

Influence of Salts and Aggregation of Gluten Proteins on Reduction and Extraction of High Molecular Weight Glutenin Subunits of Wheat¹

S. R. Bean² and G. L. Lookhart²⁻⁴

ABSTRACT

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High-molecular weight glutenin subunits (HMW-GS) of wheat were extracted by various combinations of reducing agents, salt solutions, and solvents. Preferential extraction of 1D-encoded HMW-GS occurred when flours were extracted with Tris-HCl SDS buffer at pH 6.8 containing 6% mercaptoethanesulfonic acid sodium salt (MESNA) and analyzed by SDS-PAGE. Similar effects were also found when dithiothreitol or β -mercaptoethanol were used in conjunction with nonchaotropic salts. If flours were first extracted with 50% 1-propanol, the extraction procedure

yielded all HMW-GS, even in the presence of MESNA or high levels of salts. Addition of alcohols or chaotropes to the Tris buffer solutions containing MESNA or of solutions containing salt also extracted all HMW-GS. The HMW-GS reported most important in baking quality were found preferentially extracted by nonchaotropic salts and reducing agents. This is related to gluten aggregation and the gliadin-glutenin interaction and structure.

Wheat contains many types of proteins, though four major classes are traditionally recognized based on the work of Osborne (1907): albumins, globulins, gliadins, and glutenins. It is the gluten proteins (gliadins + glutenins) that are responsible for the unique ability of wheat to be made into bread (Finney 1943). For this reason, gluten proteins have been widely studied with much recent emphasis on the high molecular weight glutenin subunits (HMW-GS).

To extract HMW-GS from wheat, several solvents can be used in conjunction with reducing agents, which reduce disulfide bonds and solubilize HMW-GS. Traditionally, β -mercaptoethanol (BME), dithiothreitol (DTT), or dithioerythritol (DTE) are used as reducing agents in SDS buffers or in aqueous alcohols. BME, however, has a strong odor and can be unpleasant to work with. DTT and DTE are more expensive than BME, but do not have its strong odor and can be used in lower concentrations.

The sodium salt of mercaptoethanesulfonic acid (MESNA) has been proposed as a less expensive substitute for DTT and DTE without the odor of BME (Singh 1994). MESNA is a commercially available, inexpensive, and odorless solid. Initial studies with MESNA were begun because of its potential as an inexpensive reducing agent useful for large-scale extractions of glutenin, and initially this article was to describe and compare the effects of three reducing agents (BME, DTT, and MESNA). During initial comparisons, we found that MESNA, under certain conditions, extracted mainly 1D-coded HMW-GS. To explain this phenomenon, we have tested many solvent systems and extraction conditions, and this information will be the main focus of this article.

MATERIALS AND METHODS

Reagents

All chemicals were reagent grade or better and purchased from Sigma Chemical Co., St. Louis, MO. Solvents were purchased from Burdick and Jackson, Muskegon, MI.

¹ Cooperative investigations, U.S. Department of Agriculture, Agricultural Research Service and the Department of Grain Science and Industry, Kansas State University. Contribution 98-2-J, Department of Grain Science and Industry, Kansas State Agricultural Experiment Station, Manhattan, KS 66506.

² Research assistant and adjunct professor, respectively, Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506.

³ Research chemist, USDA-ARS, Grain Marketing and Production Research Center, Manhattan, KS 66502. Mention of trademark or proprietary products does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

⁴ Corresponding author. E-mail: george@usgmrl.ksu.edu

Wheat Samples

Flours were produced on a Brabender Quadrumat Sr. Experimental mill from wheat cultivars used in a previous study on glutenin subunit composition (Lookhart et al 1993), from the Kansas Crop Improvement Association, Manhattan, KS, and from samples provided by Walter Bushuk, University of Manitoba. Hexaploid wheat cultivars used were: Atlas 66, Cheyenne, Chinese Spring, Glenlea, Jagger, Karl, Larned, TAM 105, and TAM 107. Durum cultivars used were Cando and Vic.

Extraction Conditions

All solutions were freshly prepared before use. Flours (250 mg) were extracted once with each of the various solvents (1 mL) and analyzed within 24 hr. Gliadins were extracted for 30 min at room temperature with vortexing every 15 min. Glutenins were extracted for 1 hr at room temperature with vortex stirring every 15 min. Samples were centrifuged for 10 min at 10,000 rpm in an Eppendorf 5415C mini-centrifuge with an 18 place rotor. Extracts to be analyzed by SDS-PAGE were mixed 1:1 with a solution containing 6% sucrose, 2% SDS, and 0.05% bromophenol blue before electrophoresis. The pH 6.8 Tris-HCl SDS sample buffer used was the same as reported in Lookhart et al (1993).

SDS-PAGE

SDS-PAGE was performed by a modified Laemmli (1970) procedure (Lookhart et al 1993). Samples (20 μ L) were analyzed on 12% polyacrylamide gels at 12°C and 150V for 16 hr (with Bio-Rad Protean II apparatus, gel size 15 cm [L] \times 18 cm [W] \times 1.5 mM [thick]). Gels were stained and destained according to Lookhart et al (1993).

RESULTS AND DISCUSSION

Extraction in Tris-HCl Buffer with Various Reducing Agents

For initial comparisons of the three reducing agents, glutenins were extracted from TAM 105 flour as part of a total protein extract using a 68 mM Tris-HCl pH 6.8 buffer with 6% SDS (referred to here as Tris buffer) or from flour that had previously been extracted with 50% 1-propanol. The 50% 1-propanol preextraction was made to remove gliadins so that glutenins could be analyzed by SDS-PAGE without interference from gliadins. Both Tris buffer + 5% BME and Tris buffer + 1% DTT extracted all four HMW-GS (2, 7, 8, and 12) as expected (Lookhart et al 1993) (Fig. 1, lanes A, B, E, F). All four subunits were also seen if the flour was first extracted with 50% 1-propanol and then extracted with Tris buffer + 6% MESNA (Fig. 1, lane C). However, if the flour was not pre-extracted with 50% 1-propanol, then Tris buffer + 6% MESNA extract contained only subunits 2 + 12 with very faint levels of 7+8 (Fig. 1, lane D).

To determine whether lipids that may have been removed during preextraction of flours by 50% 1-propanol were the cause of the unique extraction found when using MESNA, defatted flour was tested. TAM 105 flour defatted by the method of Chung et al (1979) was extracted with the Tris + MESNA buffer, both with and without a preextraction by 50% 1-propanol. When no preextraction with 50% 1-propanol was made, HMW-GS 2+12 were present with faint levels of HMW-GS 7 + 8, as in Fig. 1. When flour was first extracted with 50% 1-propanol, all four HMW-GS were present (data not shown). Therefore, lipids are clearly not involved in the unique extraction with MESNA-containing buffers.

Additional wheat samples were also tested at this point, to determine whether the unique extraction of primarily 1D-encoded HMW-GS was related only to TAM 105 or not. Flours of four cultivars containing 1D HMW-GS 5 + 10 (U.S. hard red winter wheats Jagger, Karl, and Cheyenne, and Canadian cultivar Glenlea); four cultivars containing 1D HMW-GS 2 + 12, (hard red spring cultivar Chinese Spring, U.S. hard red winter wheats Larned and TAM 107, and U.S. soft red winter cultivar Atlas 66); and two U.S. durum cultivars (Vic and Cando HMW-GS 6 + 8) (Lookhart et al 1993) were extracted with Tris buffer + 6% MESNA and analyzed by SDS-PAGE. The only HMW-GS found in the SDS-PAGE patterns when flours of Karl, Glenlea, or Cheyenne (Fig. 2, lanes A–C, respectively) were extracted with Tris buffer + 6% MESNA were the 1D subunit set 5+10. No HMW-GS were found on extraction of Jagger flour (Fig 2, lane D) but lower molecular weight proteins were noted in amounts similar to that seen in the other cultivars examined. It is unknown why no HMW-GS from Jagger were extracted with Tris buffer + 6% MESNA. When Jagger was extracted with 50% 1-propanol before extraction with Tris buffer + 6% MESNA, all five HMW-GS were present (data not shown). Additional studies are required to determine whether

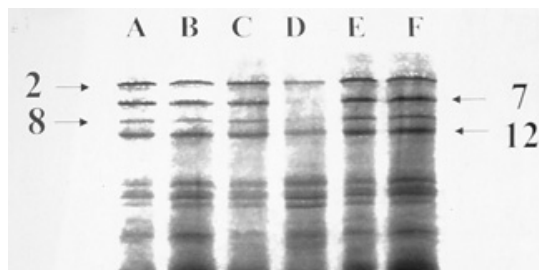


Fig. 1. SDS-PAGE (12% acrylamide) patterns of TAM 105 flours extracted with Tris-HCl (pH 6.8) solution containing SDS (Tris buffer) and either 5% β -mercaptoethanol (BME) (lanes A and B), or 6% mercaptoethanesulfonic acid sodium salt (MESNA) (lanes C and D), or 1% dithiothreitol (DTT) (lanes E and F). The Tris buffer plus reducing agent extracts in lanes A, C, and E were from flours preextracted with 50% 1-propanol and those in lanes B, D, and F were from flours **not** preextracted with 50% 1-propanol. High-molecular weight glutenin subunits (HMW-GS) 2, 7, 8, and 12 are shown in all lanes except lane D, where only GS 2 and 12 are visible.

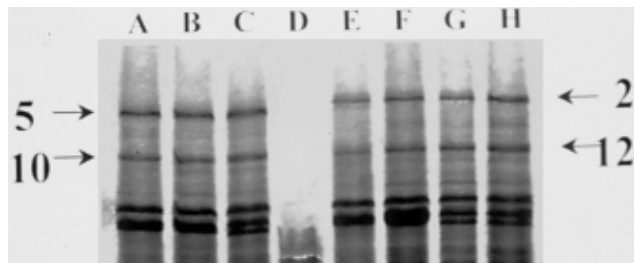


Fig. 2. SDS-PAGE (12% acrylamide) patterns of Tris buffer containing 6% mercaptoethanesulfonic acid sodium salt (MESNA) extracts of Karl, Cheyenne, Glenlea, Jagger, Chinese Spring, Larned, TAM 107, and Atlas 66 flours (lanes A–H, respectively).

there is something unique about the 1D HMW-GS in the cultivar Jagger. The SDS-PAGE patterns of Tris buffer + 6% MESNA extracts exhibit normal levels of HMW-GS 2+12 for the four cultivars analyzed with known 1D HMW-GS 2+12 (Lookhart et al 1993): Chinese Spring, Larned, TAM 107, and Atlas 66 (Fig. 2, lanes E–H).

SDS-PAGE analyses of comparable extracts of durum cultivars revealed no HMW-GS (data not shown). This behavior is as expected from analyses of the other cultivars, because tetraploid durum wheats have no 1D subunits. However, 1B-encoded durum HMW-GS 6 + 8 (Lookhart et al 1993) were extracted if the durum flours were first extracted with 50% 1-propanol (data not shown). Thus, Tris buffer + 6% MESNA extracts, at most, the 1D HMW-GS from flour samples.

Preextraction with Other Solvents

Because preextraction of flour with 50% 1-propanol appeared to influence subsequent extraction of HMW-GS when using Tris buffer + MESNA, other typical gliadin solvents (25 mM acetic acid, 0.005M lactic acid, and 4M urea) were used to extract flours before glutenin extraction with MESNA solutions. SDS-PAGE analysis of Tris buffer extracts containing either 5% BME, 1% DTT, or 6% MESNA from flours preextracted with any of these three solvents revealed all four HMW-GS (data not shown) at similar intensities for all solvents. Therefore, removal of gliadins with all four solvents allows the HMW-GS to be solubilized with Tris buffer + MESNA.

Solvent Effects

Replacement of the Tris buffer with typical gliadin solvents (50% 1-propanol, 25 mM acetic acid, 0.005M lactic acid, and 4M urea) was also tested. When flours were extracted with any of the three reducing agents (BME, DTT, or MESNA) in 50% 1-propanol or 4M urea, all four subunits were found in all extracts, whether or not flours were first extracted with 50% 1-propanol (data not shown). The less ionic solvents (propanol and urea) solubilized all HMW-GS with any of the reducing agents. When 25 mM acetic acid or 0.005M lactic acid was used, all HMW-GS were extracted with either BME or DTT (Fig. 3, lanes A, B, E, F), but no HMW-GS were extracted by 25 mM acetic acid (Fig. 3, lanes C, D) or 0.005M lactic acid containing 6% MESNA (lactic acid data not shown), with or without preextraction of flours. The more ionic solvents (acetic acid and lactic acid) with MESNA were not able to solubilize any HMW-GS.

Because MESNA extracted all four HMW-GS with 50% 1-propanol (data not shown) and none with 25 mM acetic acid (Fig. 3), a solution combining both solvents was tested. When flours were extracted with 25 mM acetic acid + 50% 1-propanol and any of the three reducing agents, all four HMW-GS were extracted inde-

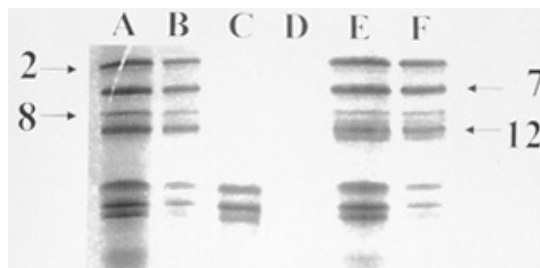


Fig. 3. SDS-PAGE (12% acrylamide) patterns of TAM 105 flours extracted with 25 mM acetic acid containing 5% β -mercaptoethanol (lanes A and B), 6% mercaptoethanesulfonic acid sodium salt (MESNA) (lanes C and D), or 1% dithiothreitol (DTT) (lanes E and F). Lanes A, C, and E show the effect of 25 mM acetic acid buffer plus a reducing agent on the extraction of flours **not** preextracted with 50% 1-propanol. Lanes B, D, and F show the effect of 25 mM acetic acid buffer plus reducing agent extraction of flours preextracted with 50% 1-propanol.

pendent of preextraction of the flours with 50% 1-propanol (data not shown). This shows that the reducing agents were effective at the pH of 25 mM acetic acid and points to 1-propanol and urea as important factors involved in the unique extraction properties of MESNA.

Effect of Salt

One explanation for the unique extraction of primarily 1D HMW-GS by Tris buffer + MESNA may be due to the high sodium levels present. A solution of 6% MESNA contains $\approx 0.4M$ sodium (6% SDS adds another $\approx 0.2M$). Singh et al (1991) and Fu and Sapirstein (1996) reported that salt extraction before gliadin extraction may aggregate gliadins to glutenins, thereby decreasing subsequent solubility in aqueous alcohols. Dupuis et al (1996) also reported that the use of salt solutions during typical Osborne extraction procedures caused gliadins to be tightly bound to glutenins. Fu et al (1996) recently reported that treatment of gluten with NaCl solutions altered subsequent protein solubility. They speculated that the salt induced aggregation between gliadins and glutenins and also induced conformational changes in the proteins. Later when the salt was removed, the gliadin-glutenin interactions were lessened and the proteins were then accessible to water and rendered more soluble. These authors point to the fact that certain ionic solvents may reduce gluten solubility by aggregating gliadins with glutenins. If this is the case, then 6% MESNA solutions may be causing aggregation of gliadins to glutenins due to the high levels of sodium present with the MESNA. We believe that the aggregation of gliadin and glutenin may decrease the solubility of the large aggregated complex of gliadin and glutenins. It is important to note that the unique extraction noted in this article takes place in the presence of very high levels of salt, conditions which promote hydrophobic interactions and protein aggregation (Creighton 1983). This would also explain why all four HMW-GS of TAM 105 were seen if the gliadins were first removed by 50% 1-propanol before the MESNA was used. If gliadins are not present, they cannot be aggregated to the glutenins and form large insoluble complexes. To test if the sodium concentration of the MESNA solutions were responsible for the extraction of primarily 1D HMW-GS a series of experiments were conducted.

The first factor tested was the concentration of MESNA used in the Tris buffer. Levels were varied from 1 to 12% (Fig. 4, Lanes A-E, respectively). All four HMW-GS were extracted with 1% MESNA (Fig. 4, lane A), but higher MESNA concentrations extracted mainly subunits 2 + 12 with faint levels of 7 and 8 visible. This data seems to suggest that it is the sodium level in the MESNA containing buffers that is responsible for the unique extraction of primarily 1D HMW-GS. This would explain why lower concentrations of MESNA (with lower sodium levels) extracted all four HMW-GS, but higher concentrations did not.

If the unique extraction of primarily 1D HMW-GS was simply due to increased sodium content of MESNA solutions, it should be possible to mimic this with other salts and reducing agents. If this effect could not be mimicked, then the extraction of primarily

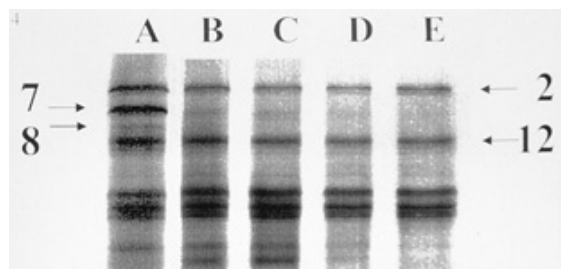


Fig. 4. SDS-PAGE (12% acrylamide) patterns of TAM 105 flours extracted with Tris buffer containing 1, 3, 6, 9, or 12% mercaptoethanesulfonic acid sodium salt (MESNA) (lanes A-E, respectively).

1D HMW-GS must be related to unknown unique properties of MESNA. For this reason, we elected to test DTT as a reducing agent in conjunction with several different salts.

The first salt tested was sodium sulfate. Flours were extracted with 1% DTT in Tris buffer with 1, 3, or 6% sodium sulfate added. When 1 or 3% sodium sulfate was added to Tris buffer containing 1% DTT, SDS-PAGE showed only what appeared to be HMW-GS 12 (Fig. 5, lanes A and C). Preliminary reviewers have pointed out that the band labeled as HMW-GS band 12 in Fig. 5 (lanes A and C) could possibly be a HMW globulin. We currently have no evidence indicating its true identity. If flour was first extracted with 50% 1-propanol, however, all four HMW-GS were visible (Fig. 5, lanes B and D). Again as with MESNA, when flours were first extracted with 50% 1-propanol, all four HMW-GS were extracted.

When 6% sodium sulfate was used with Tris buffer + 1% DTT to extract flour, no HMW-GS were extracted (Fig. 5, lane E), but again, when flour was first extracted with 50% 1-propanol, all HMW-GS were present (Fig. 5, lane F), though faint.

Sodium sulfate was also added to solutions of 50% 1-propanol + 1% DTT and the above experiment was repeated. When 50% 1-propanol+1% DTT was used in place of the Tris buffer, all HMW-GS were soluble, independent of preextraction of the flour (data not shown). Even in the presence of high salt concentrations, HMW-GS were soluble in alcohol + DTT.

As a note, reviewers questioned the role that residual solvent (particularly propanol) left in the pellet from the original gliadin extraction may have on subsequent glutenin extractions. It is possible that residual propanol may influence the solubility of the 1D HMW-GS in the presence of the MESNA solutions. It was suggested that gliadins be extracted with 50% 1-propanol and the

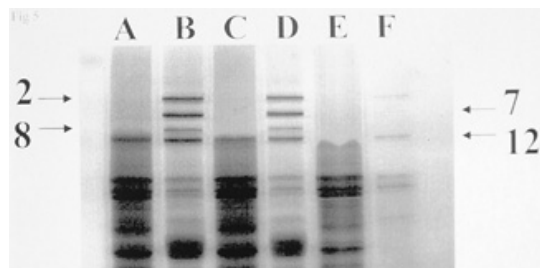


Fig. 5. SDS-PAGE (12% acrylamide) patterns of TAM 105 flours extracted with Tris buffer containing 1% dithiothreitol (DTT) and 1% Na₂SO₄ (lanes A and B), 3% Na₂SO₄ (lanes C and D), or 6% Na₂SO₄ (lanes E and F). Lanes A, C, and E show the effect of Tris buffer containing 1% DTT plus Na₂SO₄ on the extraction of flours **not** preextracted with 50% 1-propanol. Lanes B, D, and F show the effect of Tris buffer containing 1% DTT plus Na₂SO₄ on the extraction of flours preextracted with 50% 1-propanol.

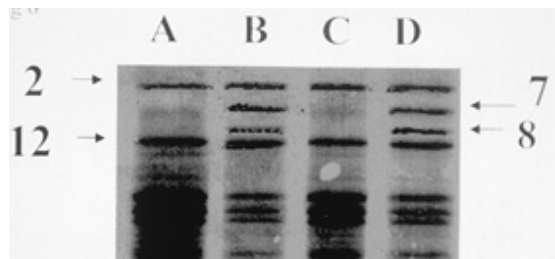


Fig. 6. SDS-PAGE (12% acrylamide) patterns of TAM 105 flours extracted with Tris buffer containing 1% dithiothreitol (DTT) and 1% NaCl (lanes A and B) or 3% NaCl (lanes C and D). Lanes A and C show the effect of Tris buffer containing 1% DTT plus NaCl on the extraction of flours **not** preextracted with 50% 1-propanol. Lanes B and D show the effect of Tris buffer containing 1% DTT plus NaCl on the extraction of flours preextracted with 50% 1-propanol.

propanol evaporated and then the Tris buffer + 6% MESNA tested. In response to this comment, 250 mg of TAM 105 flour was extracted with 50% 1-propanol and the pellet was then frozen and lyophilized to remove all propanol from the pellet before extraction with Tris buffer + 6% MESNA. Glutenins extracted with Tris buffer + 6% MESNA following this procedure showed identical SDS-PAGE patterns to all the original extracts. That is all four HMW-GS were seen on the gels, despite the presence of the MESNA (i.e., 0.4M sodium). This indicates that the important factor is the removal of gliadins and not simply an artifact of residual propanol.

Hofmeister Series Extraction Effects

Next, a number of salts from the Hofmeister series were selected for testing with the Tris buffer + 1% DTT and also 50% 1-propanol + 1% DTT. Because sulfate is the weakest chaotrope and sodium is intermediate as a cation, three strengths of anions were tested: sulfate (weakest chaotrope), chloride (medium), and thiocyanate (strongest chaotrope). Strong chaotropes have water structure-disrupting properties and therefore increase solubility of the gluten aggregate through increased interactions between non-polar amino acid residues and water molecules. Conversely, weak chaotropes decrease solubility through increased aggregation (Creighton 1983).

As noted above, when sodium sulfate (1, 3, or 6%) was included in the Tris buffer, all four HMW-GS were not extracted. However, all four HMW-GS were extracted from flours with DTT and 1, 3, or 6% sodium sulfate in 50% 1-propanol. Therefore, the propanol can overcome the aggregative properties of 1, 3, or 6% sodium sulfate.

When Tris buffer + DTT extracts contained 1 or 3% sodium chloride (6% was insoluble), only HMW-GS 2 + 12 were extracted (Fig. 6, lanes A and C), but again all four HMW-GS were found when flour was first preextracted with 50% 1-propanol (Fig. 6, lanes B and D). Chloride is a stronger chaotropic agent than sulfate, and causes less aggregation of gluten proteins than sulfate. This agrees with the lyotropic effects of neutral salts on gluten proteins as discussed by Preston (1981, 1984). SDS-PAGE patterns of proteins extracted from flours with 1 or 3% NaCl plus 50% 1-propanol +1% DTT exhibited all four HMW-GS (data not shown), demonstrating that the solubility of HMW-GS in 50% 1-propanol is less affected by the presence of salt than are SDS or dilute acid solutions. Even though overall protein solubility in organic solvents is less affected by salts than in SDS or dilute acid solutions, the solubility of specific proteins appears, at least in part, related to the gliadin-glutenin aggregation.

Extraction of flours with Tris buffer +DTT containing 1, 3, or 6% sodium thiocyanate extracted all HMW-GS independent of preextraction with 1-propanol (data not shown). Similarly, when the Tris buffer solution was replaced with 50% 1-propanol, all HMW-GS were extracted from flours. The chaotropic anion thiocyanate disrupts the water structure and the protein complex, hence facilitating extraction of HMW-GS.

Gel Layer

During extraction of flours with sodium sulfate, sodium chloride, or MESNA in Tris buffer, a yellowish-sticky gel layer was noted on the top of the flour centrifugate. (The layer also was found with defatted flour, but its color was lighter.) This layer readily solubilized in 50% 1-propanol, with or without 1% DTT. SDS-PAGE showed it contained proteins with all four HMW-GS, plus lower molecular weight proteins (data not shown). This gel layer may be similar to that noted by Mecham et al (1972), or possibly could be insoluble complexes between gliadins and glutenins. This idea is supported by the observation that the gel layer was not seen (or was much reduced in size) when gliadins were first extracted from the flour before salt containing solutions were used to extract glutenins.

Water Solubles

Recently Fu and Sapirstein (1996a) reported that 50% 1-propanol was effective in removing nearly all monomeric proteins from flour, including the albumins and globulins. Because albumins were removed during extraction of the flours with 50% 1-propanol, flours were also preextracted with water and then extracted with Tris buffer + 6% MESNA to determine whether albumins or other water-soluble material could be affecting extraction of the HMW-GS. After extracting with water, Tris buffer + MESNA extracted only HMW-GS 2 + 12 (SDS-PAGE data not shown). It appears, therefore, that the albumins or other water-soluble materials are not involved in the unique extraction of the 1D HMW-GS by MESNA. However, Tris buffer +MESNA extracted all HMW-GS from hand-washed gluten, whether or not the gluten had been previously extracted with 1-propanol. In gluten that was not pre-extracted, however, HMW-GS bands were faint. Shogren et al (1969) found that after repeated extractions with water, gliadins were eventually solubilized. Fu et al (1996) also reported that some polymeric glutenins can be extracted from flour with water. It is possible that during hand-washing of gluten, enough gliadins or polymeric glutenins are removed to allow MESNA solution to extract small amounts of HMW-GS.

SUMMARY

The results in this study may explain how gluten proteins interact with each other to influence their solubility and macromolecular structure. A backbone composed of glutenin subunits (high molecular weight or low molecular weight) linked together through disulfide bonds in one of several possible forms is known to exist in wheat (for complete review, see Shewry et al 1992). This core or backbone may be covered with gliadins that interact through hydrophobic, hydrogen, and ionic bonds with the glutenin core. Some of the D genome HMW-GS may not participate in this aggregated complex to the same extent as other proteins and, thereby, may be more accessible to other compounds present in the flour. These HMW-GS may, therefore, be most important in interactions with other flour components. Although not investigated here, it would also be interesting to note whether any D-genome LMW-GS have different extraction properties in the presence of salts.

The addition of nonchaotropic salts to extraction buffers may cause strong aggregation and binding of gliadins to the glutenin backbone, which could interfere with complete solubilization of glutenins by SDS. Chaotropic salts, alcohols, and urea can disrupt this aggregation (or may not allow it to form) and allow complete extraction of HMW-GS. SDS appears unable to break the strong aggregation of gliadins and glutenins in the presence of high levels of nonchaotropic salts. MESNA, as a sodium salt, tends to act like a nonchaotropic neutral salt, extracting all HMW-GS only in the presence of alcohols, urea, or after the gliadin aggregation to the gluten macromolecule has been at least partially disrupted by an alcohol or chaotrope. Water-soluble alcohols tend to disrupt hydrophobic forces that stabilize protein structure by changing the water solvation on the protein surface, whereas SDS appears incapable of breaking the salt induced aggregation and solubilizing all four HMW-GS. It should be noted that many typical gliadin solvents will also extract some glutenin, it may be that the removal of small amounts of glutenin from the gluten complex is also important in allowing subsequent extraction of HMW-GS from salt induced aggregates. At this time, it not known whether the extraction of all HMW-GS after preextraction with 1-propanol is due to solely to the removal of gliadins from the gluten complex or through by a disruption in the protein conformation brought about by exposure to solvents during the gliadin extraction (such as propanol or acetic acid). It is also possible that both these factors are important. Experiments to clarify these points are underway.

The idea of salt induced aggregation of gliadins to glutenins is supported by Preston (1981), who found that at low salt levels the solubility and aggregation of gluten were determined by ionic interactions, but at high salt levels solubility and aggregation depended on hydrophobic interactions. Furthermore, Preston (1981, 1984) found that gluten proteins varied widely in their hydrophobic properties and suggested that hydrophobic interactions are important in determining the physical properties of dough.

The effects of salts on wheat proteins and dough and baking properties has been studied for many years (Galal et al 1978, Bakhoun and Ponte 1982, Salovaara 1982, Holmes and Hoseneý 1987, Preston 1989, He et al 1992, Kim and Bushuk 1995). These studies indicate the impact that salt-induced aggregation or disruption of gluten structure can have on bread properties. Several authors have also found differences in hydrophobicity or aggregation behavior of gluten prepared from good and poor quality wheats (Huebner 1970, Arakawa and Yonezawa 1975, Arakawa et al 1976, Chung and Pomeranz 1979), again pointing to the potential importance of hydrophobic interactions in gluten and aggregation properties of gluten proteins. Given that flours of different qualities exhibit different hydrophobicities, it is reasonable to think that variations in quality may be in part due to differences in the tendency of the proteins to interact hydrophobically between good and poor quality wheats, this may extend down to the individual proteins, with 1D subunits differing from other HMW-GS.

CONCLUSIONS

Preferential extraction of 1D HMW-GS were found in extracts of flour when Tris buffers containing 6% MESNA were used. This was attributed to the high salt levels of the MESNA solutions. These affects could be mimicked by addition of nonchaotropic salts to conventional reducing agents, such as DTT in Tris buffers. This unique extraction of 1D HMW-GS was attributed to salt-induced aggregation of the gluten proteins. Preextraction of flours with 50% 1-propanol or other chaotropes allowed all HMW-GS to be extracted, possibly due to removal of gliadins, thus preventing aggregation between gliadins and glutenins.

Previous studies of the actions of salts on dough strength and baking properties of wheat point to the importance of gluten aggregation. Our present study indicates that 1D HMW-GS may not participate in this aggregation between gliadins and glutenins to the same extent as 1B HMW-GS. This is particularly interesting because 1D subunits are thought to be the most important HMW-GS in terms of quality (Payne et al 1979). Fu and Sapirstein (1996b) have also reported unique extraction properties of 1D HMW-GS, making this protein important for further study. Although salt levels used in this study were higher than in bread, these results do raise interesting questions about interactions between gliadins and glutenins, particularly the 1D HMW-GS. Preferential extraction of 1D HMW-GS in the presence of nonchaotropic salts may prove useful in general protein studies and for rapid screening for the presence of particular 1D HMW-GS (i.e., quickly differentiate 5 + 10 from 2 + 12 without interference from other HMW-GS).

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