Changes in Flour Proteins During Dough Mixing[1]

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ABSTRACT

Changes in extractability and gel filtration of wheat flour proteins during dough mixing were studied. Protein was extracted from flours and doughs with 1% sodium dodecyl sulfate (SDS, pH 7.0). Only 72.6% of the total nitrogen was extracted from a high-protein flour, but dough mixing increased the extractability of its protein to 95%. The additional protein extracted as a result of mixing was mainly glutenin, as shown by SDS-polyacrylamide gel electrophoresis. The molecular size distribution of the SDS-soluble protein was studied by gel filtration on a Sepharose CL-4B column with 0.05*M* Tris-HCl buffer (pH 7.0) containing 0.1% SDS for elution. The material eluted at the void volume on the column contained no protein. Glutenin extracted as a result of optimum mixing was eluted near but not at the void volume of the column. Similar elution profiles were obtained from SDS extracts of doughs that were mixed with rheologically active compounds such as *N*-ethylmaleimide, potassium iodate, fumaric acid, or ferulic acid. The increase in extractability of protein during dough mixing was not related to a decrease in protein size.

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The amount of protein extracted from doughs with dilute acetic acid increases because the size of the protein decreases during dough mixing (Mecham et al 1962, Tanaka and Bushuk 1973a, Tsen 1967). That mechanism was supported by evidence that extractability increased markedly when doughs were treated with sulfhydryl-blocking reagents or iodate (Mecham 1968, Tanaka and Bushuk 1973a, Tsen 1969).

Recent work with sodium dodecyl sulfate (SDS) as a solvent (Danno and Hoseney 1981) showed a similar increase in protein solubility caused by mixing. The additional protein had a high viscosity. The protein extracted from overmixed doughs had a lower viscosity. Thus, that solubility apparently increases before the proteins are reduced in size.

The following work was undertaken to determine whether changes in molecular size and protein solubility as a result of mixing were related.

MATERIALS AND METHODS

Flour and Chemicals

The following flours were used in this study: SRA (19.5% protein, 0.66% ash), KSU (10.0% protein, 0.37% ash), and UP flour (11.9% protein, 0.47% ash). SRA flour was experimentally milled from a wheat variety furnished by Seed Research Associates, Scott City, Kansas, and KSU flour was milled on the KSU experimental mill from a composite of hard winter wheats; UP was a commercially milled patent flour. None of the flours was defatted. The water-insoluble fraction of SRA flour, gluten plus starch (G + S), was prepared according to the method of Schroeder and Hoseney (1978). SDS (99% pure) was obtained from Polysciences Inc., Warrington, PA. All other chemicals were of reagent grade.

Doughs

A 10-g mixograph was used to mix the doughs (Finney and Shogren 1972). After being mixed, the doughs were immediately frozen, lyophilized, and finely ground. Rheologically active reagents were dissolved in dough water; fumaric acid and ferulic acid solutions were neutralized to pH 7.0 with 0.5*N* sodium hydroxide.

Protein Extraction

Proteins were extracted from flour and dough without mechanical stirring or homogenization because violent mixing during extraction increases solubility of glutenin from wheat flour (Danno 1981). Flour or ground dough (500 mg) was weighed directly into a 50-ml centrifuge tube, and 15 ml of 1% SDS (pH 7.0) was added. The slurry was gently stirred by hand with a glass rod for 2 hr at 10-min intervals at room temperature. The slurry was then centrifuged 10 min at 10,000 × *g* at 25° C. The supernatant was decanted to another centrifuge tube and recentrifuged 20 min at 17,000 × *g* at 25°C. The clear supernatant was decanted and designated the SDS-soluble fraction. The two residues (sediments) were combined and dispersed in 10 ml of 1% SDS containing 1% 2-mercaptoethanol (ME, pH 7.0). After standing 2 hr at room temperature, the slurry was centrifuged 20 min at 17,000 × *g* at 25°C. The clear supernatant was designated 2-ME-soluble fraction. The final residue was essentially a starch fraction. The procedure is summarized in Fig. 1. Protein (N × 5.7) of the supernatant was determined by a micro-Kjeldahl method and protein of starch residue by a macro-Kjeldahl method.

SDS-Polyacrylamide Gel Electrophoresis (PAGE)

SDS-PAGE was performed on 10% polyacrylamide gel slabs (0.75 mm thick) with the discontinuous buffer system of Laemmli (1970). The protein samples were mixed with an equal volume of solution containing 1% SDS, 4% 2-ME, 0.125*M* Tris (pH 6.8), 0.005% bromophenol blue, and 20% glycerol. The protein solutions were heated at 80° C for 5 min before they were applied on gels. The gels were stained with 0.04% Coomassie brilliant blue R-250 in 10%

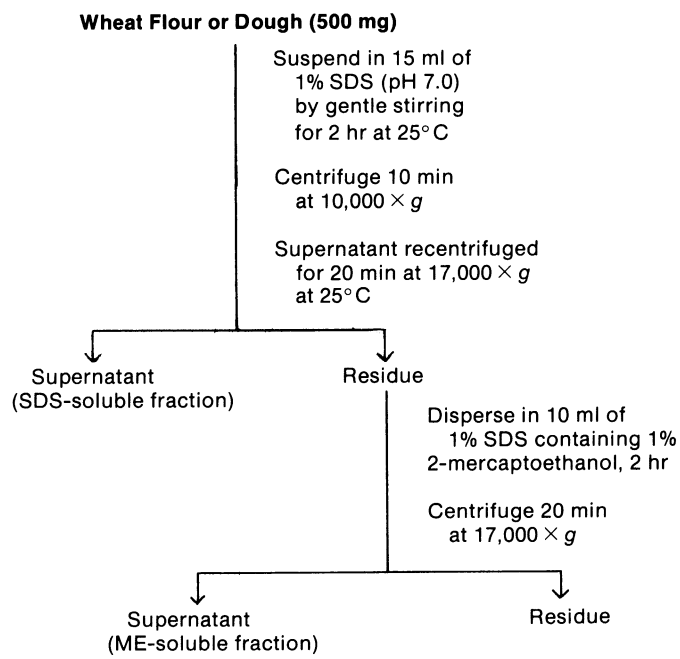

Fig. 1. Scheme for extracting proteins from wheat flours and doughs.

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0009-0352/82/04024905/$03.00/0
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trichloroacetic acid for at least 20 hr at 40°C and then destained with 7% acetic acid. The gels were scanned at 570 nm with a Spectrodensitometer model SD 3000 (Schoeffel Instrument Co.).

Gel Filtration

The Sepharose CL-4B (Pharmacia) column (2.5 × 41 cm) was equilibrated with 0.05*M* Tris-HCl buffer (pH 6.8) containing 0.1% SDS and 0.02% sodium azide. Three milliliters of protein solution (15 to ~20 mg of protein) was applied to the column, and the flow rate was 15 ml/hr at room temperature. Proteins in the effluent were determined by absorbance at 280 nm with an LKB UV-monitor, Uvicord II.

Carbohydrate Determination

The phenol-sulfuric acid method of Dubois et al (1956) was used to estimate the carbohydrate content of column chromatography eluate. Absorbance was measured at 480 nm.

RESULTS

Protein Extractability

Proteins from wheat flours and mixed doughs were extracted with 1% SDS (pH 7.0) under gentle stirring at room temperature. As shown in Table I, the amount of protein extracted with 1% SDS was increased as a result of mixing. Only 72.6% of the total flour nitrogen was extracted from SRA flour; however, when the flour was mixed to a dough (3 min), 93.5% of the total nitrogen was extracted. Extracted protein from the various SDS-soluble fractions of KSU flour, commercial patent flour, and gluten plus starch of SRA flour was consistently less than the extracted protein from SRA flour; however, extracted protein from all the overmixed doughs (15 min mixing) was fairly constant (94–96%).

TABLE I
Percentages of Nitrogen Extracted from Wheat Flour and Dough

Dough Sample Mixing Time (min)	Fractions		
	SDS-Soluble[a] (%)	ME-Soluble[b] (%)	Residue (%)
KSU flour	63.8	33.5	2.7
4	89.7	8.0	2.3
15	93.9	3.5	2.5
UP flour[c]	66.0	31.1	2.9
15	93.7	3.8	2.5
SRA flour	72.6	25.9	1.5
3	93.5	4.5	2.0
15	95.0	3.1	1.9
SRA, G + S[d]	69.6	28.4	2.0
5	87.3	10.6	2.1
15	94.9	3.4	1.7

[a] Soluble in sodium dodecyl sulfate.
[b] Soluble in sodium dodecyl sulfate plus 2-mercaptoethanol.
[c] Untreated patent flour.
[d] Gluten plus starch fraction from SRA flour.

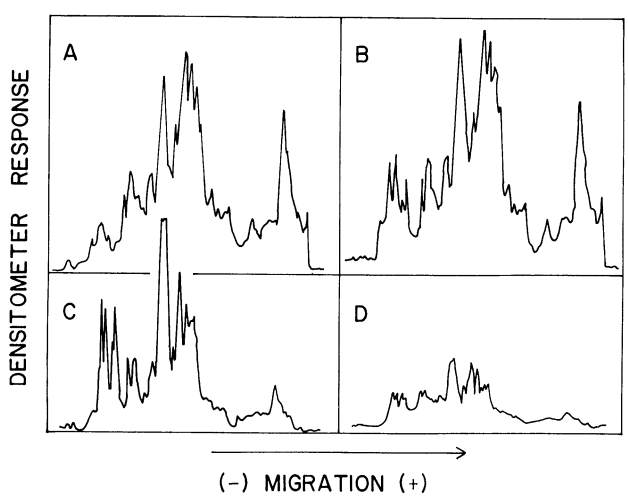

Fig. 2. Densitometer tracings of sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoretic patterns from fractions soluble in SDS and mercaptoethanol (ME). **A**, SDS extract of flour; **B**, SDS extract of overmixed dough; **C**, SDS + ME extract of flour; **D**, SDS + ME extract of overmixed dough.

Figure 2 shows densitometer tracings of the SDS-PAGE patterns of the reduced proteins from extracts of SRA flour and overmixed dough. The patterns show that the slower-moving high molecular weight proteins were not extracted from flour with SDS; however, they were almost all extracted from mixed dough. Attempts to study SDS extracts by SDS-PAGE failed, apparently because the high molecular weight proteins were too large to migrate in the SDS-PAGE system unless the protein was first reduced with ME. Similar results were obtained from the KSU flour, the patent flour, and the gluten plus starch from SRA flour (data not shown). Our results are consistent with the work of Danno (1981), who found that the SDS-insoluble protein of flour was mainly glutenin.

Molecular Size Distribution of SDS-Soluble Fraction

A Sepharose CL-4B column was used to determine the molecular size of SDS-soluble proteins extracted from SRA flour and its doughs. Satisfactory results were obtained with 0.05*M* Tris-HCl buffer (pH 6.8) containing 0.1% SDS and 0.02% sodium azide as an eluting buffer (Fig. 3). The eluted proteins were analyzed by SDS-PAGE after their disulfide bonds were reduced (Figs. 4–6).

The gel filtration pattern from the SDS extract of SRA flour

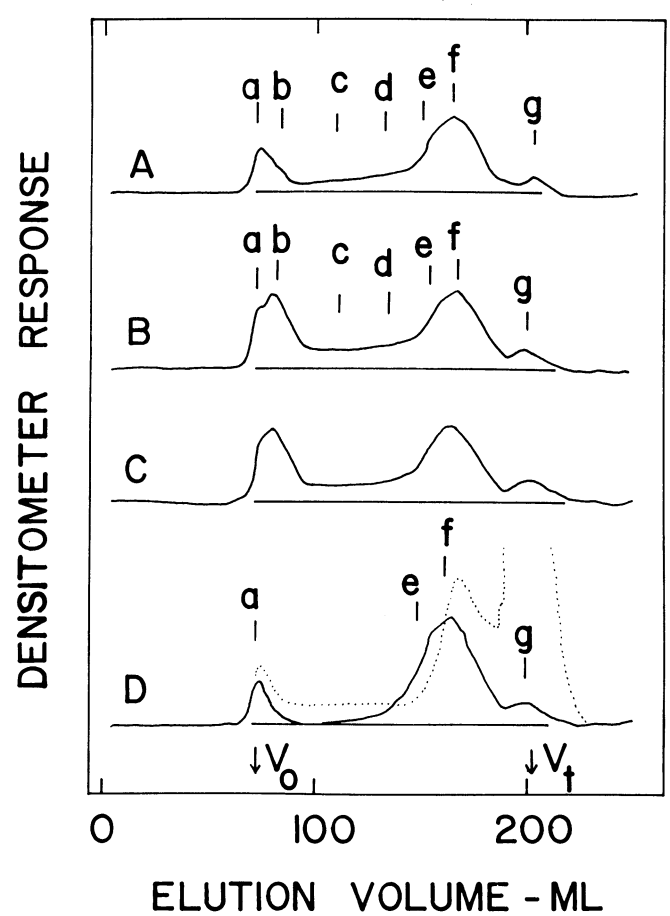

Fig. 3. Gel filtration patterns of the sodium dodecyl sulfate (SDS) extracts from SRA flour and its doughs eluted on a Sepharose CL-4B column with 0.05*M* Tris-HCl buffer (pH 6.8) containing 0.1% SDS and 0.002% sodium azide. **A**, flour; **B**, dough mixed 3 min; **C**, dough mixed 15 min; **D**, mercuric-chloride treated sample of the SDS extract from dough mixed 15 min. Solid line indicates protein (280 nm). Dotted line indicates carbohydrate (480 nm); a, b, c, d, e, f, and g correspond to samples taken in Figs. 4–7.

gave three peaks (Fig. 3A). The first peak (a) was eluted at the void volume, which indicates that the protein has a molecular weight of more than 5,000,000 (Pharmacia Handbook). Similar elution profiles were obtained by Huebner and Wall (1976). They extracted wheat flour with an AUC solvent of 0.1 *M* acetic acid, 3 *M* urea, and 0.01 *N* hexadecyltrimethylammonium bromide and fractionated the protein on a Sepharose 4B column using 5.5 *M* guanidine hydrochloride as elution solvent.

Gel filtration patterns from the SDS extracts of SRA doughs gave a new peak (b) eluting slightly after the void volume (Fig. 3B and C). Proteins contained in the new peak were confirmed by

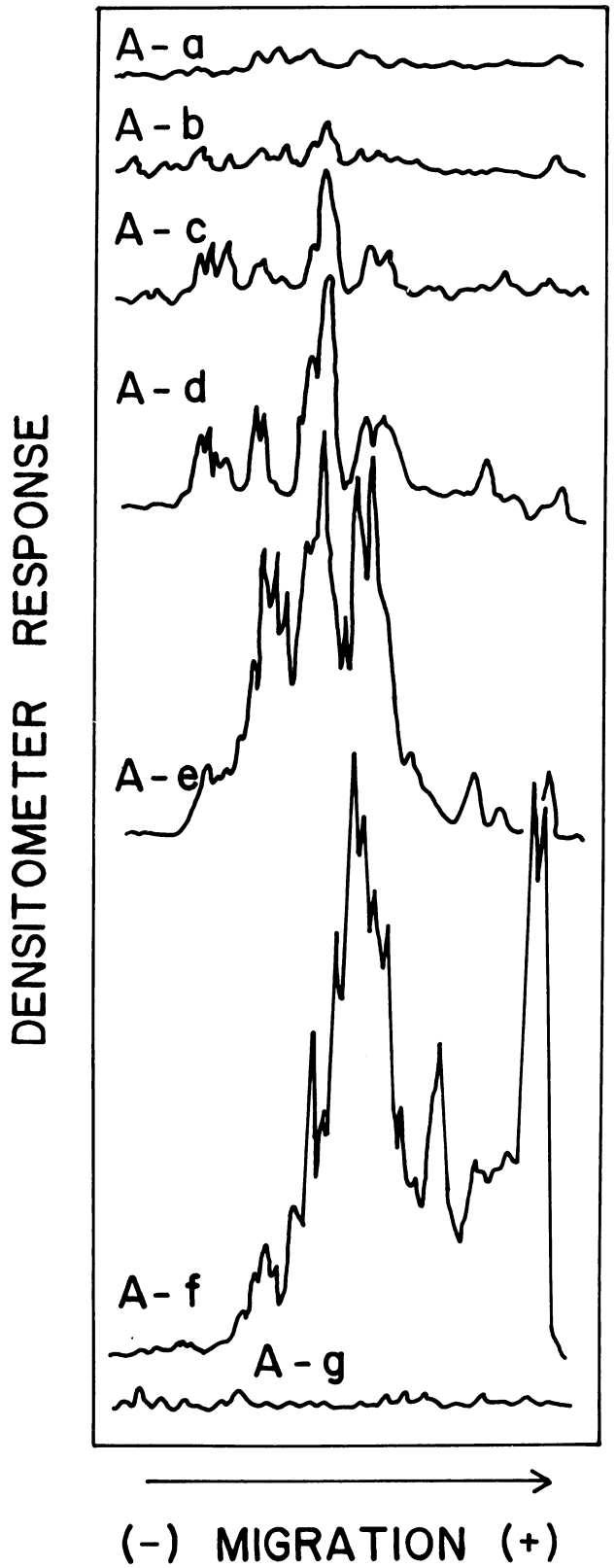

Fig. 4. Densitometer tracings of sodium dodecyl sulfate gel electrophoretic patterns from the fraction obtained from Fig. 3A. A-a through A-g correspond to extracts in Fig. 3A.

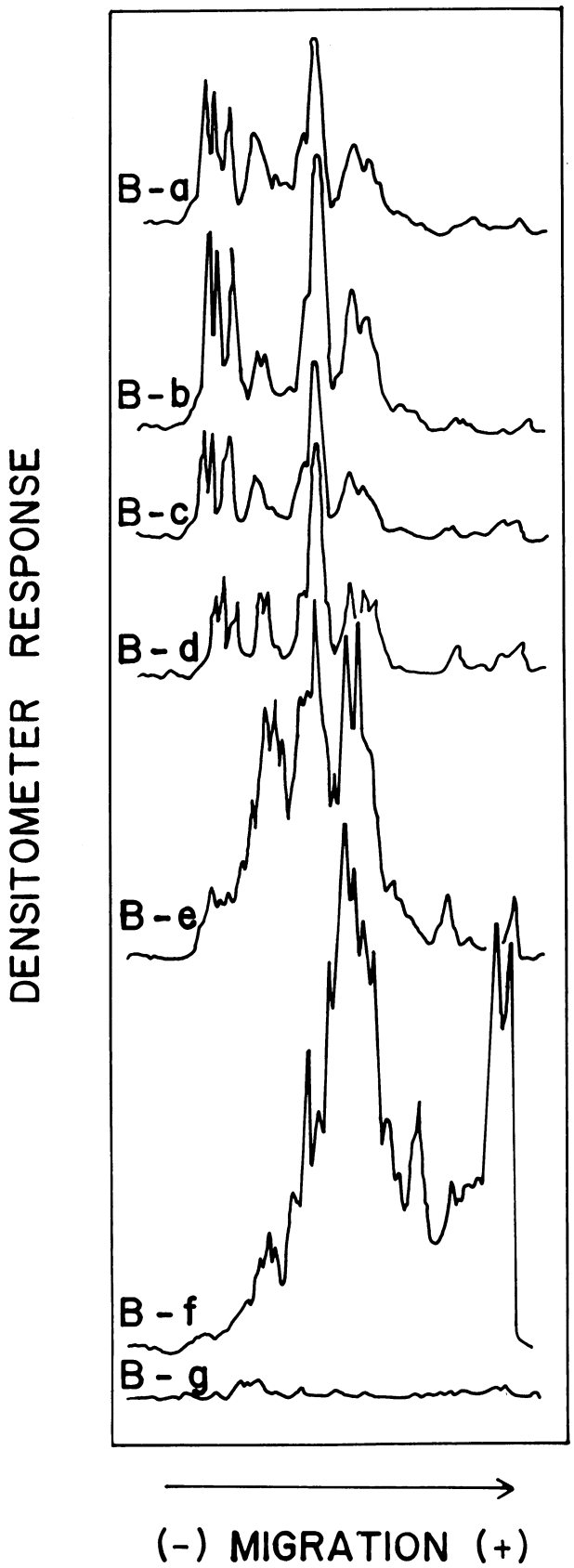

Fig. 5. Densitometer tracings of sodium dodecyl sulfate gel electrophoretic patterns from the fractions obtained in Fig. 3B. B-a through B-g correspond to extracts in Fig. 3B.

SDS-PAGE to be glutenin (Fig. 5B). This indicates that glutenin can be extracted with SDS; however, the protein retains a large molecular size.

Most of the proteins in fractions A-e and B-e (Figs. 4 and 5) were precipitated with 70% ethanol and eluted near the void volume on Sephadex (G-100); however, this fraction differs from glutenin in its SDS-polyacrylamide gel patterns (Danno 1981). Fractions f and g (Figs. 3–5) were gliadin and nonprotein materials, respectively.

Disulfide bonds of SDS-extracted proteins from dough mixed 3 min (Fig. 3B) were cleaved with mercuric chloride according to the method of Danno et al (1975). As shown in Fig. 3D, the glutenin peak (b) eluting near the void volume (3Bb), and the flat area following that peak (containing fraction c and most of d) disappeared after cleavage of the disulfide bonds; however, the first peak (a) remained at the void volume after treatment with mercuric chloride. Relatively little protein was found in the first peak by either SDS-PAGE electrophoresis (Fig. 6) or ultraviolet (UV) absorption (Fig. 7). The spectrum of this peak resembled that of wheat pentosans (Yeh et al 1980). The void-volume peak (Fig. 3) contained carbohydrates and might be pentosans and/or other material. Analogous results were obtained in the extract from flour (data not shown).

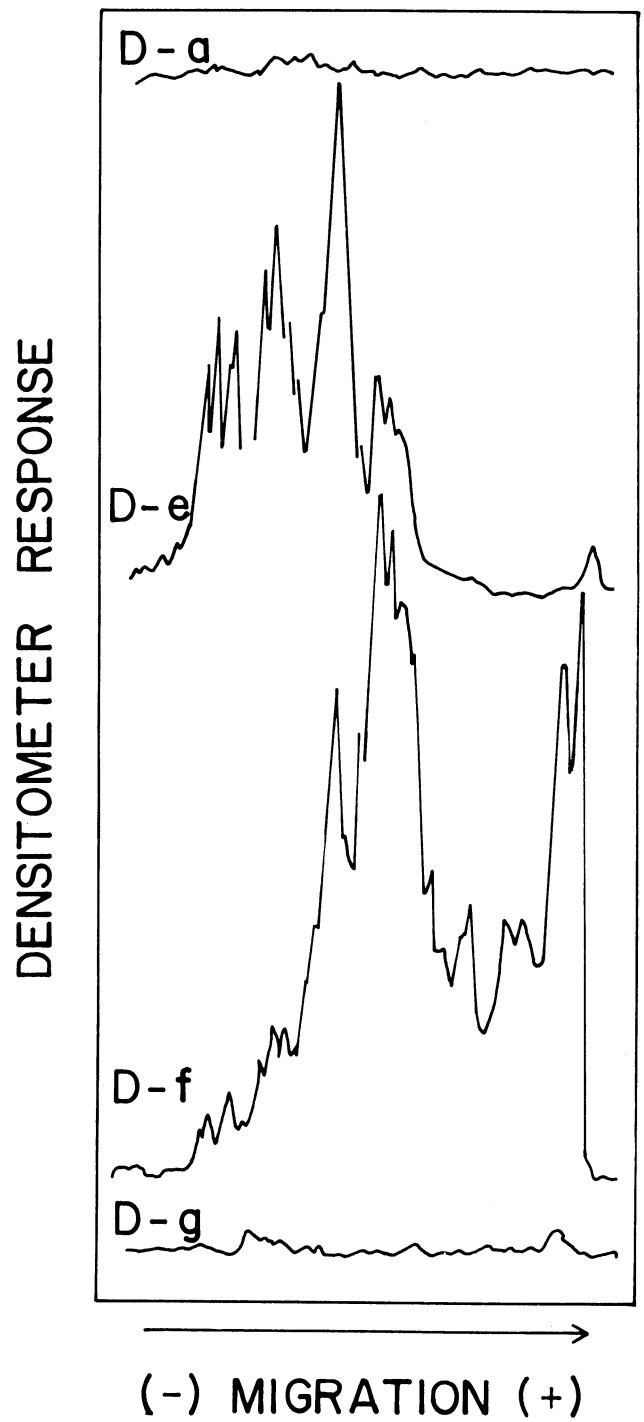

Fig. 6. Densitometer tracings of sodium dodecyl sulfate gel electrophoretic patterns from the fraction obtained in Fig. 3D. D-a, D-e, D-f, and D-g correspond to extracts in Fig. 3D.

Effect of Rheologically Active Compounds

SDS extracts from SRA doughs that were mixed with potassium iodate, potassium bromate, cysteine, *N*-ethylmaleimide (NEMI), fumaric acid, or ferulic acid, then subjected to gel filtration. Despite the presence of these rheologically active compounds, the elution profiles of the extracts were essentially the same as those obtained from the control SRA dough (Fig. 3).

DISCUSSION

Proteins from SRA flour and mixed doughs were extracted with SDS; the amount of protein extracted increased from 72.6 to 95.0% after mixing. Similar results were obtained by Mecham et al (1962), Tsen (1967), and Tanaka and Bushuk (1973b), using dilute acetic acid as a solvent.

When proteins were extracted with SDS from SRA flour, the extract contained no appreciable amounts of glutenin; however, proteins in the residue were mainly glutenin (Danno 1981, Danno et al 1976). Gel filtration chromatography on a Sepharose CL-4B column showed that the extract from SRA flour gave three peaks; the peak eluted at the void volume but did not contain appreciable amounts of protein, as shown by either SDS-PAGE after reduction of its disulfide bonds or UV-absorption.

The additional protein extracted after mixing was glutenin, as shown by SDS-PAGE after reduction of the disulfide bonds with ME. SDS is a good solvent for protein because it disrupts both hydrogen and hydrophobic bonds. Glutenin protein solubilized as a result of mixing retained a large molecular weight in SDS but gave a much smaller molecular weight after being reduced with mercuric chloride or ME. This finding strongly suggests that

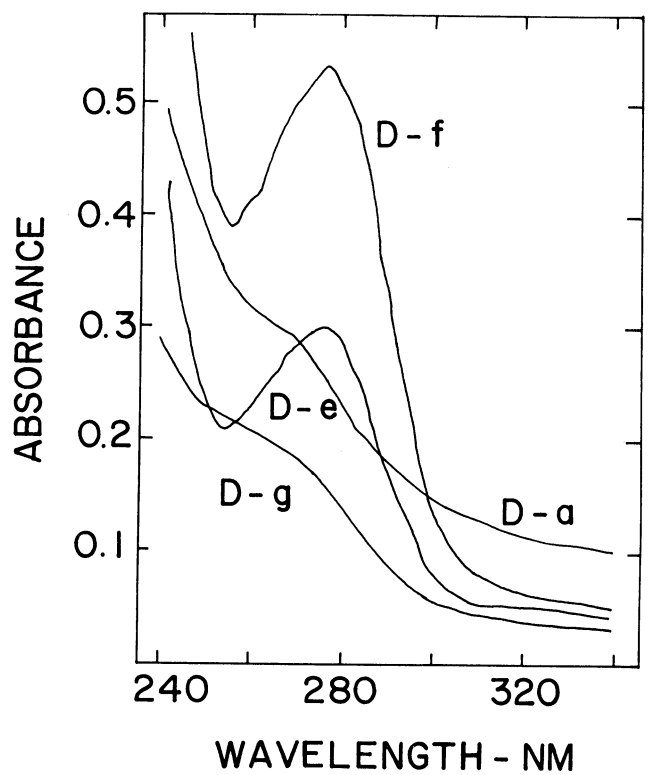

Fig. 7. Ultraviolet absorption spectra of fractions obtained from Fig. 3D. D-a, D-e, D-f, and D-g correspond to extracts in Fig. 3D.

glutenin has intermolecular disulfide bonds. The completely intramolecular disulfide bonded structure suggested by Kasarda et al (1976) does not appear to be valid unless noncovalent bonds exist that are not broken with SDS.

Gluten extracted with SDS from optimally mixed dough was shown by gel filtration to be of a high molecular weight, having eluted near the void volume as a sharp peak on a Sepharose CL-4B column. Similarly, SDS extracts from overmixed doughs gave essentially the same elution profile, even though those proteins had a lower viscosity (Danno and Hoseney 1982). Similar results were also obtained from SDS extracts of doughs mixed in the presence of such rheologically active reagents as potassium iodate, fumaric acid, ferulic acid, or NEMI. Although the few disulfide bonds broken during overmixing (Sidhu et al 1980) are plentiful enough to affect viscosity, they cannot be seen by gel filtration. The Sepharose column, not particularly sensitive to changes in molecular weight, may not reflect those changes.

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[Received July 23, 1981. Accepted February 4, 1982]