Wheat Glutenin: Effect of Dissociating Agents on Molecular Weight and Composition as Determined by Gel Filtration Chromatography

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Gel filtration chromatography of wheat glutenins on 4% agarose beads (Sepharose CL-4B) in hydrophobic bond-breaking solvents indicates that the average molecular weight (mol wt) of the dissociated native glutenin molecules may not be as large as previously determined by chromatography using 8 M urea or 6 M guanidine hydrochloride in acid buffer. The high molecular weight of native glutenin has been attributed to disulfide bonds between polypeptide chains since its molecular weight is lowered after the protein is reduced. However, in pH 9.8 buffer containing 0.2% sodium dodecyl sulfate, some of the native glutenin elutes at the same volume as gliadin (mol wt 37 000), some at the void volume (mol wt 10 million), but most at intermediate volumes, which suggests a range of molecular weight. In 0.1 M sodium dodecanoate at pH 9.9 in low ionic strength, more of the protein was retarded during agarose gel chromatography, thereby indicating that this solvent was more effective in dissociating the protein. The lower molecular weight dissociated protein was not gliadin. It must associate with the higher molecular weight glutenins by hydrophobic bonding and is not linked to it by disulfide bonds.

Wheat glutenin, the alcohol-insoluble fraction of gluten protein, contributes greatly to the cohesive elastic properties of dough as evidenced by the fact that it hydrates to a rubbery mass in water. The unique properties of glutenin have been attributed to its amino acid composition. The large amounts of nonpolar residues tend to engage in hydrophobic bonding and uncharged polar groups can participate in hydrogen bonding. Glutenin has a deficiency of charged amino acid residues that favor solution in aqueous systems. Furthermore, glutenin is believed to consist of a range of high-molecular-weight proteins formed by union of lower molecular weight polypeptide chains through disulfide bonds (Huebner and Wall, 1976). The apparent asymmetric structure of these large molecules may facilitate the association of the proteins (Taylor and Cluskey, 1962). Some claims, that these large polymers were artifacts of isolation or dough mixing and that glutenin was formed from gliadin subunits, were dispelled by demonstration of different composition of the gliadin and glutenin subunits as shown by sodium dodecyl sulfate–polyacrylamide gel electrophoresis ($NaDodSO_4$–PAGE) and amino acid analyses (Bietz and Wall, 1972; Huebner and Wall, 1974; Huebner et al., 1974). Recent reviewers (Kasarda et al., 1976) have suggested that disulfide bonds may not give rise to extremely large molecules of glutenins, but that large molecular aggregates are primarily due to noncovalent links by hydrogen or hydrophobic bonds.

Analytical ultracentrifugation studies conducted in various disaggregating solvents indicate that glutenin is heterogeneous in molecular weight; values were reported from less than 100 000 to over 2 or 3 million (Jones et al., 1961; Taylor and Cluskey, 1962; Nielsen et al., 1962). Because of its heterogeneity and insolubility, accurate determinations of the molecular weight of glutenin by analytical ultracentrifugation have been difficult. There have been some reports that, in suitable dissociating solvents, glutenin may exhibit low molecular weight. Stanley et al. (1968) reported that in a strongly acidic solution of phenol, acetic acid, and water (1:1:1) glutenin behaved as a small molecule (mol wt 50 000) in the ultracentrifuge. More recently, Kobrehel and Bushuk (1978) dissolved glutenin in sodium stearate and sodium dodecanoate solutions and suggested that ultracentrifugation in those solvents indicated a low molecular weight.

Gel filtration chromatography for molecular weight study of proteins is advantageous since it separates the molecules mainly by their size in quantities which allow one to perform additional studies on them. Use of improved agarose gel as a filtration medium has extended the range of separation to include larger molecules. Using this system with a strongly disaggregating solvent, 5.5 M guanidine hydrochloride (GHCl), we observed heterogeneous glutenin molecules with molecular weights as large as 20 million (Huebner and Wall, 1976). In the present report we describe examination of the molecular weight distribution of glutenin by comparing its gel filtration behavior in different disaggregating solvents such as GHCl, urea, sodium dodecyl sulfate ($NaDodSO_4$), and sodium dodecanoate (NaDod). For comparison, reduced glutenin was also chromatographed on the agarose columns to verify the elution positions and approximate molecular weights of the subunits. Combined fractions of peaks of different molecular weight were analyzed for their amino acids and reduced subunits by $NaDodSO_4$–PAGE to establish compositional differences among the separated fractions.

MATERIALS AND METHODS

Glutenin Preparation. The glutenin sample was isolated from a hard red winter wheat (Ponca) according to the method of Jones et al. (1959). A gluten ball was dispersed in 0.05 N acetic acid. The solution was clarified by centrifugation and heated to 95 °C to inactivate proteolytic enzymes. Addition of ethanol to 70% by volume and adjustment of the pH to 6.5 precipitated glutenin. The precipitated fraction was redispersed in 0.05 N acetic acid and reprecipitated to purify it further. This twice precipitated glutenin was used for these experiments. For the experiments with reduced glutenin, the protein was reduced with mercaptoethanol and alkylated with 4-vinylpyridine to yield *S*-pyridylethylglutenin (PE-glutenin) as previously described (Huebner and Wall, 1974). The 70% ethanol-insoluble PE-glutenin was prepared according to the procedure of Bietz and Wall (1973).

Reagents. The GHCl used was an ultrapure product from Schwarz/Mann. Urea (analytical reagent from Mallinckrodt Chem. Co.) was dissolved in distilled water and passed over a mixed bed resin (AG 501-X8D Bio-Rad) to remove cyanates and other trace impurities just before using. The NaDod was from Sigma Chemical, grade IV. Due to the presence of a fine cloudy suspension, the Na-

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Dod solution was first centrifuged at low speed or filtered through folded Whatman No. 2^v paper. It was necessary to keep CO_2 from the solution by storing under nitrogen or making up a new batch each day to prevent a decrease in pH and a cloudly precipitate from forming. $NaDodSO_4$ was Sequanal grade from Pierce Chem. Co. All the solvents were filtered through an 0.8 μm Millipore filter which prevented any eventual obstruction of the column. This purification step was especially essential with the NaDod solvent. Finally, the solvents were degassed under partial vacuum to prevent the formation of air bubbles in the gel filtration column.

The acrylamide and *N,N*-methylenebis(acrylamide) were from Eastman. Other chemicals used were ACS grade or best available. The following proteins from Schwarz/Mann (molecular weight in parentheses) were used as molecular weight markers for $NaDodSO_4$–PAGE: ribonuclease (13700), ovalbumin (43000), and serum albumin (68000). Proteins used as molecular weight markers on column chromatography were from Pierce Chem. Co.: catalase (240000), aldolase (158000), and albumin (45000).

Gel Filtration. Purified cross-linked agarose gel beads (Sepharose CL-4B, Pharmacia) were used as the gel filtration medium in a column 2.5 × 74 cm. Columns were operated with upward flow. Generally 20–30 mg of protein was partly dissolved in 6–8 mL of the solvent by stirring with a magnetic stirrer for 2 h. The samples were centrifuged at approximately 9000*g* for 8–10 min to remove undissolved protein (which appeared clear and gel-like) that would curtail flow if applied to the column. The insoluble material was redispersed and stirred in additional buffer (4–6 mL) for 1 h and recentrifuged. The supernatants were applied in sequence to the column so that the total sample volume was about 14 mL. With a flow rate of 8–10 mL/h, sample application took about 1–1.5 h.

The eluant was continuously monitored with a Schoeffel ultraviolet (UV) spectrophotometer at 276 nm or each tube read on a Beckman DU spectrophotometer using 1-cm cuvettes. Effluent corresponding to each absorbance peak was combined, dialyzed exhaustively against 0.03 M acetic acid, and lyophilized. However, fractions containing soap were initially dialyzed against dilute NH_4OH at a pH between 9.0 and 10.0; after about 5 days, the dialysis medium was changed to dilute acetic acid and dialysis was continued for 2 days before lyophilization. Since some of the soap appeared to be bound to the protein, the dried sample was dispersed in 3–4 mL of 100% ethanol plus a drop of glacial acetic acid. After centrifugation in a desk-top clinical centrifuge, the ethanol that contained most of the soap was decanted and the precipitated protein was dissolved in a few milliliters of 0.05 N acetic acid and again lyophilized. Only a trace of protein was detected in the ethanol extract.

$NaDodSO_4$–PAGE. The samples were analyzed by $NaDodSO_4$–PAGE on vertical slab gels by a modification developed by Payne and Corfield (1979) of the method of Studier (1973). Glass plates used to form the gels were 14 × 14 cm. Lucite spacers were used to give a gel 3 mm thick. The separation gel was 17% polyacrylamide in pH 8.9 Tris-HCl buffer containing 0.05% $NaDodSO_4$. The separation gel extended to within 2.5 cm of the top of the glass. After that gel was polymerized, a stacking gel prepared from 3% polyacrylamide in pH 6.8 Tris-HCl buffer containing 0.1% $NaDodSO_4$ was added. Ten wells, each 5 mm wide, were formed in the stacking gel with a lucite comb so that each well extended down to within 1 cm of the separation gel. About 250 μg of protein in 25 μL was applied to each well. Safranin O was mixed with the sample solution to serve as a marker. Filter paper was used to connect the top of the gel to the cathode buffer chamber, while the bottom of the gel was immersed in the anode buffer. Both anode and cathode buffers consisted of pH 8.3 Tris–glycine buffer containing 0.1% $NaDodSO_4$ and 0.002 M EDTA. Gels were run for 16–17 h at constant current without cooling. The gels were stained with Coomassie blue and destained as described by Koenig et al. (1970).

Table I. Percent of Glutenin Soluble in Different Solvents

solvent[a]	glutenin[b]	% soluble[c]
6 M GHCl, pH 5.5	25 mg	25–30
6 M GHCl, pH 3.2	30 mg	45–50[d]
7 M urea, pH 3.4	25 mg	30–40
7 M urea, pH 9.9	25 mg	45–50
0.1 or 0.2% $NaDodSO_4$, pH 9.1 or 9.8	30 mg	75–80
0.1 M NaDod, pH 9.9	30 mg	75–85

[a] GHCl = guanidine hydrochloride, $NaDodSO_4$ = sodium dodecyl sulfate, NaDod = sodium dodecanoate. [b] Quantity of protein used for column chromatography. Procedure given under gel filtration in the Methods section. [c] Due to difficulties handling the sticky glutenin, the small samples, and the problems in removing all the $NaDodSO_4$ and soap, the calculated percent soluble is only an estimate in 12–14 mL of solvent. [d] Not run on a column and remaining insoluble material not redissolved a second time as for the other samples.

Amino Acid Analysis. The amino acid analyses of the proteins were carried out as described previously (Huebner et al., 1974) by the method of Liu and Chang (1971). The samples were hydrolyzed with 3 M toluenesulfonic acid in evacuated sealed ampules at 105 °C for 24 h. Analyses were carried out on a Beckman automatic analyzer. Calculations were by computer according to the method of Cavins and Friedman (1968). Due to the limited availability of samples, only one 24-h hydrolysis was run for each sample.

RESULTS

Solubility of Glutenin in Various Solvents. Although GHCl is considered a good hydrogen-bond breaking agent, less than one-third of the glutenin was soluble in 6 M solutions of it at pH 5.5 (Table I). GHCl appeared to disperse the glutenin to form a clear solution; however, centrifugation of the sample at over 9000*g* removed insoluble gel-like material. Lowering the pH of the 6 M GHCl solution to pH 3.2 by addition of 0.03 M acetic acid increased the solubility of glutenin in it. Concentrated urea solutions (7–8 M) were more effective solvents than the GHCl, especially at the alkaline pH (9.9). GHCl was added to the urea solutions to elevate the ionic strength to prevent adsorption of the protein to the agarose column. Previous experience with Sepharose indicated that 0.1 M NaCl or GHCl solution was sufficient to minimize adsorption. Solutions of $NaDodSO_4$ and NaDod were good solvents for glutenin, with NaDod dissolving the highest percentage of glutenin (Table I). Evidently the aggregation of glutenin molecules is partially maintained by hydrophobic bonds. Naturally, other conditions such as a larger volume of solvent would give a higher percentage of solubility; this procedure merely shows that these solvents gave different results and not all of the protein is being applied to the column.

Chromatography of Native Glutenin. The elution profiles of wheat glutenin separated on Sepharose CL-4B columns with various solvents varied as shown in Figure 1. Approximate molecular weights of proteins eluting at different volumes, given at the top of the figure, are based

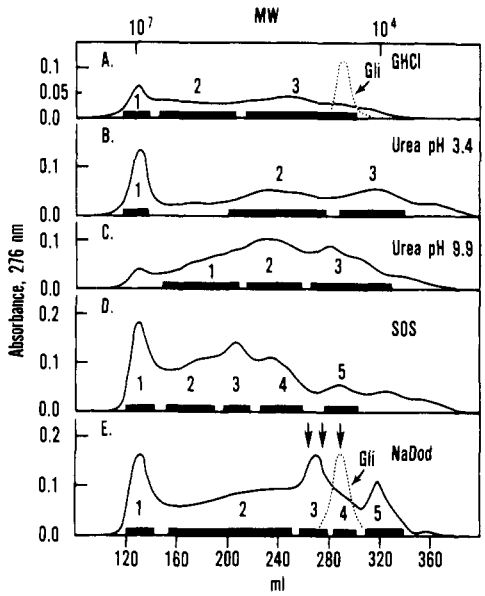

Figure 1. Gel filtration on Sepharose CL-4B of glutenin in various dissociating solvents. Quantity of samples used is given in Table I. (A) Solvent: 6 M GHCl, 0.05 NaAc, pH 5.5; elution position of gliadin is shown by dotted line; (B) solvent: 7 M urea, 1 M GHCl, 0.05 N lactic acid, pH 3.4; (C) solvent: 7 M urea, 0.1 M GHCl, 0.002 M Na_2CO_3, pH 9.9; (D) solvent: 0.2% $NaDodSO_4$, 0.05 M Tris-borate, pH 9.8; (E) solvent: 0.1 M NaDod, 0.001 M Na_2CO_3, pH 9.9. Elution position of three standard molecular weight marker proteins are shown by the three arrows, respectively: catalase 240 000, aldolase 158 000, and albumin 45 000. Position for gliadin is shown by dotted line.

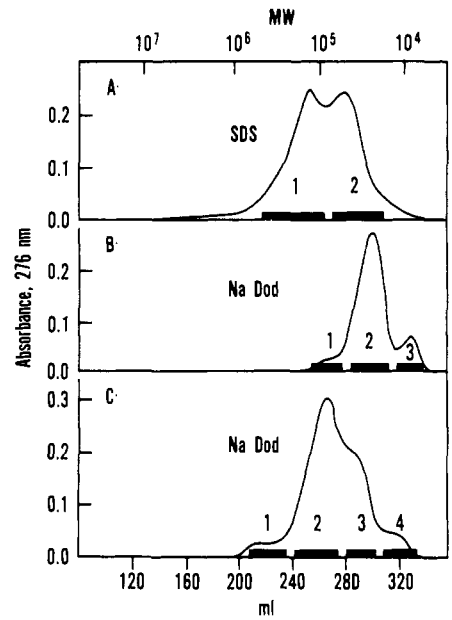

Figure 2. Gel filtration on Sepharose CL-4B of reduced glutenin and gliadin: (A) 30 mg of PE-glutenin: solvent; 0.1% $NaDodSO_4$, 0.05 M Tris-borate, pH 8.9; (B) 20 mg of gliadin: solvent; 0.1 M NaDod, 0.001 M Na_2CO_3, pH 9.9; (C) 21 mg of 70% ethanol insoluble PE-glutenin: solvent; 0.1 M NaDod, 0.001 M Na_2CO_3, pH 9.9. Sample applied in 6–8 mL.

on the manufacturers data for globular proteins. The indicated effective fractionation range is from a maximum of 10 million molecular weight at the void volume to approximately 20 000. Because of the difference in conformation of globular proteins and the glutenin proteins (Taylor and Cluskey, 1962) and in the different solvents at varied pH's, these approximate molecular weight values could be considerably different than those for the glutenin proteins eluting at a given volume; therefore, these values are cited only as an aproximate guide. Standard proteins are, therefore, included in Figure 1 for additional reference.

GHCl (6 M), an effective hydrogen bond-breaking solvent, was used initially as eluant (Figure 1A). A wide molecular weight range of the soluble proteins was indicated. The elution profile was fairly flat except for a peak at the void volume (Figure 1A). When glutenin was applied in 7 M urea at pH 3.4, more protein was eluted from the column; while some of the increase may be attributed to slightly greater solubility in this solvent as compared to GHCl, urea apparently disaggregated more of the lower molecular weight glutenin. The increased protein eluted both in the void volume and at volumes indicating that lower molecular weight proteins were present (Figure 1B). Upon shifting to a higher pH urea solution (pH 9.9), less protein eluted at the void volume and more came off at the intermediate molecular weight range (Figure 1C). The higher pH evidently favored dissociation of protein aggregates but possibly changed the conformational structure, causing some of the proteins to be eluted sooner.

Although $NaDodSO_4$ solution dissolved more protein than either urea or GHCl, the elution profile of glutenin separated in $NaDodSO_4$ at pH 9.8 indicated the presence of a large amount of high molecular weight protein as well as intermediate sized glutenin molecules (Figure 1D). Separated low-molecular-weight proteins were not evident in large amounts. The elution profile for glutenin dissolved in NaDod at pH 9.9 (Figure 1E) differs from those of the other solvents by having more lower molecular weight components, but it still indicates that most of the glutenin is high-molecular-weight protein. This profile confirms earlier reports (Kobrehel and Bushuk, 1977) that NaDod is a superior solvent for glutenin and is probably the best disaggregating agent tested in this study. The concentration of soap and the pH of the solvent are critical. Preliminary studies with 0.01 M NaDod plus 0.01 to 0.02 M Tris borate buffer at pH 9.1 gave erratic results with large absorbances at or near the void volume which contained very little protein. It was established that at pH 9.1, the soap–protein complex precipitated in certain fractions to yield a turbid solution with a false high absorbance at 276 nm. Upon raising the pH above 9.5, the solution cleared and gave absorbance readings proportional to the amount of protein present. The peaks eluting at the void volume and the last peaks of the $NaDodSO_4$ and NaDod columns (Figures 1D and 1E) had slightly higher total absorbances than accounted for by recovered proteins; however, a considerable percentage of protein could have been lost in the various operations required to recover the small fractions.

Chromatography of Reduced Glutenins and Gliadin. After disulfide bonds had been broken and the sulfhydryls alkylated, the glutenin dissolved quickly and completely in all solvents tested in this study. The separation of reduced glutenin on Sepharose CL-4B columns in 0.1% $NaDodSO_4$ solution at pH 9.1, shown in Figure 2A, indicates that the protein is partially resolved into two peaks of different molecular weight components. These results contrast with earlier findings (Huebner and Wall, 1974) that in 4 M urea solution separation of reduced alkylated glutenin on Sephadex G-200 yielded three peaks—A, B, and C. Fraction A, eluting at the void volume of PE-glutenin in 4 M urea, consists of aggregated protein that was shown to be dissociated by $NaDodSO_4$–PAGE (Huebner and Wall, 1974). Elution of the reduced and alkylated glutenin in NaDod from Sepharose CL-4B yields no high-molecular-weight components (Figures 2A and 2C)

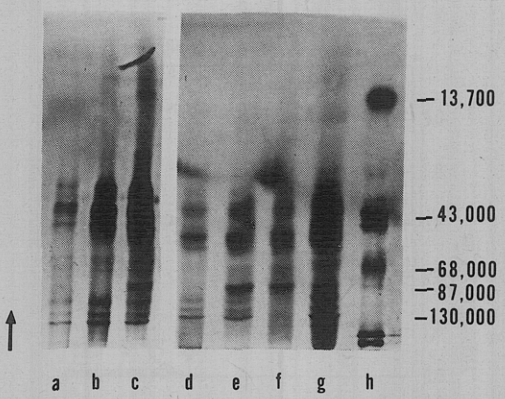

Figure 3. NaDodSO$_4$–PAGE patterns of samples from Figure 1A,B; (a–c) fractions 1–3 from Figure 1A; (d–f) fractions 1–3 from Figure 1B; (g) glutenin (h) standard protein calibration mixture.

such as those eluting at the void volume (130 mL, Figures 1A–E) or just subsequently during gel filtration of the native protein (Figure 1). In NaDodSO$_4$ or NaDod solutions, material comprising fraction A (above) (Huebner and Wall, 1974) elutes under the other two peaks (Figures 2A and 2C) since it consists of lower molecular weight polypeptides. Thus, in these latter solvents no significant aggregation of the reduced protein occurs.

Gliadin, upon chromatography on Sepharose CL-4B in NaDod, elutes as mainly one component in the molecular weight range of 35–38 000 with adjacent trace amounts of higher and lower molecular weight material (Figure 2B). The gliadin elutes long after the bulk of glutenin leaves the column under identical conditions (Figure 1E). Thus, it is apparent that glutenin does not consist of aggregates of gliadin components.

The 70% ethanol-insoluble fraction of PE-glutenin separated as a major peak with three closely associated smaller protuberances in NaDod solvent upon gel filtration (Figure 2C). Alcohol-insoluble PE-glutenin lacks most of the gliadin-like fraction of reduced glutenin, which accounts for the decrease in the size of peak 3 in Figure 2C compared to peak 2 in Figure 2A. Apparently, proteins elute slightly earlier in the NaDodSO$_4$ buffer than in the NaDod. With a NaDodSO$_4$ buffer, a higher pH of 9.8, and the same quantity of protein, the difference is even larger (results not shown). The small difference in elution volumes is probably the result of protein conformational differences in the two solvents. The major peak of the 70% ethanol-insoluble glutenin (Figure 2C) elutes in the same region as fraction 3 of whole glutenin (Figure 1E).

NaDodSO$_4$–PAGE. The NaDodSO$_4$–PAGE patterns of the chromatographic fractions eluted in GHCl at pH 5.5 show some small but important differences (Figures 3a–c). The polypeptide band corresponding to around mol wt 87 000 appears to be more intense in fraction 3c, and a number of low-molecular-weight (10 000 to 30 000) components are present. The amount of 87 000 mol wt components also increases in the lower molecular weight fractions eluted in 7 M urea at pH 3.4 (Figures 3d–f). Conversely, the highest molecular weight subunits are absent in the last fraction (Figure 3f). Evidently, the degree of disulfide linkage varies among the subunits.

In Figures 4a–d are shown the NaDodSO$_4$–PAGE patterns of fractions separated with 7 M urea pH 9.9 (from Figure 1C). Differences occur among subunits present in each fraction. Nearly all of the higher molecular weight components are missing in the last fraction, further confirming the idea that these subunits are probably disulfide linked. NaDodSO$_4$–PAGE patterns of PE-glutenin of the NaDodSO$_4$-insoluble material (glutenin which was not

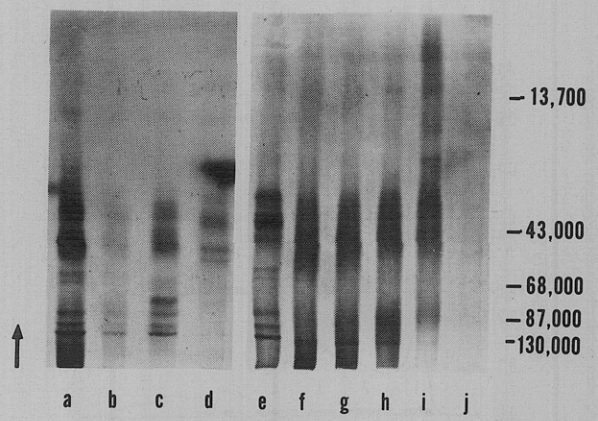

Figure 4. NaDodSO$_4$–PAGE patterns of samples from Figure 1C,D; (a) insoluble glutenin (Table I); (b–d) fractions 1–3 from Figure 1C; (e) insoluble glutenin (Table I); (f–j) fractions 1–5 from Figure 1D.

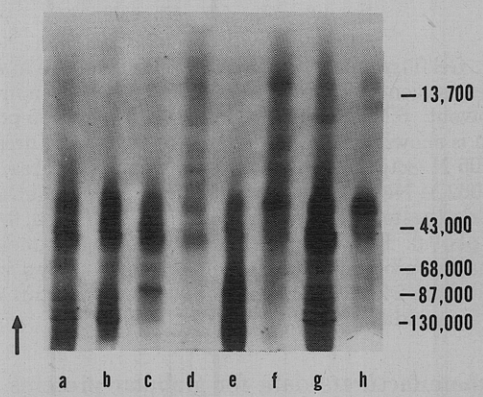

Figure 5. NaDodSO$_4$–PAGE patterns of samples from Figure 1E: (a–d) fractions 1–3, 5, (e,f) fractions 3 and 5 without mercaptoethanol, (g) glutenin, (h) gliadin.

soluble, Table I) and fractions separated on the NaDodSO$_4$ pH 9.8 column are shown in Figures 4e–j. Pattern 4e indicates that the insoluble native protein contains the total subunit structure of glutenin, suggesting that this material results from extensive disulfide linkage of all the polypeptides. The reason for the extensive streaking in patterns f–i is unknown. Fraction 4i contains mainly lower molecular weight proteins.

Figures 5a–d shows the NaDodSO$_4$–PAGE patterns of fractions from native glutenin separated on an agarose column in NaDod pH 9.9 (Figure 1E). The difference in the NaDodSO$_4$–PAGE pattern indicates that NaDod does dissociate some of the glutenin to yield some lower molecular weight proteins. Fraction 3 (Figure 5c) contains, in addition to 87 000 mol wt subunits, polypeptides in the 50 to 30 000 mol wt range, whereas fraction 5 (Figure 5d) contains, in addition to some of the latter subunits, still lower molecular weight materials. The patterns in Figures 5e and 5f are of the same fractions as in 5c and 5d, but without mercaptoethanol to reduce the proteins upon NaDodSO$_4$–PAGE. These patterns of the unreduced proteins indicate that in pattern e, the 30 to 50 000 mol wt are primarily disulfide linked, which restricts their mobility in the gel so they remain close to the origin. These results establish that even some of the lower molecular weight proteins in native glutenin exist as disulfide-linked dimers and trimers of smaller subunits. However, the components of fraction 5 (Figure 1E) migrate readily into the gel as low-molecular weight components as shown in pattern f (Figure 5). Some of these proteins have molecular weights similar to gliadin (h), but others

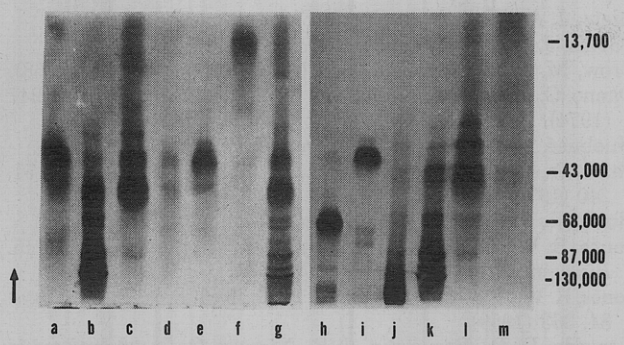

Figure 6. $NaDodSO_4$–PAGE patterns of samples from Figures 2A–C: (a) gliadin; (b,c) fractions 1 and 2 from Figure 2A, (d–f) fractions 1–3 from Figure 2B; (g) glutenin, (h) bovine albumin, (i) ovalbumin, (j–m) fractions 1–4 from Figure 2C.

migrate as lower molecular weight proteins.

Although the reduced glutenin peaks are not completely resolved by gel filtration in $NaDodSO_4$ solution (Figure 2A), the $NaDodSO_4$–PAGE patterns shown in Figures 6b and 6c indicate fractionation of high- and low-molecular-weight components. The elution volumes of the proteins from the Sepharose column in $NaDodSO_4$ are consistent with the molecular weight established by $NaDodSO_4$–PAGE, indicating that little or no aggregation of the subunits occurs in $NaDodSO_4$ solution. Previously, Sephadex separation of reduced glutenin in 4 M urea yielded a high-molecular weight aggregated fraction that is dissociated in $NaDodSO_4$ or NaDod. The subunits of this fraction appear to be distributed through the two fractions obtained on the $NaDodSO_4$ separation as evidenced by the $NaDodSO_4$–PAGE analysis of the fractions (Figures 6b and 6c). The effectiveness of $NaDodSO_4$ in dissociating these proteins would indicate that they were probably associated by hydrophobic bonds.

The $NaDodSO_4$–PAGE pattern of gliadin fractions (Figures 6d and 6f) separated on Sepharose columns with NaDod solutions (Figure 2B) indicates that the higher molecular weight fraction contains 44 000 mol wt subunits that are prevalent in glutenin. The bulk of the main gliadin fraction consisted of 35 to 38 000 mol wt proteins. A trailing fraction consisted of very low-molecular-weight proteins. Despite the broad range of molecular weight separation obtainable on this column, good resolution is also obtained even at this low-molecular-weight range.

The fractions of the 70% ethanol-insoluble portion of PE-glutenin obtained by gel filtration in NaDod solvent (Figure 2C) were analyzed by $NaDodSO_4$–PAGE with results given in Figures 6j–m. Fraction 1 (j) did not migrate far from the origin, indicating that a small fraction of very high molecular weight, possibly 200 000 to 600 000 according to the Figure 2C, was either not reduced or it consists of some longer chain polypeptides. Fraction 2 (k) consisted of high-molecular-weight subunits from 130 000 to 60 000 molecular weight, while fraction 3 consisted mainly of 44 000 and 37 000 subunits. Fraction 4 (pattern m) contained low molecular weight proteins. Proteins that constitute the aggregated fraction A in 4 M urea are evident in all four of these fractions.

Amino Acid Analyses. The amino acid analysis of four fractions of native glutenin obtained by separation on Sepharose using NaDod as solvent was determined (Table II). The fractions differ only slightly in composition. Fraction 5 contains slightly more tryptophan, lysine, and arginine and less glutamic acid than the other fractions. This composition would suggest that this fraction may be richer in the subunits that constitute the aggregated fraction A of the PE-glutenin separated in 4 M urea.

Table II. Amino Acid Analyses of Wheat Glutenin Fractions Separated by Gel Filtration[a]

amino acid	fractions from Figure 1E				A fraction PE-Glu[b]
	1	2	4	5	
Trp	0.3	0.5	0.5	0.7	0.4
Lys	2.0	1.3	1.5	2.1	4.5
His	1.6	1.4	1.8	1.6	2.0
Arg	2.7	2.3	2.7	2.8	3.6
Asp	3.6	2.4	2.8	4.2	8.6
Thr	3.5	3.3	3.2	3.6	4.5
Ser	7.4	7.6	7.8	7.7	7.1
Glu	29.8	33.0	32.6	28.8	16.4
Pro	11.7	12.8	13.9	12.7	7.3
Gly	10.7	11.1	6.0	5.8	8.1
Ala	4.4	3.6	3.6	4.5	7.9
1/2-Cys	1.3	0.8	1.5	1.8	1.5
Val	3.5	3.2	3.8	4.3	5.6
Met	1.5	1.4	1.6	2.1	2.4
Ile	2.7	2.4	3.2	3.5	3.8
Leu	6.9	6.6	7.3	7.7	9.3
Tyr	3.7	3.5	2.1	2.0	3.0
Phe	2.8	2.6	4.2	3.9	4.3

[a] Values shown are as molar percent of the total protein. [b] Sample obtained by method of Huebner and Wall (1974) (Figure 1).

Fractions 1 and 2 show high contents of glycine, which are present in the high-molecular-weight subunits (Fraction B, 4 M urea separation). This observation indicates that the high-molecular-weight proteins contain higher levels of the larger subunits than other fractions of native glutenin and confirms $NaDodSO_4$–PAGE analyses that show that the polypeptide subunits are not uniformly distributed in glutenin.

DISCUSSION

It was observed in these experiments that it was not possible to completely resolubilize isolated purified glutenin even in strong dissociating solvents such as 6 M GHCl or 0.2% $NaDodSO_4$ except at very low protein concentrations. In most previous gel filtration studies, whole gluten was employed and the native glutenin was still mixed with gliadin and other proteins. Whether elimination of 70% ethanol-soluble proteins or whether drying of the protein was responsible for the poor solubility of the isolated glutenin was not determined. Previously Crow and Rothfus (1968) tried to separate native glutenin on Bio-Gel P-300, another gel filtration medium, in 8 M urea. They found that part of the glutenin was not soluble, and the solution was very viscous and difficult to pass through the column. The possibility remains that handling of glutenin favors formation of additional intermolecular disulfide bonds. However, Danno et al. (1974) found that $NaDodSO_4$ did not completely extract all protein from flour and that the $NaDodSO_4$-insoluble protein contained subunits like those in glutenin after reduction. Possibly glutenin may be completely dissolved in dissociating agents in very dilute solutions, further suggesting that aggregation is highly concentration dependent.

The observation that low-molecular-weight proteins could be dissociated from glutenin by $NaDodSO_4$ during gel filtration was first made by Redman (1973). Khan and Bushuk (1977) observed that purified components of unreduced native glutenin migrated into the gel as low-molecular-weight proteins. They concluded that these proteins were associated to other glutenins by hydrophobic bonds. During chromatography of whole gluten in 6 M GHCl, some of these low-molecular-weight proteins may have eluted with the gliadin fraction (Huebner and Wall, 1976). The present study demonstrates that $NaDodSO_4$ or NaDod released more of the glutenin protein as low-

molecular-weight components during gel filtration separation than do urea or GHCl. Since the dissociated proteins are of different molecular weights or are not completely dissociated even in $NaDodSO_4$, they have not been isolated into pure components.

Whether or not these low-molecular-weight fractions separated from glutenin should be classified as glutenin is debatable. The A fraction separated from PE-glutenin is a major contributor to the low-molecular-weight fractions. Thus it appears that the A fraction may not be bonded by intermolecular disulfide links to the B and C fractions (Huebner and Wall, 1974). If it is mainly associated by hydrophobic groups to glutenin, it may be an important factor in gluten aggregation and rapid protein interactions. It has been speculated that this protein is derived from membrane proteins (Simmonds, 1971).

The evidence obtained in these studies still indicates that glutenin is composed of a mixture of components differing in molecular weight. Most of these components are composed of polypeptide chains intermolecularly linked by disulfide bonds to yield chains ranging from 100 000 to 10 million mol wt. From $NaDodSO_4$ patterns of the reduced protein it appears that the different molecular weight fractions vary in subunit composition. The fractions around 100 000 consist mainly of a few C fraction polypeptides. The highest molecular weight fraction consists of the C fractions and the higher molecular weight B subunits, whereas intermediate range fractions contain varying amounts of the lower molecular weight B and C subunits. These results may explain the diversity of glutenin molecular weight and are consistent with previous observations of variations in subunit composition of glutenin solubility fractions (Bietz and Wall, 1973).

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Chemical Coagulation of Industrial Animal Blood Using Aluminum Sulfate, Zinc Sulfate, Methanol, and Acetone

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Industrial whole animal blood samples were treated, under conditions of low dilution, with the protein coagulants aluminum sulfate, zinc sulfate, methanol, or acetone. Aluminum sulfate or methanol treatment resulted in quantitative removal of protein from blood at acidic pH, using the procedure tested. Zinc sulfate also quantitatively removed protein from diluted blood, under basic pH conditions. Acetone was not nearly as effective a protein coagulant as the other reagents tested. The metallic salts used as coagulants produce protein–coagulant complexes which retain significant amounts of water. Similarly, protein coagulated using organic solvents contains significant amounts of solvent. It is concluded that cold blood processing by chemical coagulation holds promise as an economical technique for the production of dried blood suitable for use as an animal feed ingredient.

One of the major byproducts of the meat processing industry is animal blood. Protein is normally reclaimed from animal blood using whole blood drying or steam coagulation techniques (Kramer et al., 1978). Whole blood drying necessitates a large investment in heat energy and results in reduction in the levels of lysine, methionine, and cystine, as well as diminished digestibility of the protein product (Waibel et al., 1977; Kramer et al., 1978). Steam coagulation of blood, followed by separation of the coagulated solids, often results in nonquantitative protein removal and attendant sewage effluent problems (Sanders, 1948). A different approach to blood treatment lies in the development of techniques to remove protein directly from blood by chemical coagulation. We have reported that

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