Studies of Glutenin Solubilized in High Concentrations of Sodium Stearate[1]

R. J. WASIK[2], H. DAOUST[3], and C. MARTIN[2]

Cereal Chem. 56(2):90-94

ABSTRACT

Solubilization of freeze-dried glutenin in water in the presence of sodium stearate at 21 and 45°C was studied by viscometry and electrophoresis at temperatures between 30 and 50°C. Specific viscosity determined at 50°C and at one glutenin concentration indicates that, after incubation at 45°C for 48 hr, the protein molecules are disaggregated into smaller particles that do not appear to change in size or shape between 30 and 50°C. Sodium dodecylsulfate-polyacrylamide gel electrophoresis patterns of glutenin solubilized by sodium stearate differ both quantitatively and qualitatively from the pattern obtained from glutenin solubilized by disulfide bond cleavage with β-mercaptoethanol, suggesting that disulfide bonds are not cleaved during solubilization in sodium stearate. Solubilization of glutenin in water in the presence of soaps can be explained by peptization of glutenin through the formation of protein-detergent complexes that are soluble in water. It is unnecessary to postulate the presence of many intermolecular disulfide bonds for the insolubility of glutenin in pure water or other mild solvents.

Glutenin is the most important protein fraction in wheat destined for bread making (Bushuk 1974). It is the only constituent that possesses the viscoelasticity to which wheat owes its industrial importance (Ewart 1972).

In 1972 Bietz and Wall (1972) demonstrated by electrophoresis in the presence of sodium dodecyl sulfate that glutenin, after reduction with β-mercaptoethanol, is a mixture of about 15 different subunits in the range of 133,000 to 11,600 Daltons. This has been confirmed by different investigators (Kasarda et al 1976, Orth and Bushuk 1973b, Wasik and Bushuk 1975).

There have been several refinements in methods of preparing glutenin (Kasarda et al 1976), but its limited solubility has largely restricted research to studies on the subunits produced by disulfide reduction (Bietz and Wall 1972, Orth and Bushuk 1973b, Wasik and Bushuk 1975). Two theories have been advanced for the structure of glutenin. Ewart (1972, 1977) suggested an idea similar to that proposed by Nielsen et al (1962) and Beckwith and Wall (1966), a belief that glutenin is held together primarily by intermolecular disulfide bonds. This hypothesis is supported by a large collection of research on glutenin in which the disulfide bonds were cleaved.

Kasarda et al (1976), however, stressed the hypothesis that the disulfide bonds within glutenin are intramolecular. These intramolecular disulfide bonds arrange the conformation of subunits in a manner that allows noncovalent (hydrophobic) bonding to other subunits with the correct conformation. This hypothesis was supported by the discovery that glutenin could be solubilized at high concentrations of sodium stearate or sodium palmitate (Kobrehel and Bushuk 1977). This suggests that hydrophobic interactions are involved since the solubilization technique is not believed to disrupt disulfide bonds.

This article presents results showing that glutenin is extensively disaggregated when dissolved in high concentrations of sodium stearate and that the subunits produced differ from those produced by disulfide cleavage.

MATERIALS AND METHODS

Glutenin

The glutenin used was isolated from flour of the Canadian hard red spring wheat cv. Manitou by the pH-precipitation method of Orth and Bushuk (1973a) and freeze-dried.

Chemicals

All chemicals used were reagent grade. Water was distilled and deionized before use in all cases.

Solubilization Procedure for Sodium Stearate

Glutenin samples were prepared with sodium stearate or sodium dodecyl sulfate (SDS) according to the method of Kobrehel and Bushuk (1977). Samples (25 mg of glutenin and 20 mg of soap in 5.0 ml of H_2O) were mixed for 24 and 48 hr at 21 and 45°C.

Viscosity Measurements

A Cannon-Ubbelohde, semimicro, calibrated, dilution viscometer, size 75, was used to measure the viscosities of the glutenin samples. The viscometer was immersed in a circulating water bath controlled to ±0.01°C.

Viscosity measurements were made three or more times on each sample at any given set of conditions. Viscosities were recorded at 30, 40, and 50°C. Precision was always better than ±1% of the values reported.

pH Measurements

The pH of the glutenin solutions was measured with a Sargent-Welch Model LSX pH meter equipped with a Sargent-Welch model S-30072-15 glass electrode. The pH meter was calibrated with three Fisher Scientific buffers certified at pH 10.00, 7.00, and 4.00 at 25°C. Calibration was checked every day before making any measurements.

Sample Preparation for Electrophoresis

Glutenin samples (1 ml) previously dissolved in sodium stearate and water were pipeted into small glass culture tubes, SDS or β-mercaptoethanol, or both, was added, and these samples were then incubated for 24 hr at 37°C in a shaking water bath. Glutenin samples for electrophoresis without sodium stearate were prepared as outlined by Khan and Bushuk (1977).

Molecular-weight markers covering the range of 265,000 to 14,300 Daltons, obtained from BDH Chemicals Ltd., were used to calibrate the subunits of reduced bovine thyroglobulin, Type 1, obtained from Sigma Chemical Co. Both BDH markers and thyroglobulin were dissolved in the same reducing buffer and were not alkylated. Reduced thyroglobulin was run in at least one slot of every gel.

Electrophoresis

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described previously (Wasik 1978) with and without reduction with β-mercaptoethanol. After electrophoresis, the gels were stained with Coomassie Brilliant Blue R

[1]Paper 349 of the Food Research Institute, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6.

[2]Food Research Institute, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6.
[3]Department of Chemistry, University of Montreal, Montreal, Quebec, Canada H3C 3V1

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Association of Cereal Chemists, Inc., 1979.

(Khan and Bushuk 1977), photographed on high-contrast film, and scanned at 570 nm in a Gilford Model 2400 spectrophotometer equipped with a model 2410 linear transport. All scans were made at the same scale setting (0–1) for optical density.

RESULTS AND DISCUSSION

Glutenin (5 mg/ml) solubilized in water in the presence of sodium stearate according to the method of Kobrehel and Bushuk (1977) became turbid within an hour after preparation if left undisturbed or within a day with mild agitation at 21°C. If this turbid suspension were heated to 45°C, it clarified almost instantaneously. If heating were continued to 100°C, a precipitate formed, settled, and left a clear supernatant.

These observations, plus a desire to determine what happens to glutenin when it is solubilized in the presence of sodium stearate, led to the examination of the viscosities of these solutions or suspensions.

Viscosity Measurements

The first viscosity experiments were performed on solutions prepared according to the method described by Kobrehel and Bushuk (1977) except that mixing was done at 45°C. Kjeldahl determinations of the amount of protein solubilized at 21 and 45°C revealed that slightly more glutenin was solubilized on the first extraction at 45°C than at 21°C. A second (1 ml) water extraction of the residue, as recommended by Kobrehel and Bushuk (1977), eliminated any differences in solubilization between 21 and 45°C, and both the 21 and 45°C methods solubilized at least 95% of the glutenin.

Initially, all viscosity measurements were conducted at 50°C to prevent the onset of turbidity while samples were in the viscometer. At this temperature, the solution was clear and had an initial specific viscosity (ηsp.) of 0.20 in relation to a sodium stearate solution (4.0 mg/ml). The viscosity decreased during the next 24 hr and eventually reached a stable reading of 0.6304 centistokes (ηsp. = 0.14). The change in viscosity with time for glutenin in sodium stearate is illustrated in Fig. 1.

We later discovered that if samples were mixed for 48 hr at 45°C a stable viscosity reading could be achieved within 3 hr of equilibration at 50°C in the viscometer. Experiments to determine the effect of temperature on the viscosity of glutenin were only begun after a constant viscosity was established at 50°C (ηsp. = 0.14).

Table I summarizes the data from these experiments and shows the viscosities of water and sodium stearate in water. On cooling from 50 to 40 or 30°C, solutions that attained a stable viscosity at 50°C became turbid. Surprisingly, the specific viscosities of these solutions were stable after 1 hr of equilibration at 40 and 30°C.

Reheating solutions from 30 or 40 to 50°C clarified the solutions and returned the viscosity to the original value at 50°C. Turbidity at 30 and 40°C is likely due to the formation of soap micelles that are disaggregated upon heating. No significant variation in pH was found among the samples reported in Table I.

Sodium stearate solutions of glutenin were then made up to 1% in β-mercaptoethanol and their viscosities measured at 50°C.

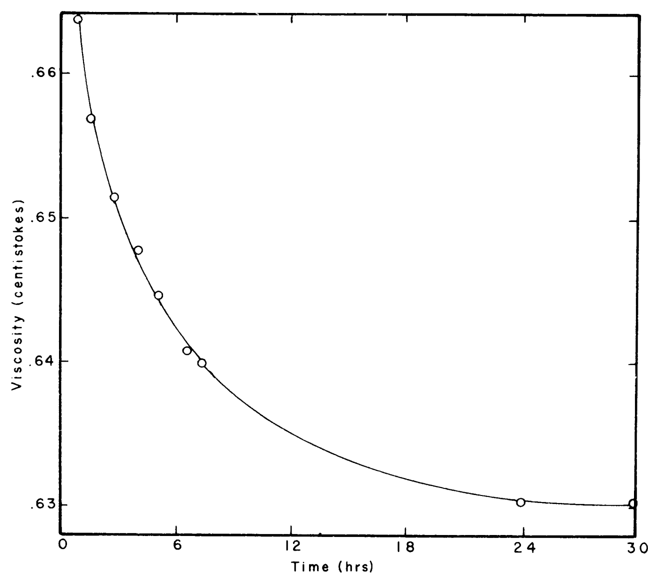

Fig. 1. A plot of viscosity (centistokes) of a solution of (5.0 mg/ml) glutenin and (4.0 mg/ml) sodium stearate against time.

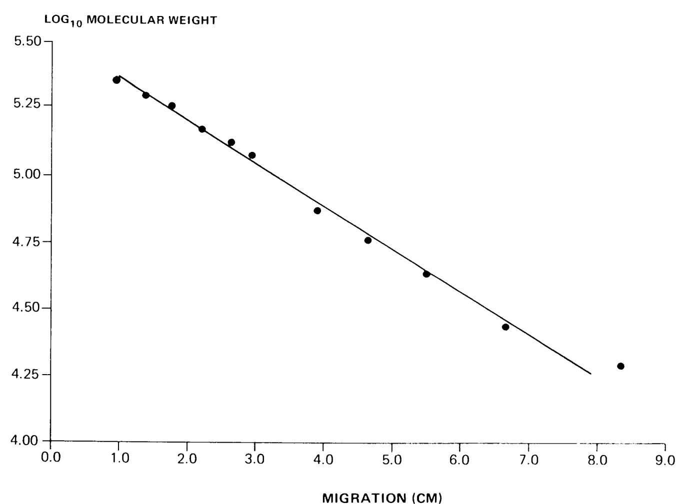

Fig. 2. A plot of migration (cm) from the origin of the gel against $\log_{10}$ of the molecular weight of the subunits of reduced bovine thyroglobulin.

TABLE I
Effect of Temperature on the Viscosity of Freeze-Dried Glutenin Dissolved in Water at 45°C in the Presence of Sodium Stearate (5 mg Glutenin and 4 mg Sodium Stearate per 1 ml Water)

	Solvents		Solutions			
Temperature (°C)	Water Centistokes	Sodium[a] Stearate Centistokes	Glutenin Centistokes	ηsp.[b]	pH	Appearance of Glutenin Solution
50	0.5632	0.5552	0.6304	0.14	9.5	Clear
40	0.6720	0.6640	0.7632	0.15	9.6	Slightly turbid
30	0.8912	0.8192	0.9328	0.14	9.6	Extremely turbid
50 (reheated from 30°C)	0.5632	0.5552	0.6304	0.14	9.5	Clear

[a]4 mg sodium stearate per 1 ml water.

[b]ηsp. = specific viscosity = $\frac{\eta \text{ solution}}{\eta \text{ solvent}} - 1.$

Addition of β-mercaptoethanol reduced the equilibrium specific viscosity from 0.14 to 0.09 relative to a sodium stearate solution (4 mg/ml and 1% β-mercaptoethanol). This significant decrease in viscosity suggests that glutenin previously disaggregated by sodium stearate undergoes further molecular-weight reduction when disulfide bonds are cleaved with β-mercaptoethanol. This could also be taken to suggest that disulfide bonds are not cleaved in the process of sodium stearate solubilization. Increasing the concentration of β-mercaptoethanol beyond 1% did not further decrease the viscosities of these solutions, indicating that disulfide cleavage was complete.

Viscosities much higher in magnitude than those reported for the aqueous sodium stearate solutions could be expected if glutenin (mol wt > 2,000,000) (Kasarda et al 1976) was intact when in solution or suspension in the presence of this soap. The low viscosities reported in Table I for glutenin solutions indicate that the glutenin was extensively disaggregated.

Sodium Dodecylsulfate-Polyacrylamide Gel Electrophoresis

The relationship between the distance the subunits of bovine thyroglobulin migrated from the origin of the gel against the $\log_{10}$ of their molecular weight is illustrated in Fig. 2. This relationship is

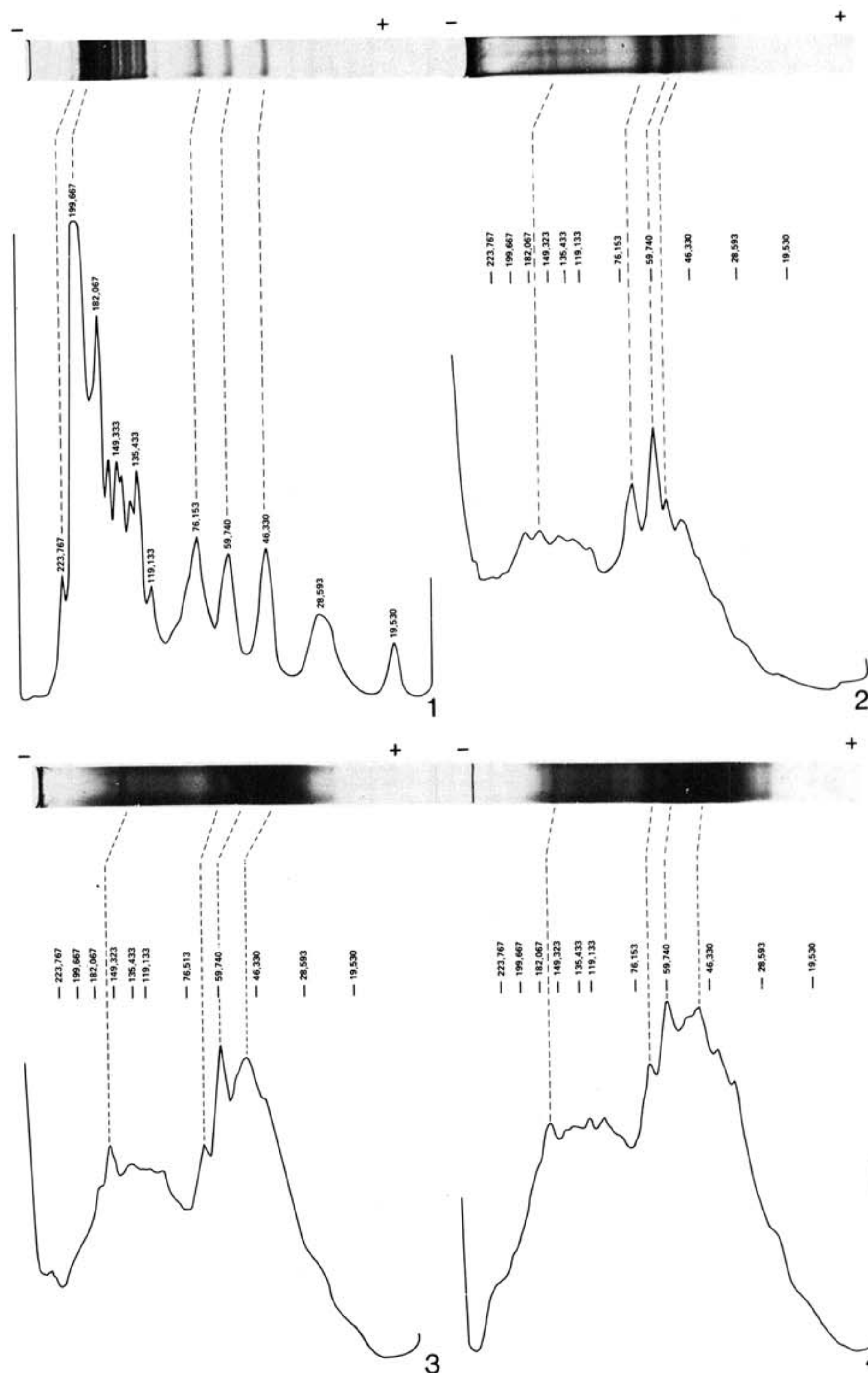

Fig. 3. 1, SDS-PAGE pattern of reduced bovine thyroglobulin; 2, SDS-PAGE pattern of glutenin solubilized for 24 hr at 21°C; 3, SDS-PAGE pattern of glutenin solubilized for 24 hr at 45°C; 4, SDS-PAGE pattern of glutenin solubilized for 48 hr at 45°C.

linear and also quite reproducible. On the average, molecular weights of this standard never varied more than ±3% regardless of the operator.

Figure 3-1 shows the SDS-PAGE pattern of the reduced thyroglobulin and its densitometric trace. At least 12 subunits can be identified easily. Once the molecular weights of these subunits are determined with appropriate standards, determining the molecular weight of an unknown run under the same conditions is no longer a problem.

If disulfide bonds are cleaved during solubilization, the SDS-PAGE pattern of the soluble glutenin would be expected to resemble that of glutenin reduced by β-mercaptoethanol. To check this hypothesis and also to assess the degree of disaggregation, the SDS-PAGE patterns of glutenins that were solubilized at 21 and 45°C for 24 hr and 45°C for 48 hr were compared, with and without reduction by β-mercaptoethanol. Incubating the samples at 50°C for 3 hr did not produce a detectable change in the electrophoresis patterns. This step was therefore omitted in later experiments. The patterns were then compared with the SDS-PAGE pattern of glutenin reduced with β-mercaptoethanol without prior sodium stearate treatment. The results of these experiments appear in Figs. 3 and 4.

Figure 3-2 illustrates the SDS-PAGE pattern of glutenin solubilized at 21°C for 24 hr or according to Kobrehel and Bushuk (1977). Figures 3-3 and 3-4 show the SDS-PAGE patterns of glutenin solubilized at 45°C for 24 and 48 hr, respectively. Glutenin solubilized at 21°C for 24 hr (Fig. 3-2) is not disaggregated to the same extent as glutenin mixed at 45°C for 24 hr (Fig. 3-3). The latter sample had considerably less immobile material remaining at the origin of the gel as well as a lower overall subunit molecular-weight distribution. The samples solubilized at 45°C for 48 hr (Fig. 3-4) completely entered the gel and had the lowest overall subunit molecular-weight distribution. Glutenin mixed and incubated with SDS before electrophoresis did not enter the gel, indicating that the disaggregation observed for sodium stearate was due to sodium stearate and not to the SDS (Fig. 4-1).

The proportions, number, and molecular weights of the proteins in Fig. 3-2, 3-3, and 3-4 differ significantly from one another and probably represent different degrees of disaggregation of the glutenin complex. The finding that glutenin mixed for 24 hr at 45°C (Fig. 3-2) required an additional 24 hr of incubation at 50°C to achieve a stable viscosity can now be rationalized on the grounds that the glutenin had not become completely disaggregated. Glutenin solubilized at 45°C for 48 hr (Fig. 3-4) was more completely disaggregated.

The SDS-PAGE patterns in Figs. 3-2, 3-3, and 3-4 differ qualitatively and quantitatively from the pattern obtained from glutenin solubilized by disulfide bond cleavage (Fig. 4-2). At least 24 subunits can be counted in the SDS-PAGE pattern for glutenin solubilized in sodium stearate at 45°C for 48 hr (Fig. 3-4), but only 15 subunits can be counted in the SDS-PAGE pattern for glutenin solubilized by reduction with β-mercaptoethanol (Fig. 4-2). The overall molecular-weight distributions of the subunits shown in Figs. 3-4 and 4-2 are also quite different. The apparent molecular-weight distribution of subunits produced by mercaptan reduction (Fig. 4-2) is lower than those produced with sodium stearate (Fig. 3-4).

The results of the addition of β-mercaptoethanol to glutenin solubilized in sodium stearate alone are illustrated in Fig. 4-3. β-Mercaptoethanol was added to glutenin solubilized in sodium stearate at 21°C for 24 hr (Fig. 4-3), 45°C for 24 hr, and 45°C for 48 hr. The densitometric traces for all these samples were identical. Disulfide bond cleavage of glutenin with β-mercaptoethanol first disaggregated by sodium stearate produced essentially the same SDS-PAGE pattern as obtained by treatment with β-mercaptoethanol alone (Fig. 4-2). This finding supports the results obtained from the viscosity experiments on the effect of β-mercaptoethanol on sodium stearate solutions of glutenin.

Solubilization of glutenin in the presence of sodium stearate can be explained by peptization, ie, dispersion of the insoluble material by specific physicochemical interactions with a detergent. It is well known that long-chain fatty acids or their alkali salts (soaps) tend to form spherical micelles in water with their hydrophilic groups extending out of the micelles. In the presence of an insoluble hydrocarbon compound, soap micelles are partly broken to form new micelles around the compound molecules through

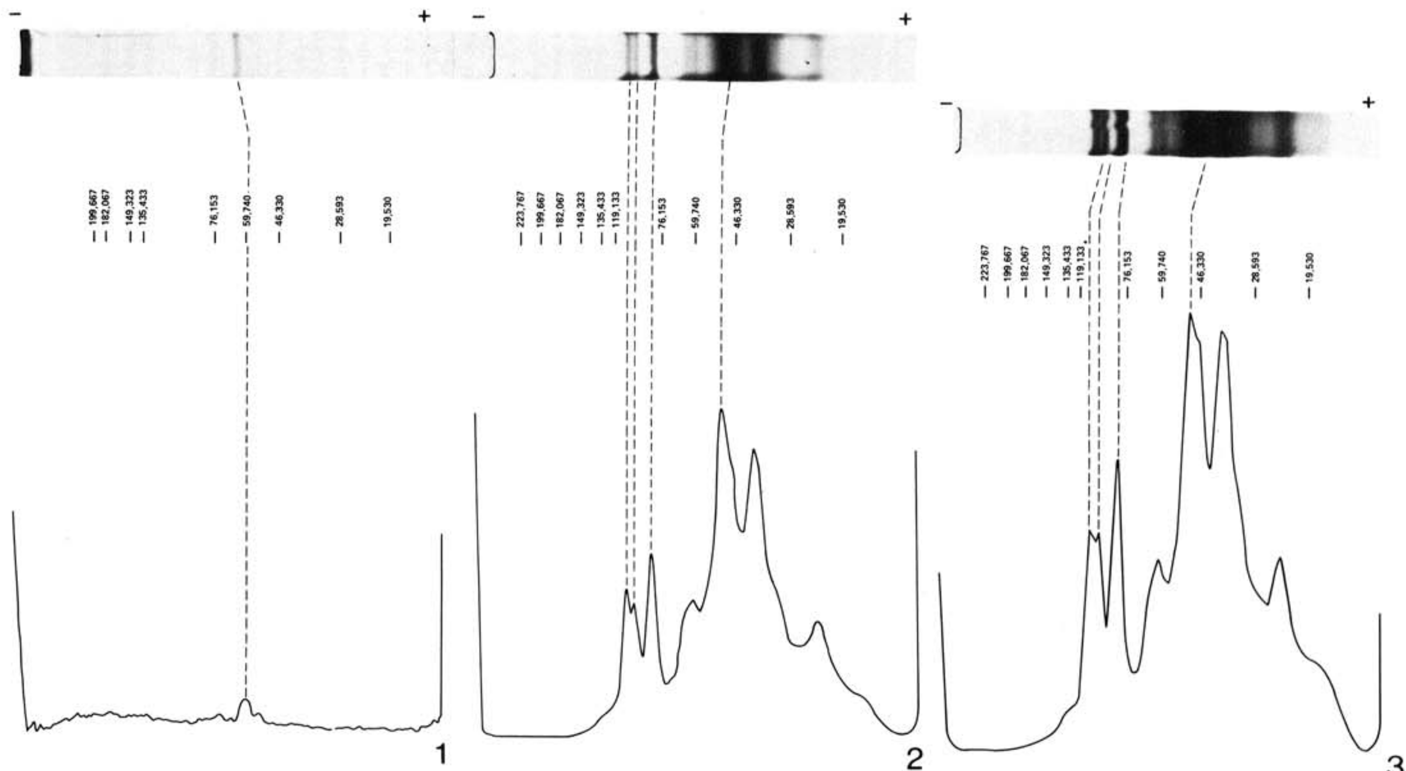

Fig. 4. 1, SDS-PAGE pattern of glutenin incubated in SDS for 48 hr at 45°C; 2, SDS-PAGE pattern of glutenin solubilized by β-mercaptoethanol alone; 3, SDS-PAGE pattern of glutenin previously solubilized with sodium stearate for 24 hr at 21°C and subsequently treated with β-mercaptoethanol.

hydrophobic interactions. The longer the hydrophobic chain of the soap the better its solubilization effect on insoluble particles (Jirgensons and Straumanis 1954). In a protein, solubilization in the presence of detergent is due to complex formation between protein and detergent. Such complexes might result from electrostatic interactions between the charged groups along the polypeptide chain and the hydrophilic part of soap molecules; the complexes are then made soluble by interactions between the hydrophobic chains of the soap molecules bound to the polypeptide chain and hydrophobic chains of the free soap molecules (Lundgren 1945, Putman 1948).

The presence of charged groups along the polypeptide chain is not essential for complex formation with the detergent. The large hydrophobic parts of the detergent ions can combine with some hydrophobic parts of the peptide chain to form micellelike regions (Jirgensons 1950, Tanford 1968).

A possible explanation of the fragmentation of glutenin upon solubilization in the presence of soap may be that, first, the tightly folded polypeptide chains in the insoluble protein superstructure are penetrated by a few large detergent anions by electrostatic interaction as described, forcing the chains apart to allow more detergent molecules to enter the glutenin particles. Then micellelike regions around hydrophobic groups are formed and this results in the breaking of all the noncovalent side-chain linkages.

To solubilize glutenin in water, a large relative concentration of soap is needed since detergents are believed to combine stoichiometrically with proteins to form complexes through electrostatic forces (Putman 1948), and more than one soap molecule might be involved in the formation of micellelike regions around hydrophobic groups. It is unlikely, however, that a soap could break a chemical bond such as a disulfide bond.

ACKNOWLEDGMENTS

We thank W. Bushuk of the Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada, for the sample of Manitou flour, and G. Morris of the Chemistry and Biology Research Institute, Agriculture Canada, Ottawa, Ontario, for his analytical services.

LITERATURE CITED

BECKWITH, A. C., and WALL, J. S. 1966. Reduction and reoxidation of wheat glutenin. Biochim. Biophys. Acta 130:155.

BIETZ, J. A., and WALL, J. S. 1972. Wheat gluten subunits: Molecular weights determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cereal Chem. 49:416.

BUSHUK, W. 1974. Glutenin: Functions, properties and genetics. Baker's Dig. 14:14.

EWART, J. A. D. 1972. A modified hypothesis for the structure and rheology of glutenins. J. Sci. Food Agric. 23:687.

EWART, J. A. D. 1977. Re-examination of the linear hypothesis. J. Sci. Food Agric. 28:191.

JIRGENSONS, B. 1950. Denaturation of plant proteins. III. Optical rotations, viscosity, and reducing capacity of denatured proteins. J. Polym. Sci. 5:179.

JIRGENSONS, B., and STRAUMANIS, M. E. 1954. A short textbook on colloid chemistry. Wiley: New York.

KASARDA, D. D., BERNARDIN, J. E., and NIMMO, C. L. 1976. Wheat proteins, p. 158. In: Advances in Cereal Science and Technology, ed. by Y. Pomeranz. Am. Assoc. Cereal Chem.: St. Paul, MN.

KHAN, K., and BUSHUK, W. 1977. Studies of glutenin. IX. Subunit composition by sodium dodecylsulfate-polyacrylamide gel electrophoresis at pH 7.3 and 8.9. Cereal Chem. 54:588.

KOBREHEL, K., and BUSHUK, W. 1977. Studies of glutenin. X. Effect of fatty acids and their sodium salts on solubility in water. Cereal Chem. 54:833.

LUNDGREN, H. P. 1945. Synthetic protein fibers from protein-detergent complexes. Text. Res. J. 15:335.

NIELSEN, H. C., BABCOCK, G. E., and SENTI, F. R. 1962. Molecular weight studies on glutenin before and after disulfide-bond splitting. Arch. Biochem. Biophys. 96:252.

ORTH, R. A., and BUSHUK, W. 1973a. Studies of glutenin. I. Comparison of preparative methods. Cereal Chem. 50:106.

ORTH, R. A., and BUSHUK, W. 1973b. Studies of glutenin. II. Relation of variety, location of growth, and baking quality to molecular weight distribution of subunits. Cereal Chem. 50:191.

PUTMAN, F. W. 1948. The interactions of proteins and synthetic detergents. Adv. Protein Chem. 4:80.

TANFORD, C. 1968. Protein denaturation. Adv. Protein Chem. 23:121.

WASIK, R. J. 1978. Relationship of protein composition of durum wheat with pasta quality and effects of processing and cooking on these proteins. C.I.F.S.T. J. 11:129.

WASIK, R. J., and BUSHUK, W. 1975. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of reduced glutenin of durum wheats of different spaghetti-making quality. Cereal Chem. 52:328.

[Received March 6, 1978. Accepted July 12, 1978]