STUDIES OF GLUTENIN. XI. NOTE ON GLUTENIN SOLUBILIZATION WITH SURFACTANTS IN WATER[1]

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Cereal Chem. 55(6): 1060-1064

We (1) have shown that glutenin (unreduced) can be dissolved in solvents comprising distilled water and sodium salts of some fatty acids. The dissolving capacity of soaps increased with the length of the carbon chain. In the presence of small ions, soaps were less effective in dissolving glutenin. Further studies, reported in this note, have been made to obtain more information concerning the interaction between soaps and glutenin, and to compare the ability of different types of surfactants to dissolve glutenin.

MATERIALS AND METHODS

Glutenin isolated from flour of hard red spring wheat cv. Manitou by pH precipitation (2) and freeze-dried was identical to that used in the previous study (1).

The following surfactants were tested for their ability to dissolve glutenin: 1) cationic surfactants—hexadecyltrimethylammonium chloride (HACl), hexadecyltrimethylammonium bromide (HABr), and dodecylamine hydrochloride (DHCl); 2) anionic surfactants—sodium desoxycholate (SDCh) and sodium dodecyl sulfate (SDS); 3) nonionic surfactants—Tween 80 and Span 60; and 4) miscellaneous surfactant—lecithin.

Solubilization of glutenin was performed as described previously (1). Protein ($N \times 5.7$) was determined by the micro-Kjeldahl method. Dissolved glutenin was precipitated by adding varying amounts of NaCl or $CaCl_2$ to 1 ml of clear glutenin solution. Precipitated glutenin was recovered by centrifugation at 4° C for 15 min at 17,000 × *g*, washed twice with 2 ml of distilled water, and centrifuged each time. After each centrifugation, the supernatant was discarded. For fatty acid analysis, the soaps in the precipitated glutenin were acidified with 0.2*N* HCl. The fatty acids were converted to methyl esters with boron trifluoride-methanol (3) and analyzed by gas chromatography. A Perkin Elmer model 3920 B gas chromatograph with flame ionization detector and tubular glass column (60 m × 0.36 mm) coated with Silar-5 CP was used. The injection port, column, and detector temperatures were 260°, 190°, and 280° C, respectively. The carrier gas was helium, 7 ml/min. Peak areas were measured with electronic digital integrator CSI-204.

Sedimentation velocity of dissolved glutenin was calculated from data obtained at 46,000, 50,000, and 56,000 rpm, 20° C, using the synthetic boundary cell and Schlieren optics of the Beckman analytic ultracentrifuge. Photographs were taken at 4-min intervals. Ultracentrifugations lasted 35–40 min.

[1]Contribution No. 513 with financial assistance from the National Research Council of Canada.
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0009-0352/78/000117$03.00/0

RESULTS AND DISCUSSION

Table I shows the solubility of glutenin in distilled water in the presence of various surfactants. For the surfactants tested, the conditions of solubilization were not optimized to determine the maximum solubilizing effect. The purpose of these experiments was only to see whether the surfactants had any solubilizing action similar to that of soaps. For this reason, the conditions at which glutenin was completely dissolved with sodium stearate (1) were used (5 mg of glutenin, 4 mg of soap, 1 ml of water).

The nonionic surfactants and lecithin (miscellaneous surfactant) were the least efficient in solubilizing glutenin under the conditions used. Of the anionic surfactants, SDS was considerably more effective than SDCh. With the cationic surfactants, the difference in efficiency between HACl and HABr may be due to the difference in their purity; HACl was highly purified and HABr was of technical grade. In the case of HACl, HABr, DHCl, SDCh, and SDS, the undissolved glutenin had the appearance of a transparent gel, indicating that considerable interaction between these surfactants and glutenin occurred. The pH values of glutenin solutions (Table I) indicate that pH probably had little influence on the differences in glutenin solubilization by the different surfactants.

Glutenin solubilized with surfactants can be reprecipitated by adding neutral salts to the solution. With NaCl, the precipitation of glutenin dissolved by sodium dodecanoate (1) was complete at a salt concentration of about 0.5*M*. $CaCl_2$ was more effective—precipitation was complete at a $CaCl_2$ concentration of 0.05*M*. These results appear somewhat contradictory, since all wheat proteins, including gliadins, have been reported to be soluble in salt solutions to some extent. According to Mecham et al (4), the presence of salt (NaCl) in the 0.01*N* acetic acid solvent decreased the amount of flour nitrogen solubilized only slightly; however, some glutenin was solubilized. Various authors (5–7) have

TABLE I
Glutenin Dissolved With Different Surfactants in Distilled Water and pH of Resulting Solutions

Surfactant	Glutenin Dissolved (%)	pH
Cationic		
Hexadecyltrimethyl ammonium chloride	90	5.9
Hexadecyltrimethyl ammonium bromide	68	5.9
Dodecylamine hydrochloride	70	5.6
Anionic		
Sodium desoxycholate	45	7.3
Sodium dodecyl sulfate	82	7.3
Nonionic		
Tween 80	28	7.1
Span 60	0	...
Miscellaneous		
Lecithin	6	...

discussed the solubility of gliadin in salt solutions. The precipitation of glutenin may be the consequence of an interaction between the salt and the surfactant or between the salt and the surfactant-glutenin complex or both. In any case, the interaction of glutenin with salt ions warrants further investigation. The salt ions can be washed out from the precipitates with distilled water, and the precipitated glutenin can be resolubilized with surfactants in distilled water like the original freeze-dried glutenin. These experiments on resolubilization of glutenin were performed only on glutenin solubilized with sodium dodecanoate.

An attempt was made to determine the strength of the interaction (binding) between the soap and glutenin that produces the solubilization. Experiments were performed using increasing quantities of sodium dodecanoate (1, 2, 3, and 4 mg) to solubilize 5 mg of freeze-dried glutenin. Sodium dodecanoate was chosen because of its high solubility in water. Gas chromatographic analysis showed that the amount of sodium dodecanoate in the precipitated glutenin increased when a higher amount of soap was used for the solubilization (Fig. 1). Thus, when 2, 3, and 4 mg of sodium dodecanoate was used to solubilize 5 mg of glutenin, the reprecipitated glutenin contained 11, 20, and 32 times, respectively, more dodecanoic acid than the original glutenin (Fig. 1). The fact that a specific amount of soap cannot be washed out with water indicates an extremely strong interaction between glutenin and the soap.

The increasing amount of fatty acid bound to glutenin suggests that the

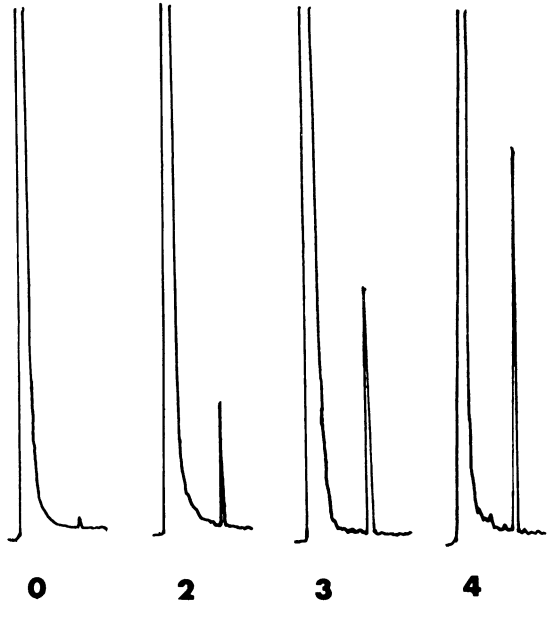

Fig. 1. Gas chromatograms of dodecanoic acid in precipitated, washed, and freeze-dried glutenin from solutions with increasing amounts of sodium dodecanoate. Number under each chromatogram indicates milligrams of sodium dodecanoate added to 5 mg dry glutenin. Larger peaks, solvent; smaller peaks, dodecanoic acid.

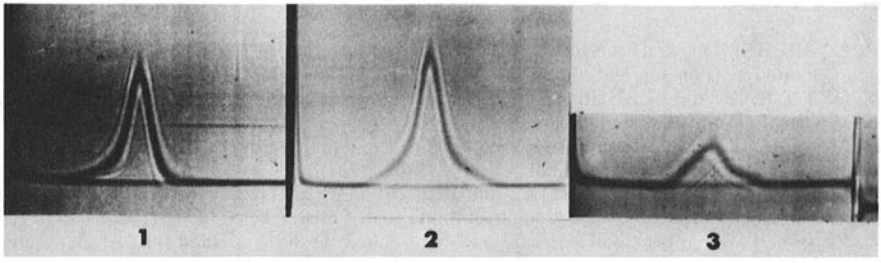

Fig. 2. Sedimentation patterns of glutenin solutions (5 mg glutenin, 1 ml of water) with 1) 4 mg sodium stearate, 2) 4 mg sodium dodecanoate, and 3) 2 mg sodium dodecanoate. Photographs were taken 30 min after start of centrifugation.

aggregates formed are of different size, depending on the initial concentration of soap used to dissolve the glutenin. Another possible explanation is that glutenin contains different types of hydrophobic zones, which become accessible to the surfactants as the concentration of the surfactant increases.

Sedimentation velocities of glutenin dissolved in soap solution were determined to obtain information on the size of the surfactant-glutenin aggregate. The $S_{20,w}$ values obtained from sedimentation experiments on solutions from 2 and 4 mg of sodium dodecanoate and 4 mg of sodium stearate with 5 mg of glutenin were 5.33×10^{-13}, 3.88×10^{-13}, and 3.38×10^{-13}, respectively. For $S_{20,w}$ values of 3.38×10^{-13} to 3.88×10^{-13}, the estimated average molecular weight of globular proteins is about 40,000–45,000 and for a value of 5.33×10^{-13}, about 75,000–80,000 (8). The $S_{20,w}$ value alone is not an accurate measure of molecular weight, but it gives a useful estimate if a particular shape is assumed. The $S_{20,w}$ values obtained in this study suggest the presence of protein aggregates of different size, depending on the quantity of soap used for glutenin solubilization. The size appears to decrease as the amount of soap increases. The shape of the Schlieren patterns (Fig. 2) suggests a fairly homogeneous particle size, especially when 4 mg of sodium stearate or sodium dodecanoate was used to solubilize the glutenin. Since all of the glutenin was dissolved in the case of sodium stearate, the above sedimentation velocity results suggest that glutenin comprises subunits of relatively low molecular weight (40,000–45,000). The estimated molecular weights are based on the assumption that the subunits are globular in shape; this remains to be confirmed. Previous reports indicate much higher molecular weights (up to 3 million) for glutenin (9–11). Molecular weights of subunits of reduced glutenin, determined by SDS polyacrylamide gel electrophoresis, range from 13,000 to 134,000 (12–14). The discrepancies in the published molecular weights of glutenin could result from the marked tendency of glutenin subunits to aggregate (15). In the presence of soaps, the hydrophobic interactions, which stabilize the aggregates, can apparently be disrupted without prior reduction of disulfide bonds (1).

Acknowledgment

We are grateful to Dr. E. Wasowicz (now at University of Agriculture, Poznan, Poland) for performing the gas chromatographic analyses.

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[Received February 7, 1978. Accepted July 26, 1978]