yield mainly 44,200-MW subunits and lesser amounts of 36,500-MW subunits. Previous studies

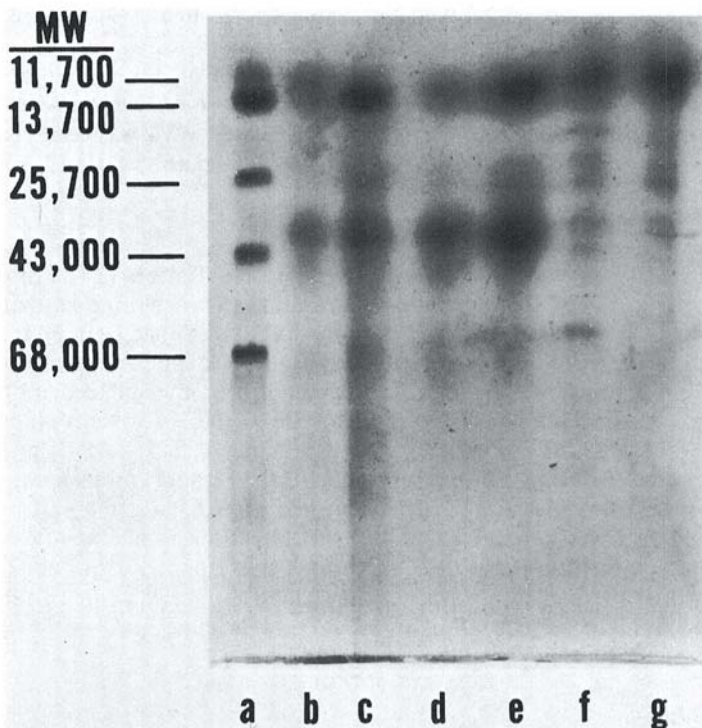


Fig. 2. SDS-polyacrylamide gel electrophoretic separation of: a) standard protein calibration mixture; b) whole gliadin; c) aminoethylgliadin; d) whole gliadin complexed with SDS in 8M urea; e) "purified gliadin" from Sephadex G-100; f) albumins; and g) globulins. See text for details. MW's of reference proteins are indicated.

(2,4) showed the native excluded proteins to have average MW's of 104,000 to 125,000; reduction decreased their MW to 37,000, indicating that intermolecular disulfide bonds occur in the native proteins. These results, along with amino acid analyses and starch-gel electrophoresis, suggested that these proteins were low-MW glutenins. Now, however, SDS electrophoresis reveals that these proteins are fairly homogeneous in subunit size distribution compared to reduced glutenin (see the following section). The high-MW proteins in whole gliadin are thus uniquely different from either whole glutenin or purified gliadin. These proteins are relatively minor components of whole gliadin (7.7% was recovered in fractions a to c, Fig. 3), but could have a significant effect on properties of the whole gluten complex.

Fractions d to f (Fig. 3) contain the Ω -gliadins, which SDS electrophoresis shows to have subunit MW's of 69,300 and 78,100; these values agree with MW's of 73,000 to 74,000 reported for similar proteins by Booth and Ewart (28). Reduced fractions d to f also contain significant amounts of polypeptides of MW's 44,200 and 36,500; these may be subunits of dimers, the MW's of which would be near that of the Ω -gliadins, causing them to have the same elution volume from Sephadex G-100.

Proteins containing 36,500-MW polypeptides are eluted nearly free of larger protein molecules containing 44,200-MW subunits in the purified gliadin peak (Fig. 3, g-j; Fig. 2e). The pooled "purified gliadin" (Fig. 2e) still contains small amounts of Ω -gliadin, as well as the 11,400- and 25,600-MW polypeptides which elute at the end of the chromatogram (Fig. 3, i and j). A smaller sample must be used to separate these polypeptides completely from gliadin on Sephadex G-100 (29).

The 11,400-MW polypeptide in the "purified gliadin" fraction exhibits the same mobility as the major polypeptides of albumin and globulin (Fig. 2, f and g). Since the 11,400-MW components from purified gliadin elute from sulfoethyl cellulose at a high salt concentration and from G-100 near the total column volume (Fig. 3), it is likely, but not proved, that they are albumins or globulins which may have been trapped in, or bound to, the gluten matrix. The occurrence of albumins in gliadin was also noted by Booth and Ewart (28). The function of the 11,400-MW polypeptides remains to be established. They appear to be major contaminants of purified gliadin (Fig. 2e) but actually constitute only 5 to 10% of its total weight². Increased electrostatic binding of the Coomassie blue dye to these proteins apparently results from their higher content of basic amino acids.

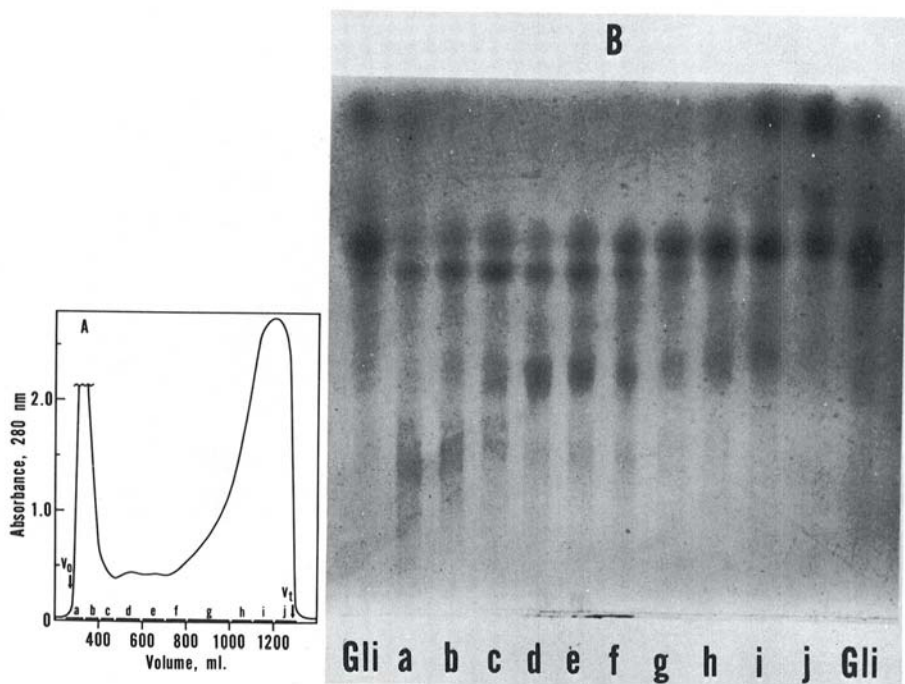


Fig. 3. A, Chromatographic separation of 2.15 g. of whole gliadin on Sephadex G-100 (4.2 \times 93-cm.); 0.1N acetic acid was the eluting solvent. Heavy lines on abscissa indicate combined fractions. Void volume (V_0) and total volume (V_t) of the gel bed are indicated. B, SDS-polyacrylamide gel electrophoresis of Sephadex G-100 fractions a-j. Whole gliadin (Gli) is included for comparison.

²F. R. Huebner, personal communication, based on recoveries from sulfoethyl cellulose (9).

SDS Electrophoresis of Purified Gliadin Proteins. Owing to the large number of gliadin proteins, small MW differences among them may not be apparent upon SDS electrophoresis of the mixture. SDS-electrophoretic examination of individual gliadins reveals that such differences do occur. SDS-gel patterns of Comanche gliadin-sulfoethyl cellulose fractions (9) and purified Ponca gliadins are reproduced in Fig. 4. For example, in the sulfoethyl-cellulose fractions, one β -gliadin (in fraction 5) has an unusually high MW, and gliadins in fractions 6 to 9 and 11 differ slightly in MW's. Fraction 11, eluted with a high salt concentration, contains albumin or globulin subunits as well as an α -gliadin component. Generally, however, most components in "purified gliadin" have MW's near 36,500. Similarly, some differences in MW's are revealed by SDS electrophoresis of individual Ponca gliadins. γ_3 -Gliadin has a higher MW than γ_1 -gliadin, in agreement with the higher minimum MW deduced from amino acid analyses (3). The α -7-gliadin, which migrated as a single band upon starch-gel electrophoresis, can now be seen to contain three or more proteins.

Thus, SDS electrophoresis is useful for relating each component to the mixture it was derived from, for monitoring separations, and as a criterion of purity, as well as for the determination of MW's. It can also be used for comparing similar

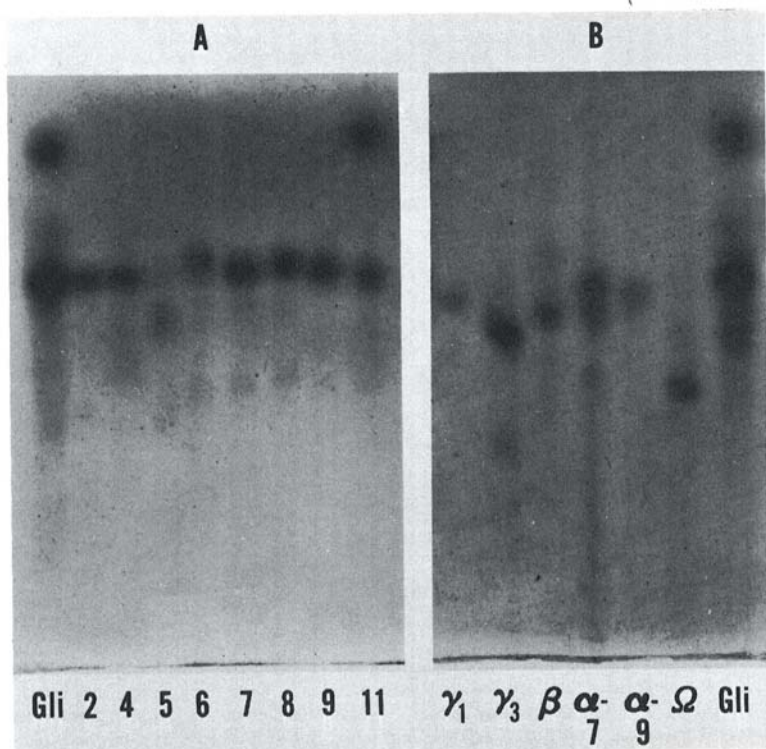


Fig. 4. SDS-polyacrylamide gels of gliadin fractions and components. A, Whole gliadin (Ponca) and sulfoethyl-cellulose fractions 2, 4-9, and 11 from Comanche gliadin (9). B, Ponca γ_1 , γ_3 , β , α -7-, and α -9-gliadins, Brevor Ω -gliadin, and whole Ponca gliadin.

proteins. For example, the MW of Brevor Ω -gliadin (Fig. 4) is consistent with that of Ponca Ω -gliadins.

SDS Electrophoresis of Unreduced Gliadin Proteins. MW's determined by SDS electrophoresis are valid only for reduced polypeptides, but some information can also be gained from electrophoresis of unreduced protein-SDS complexes on gels containing no sodium sulfite. Dunker and Rueckert (19) showed that mobilities of SDS complexes of reduced and nonreduced single-chained proteins are similar. This similarity results from a fortuitous balance between increased mobility, due to greater SDS binding resulting in increased negative charge, and decreased mobility caused by greater frictional resistance as chains unfold. We found mobilities of the 36,500-MW gliadin polypeptides, of the Ω -gliadins, and of the 11,400-MW albumin or globulin polypeptides to be practically unchanged by reduction when compared to the unreduced proteins. These results confirm the intramolecular nature of disulfide bonds in purified gliadin and Ω -gliadin, and indicate that the albumins or globulins are not joined through disulfide bonds in their native states. SDS electrophoresis of the unreduced high-MW proteins in whole gliadin (first peak from G-100), however, revealed no 44,200-MW polypeptides; but a streaked band present in the high-MW range confirmed the intermolecular nature of disulfide bonds in these proteins.

Subunits of Glutenin

Number and MW's of Subunits. SDS electrophoresis reveals a more complex subunit distribution for reduced glutenin (Fig. 5, a and c) than for gliadin (Fig. 5d); and many glutenin subunits are of higher MW's. Subunits of at least 15 unique MW's occur in glutenin. Their calculated MW's are as follows: band 1, 133,000; band 2, 124,000; band 3, 102,100; band 4, 87,200; band 5, 79,100; band 6, 71,000; band 7, 64,300; band 8, 49,400; band 9, 44,600; band 10, 42,200; band 11, 36,000; band 12, 32,600; band 13, 27,500; band 14, 18,000; and band 15, 11,600. Comparison of the SDS electrophoresis patterns of gliadin and glutenin (Fig. 5, c and d) reveals that glutenin bands 9 and 11 correspond fairly closely in MW's to the two major gliadin polypeptides. In addition, glutenin band 8 corresponds in MW to a minor polypeptide in gliadin. Visually, the mobilities of these corresponding bands are identical; slight calculated numerical-MW differences occur, but are not significant, and could be due to band spreading caused by concentration differences.

The SDS-gel patterns of aminoethylglutenin (Fig. 6c), cyanoethyl glutenin (13), glutenin oxidized with performic acid (10), and *S*-sulfo-glutenin (10), in which all disulfides had been cleaved and sulfhydryls derivatized before complexing with SDS, were all equivalent to the gel pattern of native glutenin (Fig. 6, a and b). Thus, reductive cleavage of disulfides during sample preparation is quantitative. Denaturation with 1% SDS was also complete since addition of 8M urea (Fig. 6d) or 6M guanidine hydrochloride produced no differences in number and relative intensities of bands. Electrophoresis at an alternate pH (7.1) also gave essentially the same pattern. Association of gluten proteins during electrophoresis has not been observed, and MW comparisons have not indicated a polymeric nature of any high-MW glutenin polypeptides. All commonly used criteria, therefore, demonstrate that high-MW glutenin subunits are neither associated forms nor undenatured proteins with remaining disulfide bonds.

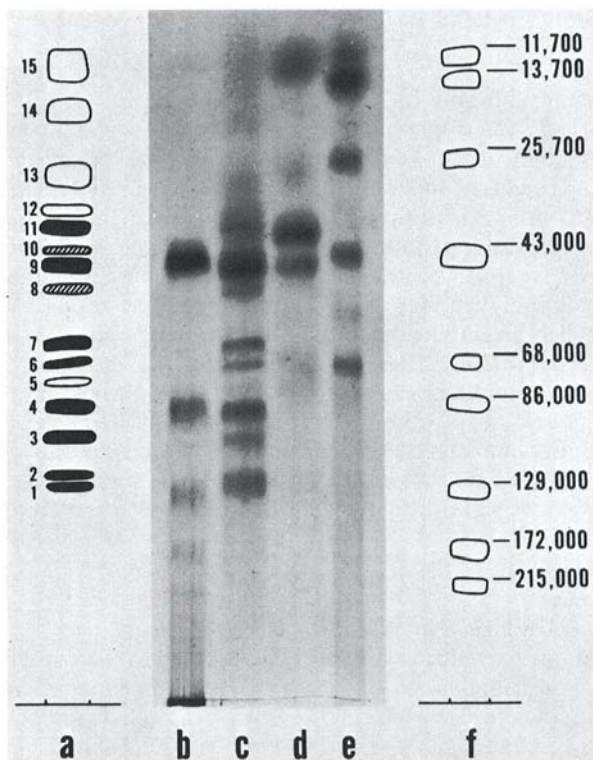


Fig. 5. Comparison of SDS-electrophoresis patterns of glutenin and gliadin with those of proteins of known MW's: a) diagram and numbering system used in text for glutenin subunits, with prominent bands shaded; b) polymeric ovalbumin; c) glutenin; d) gliadin; e) standard protein calibration mixture; and f) composite diagram and MW's of components of calibration mixtures.

Examination of Glutenin Fractions by SDS Electrophoresis.

Aminoethylglutenin has been divided into four fractions by selective precipitation with copper salts (12); each fraction has now been characterized more completely in terms of number and MW's of subunits and has been related to whole aminoethylglutenin by SDS electrophoresis (Fig. 6, e-h). For example, fraction A (Fig. 6e) contains only the high-MW polypeptides 1 to 3 in significant amounts; the unique amino acid composition of this fraction (12) is a further indication that high-MW glutenin polypeptides are not formed by association of lower-MW components. Previous estimates of the MW of subunits in fraction A (12) also support the values obtained by SDS electrophoresis. Similarly, fraction B (Fig. 6f) is enriched in certain subunits; it is apparent that salt precipitation is useful as an initial step in purification of these polypeptides.

In SDS gels of fractions C and D (Fig 6, g and h), the two fastest bands are the most intense, but bands representing three larger subunits are also present. The low-MW polypeptides may be albumins or globulins (as in Fig. 2, f and g), which may occur with glutenin (30,31). The amino acid composition of fractions C and D

(12), however, is similar to that of aminoethylglutenin, but not of the water-soluble gluten proteins (32). It is necessary to isolate these low-MW polypeptides to determine whether they are major gluten components or minor albumins or globulins exhibiting increased dye-binding.

In addition to characterizing these aminoethylglutenin fractions, SDS electrophoresis is also valuable for monitoring other preliminary separations of glutenin proteins and subunits.

Search for Cross-Links in Reduced Glutenin. Because of the high MW's observed for some reduced glutenin-SDS complexes, several attempts were made to demonstrate remaining labile nondisulfide cross-links in alkylated-reduced glutenin. Since neither hydrazine nor hydroxylamine produced additional cleavage, esterlike

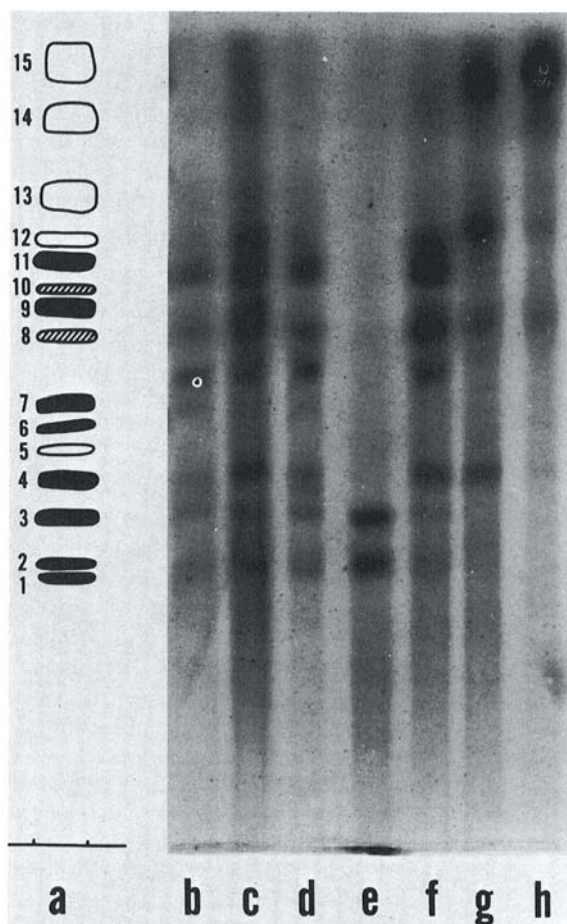


Fig. 6. SDS-polyacrylamide gel of various glutenin preparations and subfractions: a) diagram and numbering system used in text for glutenin subunits, with prominent bands shaded; b) glutenin; c) aminoethylglutenin; d) glutenin complexed with SDS in 8M urea; and e-h) aminoethylglutenin-salt precipitation fractions A-D, respectively.

bonds (22) involving aspartyl residues must be absent in aminoethylglutenin. The extensive destruction of all aminoethylglutenin electrophoretic bands, caused by alkali as incubation time or alkali concentration increased, indicated a peptide-bond hydrolysis as noted by Murphy (33). There was no indication of simultaneous destruction of high-MW polypeptides and formation of low-MW ones. Similarly, alkaline sodium borohydride (23), a test for serine or threonine glycosidic linkages, destroyed nearly all intact subunits in aminoethylglutenin as demonstrated by SDS electrophoresis. These results support the concept that high-MW glutenin subunits are single-chained, but do not rule out the possibility that they consist of smaller subunits joined by stable covalent bonds of undetermined nature.

Comparisons of Glutenins from Different Varieties. Differences among reduced glutenin subunits between varieties and classes of wheat have been demonstrated by starch-gel electrophoresis (24,34), but the relationship between quality and glutenin structure is still obscure. SDS electrophoresis is also useful for examining varietal differences between glutenins (Fig. 7). Much similarity between the SDS-electrophoretic patterns of subunits from glutenins of different classes and varieties exists, but distinctive differences do occur. For example, Red Chief and Comanche (Fig. 7, c and d), which are hard red winter wheats of poor and good breadmaking potentials, respectively, differ in the amount of protein in bands 6 and 7. The semidwarf hard red spring variety Red River 68 (Fig. 7f) contains more material in bands 3 and 9 than the hard red spring variety Selkirk (Fig. 7e). The durum (Fig. 7g) is very deficient in bands 1, 2, and 12; and the club and soft red winter varieties Omar and Seneca (Fig. 7, h and j) are deficient in bands 3 and 6. Further comparisons by this method may be useful in revealing the genetic background of glutenin subunits, and may indicate whether any relationship exists between gluten quality and the presence or quantity of certain glutenin polypeptides.

Equilibrium Dialysis

Mobility of polypeptides during SDS electrophoresis is related to MW because a constant SDS-protein binding ratio gives the rodlike complex a constant charge per mass unit (20). Many proteins hydrophobically bind identical amounts of SDS (1.4 g. SDS per g. protein) (27) and exhibit the linear relationship between log MW and mobility. The large number of hydrophobic amino acid residues in gliadin and glutenin (6), however, necessitated examining their binding ratios to ensure that determined MW's were accurate to within the 5 to 10% maximum error, since excessive binding could possibly lead to elevated MW estimations.

Equilibrium dialysis was carried out on glutenin, aminoethylglutenin, and purified gliadin (Fig. 3g); ovalbumin and serum albumin served as standards. The SDS-protein ratios (g./g.) were: gliadin, 0.92; glutenin, 0.84; aminoethylglutenin, 0.91; ovalbumin, 0.82; and serum albumin, 0.97. Although values are lower than maximum, the gluten proteins bound no more SDS than did the standards; evidently then, the MW's determined for gliadin and glutenin subunits are valid.

GENERAL DISCUSSION

The gliadin and glutenin subunits resolved by SDS electrophoresis are the smallest obtainable by complete denaturation and disulfide-bond cleavage. It is unlikely that these subunits are either associated or polymeric forms, since high-MW



Fig. 7. SDS-polyacrylamide gel of glutenin from several varieties and classes: a) diagram and numbering system used in text for Ponca glutenin subunits, with prominent bands shaded; b) Ponca (hard red winter); c) Red Chief (hard red winter); d) Comanche (hard red winter); e) Selkirk (hard red spring); f) Red River 68 (hard red spring); g) Wells (durum); h) Omar (club); i) Brevor (soft white winter); and j) Seneca (soft red winter).

glutenins (bands 1 to 3, Fig. 6e) have unique amino acid compositions, their MW's do not seem to be integral multiples of lower values, and no remaining cross-links could be detected. All MW's are also reproducible and accurate (as demonstrated by equilibrium dialysis); so SDS electrophoresis is, by all criteria, a valid technique for the determination of number and MW's of gluten-protein subunits. It is also useful, as described above, for monitoring and predicting fractionations of mixtures, and may possibly be used as a preparative technique.

These studies have shown that most gliadin proteins are single-chained and have MW's near 36,500. Two or more single-chained Ω -gliadins of MW's 69,300 and

78,100 also occur in whole gliadin, as well as proteins of MW 11,400, which may be albumins or globulins. Polypeptides of MW 44,200, together with 36,500-MW subunits, occur in whole gliadin as disulfide-linked components of higher-MW proteins. In the Sephadex G-100 separation of whole gliadin, proteins separating with Ω -gliadins appear to be dimers of these subunits, whereas the high-MW protein fraction in whole gliadin contains trimers and possibly higher-MW polymers of predominantly 44,200-MW subunits. The 44,200-MW polypeptides are thus of special interest, since they may promote disulfide bonding between subunits. If the subunit of MW 44,600 in glutenin is similar to the 44,200-MW subunit in whole gliadin, it could contribute significantly to intermolecular disulfide bonding of glutenin polypeptides.

The MW's determined in this study for reduced gliadin polypeptides agree with reported values of 30,000 to 50,000 (4,5,6,8) for the major gliadin components, and with minimal MW's of 16,000 to 18,000 determined for γ -gliadins by amino acid analyses (3) if two residues rather than one residue of the limiting amino acid are present. Our data are also consistent with reported MW's of Ω -gliadins (28), and with reduced gliadin MW's of 69,000, 43,000, and 30,000 determined by Meredith and Wren (7) by Sephadex G-200 chromatography in a dissociating solvent system. The data reported here are not consistent with the weight-average MW of 22,000 obtained for reduced purified gliadin by sedimentation equilibrium (2).

SDS electrophoresis reveals that glutenin is composed of subunits of at least 15 distinct MW's, ranging from 11,600 to 133,000. MW's of the largest, fully reduced, glutenin subunits agree with values in excess of 100,000 obtained by gel filtration (11,12,13) and are larger than for most single-chained proteins. No other intermolecular cross-links [a possibility noted for glutenin by Meredith (35) and by Ewart (36)] were detected, however; but further studies are necessary to resolve this question fully. Our results also indicate that the relatively homogeneous MW of 20,000 for glutenin subunits as measured by analytical ultracentrifugation (10) apparently resulted from rupture of peptide bonds by the alkaline solvent (0.03M ammonium hydroxide) used.

Genetic considerations suggest that gliadin and glutenin proteins could conceivably have originated from the same precursor; indeed, considerable chemical similarity (along with unique differences) has been demonstrated between proteolytic digests of the two groups of proteins (15,16). Comparison of gliadin and glutenin subunits by SDS electrophoresis (Fig. 5) has now revealed that three glutenin subunits apparently have counterparts of similar MW's in gliadin and could, therefore, be identical. The marked MW differences observed between other glutenin subunits and gliadin, however, suggest that in most subunits, any regions of similar sequence must occupy part of, rather than the entire length of, the polypeptides, or that the subunits are uniquely different.

Some hypotheses have considered gliadin and glutenin to each be relatively homogeneous in subunit composition, and have failed to consider their previously demonstrated variability. The technique of SDS electrophoresis has now led to a more accurate understanding of the distribution of subunits within these proteins, and has emphasized the heterogeneity that exists. Also defined more completely are atypical subunits, such as those of high MW in glutenin and the 44,200-MW polypeptides in whole gliadin, which may significantly affect the functional properties of the whole gluten complex.

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