

Isolation and Functionality Testing of Low Molecular Weight Glutenin Subunits

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ABSTRACT

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Various protein fractionation techniques have been applied to the isolation and purification of milligram quantities of low molecular weight glutenin subunits (LMW-GS). No single technique was applicable to the purification of the majority of the subunits. Partial purification of certain LMW-GS was obtained using ion-exchange chromatography and reversed-phase HPLC. Preparations containing α - and γ -type subunit sequences did not strengthen dough when incorporated into a base flour, whereas

preparations containing a subunit with an N-terminal methionine residue (METSHIPGL-) did. Using preparative isoelectric focusing over a narrow pH range, it was possible to purify (to $\approx 90\%$ purity) a B subunit that also had the N-terminal sequence of METSHIPGL-. This polypeptide, when incorporated into a base flour, had a dough strengthening effect in mixing trials, but less so than an equivalent amount of a high molecular weight glutenin subunit.

The glutenin proteins of wheat play a key role in determining the unique viscoelastic properties of wheat flour doughs. Although the low molecular weight glutenin subunits (LMW-GS) are more abundant than are the high molecular weight glutenin subunits (HMW-GS), they are less well characterized and the effects of individual components have not been studied. Correlations obtained between particular LMW-GS alleles and dough extensibility and resistance suggest that these polypeptides are important (Gupta et al 1989, Gupta et al 1991, Khelifi and Branlard 1992), so further study of their functional roles in dough is required. Study of effects of addition of individual gliadins and incorporation to the gluten polymer of glutenin subunits to a base flour has been possible because of the availability of the small 2-g mixograph that does not require large quantities of purified protein for assessment (Rath et al 1990, Bekes et al 1995). However, the close sequence homologies, low aqueous solubilities, and low charge of the LMW-GS have made their purification in quantities above several milligrams difficult.

The nomenclature for the major LMW polypeptides of glutenin has been based on their mobility in SDS-PAGE, namely as B and C-type LMW polypeptides (Gupta and Shepherd 1990). However, there is growing evidence of similarity of groupings of LMW polypeptides based on sequence studies. Indeed, Lew et al (1992) suggested that the different classes of sequence for the LMW polypeptides might be a better way to describe them than their mobility in SDS-PAGE. Six main sequence types of LMW-GS have been defined on the basis of N-terminal sequence: SHIPGLERPSGL-, METSHIPGL-, METS(R)CIPGL-, and those that closely resemble the α -, γ -, and ω -type gliadins in sequence. The latter three have an odd number of cysteine residues, which allows their incorporation into glutenin by means of an intermolecular disulfide bond (Kasarda 1989). Two major types of LMW-GS, LMW_s and LMW_m, have been described (Lew et al 1992, Masci et al 1995) where the subscript designation refers to the first amino acid in the sequence. The relationships between these different forms of nomenclature are shown in Fig. 1. Our eventual objective is to isolate a representative subunit of each sequence type and study the effects on dough properties. Although genes for a few LMW-GS from wheat have been isolated, the polypeptides expressed by them are not thought to be quantitatively major ones (Lew et al 1992). Recently, two LMW-GS genes were isolated

from the A-genome progenitor, *Triticum boeoticum*. After heterologous expression of the genes in bacteria, the encoded polypeptides increased mixing time (Y. K. Lee, F. Bekes, M. K. Morell, R. B. Gupta, and R. Appels, unpublished data). These polypeptides had a cysteine residue at position 5 in the N-terminal sequence. It is possible that LMW-GS with two cysteine residues (one at the N- and another at the C-terminal) that are available for intermolecular disulfide bond formation could act as "chain extenders." In order to find a method suitable for the preparation of sufficient material from polypeptides of defined N-terminal sequence, we surveyed a range of preparative purification techniques.

MATERIALS AND METHODS

Plant Samples

The Canadian wheat variety Roblin and a spontaneous double null in Roblin lacking the glutenins and gliadins encoded by the short arms of chromosomes 1B and 1D (Roblin 1A) were provided by N. K. Howes (formerly of Agriculture Canada, Winnipeg, MB). The material was expanded under quarantine by single-seed descent and its homogeneity was checked by SDS-PAGE.

Preparation of LMW-GS from Flour

Wholemeal flour (60 g) was extracted four times with 12.5 mL/g of 50% iso-propanol for 30 min each at 20–23°C with constant mixing to remove gliadin. Glutenin subunits were extracted from the residue using 50% iso-propanol-80 mM Tris-HCl, pH 8.0, containing 1% dithioerythritol (300 mL) at 65°C for 1 hr with occasional shaking. After centrifugation, the HMW-GS in the supernatant were precipitated using acetone (added to 40%, v/v) and after 30 min they were removed by centrifugation (Melas et al 1994). The supernatant was dialyzed extensively against 0.1 mM acetic acid to retain functionality (Skerritt et al 1996). For all applications except ion-exchange chromatography, the dialyzed material was freeze-dried and stored at 4°C.

Ion-Exchange Chromatography

Dialyzed LMW-GS extracted from 60 g of Roblin 1A wholemeal was adjusted with acetic acid to pH 4.6 and dithiothreitol (DTT) added to 20 mM. Any precipitate formed was removed by centrifugation (10,000 \times g, 20 min). The sample was applied to a column (2.6 \times 40 cm) of SP Sepharose Fast Flow (Pharmacia, Uppsala, Sweden) equilibrated in 10 mM glycine-acetate, 50% iso-propanol, pH 4.6, containing 2 mM DTT at 60 mL/hr. After washing the column with this buffer for 2 column volumes, a linear 0–0.15M sodium chloride gradient in the same buffer was developed over eight column volumes. A portion of selected fractions was mixed with acetone (80%, v/v) at –20°C overnight to precipitate protein and centrifuged at 20,000 \times g (4°C, 15 min). The

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pellet was redissolved in sample loading buffer, heated at 95°C for 5 min, and centrifuged (14,000 × *g*, 5 min). An aliquot of the supernatant containing reduced LMW-GS was analyzed by SDS-PAGE. Fractions containing similar proteins were pooled, dialyzed against 1 mM acetic acid, and freeze-dried for subsequent reanalysis.

Preparative RP-HPLC

A 218TP1010 1 × 25 cm semipreparative column (Vydac, Hesperia, CA) with attached 218TP guard column heated to 50°C and equilibrated with a mixture of 30% acetonitrile (ACN) in water containing 0.1% (v/v) trifluoroacetic acid (TFA) (Pierce, Rockford, IL) in water and 0.1% TFA in acetonitrile 70:30, was used to separate LMW-GS. Freeze-dried LMW-GS from Roblin 1A were dissolved at 50 mg/mL in 50% aqueous *n*-propanol, 80 mM Tris-HCl, pH 7.5, containing 1% (w/v) DTT, heated for 30 min at 65°C, and filtered through a 0.45-μm polyvinylidene difluoride syringe filter (Gelman, Ann Arbor, MI). Aliquots (100 μL) were injected onto the column and elution was with a gradient of 30–57% acetonitrile over 120 min at a flow rate of 3 mL/min with peak detection at 210 nm. Data acquisition and analysis and system control was through Delta software version 5.0 (Digital Solutions, Margate, Queensland, Australia) using Waters model 510 pumps, a model 710B automatic sampler, and model 490 detector. A total of 18 peaks was obtained and the composition of each peak was analyzed. Peaks collected from each of five consecutive runs were pooled, dialyzed against 1 mM acetic acid, and freeze-dried for subsequent analysis by SDS-PAGE.

Preparative Isoelectric Focusing (IEF)

Freeze-dried LMW-GS from Roblin 1A (250 mg) were dissolved in 50% *n*-propanol (53 mL) containing 1% DTT and heated at 65°C for 30 min. After cooling, ampholyte 2% (v/v), pH 4–6 (Pharmacia), was added and mixed. The sample was loaded into a Rotofor solution phase IEF apparatus (Bio-Rad, Hercules, CA) and cooling was provided by recirculating water at 4–6°C. The initial fractionations were performed at 12 W constant power for up to 4 hr. Twenty fractions were harvested and the pH of each fraction was measured; an aliquot was analyzed by SDS-PAGE. Suitable fractions were pooled, diluted to 55 mL with aqueous *n*-propanol containing DTT (to 1%, w/v, final), fresh electrode buffer was added, and the samples were refractionated in the Rotofor appa-

ratus. Power was adjusted to between 8 and 12 W to keep the voltage <2,500 V. Analyses of the fractions were performed as for the initial fractionation.

Amino Acid Sequencing

Sequencing was performed with a model 494 protein sequencer (Applied Biosystems, Foster City, CA). Aliquots of 50–100 μL of total glutenin extracts from Roblin and Roblin 1A were loaded onto SDS-PAGE gels in which the resolving gel had been polymerized two to three days ahead of use to remove free acrylic acid. Thioglycolic acid (2 μM) was added to the top tank during electrophoresis and after staining bands were excised. Protein was eluted from the gel fragments by adding 150 μL of 0.2M Tris-HCl, pH 8.5, containing 2% SDS (for 2–3 hr at room temperature) and diluted to 500 μL with water. The liquid was removed and placed onto a polyvinylidene difluoride disk (prewetted in 100% methanol) and air-dried overnight. Analyses of the sequence data were performed by Applied Biosystems 610 software model 610A data analysis program and independently by the operator. Average repetitive yields were >90% and indicated good confidence in the deduced sequence together with correspondence between the sequence determined instrumentally and by the experienced operator.

SDS-PAGE

Total glutenin subunits were prepared from flour (40 mg) after 30-min preextractions at 20–25°C in 1.5 mL of 50% (v/v) aqueous *n*-propanol. The residue was extracted with 125 mM Tris-HCl, pH 7.5, containing 1% (w/v) DTT for 30 min at 65°C. After centrifugation (10 min at 15,000 × *g*), the polypeptides in the supernatant were alkylated with 4-vinylpyridine (2.8%, v/v) at 65°C for 15 min and diluted with an equal volume of sample loading buffer (250 mM Tris-HCl, pH 7.5, 4% [w/v] SDS, 20% [v/v] glycerol with 4% [v/v] mercaptoethanol). The alkylated glutenin subunits were fractionated on a 12% T, 2% C polyacrylamide gel (15 cm × 17 cm × 1.5 mm), run initially at 70 V until samples had left the stacking gel then at constant voltage for 1,800 Vhr (Skerritt and Underwood 1986). Fractionated proteins were stained using colloidal Coomassie G-250 in methanol-ammonium sulfate-phosphoric acid (Neuhoff et al 1991) except for sequencing or where indicated using Coomassie R-250 in 40% (v/v) methanol, 10% (v/v) acetic acid in water.

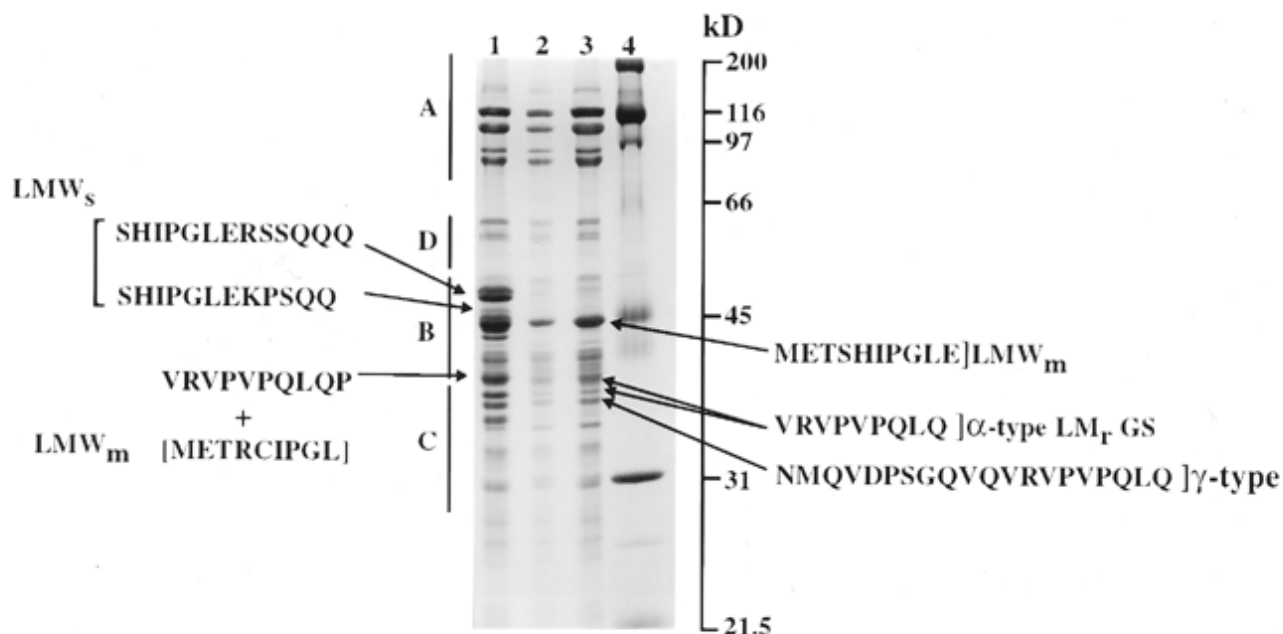


Fig. 1. SDS-PAGE separation of total reduced and alkylated glutenin subunits stained with Coomassie Blue R250 from Roblin and Roblin 1A. N-terminal sequences for selected polypeptides and LMW_s and LMW_m groupings are indicated. Tracks 1,2 = Roblin; 3 = Roblin 1A; 4 = molecular weight markers.

Dough Mixing

Mixing tests were performed with a 2-g mixograph using an established technique to incorporate glutenin subunits (Gras and Bekes 1996). The base flour used in mixing studies was a Gabo null line for HMW-GS 17+18. Various samples were used in the dough mixing experiments and include: 1) pools of ion-exchange chromatography fractions containing only a few LMW-GS of different sequence types (Table I); 2) total LMW-GS from Roblin and Roblin 1A; 3) a B-type LMW-GS purified by IEF; and 4) a HMW-GS, 1Bx7.

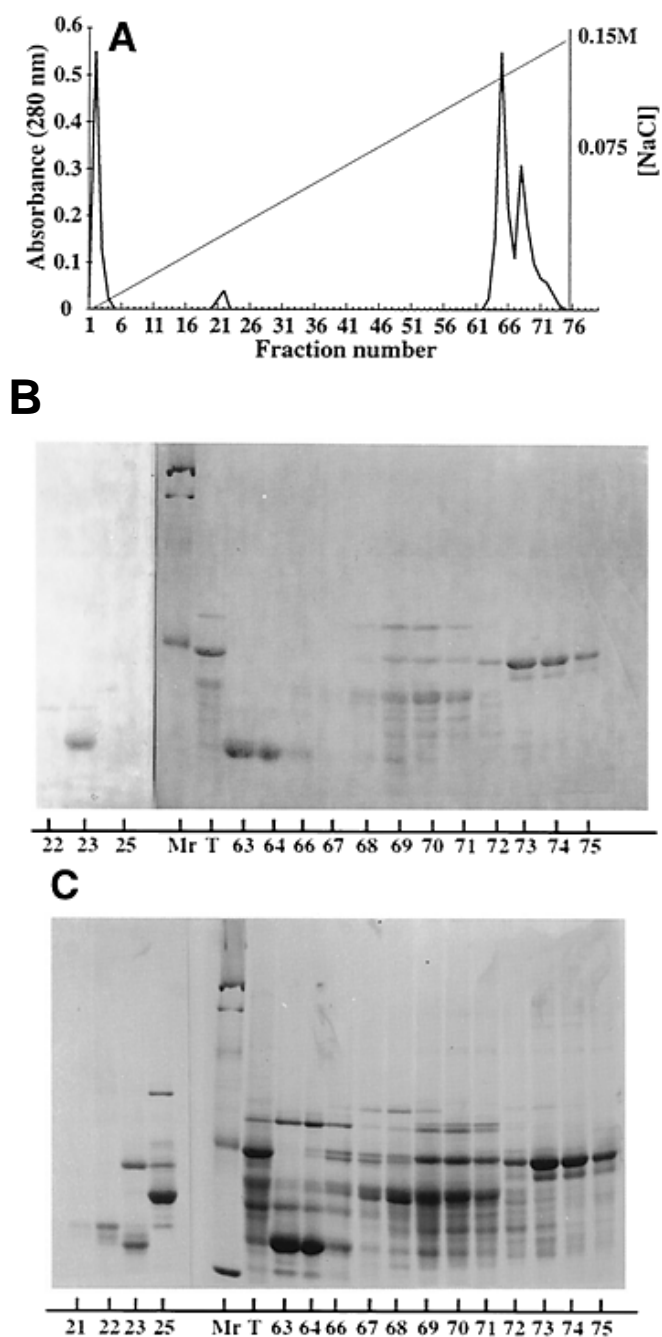


Fig. 2. Separation of low molecular weight glutenin subunits by SP-Sepharose chromatography. **A**, Absorbance (280 nm) profile from the start of the NaCl gradient. **B**, SDS-PAGE analysis of selected fractions stained with Coomassie Blue R250. **C**, Stained with colloidal Coomassie Blue G250. M_r = molecular weight markers at 31, 45, 66, 97, and 116 kDa. T = Roblin 1A.

RESULTS AND DISCUSSION

N-Terminal Amino Acid Sequencing of LMW-GS

To simplify the number of LMW-GS polypeptides from which to obtain a subunit, we chose a spontaneous null of the cultivar Roblin, which has only chromosome 1A LMW-GS. We sequenced several LMW-GS polypeptides (Fig. 1) and the results produced N-terminal sequences of the type described by Lew et al (1992). Some of the polypeptides contained a mixture of sequences due to similar mobilities on one-dimensional SDS-PAGE, and it was not possible to identify those sequences with confidence.

Ion-Exchange Chromatography

Strong cation exchangers have been used for almost 30 years to fractionate gliadins (Oh and Gehrke 1965) and have also been used for HMW-GS partial purification (Shewry et al 1984). An earlier partial purification of several hundred milligrams of gliadins using SP-Sepharose chromatography (Weegels et al 1994) suggested that this matrix may have potential for preparing large quantities of partially pure LMW-GS. To ensure good solubilities of the LMW-GS, we used 50% iso-propanol and 2 mM DTT in

TABLE I
Effect of Incorporation of Low Molecular Weight Glutenin Subunit (LMW-GS) Fractions Isolated by Ion-Exchange Chromatography on Dough Properties

Sample	Major Sequence	Mixing Time (sec)	Resistance Breakdown (%)
Roblin 1A control		81a ^a	52a
62-65	Mainly γ - and α -type	80a	50a
73-76	Mainly METSHIP-	94b	50a
67+68	α -Type and others (unknown)	102b	42b
LSD ^b		12	3

^a Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

^b Least significant difference ($P < 0.05$).

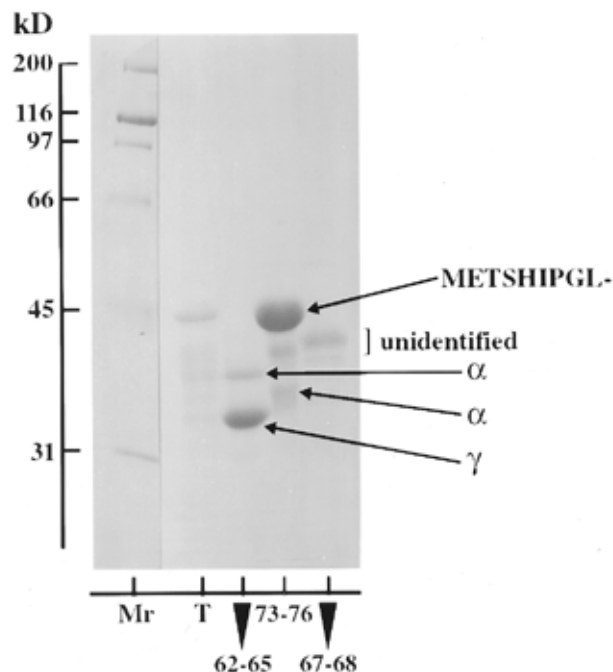


Fig. 3. Pools of like fractions from ion-exchange chromatography of Fig. 2 separated by SDS-PAGE and stained with Coomassie Blue G250. Putative sequences of polypeptides based upon mobility with reference to Fig. 1 are shown. M_r = molecular weight markers at 31, 45, 66, 97, and 116 kDa. T = Roblin 1A.

both the sample and mobile phase. Binding was obtained at pH <5.0 and resolution was optimal using a shallow linear 0–0.15M NaCl gradient. Preliminary experiments showed that applying the LMW-GS extracted from 60 g of flour as a freeze-dried preparation redissolved in column buffer resulted in strong binding of the polypeptide to the column that could not be reversed with increasing NaCl concentration. To avoid poor column recoveries, it was necessary to maintain a low level of DTT throughout the chromatography to reduce the polymerization of unalkylated LMW-GS and to avoid high NaCl concentrations (>0.5M) that caused protein precipitation. At pH 4.6, all of the LMW-GS bound to the column and no proteins were detected in the column wash by electrophoresis (data not shown). The elution profile after application of the NaCl gradient showed an absorbance peak in fractions 2–5 (<0.01M NaCl), but this did not contain protein that was detectable by SDS-PAGE (Fig. 2A). However, polypeptides with molecular weights <15,000–20,000 were not retained on the electrophoresis gel because conditions were optimized for the separation of the LMW-GS. There was a small peak in fractions 21–24, which contained two to three C-subunits (Fig. 2C). Most of the separation occurred between 0.10 and 0.14M NaCl. Several groups of polypeptides were obtained, each containing a main polypeptide with smaller amounts of other proteins; these were more apparent when the high-sensitivity protein stain (Neuhoff et al 1991) was used (Fig. 2C). When like fractions were pooled and reanalyzed, fractions 62–65 contained two C LMW-GS. The stronger staining band represented 72% of the total stain (by densitometer scan) and

based on its mobility with reference to Fig. 1 is likely to have the γ -type N-terminal sequence. The minor polypeptide aligns with an α -LMW-glutenin subunit sequence (Fig. 3). The pool created from fractions 73–76 consists of several polypeptides but the predominant one is a M_r 45,000 polypeptide representing >43% of the total protein loaded. This band aligns with a B LMW-GS from Roblin 1A with the METSHIPGL- N-terminal sequence (Fig. 1). The pool of fractions 67+68 contains several polypeptides from the B and C group. The C-type are likely to be α - and γ -types, whereas the B-type polypeptides have not been sequenced and remain to be identified. It should be emphasized, however, that these polypeptide fractions are the main components present and staining of the gel using the much higher sensitivity stain (Neuhoff et al 1991) reveals more minor polypeptides (Skerritt and Lew 1990).

The three pools of LMW-GS encoded by chromosome 1A were analyzed for the effects on dough mixing time by incorporation into the base flour (Table I). Pool 62–65, containing α - and γ -type LMW-GS sequences had no effect on peak mixing time (MT) and resistance breakdown (RBD). Pool 73–76, which contained mainly a METSHIPGL sequence, increased MT by 16%. The major polypeptide was isolated by IEF and increased MT (Fig. 1). Pool 67+68 contains polypeptides whose sequences cannot be deduced because this group of bands have not been sequenced (Fig. 1). The polypeptides in this pool increased MT by 26% and decreased RBD by 20%. These effects were produced using only 1 mg of subunit, indicating a potent effect for this group of B subunits. It is possible that this pool may contain the MET-

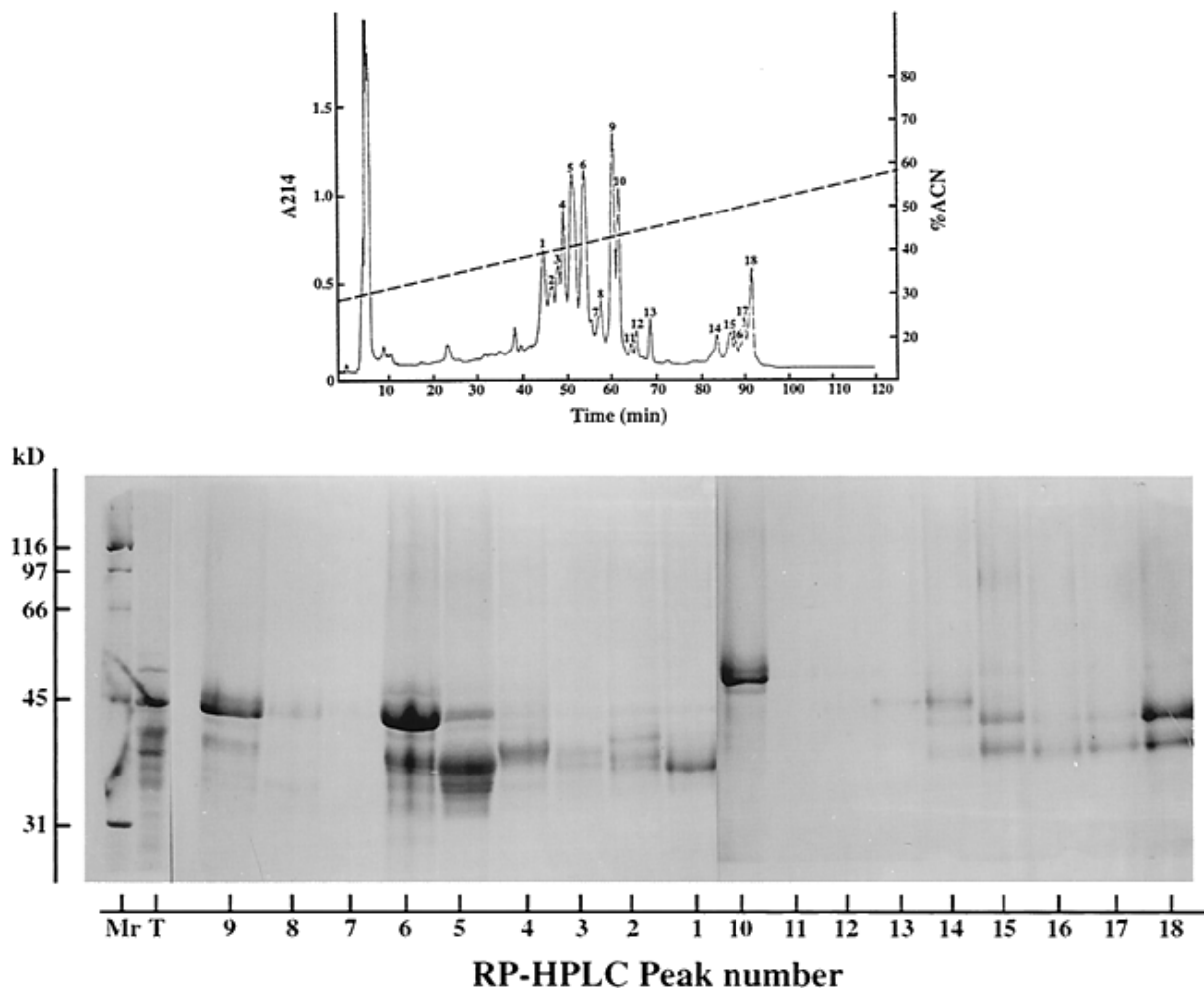


Fig. 4. A, Reverse-phase HPLC of low molecular weight glutenin subunits prepared by acetone fractionation from Roblin 1A flour. Gradient of 30–57% acetonitrile (ACN) in 0.1% trifluoroacetic acid over 120 min. Peaks collected for analysis by SDS-PAGE are numbered. B, SDS-PAGE analysis of peaks from RP-HPLC stained with colloidal Coomassie Blue G250. M_r = molecular weight markers at 31, 45, 66, 97, and 116 kDa. T = Roblin 1A.

SCIPGL- sequence. Recently, an expressed LMW-GS from *T. boeoticum* with this N-terminal sequence has been shown to increase MT by as much as the HMW-GS, Bx7 (Y. K. Lee, F. Bekes, M. K. Morell, R. B. Gupta, and R. Appels, *unpublished data*).

Semi-Preparative RP-HPLC

Alkylation was avoided since we were interested in collecting pure fractions for functionality testing in which the cysteines were unaltered. The best resolution that we obtained on the semipreparative column is shown in Fig. 4A. A doubling of the column loading to 10 mg gave poor resolution of the main peaks (4–6, 9–10), so that multiple separations were required to obtain sufficient polypeptide for analysis. However, in no peak were single bands on SDS-PAGE obtained, which is not unexpected, since very few peaks showed baseline separation (Fig. 4B). Although it was possible to separate the C and B LMW-GS, two or more polypeptides were always present in the peaks. It is more likely that success in the purification of other subunits will be possible using ion-exchange chromatography as the first step followed by RP-HPLC of the less complex polypeptide composition to isolate individual subunits (Sissons et al 1996).

Preparative Electrophoresis and Isoelectric Focusing

Recently, the separation of some reduced, alkylated LMW-GS in a one-step procedure using preparative acid PAGE was described

(Curioni et al 1995). This method is attractive as it involves minimal manipulation but uses alkylation of the subunits which alters their functionality in mixing studies. We attempted to separate reduced LMW-GS by this method but obtained poor resolution of the LMW-GS. Further work is required to improve the resolution of reduced LMW-GS using this technique.

Preparative IEF has been used to purify some HMW-GS (Khan and Bushuk 1979) using an electrofocusing column with urea as solvent. Urea has the potential to alter the pH gradient. Isocyanate formed by decomposition of urea may result in carbamylation of polypeptides (O'Farrell 1975). To overcome this, some resolution of glutenin polypeptides has been achieved using 50% *n*-propanol over a pH 3–10 gradient (Curioni et al 1990). Preliminary IEF of LMW-GS from Roblin 1A using a Rotofor apparatus and a pH 3–10 gradient in the presence of 50% *n*-propanol, showed that the best fractionation occurred over the pH range 4–7, indicating that a narrower pH range may be best (data not shown). Using a pH 4–6 ampholyte range, an almost linear pH gradient was obtained (data not shown) and this provided a good resolution of the LMW-GS in Roblin 1A between pH 4 and 5 (Fig. 5A). The protein load contains no HMW-GS that could potentially contaminate purified fractions, since the pI of some x-type HMW-GS is <pH 5 (Curioni et al 1990). Fractions in the pH range 4.16–4.40 contain two main polypeptides (M_r 35,000 and 45,000) plus minor contaminants, detectable with the high-sensitivity protein stain. Poor fractionation occurred at >pH 4.8. To further improve the separation, fractions of pH 3.80–4.64 were pooled and refractionated at 10 W constant power for 2.5 hr. The pH profile showed a much shallower linear gradient between pH 4.2 and 4.6 (not shown). This

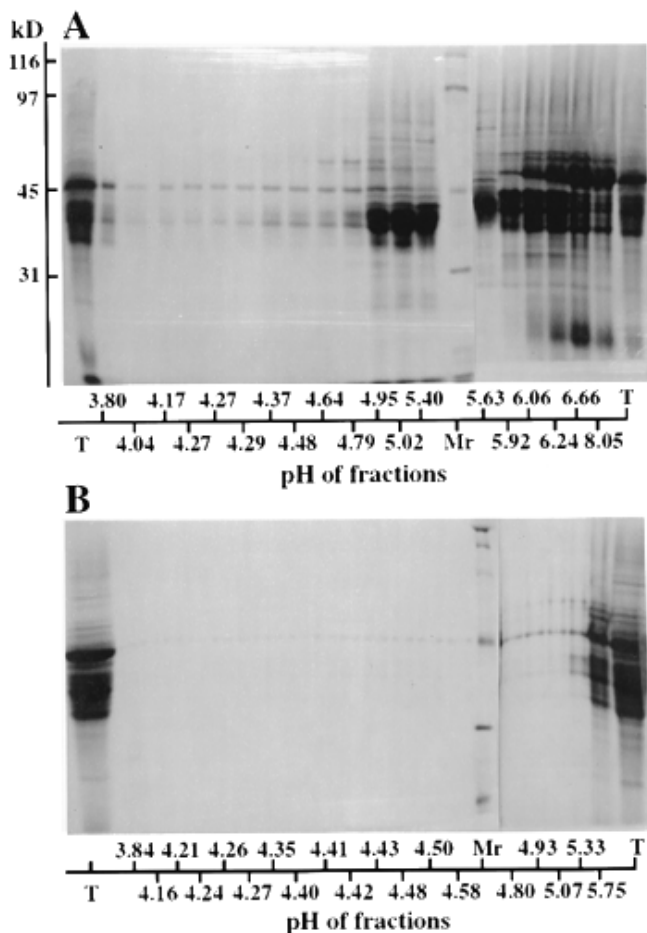


Fig. 5. Analysis by SDS-PAGE of the content of Rotofor fractions obtained from the preparative isoelectric focusing of low molecular weight glutenin subunits (LMW-GS) from Roblin 1A. Gels are stained with colloidal Coomassie Blue G250. **A**, Initial fractionation. **B**, Refractionation of fractions from pH 3.80–4.64 of the initial fractionation. M_r = molecular weight markers at 31, 45, 66, 97, and 116 kDa. T = Roblin 1A. Numbers refer to the pH of fractions 1–19 inclusive for each fractionation.

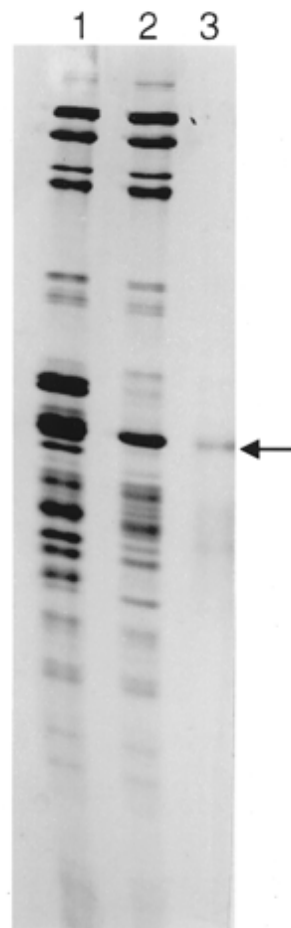


Fig. 6. SDS-PAGE analysis of B-subunit purified by isoelectric focusing (IEF). Lane 1: Roblin 1A, 1B, and 1D total glutenin. Lane 2: Roblin 1A total glutenin. Lane 3: Pool from IEF of fractions with pH 3.84–4.42 as shown in Fig. 5B.

small change in pH allowed purification of the main B LMW-GS found in Roblin 1A (Fig. 5B). To obtain sufficient material for dough testing, these IEF two-step separations were repeated several times and the resulting pool of fractions were combined, dialyzed against 0.1 mM acetic acid, and freeze-dried. Analysis of the resulting pool by SDS-PAGE revealed minor contaminants, but the M_r 45,000 polypeptide accounted for \approx 90% of total protein loaded (Fig. 6). Based on mobility, this subunit corresponds to the N-terminal METSHIPGL sequence (Fig. 1). This was confirmed by the specific recognition of this polypeptide in immunoblotting using a specific monoclonal antibody (42327 5G5) that has been prepared to this peptide sequence (data not shown).

Effects on Mixing Parameters of LMW-GS Fractions After Incorporation into a Base Flour

The effects of 5 mg of a total LMW-GS extract prepared from Roblin and the Roblin 1A double null together with purified B subunit from 1A and HMW-GS Bx7 were studied by incorporating the protein into the base flour and obtaining mixing curves using a 2-g mixograph (Bekes et al 1994). The ability of isolated, individual LMW-GS to be incorporated into the glutenin polymer has been previously demonstrated by size-exclusion HPLC analysis of the glutenin polymers extracted from doughs from a base flour and the flour containing added or incorporated LMW-GS (Y. K. Lee, F. Bekes, M. K. Morell, R. B. Gupta, and R. Appels, unpublished data). In that study, the incorporation treatment increased the proportion of polymeric protein from 36 to 41% of the total, while simple addition of the subunits slightly decreased the proportion of polymeric protein. Incorporating the HMW-GS Bx7 into a base flour had a strengthening effect, increasing MT significantly and decreasing RBD (Table II), a result consistent with earlier findings (Bekes et al 1995). Incorporation of total LMW-GS from both euploid and Roblin 1A wheats also significantly increased MT and decreased RBD but to a lesser extent than Bx7. LMW-GS from chromosome 1A tended to increase MT and decrease RBD to a lesser extent than those from Roblin, but the differences were not significant. These results contrast with those of Saperstein and Fu (1996), who found that total LMW-GS from Roblin and three other varieties caused only slight changes in mixing properties. It is possible that the differences between these results and ours relate to either the choice of the base flour, or more likely the preparation method for the subunits. Some differences include our use of a buffered solution to extract the glutenin, use of DTE rather than DDT as the reducing agent, and perhaps most importantly, a lower concentration of acetic acid for dialysis of the subunits we prepared.

Incorporation of the purified METSHIPGL- LMW-GS increased MT to a greater extent than either of the total LMW-GS preparations, but the effect was less than the Bx7 HMW-GS. The RBD for the B subunit was decreased from the control flour, but

there was no significant difference from the LMW-GS preparations from Roblin for this parameter. This difference in effects of the purified subunit from the total preparation could be because a much greater amount of this subunit was added than is present in the natural ratio for either Roblin or Roblin 1A. The larger effect on MT of the purified subunit could be due to two factors. One is its larger size compared to most other LMW-GS, since the size of HMW-GS has been correlated positively with the extent of increase in MT (Bekes et al 1995). Alternatively, the family of subunits with a N-terminal METSHIPGL- sequence may have a greater effect than other LMW-GS families. Even though this sequence does not have a cysteine available to form intermolecular disulfide bonds in the immediate N-terminal region, it has recently been shown in a bread wheat cultivar (Yecora Rojo) that LMW-GS of the METSHIPGL- sequence type have a cysteine residue in the repeat region of the sequence near the N-terminus that is available to form interchain disulfide bonding (Masci et al 1996). These types of sequences could act as chain extenders and so explain the increase in MT observed.

Differentiating the LMW-GS on the basis of the N-terminal sequences helps to identify the different subunits that can be isolated by a range of techniques. Potentially this distinction is important because it has been shown that in durum wheat the ratio of chain extenders (sequences beginning with a serine or methionine) to chain terminators (N-terminal sequences of the α - and γ -type) can influence quality (Masci et al 1995). More information is required on the functionality of each of the six main sequence types by the approaches described in this communication to clarify the roles of the LMW-GS in breadmaking quality.

ACKNOWLEDGMENTS

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TABLE II
Effect of Incorporation of Various Glutenin Fractions on Dough Properties

Sample ^a	Mixing Time (sec)	Resistance Breakdown (%)
Control flour	158.0a ^b	19.0a
HMW-GS Bx7	252.0d	10.0b
Roblin 1A, 1B, 1D total LMW-GS	191.5b	11.5c
Roblin 1A total LMW-GS	182.5b	13.0c
Isoelectric focusing purified B LMW-GS	214.5c	11.0c
LSD ^c	10.7	2.5

^a HMW-GS = high molecular weight glutenin subunits; LMW-GS = low molecular weight glutenin subunits.

^b Data are the mean of three experiments. Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

^c Least significant difference ($P < 0.05$).

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