

Studies of Glutenin. XIV. Gel Filtration and Sodium Dodecyl Sulfate Electrophoresis of Glutenin Solubilized in Sodium Stearate¹

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ABSTRACT

Cereal Chem. 56(6):513-516

Glutenin was dissolved in sodium stearate-water solvent and subjected to gel-filtration chromatography on Sephadex G-150 and G-200, using tris-hydrochloric acid-sodium dodecyl sulfate (SDS) buffer, and on Bio-gel P-300, using tris-borate-SDS buffer as eluants. One major fraction (peak I), eluting in the void volume, and two minor fractions (peaks II and III) were obtained. SDS-polyacrylamide gel electrophoresis (PAGE) showed that

the reduced proteins of peak I contained the total subunit pattern of glutenin, with subunit molecular weights ranging from 12,000 to 134,000. Peak II, contained predominantly the 35,000 and 45,000 subunits, and peak III was predominantly the 12,000 subunit. Gel-filtration and SDS-PAGE results also showed that the disulfide bonds of glutenin are not cleaved by sodium stearate.

Kobrehel and Bushuk (1977) found that glutenin could be completely dissolved in sodium stearate-water to yield a visually clear solution. They suggested that the insolubility of glutenin in other commonly used solvents is due to the very strong aggregation properties of glutenin molecules through hydrophobic interactions. Sedimentation velocity values ($S_{20,w}$) of Kobrehel and Bushuk (1978) indicated that the sodium stearate-solubilized glutenin had a molecular weight of about 40,000–50,000. However, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) results (Bietz and Wall 1972, Orth and Bushuk 1973b, Khan and Bushuk 1977) have showed that reduced glutenin contains many subunits with molecular weights greater than 40,000 or 50,000. It is not clear what happens to these large subunits in the sodium stearate solubilization. Gel-filtration (Bushuk and Wrigley 1971, Huebner and Wall 1976) and sedimentation equilibrium (Jones et al 1961) gave molecular weight values for unreduced glutenin that ranged in the millions. The size of glutenin and the manner in which its components are linked (Kasarda et al 1976, Ewart 1977, Khan and Bushuk 1979a) are, therefore, still not clearly understood.

This study was conducted, first, to determine whether cleavage of disulfide bonds (reduction) of glutenin in the presence of sodium stearate was responsible for the apparent low molecular weights ($S_{20,w}$) of solubilized glutenin as reported by Kobrehel and Bushuk (1978) and, secondly, to determine the molecular weight of the sodium stearate-solubilized glutenin, using gel-filtration and SDS-PAGE techniques.

MATERIALS AND METHODS

Preparation of Glutenin

Glutenin was prepared from flour of the Canadian hard red spring wheat cultivar Manitou by the alcohol-pH precipitation methods of Orth and Bushuk (1973a). This glutenin preparation will be referred to in this paper as total glutenin.

Gel-Filtration Chromatography

Sephadex G-150 and G-200 (Pharmacia) and Bio-gel P-300 (Bio-Rad) were hydrated and packed into 2.0×100 cm glass columns as recommended by the manufacturers. The columns were operated by the downward flow technique. The optical density of the fractions collected was measured with the Carl Zeiss PMQ II spectrophotometer. Appropriate fractions were pooled, dialyzed against distilled water, and freeze-dried.

¹Contribution No. 547, Department of Plant Science, University of Manitoba, with financial assistance from the National Research Council of Canada.

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SDS-PAGE

SDS-PAGE was performed according to Khan and Bushuk (1977, 1979b).

Reagents

Sodium stearate was obtained from Matheson, Coleman, and Bell. Acrylamide, *N,N*-methylenebisacrylamide, and *N,N,N',N'*-tetramethylethylenediamine were obtained from Eastman Chemical Company. SDS and all other chemicals were obtained from Fisher Scientific Company. All chemicals were used as purchased.

RESULTS

Selection of Solvent for Gel Filtration

Total glutenin was solubilized in sodium stearate-water (St-H₂O) and centrifuged to give a clear supernatant (Kobrehel and Bushuk 1977). The clear supernatant became turbid after a short period of standing at room temperature, and eventually a precipitate formed. The clarified sodium stearate solution of Kobrehel and Bushuk (1977) could not be used directly in gel-filtration experiments because of this instability. Therefore, a solvent had to be found with which the St-H₂O-solubilized glutenin could be combined without resultant turbidity or precipitation.

The first solvent tested was tris-hydrochloric acid (HCl)-SDS buffer of pH 8.4. When the clarified St-H₂O-glutenin solution was mixed immediately after centrifugation with an equal volume of tris buffer, and left standing at room temperature, only slight turbidity appeared after 75 hr (Fig. 1C). In the absence of tris buffer, however, turbidity appeared in the solution after as little as 5 hr at room temperature (Fig. 1A). When the St-H₂O-solubilized glutenin was centrifuged at 2°C, the turbidity of the supernatant (Fig. 1B) appeared later and was less prominent than that of the supernatant obtained at room temperature (Fig. 1A). Furthermore, when the 2°C supernatant was mixed with an equal volume of tris buffer, no turbidity was evident even after three days of standing at room temperature (Fig. 1D). Accordingly, for gel filtration, the St-H₂O-solubilized glutenin was centrifuged at 2°C. The supernatant was mixed immediately with an equal volume of 0.2M tris-HCl-SDS (0.2% w/v) buffer, pH 8.4, and then applied to the Sephadex column for chromatography. The eluant used in gel filtration was 0.1M tris-HCl-SDS (0.1% w/v).

Gel Filtration on Sephadex G-150

Glutenin was solubilized in St-H₂O and mixed (1:1) with 0.2M tris-HCl-SDS (0.2% w/v) buffer, pH 8.4. A small amount of blue dextran (Pharmacia) was added to the glutenin solution to obtain an estimate of the void volume. The mixture was applied to the Sephadex G-150 column and chromatographed. The elution profile (Fig. 2A) showed three peaks, with peak I eluting in the void volume.

SDS-PAGE of the unreduced proteins of peak I (Fig. 3A) showed that there was much protein at the point of sample application. Peak I also contained some protein components that entered the gel (indicated by faint bands); these had molecular weights above 68,000. In contrast, the SDS-PAGE pattern of unreduced total glutenin (Fig. 3B) showed protein components in the region of molecular weights 68,000 and lower. Both unreduced preparations (Fig. 3A and B) showed streaking in the region of molecular weights above 134,000. The reduced proteins of peak I (Fig. 3C) showed an SDS-PAGE pattern that was qualitatively identical to that of reduced total glutenin (Fig. 3D).

The unreduced proteins of peak II (Fig. 3E) showed a brightly stained 35,000 mol wt subunit and some faint protein bands in the 90,000 and in the 45,000–12,000 mol wt regions. Upon reduction, peak II proteins (Fig. 3F) showed a prominent 45,000 mol wt subunit, unlike the unreduced sample (Fig. 3E), and also subunits in the 35,000–12,000 mol wt region. These results indicate that peak II proteins contain higher molecular weight components that, upon reduction of their disulfide bonds, produced the well defined 45,000 mol wt subunit. Peak III (Fig. 3G) showed predominantly the lowest molecular weight subunit of glutenin. The mobility of the major subunits in pattern G is slightly lower than that of the corresponding subunits in the other patterns. This is apparently due to interference from the presence of sodium stearate, which migrated at the front edge of these protein bands.

Peak I (Fig. 2A) proteins (dialyzed and freeze-dried) were

dissolved in St-H₂O and rechromatographed on Sephadex G-150 in tris-HCl-SDS buffer. The elution profile (Fig. 2B) was identical to that obtained from the chromatography of total glutenin dissolved in St-H₂O (Fig. 2A). Three peaks were again obtained, with peak I eluting in the void volume. The SDS-PAGE patterns of the unreduced and reduced proteins of peak I (Fig. 4A and B) were qualitatively similar to those of peak I (Fig. 3A and C) from chromatography of total glutenin (Fig. 2A). The SDS-PAGE pattern of peak II from rechromatography (Fig. 4C and D) differed from peak II of Fig. 3 (E and F) in having an extremely faint 45,000 mol wt subunit. Peak III (Fig. 4E and F) contained the 12,000 mol wt subunit, as did peak III of Fig. 3(G). Reduced total glutenin (Fig. 4G) is presented for comparison.

Glutenin was also solubilized directly in 0.1 M tris-HCl-SDS (1% w/v) and chromatographed on Sephadex G-150 and the results were compared with those for glutenin solubilized in St-H₂O first and then transferred into the tris-HCl-SDS buffer. The chromatography conditions were identical for the two solutions.

The elution profile of the buffer-solubilized glutenin (Fig. 2C) also showed three peaks. Peak I eluted in the void volume; peaks II and III eluted at the same positions as peaks II and III (Fig. 2A) of St-H₂O-solubilized glutenin. The unreduced and reduced proteins of peak I (Fig. 5A and B) gave SDS-PAGE patterns similar to those of peak I of the St-H₂O-solubilized glutenin (Fig. 3A and C). The

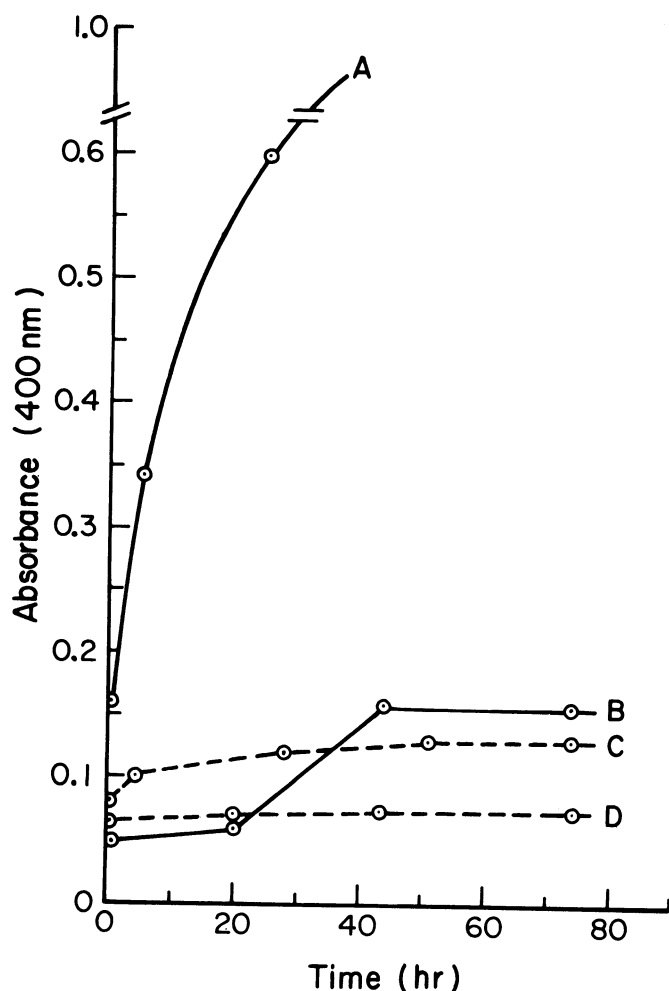


Fig. 1. Turbidity measurements of sodium stearate-water (St-H₂O)-solubilized glutenin. **A**, St-H₂O-solubilized glutenin centrifuged at room temperature; **B**, St-H₂O-solubilized glutenin centrifuged at 2°C; **C**, St-H₂O-solubilized glutenin centrifuged at room temperature and mixed (1:1) with 0.2 M tris-HCl-SDS (0.2% w/v) buffer; and **D**, St-H₂O-solubilized glutenin centrifuged at 2°C and mixed (1:1) with tris-HCl-SDS buffer.

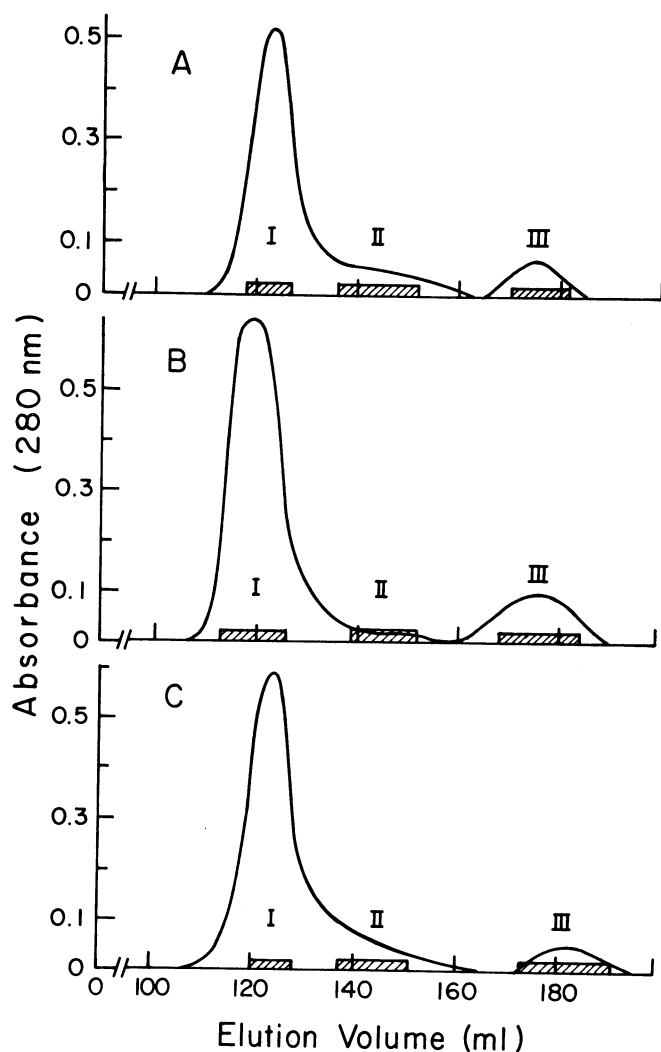


Fig. 2. Elution profiles from gel-filtration chromatography on Sephadex G-150. **A**, glutenin solubilized in sodium stearate-water; **B**, peak I (dialyzed and freeze-dried) from **A** solubilized in sodium stearate-water; and **C**, glutenin solubilized in tris-HCl-SDS. Eluant in all cases was 0.1 M tris-HCl-SDS (0.1% w/v) buffer, pH 8.4. Peaks are indicated by I, II, and III. Shaded areas indicate fractions that were pooled.

reduced proteins of peak II (Fig. 5C), however, differed from peak II of the St-H₂O-solubilized glutenin (Fig. 3F) in having substantially less of the 45,000 mol wt subunit. Peak III (Fig. 5E and F) contained predominantly the 12,000 mol wt subunit just as did peak III of the St-H₂O-solubilized glutenin (Fig. 3G). In summary, the gel-filtration results for the glutenin in buffer and the St-H₂O-solubilized glutenin are similar. The only difference is in the SDS-patterns of the peak II fraction.

Gel Filtration on Sephadex G-200

Sephadex G-200 was examined for gel-filtration of St-H₂O-solubilized glutenin because of its higher exclusion limit (>G-150), in an attempt to fractionate peak I (Fig. 2A) proteins. The elution profile (Fig. 6) showed predominantly one large peak (I), which eluted in the void volume, and a small tailing peak (II).

SDS-PAGE showed that the unreduced proteins of peak I (Fig. 7A) contained much protein that remained at the point of sample application. There were a number of faintly stained protein components in the gel and some streaking in the region of molecular weight above 134,000. The reduced proteins of peak I (Fig. 7B) gave an SDS-PAGE pattern that was very similar to that of reduced total glutenin (Fig. 7E). The SDS-PAGE pattern of the unreduced peak II proteins (Fig. 7C) showed more protein components in the 90,000–45,000 mol wt region and much fainter bands of 35,000 and 12,000 mol wt, unlike the unreduced peak II

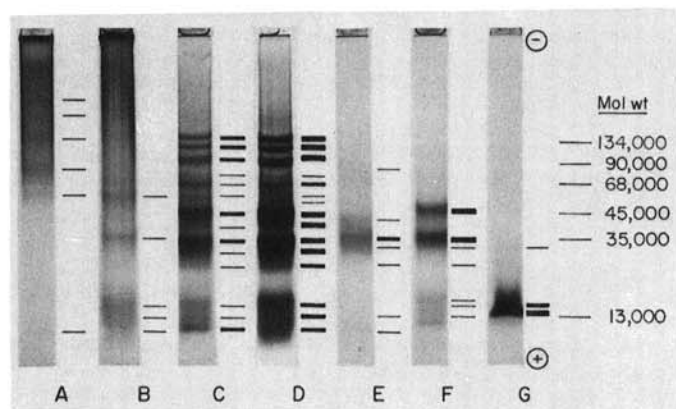


Fig. 3. SDS-PAGE of protein fractions from gel filtration on G-150 of St-H₂O-solubilized glutenin. A, unreduced peak I (Fig. 2A) proteins; B, unreduced total glutenin; C, reduced peak I proteins; D, reduced total glutenin; E, unreduced peak II proteins; F, reduced peak II proteins; and G, reduced peak III proteins.

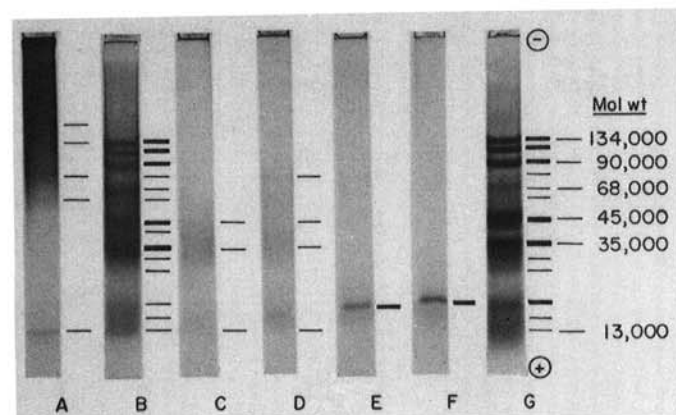


Fig. 4. SDS-PAGE of protein fractions from gel filtration on G-150 of peak I (Fig. 2A) proteins. A, unreduced peak I (Fig. 2B) proteins; B, reduced peak I (Fig. 2B) proteins; C, unreduced peak II proteins; D, reduced peak II proteins; E, reduced peak III proteins; F, unreduced peak III proteins; and G, reduced total glutenin.

proteins (Fig. 3E) obtained from G-150. Upon reduction (Fig. 7D), peak II proteins showed bands in the 45,000–12,000 mol wt region. These results are similar to those obtained with Sephadex G-150 in that the majority of glutenin eluted in the void volume, indicating that most of glutenin dissolved in St-H₂O-solvent is of considerably higher molecular weight (particle weight) than is the largest subunit of reduced glutenin.

Gel Filtration on Bio-gel P-300

Tris-borate-SDS buffer was used as an alternate solvent to the tris-HCl-SDS in gel-filtration of the St-H₂O-solubilized glutenin to examine possible solvent effects on elution profile and SDS-PAGE patterns. Because boric acid and saccharides form complexes (Whistler 1965), Sephadex cannot be used with tris-borate-SDS buffer. For this reason Bio-gel P-300 was used. Preparation of glutenin in St-H₂O and chromatographic conditions were identical to those used for gel filtration on Sephadex G-150 and G-200. The elution profile and SDS-PAGE patterns (not shown) were identical to those obtained for experiments with Sephadex G-200 and tris-HCl-SDS buffer.

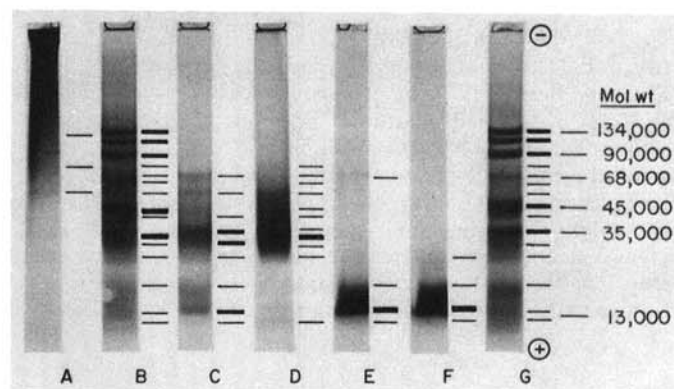


Fig. 5. SDS-PAGE of protein fractions from gel filtration on G-150 of glutenin solubilized in tris-HCl-SDS buffer. A, unreduced peak I (Fig. 2C) proteins; B, reduced peak I (Fig. 2C) proteins; C, reduced peak II proteins; D, unreduced peak II proteins; E, reduced peak III proteins; F, unreduced peak III proteins; and G, reduced total glutenin.

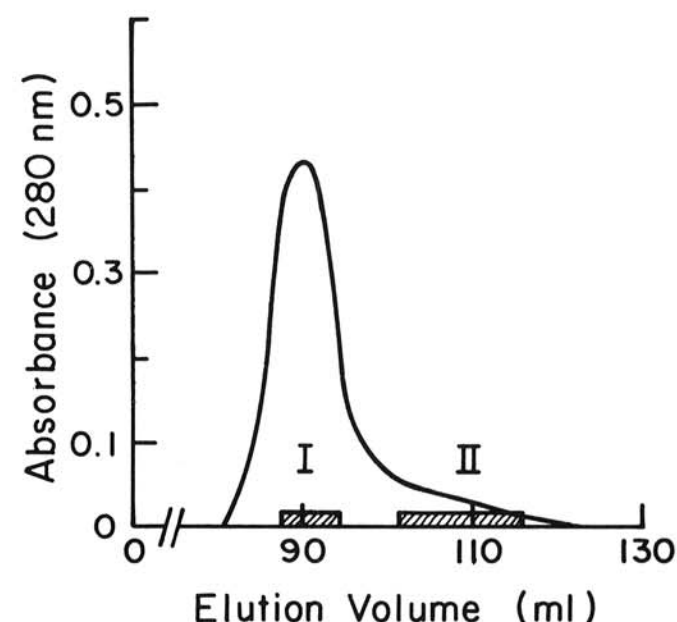


Fig. 6. Elution profile from gel-filtration chromatography on Sephadex G-200 of St-H₂O-solubilized glutenin. Eluant was tris-HCl-SDS. Peaks are indicated by I and II. Shaded areas indicate fractions that were pooled.

DISCUSSION

Gel-filtration results show that most of the sodium stearate (St-H₂O)-solubilized glutenin elutes in the void volume on Sephadex G-150 and G-200 and on Bio-gel P-300. This indicates that the proteins in the void volume fraction have a molecular weight above 200,000. Kobrehel and Bushuk (1977, 1978) postulated that the high molecular weight obtained for glutenin (Jones et al 1961, Bushuk and Wrigley 1971, Huebner and Wall 1976) was a result of incomplete dissociation of glutenin aggregates comprising subunits linked by very strong hydrophobic interactions. Gel-filtration results of this study, however, showed that even though St-H₂O solubilized glutenin to form a visually clear solution, the resulting solute particles were still so large that they were eluted in the void volume of Sephadex G-150 and G-200 and of Bio-gel P-300. Furthermore, on SDS-PAGE, essentially all the protein of this fraction remained at the point of sample application. These results also indicate that St-H₂O does not dissolve (dissociate) all the glutenin to the level of subunits obtained by chemical reduction (Bietz and Wall 1972, Orth and Bushuk 1973b, Khan and Bushuk 1977) of the disulfide bonds of the glutenin. Nevertheless, gel filtration and SDS-PAGE showed that St-H₂O-solubilized glutenin contains species with molecular weights in the range 12,000–90,000, which were previously obtained only after reduction. This supports the hypothesis, proposed previously (Khan and Bushuk 1979a), that glutenin is basically an aggregate of disulfide crosslinked molecules and single chain molecules (perhaps containing intrachain disulfide bonds). The aggregation forces are apparently disrupted by the stearate to produce the results reported in this and previous papers (Kobrehel and Bushuk 1977, 1978). The glutenin aggregate can also be disrupted to varying extents by other solvents such as the two used in the present study. The fact that SDS, like the soaps used previously, is also effective in disrupting the aggregate supports the hypothesis that

hydrophobic interactions contribute substantially to the stability of the aggregate.

The molecular weights for stearate-solubilized glutenin estimated from gel-filtration and SDS-PAGE of the present study do not agree with the value reported previously (Kobrehel and Bushuk 1978), which was estimated from the sedimentation coefficient ($S_{20,w}$). The most plausible explanation of this discrepancy is that in the ultracentrifuge, the high molecular (particle) weight protein (peak I in gel filtration) sediments to the bottom of the centrifuge cell and therefore is not seen in schlieren pattern when constant speed is reached. The value of 40,000–50,000 reported previously (Kobrehel and Bushuk 1978) could be an average for peak II and III proteins. There may be other explanations. Glutenin has many peculiar properties such as its asymmetry (Wu and Dimler 1964), so that abnormal behavior in gel filtration or sedimentation diffusion would not be unexpected.

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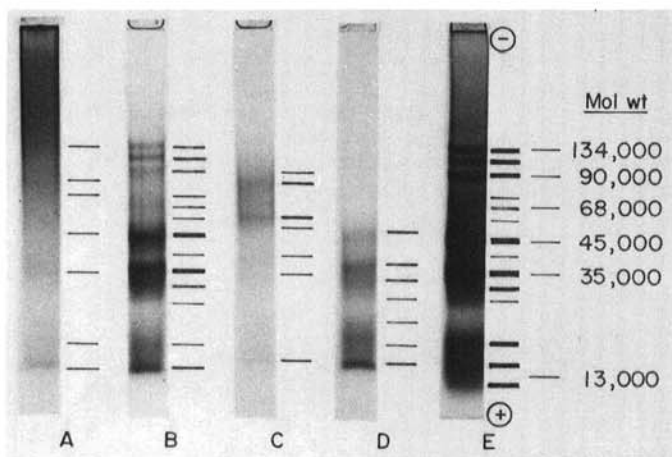


Fig. 7. SDS-PAGE of protein fractions from gel filtration on G-200 of St-H₂O-solubilized glutenin. A, unreduced proteins of peak I (Fig. 6); B, reduced proteins of peak I (Fig. 6); C, unreduced peak II proteins; D, reduced peak II proteins; and E, reduced total glutenin.

[Received January 12, 1979. Accepted April 18, 1979]