Polymeric Glutenin of Wheat Lines with Varying Number of High Molecular Weight Glutenin Subunits[1]

L. GAO and W. BUSHUK

ABSTRACT

Glutenin of wheat lines that were null for some or all of the high molecular weight (HMW) subunits was extracted with urea-sodium dodecyl sulfate (SDS) solvent from the Osborne glutenin (ethanol-insoluble) fraction. For the eight lines examined, the amount of glutenin protein ranged from 32 to 55% of the flour protein. The line with no HMW subunits had the lowest amount of glutenin, while the line with all five of the HMW subunits had the highest amount. The glutenin, dissolved in urea-SDS, was fractionated by gel filtration on Sephacryl S-500 using the same solvent as the eluent. Gel-filtration fractions were collected, partially purified by ultrafiltration and dialysis, and analyzed by SDS-polyacrylamide gel electrophoresis (PAGE). Gel-filtration results showed that there was little difference in the glutenin elution profile for the different wheat lines. The elution profile for the line that contained no HMW glutenin subunits was essentially the same as that for the control that contained all five of the HMW glutenin subunits. These results indicate that the low molecular weight (LMW) glutenin subunits by themselves can form glutenin polymers that appear to be similar in molecular size to the polymers containing both the HMW and the LMW subunits. These results suggest that the contribution of glutenin to intervarietal differences in bread-making quality in the eight wheat lines examined depends on properties other than the differences in molecular weight distribution.

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Wheat proteins are classified as albumin, globulin, gliadin, and glutenin, based on the classical, but still widely used, Osborne (1907) system. It is now accepted that gliadin (aqueous ethanol-soluble) and glutenin (aqueous ethanol-insoluble) are distinctly different classes of wheat storage proteins. Gliadins comprise molecules of single polypeptide chains, while glutenin is a group of large polymeric molecules composed of both high molecular weight (HMW) and low molecular weight (LMW) subunits linked together by interpolypeptide disulfide bonds. The subunit composition of glutenin, obtained after reduction of disulfide bonds, is well defined, but very little is known about the structure of glutenin polymers (molecules) (see Bushuk and Tkachuk 1991 for review). Elucidation of the functional role of glutenin in bread-making potential of wheat flour requires detailed information on its polymeric structure.

This article presents results of studies on the polymeric structure of glutenin of wheat lines of widely diverse bread-making quality that were genetically developed (Lawrence et al 1988) to contain gradually decreasing HMW glutenin subunits from five (normal) to zero.

MATERIALS AND METHODS

Glu-1 Null Wheat Samples

Seed of eight so-called *Glu-1* null wheat lines used in this study, designated A1 (normal HMW glutenin subunit composition 1,

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17+18, and 5+10) through to A8 (no HMW subunits), was provided by F. MacRitchie of the Commonwealth Scientific and Industrial Research Organization, Grain Quality Research Laboratory, Sydney, Australia. The nomenclature of Payne and Lawrence (1983) for the HMW glutenin subunits is used throughout this article. The grain was grown on an experimental field at the University of Manitoba. The lines were originally derived from crosses between a mutant line of the cultivar Olympic, null at the *Glu-B1* locus due to a deletion of part of the long arm of chromosome 1B, and an isogenic line of the cultivar Gabo, null at the *Glu-A1* and *Glu-D1* loci (Lawrence et al 1988). The HMW subunit composition, determined originally by Lawrence et al (1988) and confirmed in our laboratory, and the wheat and flour protein (N × 5.7) contents of the eight lines are given in Table I. The wide variation in protein content of the null lines has been attributed to the variation in vigor (Lawrence et al 1988).

Chemicals

Urea from Sigma Chemical Co. (St. Louis, MO) was used without further purification. Electrophoretically pure SDS was obtained from Bio-Rad Laboratories (Richmond, CA). All other chemicals used were of reagent grade.

Protein Determination

Total protein (N × 5.7) content of grain, flour, and glutenin preparations were determined by the micro-Kjeldahl method 46-13 of AACC (1983).

Preparation of Glutenin

Wet glutens were prepared from the flours according to AACC (1983) method 38-10. The wet gluten was used immediately for preparation of the ethanol-insoluble fraction (glutenin) as described in Gao and Bushuk (1992).

The yields of glutenin protein, as a proportion of flour protein of samples A1–A6, were 55.0, 53.2, 45.6, 53.7, 41.8, and 38.2%, respectively. The yields of gluten, and hence glutenin, from A7 and A8 flour samples were extremely variable under mechanical washing conditions. The glutenin protein yields were 17.0 and 7.8% for A7 and A8 using the approved hand-washing procedure (AACC method 38-10) and 41.6 and 32.3% using a modified, more cautious hand-washing procedure. The protein (N × 5.7, db) contents (Table I) of the glutenin preparations (hand-washing procedure for A7 and A8) ranged from 68.7% (lowest) for A8 to 77.2% (highest) for A5.

Amino Acid Analysis

The hydrolysis of glutenin for amino acid composition analysis was carried out in 6*N* HCl for 24 hr under vacuum. The composition was determined on an automated LKB analyzer using an ion-exchange separation procedure and a ninhydrin detection and quantization method (Andrews and Baldar 1985).

Gel-Filtration Chromatography

Gel-filtration conditions. Sephacryl S-500 (Pharmacia, Uppsala, Sweden), in dispersed form, was equilibrated with a solvent of 6*M* urea and 6% (w/v) SDS. The gel slurry was poured into a column (2.6 × 100 cm). The same solvent was used for elution. The column was maintained at 40°C by circulating water through the jacket around the column.

The column was operated by downward flow, and the elution was monitored continuously at 280 nm. The elution rate was 14 ml/hr. Fractions were collected by an LKB 2211 Superrac automatic fraction collector.

Before each separation, the column was calibrated for molecular weight by determining the elution volume for three reference proteins: bovine thyroglobulin (669 kDa), horse spleen apoferritin (443 kDa), and bovine serum albumin (66 kDa). The reference proteins were obtained from Pharmacia LKB (Montreal, PQ). No variation in elution volume for reference proteins was observed in duplicate experiments. At the end of each separation, the column was washed with water and NaOH solution (0.01*M*) and the eluates were concentrated (after being neutralized with 6*N* HCl for the latter eluate) and freeze-dried using the same procedures used for protein fractions. No protein was detected by SDS-PAGE in the eluates collected from two consecutive washings.

Solubilization of glutenin. Glutenin (30 mg of protein) was weighed into a 15-ml centrifuge tube and solubilized using 6*M* urea and 6% (w/v) SDS solvent as described in Gao and Bushuk (1992), with slight modification. After vortexing, following 24 hr of standing and before centrifugation, the solution was overlaid with a layer of hexane according to Payne and Corfield (1979). After centrifugation, a syringe was used to penetrate the hexane layer and remove the predetermined amount of supernatant for immediate application onto the gel-filtration chromatography column.

Fraction collection and purification. Two-hundred-drop fractions were collected and pooled into larger working fractions. The pooled fractions were concentrated on an ultrafiltration apparatus (Amicon Corporation, Lexington, MA) to remove most of the urea and dialyzed (MW cut-off of dialysis tubing, 5 kDa, from Medical Industries, Inc., Los Angeles, CA) against distilled water. For the first two days, the dialysis was performed at room temperature to prevent precipitation of SDS at refrigeration temperature. Then, the dialysis was performed in a refrigerator until no foam was evident in the dialyzate. The contents of the dialysis tube were transferred into plastic containers, frozen, and freeze-dried. The dry material was carefully transferred into containers, sealed, and stored in a freezer until used for SDS-PAGE analysis.

TABLE I
Some Properties of *Glu-1* Null Wheat Lines

Sample	High Molecular Weight Glutenin Subunits[a]			Protein Content, %		Glutenin Protein, %	
	Glu-A1	*Glu-B1*	*Glu-D1*	Wheat	Flour	Content	Yield
A1	1	17+18	5+10	9.9	9.3	72.5	55.0
A2	1	...	5+10	10.7	9.4	74.9	53.2
A3	1	17+18	...	10.6	9.6	70.6	45.6
A4	...	17+18	5+10	9.5	8.2	75.7	53.7
A5	...	...	5+10	11.1	10.0	77.2	41.8
A6	...	17+18	...	10.1	7.8	69.1	38.2
A7	1	...	...	11.4	10.4	73.0	17.0[b]
A8	...	...	...	11.3	10.0	68.7	7.8[b]

[a] Nomenclature according to Lawrence et al (1988).
[b] Values from hand-washing procedure varied widely; lowest values are reported.

SDS-PAGE Analysis

SDS-PAGE was carried out on an LKB 2001 unit according to Ng and Bushuk (1987) under unreduced and reduced (with 5% 2-mercaptoethanol in extracting buffer) conditions. The stacking and separating gel concentrations were 3.03 and 17.3%, respectively. Electrophoresis was for 45 min at 35 mA followed by 18 hr at 15 mA. Electrode buffer (maintained at 20°C) was circulated during electrophoresis.

Sample from fractions collected by gel filtration were prepared according to Ng and Bushuk (1987). The size of the samples were adjusted so that the glutenin subunit bands were of sufficient intensity for visibility and resolution. In a preliminary experiment, the samples from the excluded peak were found to contain much less protein. Accordingly, the volume of these samples applied onto the gel was doubled. The second and third fractions contained a substantial amount of protein; the volume used for their electrophoresis was halved.

RESULTS AND DISCUSSION

Characterization of the *Glu-1* Null Wheats

SDS-PAGE patterns of extracts of the eight flour samples under unreduced conditions showed very little variation among the samples (Fig. 1A). The patterns under reduced conditions (Fig. 1B) confirmed the results for the same wheat lines reported by Lawrence et al (1988). The patterns for some flour samples under reduced conditions contained faint bands in the position of the

HMW subunits that supposedly had been deleted. It is not known whether they are the regular HMW subunits, produced by a very low level of gene activity, or some other polypeptides, such as the K-bands reported by Kazemie and Bushuk (1990) or the E-bands reported by Gupta and Shepherd (1987). Their complete identity was not investigated in this study.

As expected from their glutenin contents and HMW subunit compositions, the chemical, rheological, and bread-making test results showed marked differences among the eight wheat samples (data not shown). Generally, the deletion of HMW glutenin subunits shortened the farinograph dough development time and increased the mixing tolerance index values; farinograph absorption was not affected. Extensigraph results indicated that deletion of the HMW subunits caused a drastic decrease in resistance to extension. The extensibility showed much less variation. These results are consistent with the observed decrease of Zeleny sedimentation values and loaf volume. The technological results obtained in our study are generally consistent with the results of Lawrence et al (1988).

As indicated above, it was difficult to obtain glutens from A7 and A8 (with satisfactory reproducibility) using mechanical washing procedures. The doughs were very plastic but not elastic. The reproducible gluten that was eventually obtained by the hand-washing procedure had the consistency of chewed gum. Normal elastic glutens were obtained for the other samples using both the hand-washing and the mechanical procedures.

Gel-Filtration Chromatography Results

Gel-filtration chromatography profiles of glutenin preparations from the eight samples (Fig. 2) were remarkably similar, in spite of the differences in glutenin content and HMW subunit composition of the glutenin (and rheological and baking properties). Each profile showed one excluded peak and one group of included peaks that overlapped to form a broad peak centered at about 350-ml elution volume. These profiles were generally similar to those of glutenins of Canadian bread wheats (Gao 1992).

The size of the excluded peak varied slightly among the eight samples, but the range of variation was small. Based on visual examination of the size of the peak, it has no apparent relationship to the bread-making potential of flour. This observation is contrary to current dogma, which presumes that the molecular weight distribution of glutenin is related to bread-making potential. It is possible that the resolution of the gel-filtration chromatography used in this study was not sufficient to detect subtle differences in molecular weight distribution. This point requires further study.

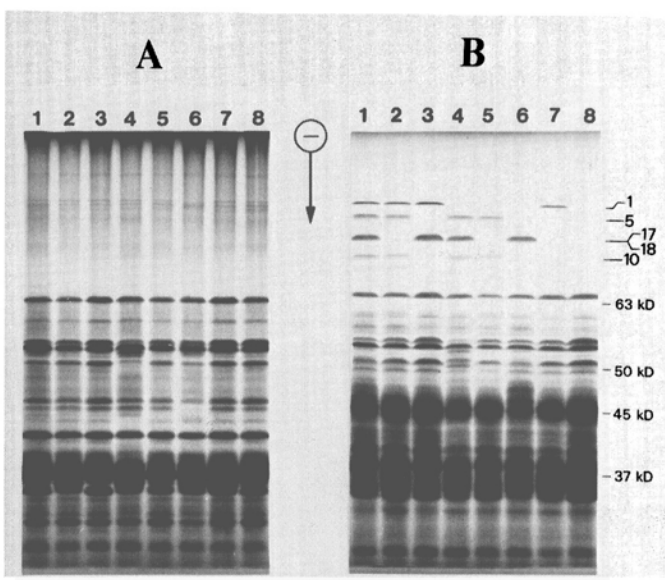

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of extracts of flour of eight Glu-1 null wheat lines used in this study (A1–A8) highlighting the variation in composition of high molecular weight glutenin subunits. **A,** unreduced. **B,** reduced with 5% 2-mereapto-ethanol in extracting buffer.

The shape of the broad included peak of the chromatography profiles varied more among the samples than the excluded peak did, although there was no obvious relationship, judging visually, between size or shape of the broad peak and the rheological or baking properties. Accordingly, we concluded that, for the wheats examined in this study, there appears to be no relationship between molecular weight distribution of the glutenin protein and bread-making potential. This observation does not invalidate the well-known relationship between the amount of acetic acid-soluble and insoluble glutenin and bread-making quality reported by Orth and Bushuk (1972). In the present study, the same amount of glutenin protein was applied onto the column.

One possible explanation of these results is that the variation in glutenin structure that is related to bread-making quality is mostly derived from noncovalent forces, which could affect solubility as observed by Orth and Bushuk (1972). Such intermolecular interaction effects would not be detected by gel-filtration chromatography of dissolved glutenin.

Earlier studies suggested that the elution profiles of solubilized proteins were indeed related to bread-making quality (Huebner and Wall 1976, Bottomley et al 1982, Field et al 1983, Singh et al 1990). In those studies, flours or glutens (not glutenin) were used. Therefore, the results were probably a reflection of the relationship between bread-making quality and the relative content of glutenin in flours. Moreover, the published results might be complicated by incomplete solubilization of the flour proteins as suggested by Huebner and Wall (1976) or by incomplete separation of gliadins and glutenin (Bottomley et al 1982).

We found that the freeze-dried material from the excluded peak of the gel-filtration chromatography contained less protein than the materials from other peaks in the same gel-filtration chromatography (judged visually by the lower band intensity on SDS-PAGE). If the nonprotein constituents in the protein extract also absorb at 280 nm, the peak area-size would not proportionally reflect the molecular weight distribution of protein in a single gel-filtration profile. However, the relative proportion of protein between different gel-filtration profiles should be comparable. To eliminate the possible errors arising from the nonprotein impurities, we recommend that further studies be carried out by monitoring the absorbance at two wavelengths or by determining the protein quantity in the fractions of gel filtration.

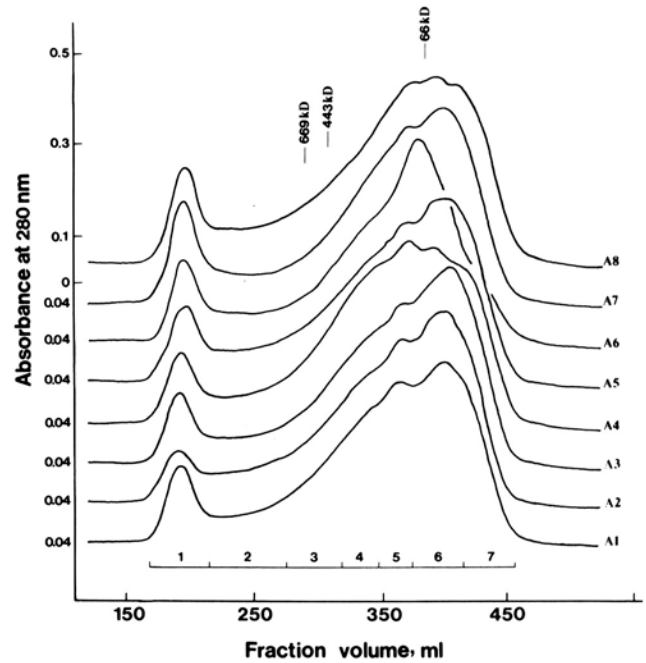

Fig. 2. Gel-filtration chromatography elution profiles of glutenin preparations of eight Glu-1 null wheat lines used in this study (A1–A8) on Sephacryl S-500 in urea-sodium dodecyl sulfate solution. **1–7,** fractions for sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

SDS-PAGE Results for Fractions from Gel Filtration

SDS-PAGE results for sample A1, the wheat line with all five (normal complement) HMW glutenin subunits, showed that the first peak contained both HMW and LMW subunits (Fig. 3B). The HMW subunits of A1 were concentrated in the middle fractions 2–5. Fraction 6 contained only trace amounts of HMW subunits.

SDS-PAGE results (Fig. 4) for the glutenin of A7 (which should contain HMW subunit 1 only) showed that the overall distribution of the HMW subunit among the fractions was very much the same as that for A1. The first peak contained only a slight amount of the subunit and somewhat more of the LMW subunits (based on the intensity of the bands); most of the HMW subunit was concentrated in fractions 2–5. It is interesting to note that, although there is only one HMW subunit in A7, still, it exists in the form of large polymeric molecules, giving the same distribution pattern across the fractions as those obtained for A1 with all five HMW subunits. The rheological and bread-making properties of the two lines were quite different, despite the similar molecular weight distribution of these glutenins.

The SDS-PAGE patterns showed that the faint bands, which were only marginally discernible in the patterns for flour extracts, were much stronger in the patterns of gel-filtration fractions (lanes 3–5, Fig. 3). This is probably due to the concentration effect of gel filtration and the greater amount of protein loaded onto the gel. The fact that the fractions containing these faint bands were the earlier fractions of gel filtration suggests that these bands may be aggregated or linked to the HMW glutenin subunits in the polymeric glutenin.

The distribution pattern for the HMW subunits for the *Glu-1* null lines A2–A6 was essentially the same as that for A1 (not shown). These results suggest that the difference in glutenin quality for breadmaking does not lie in the size distribution of polymeric molecules of glutenin but, probably, in the chemical properties of glutenin subunits contributing to the unique viscoelasticity of gluten and dough. The rheological properties of gluten (and dough) would also be influenced by the relative amount of gliadin and glutenin in the flour.

SDS-PAGE results (Fig. 5) for the *Glu-1* null line A8 (no HMW subunits) fractions from gel filtration showed LMW glutenin subunits in all fractions but mostly in fractions 1–4. The LMW subunits are mainly two groups of polypeptides (36 and 45 kDa). The pattern contains a trace amount of protein in the 58–63 kDa range. These results indicate that LMW glutenin subunits, by themselves, can form large polymeric glutenin molecules too large to enter the separating gel in SDS-PAGE under unreduced conditions (Fig. 5A). These large polymeric molecules appear to be stabilized by interpolypeptide disulfide bonds because they survived the solubilization condition in the preparation of the sample for gel filtration and the SDS-PAGE separation under unreduced conditions. This evidence supports the existence of polymeric glutenin comprising only LMW subunits.

The existence of large polymeric molecules comprised of only LMW subunits raises a question regarding the identity of glutenin molecules. Traditionally, it has been presumed that polymeric glutenin molecules comprise both HMW and LMW subunits. It has been reported that polymers composed of only LMW

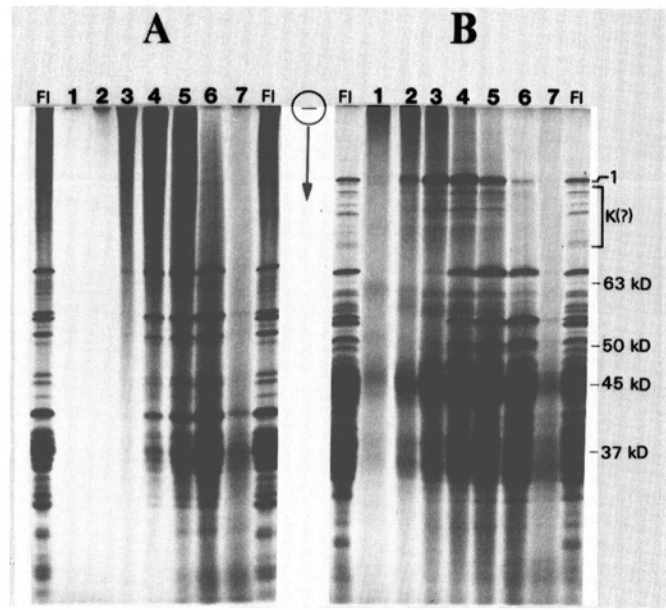

Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of fractions 1–7 from gel filtration of A7 (high molecular weight glutenin subunit 1 only) and extract of A1 flour (Fl). **A,** unreduced. **B,** reduced with 5% 2-mercaptoethanol in extracting buffer.

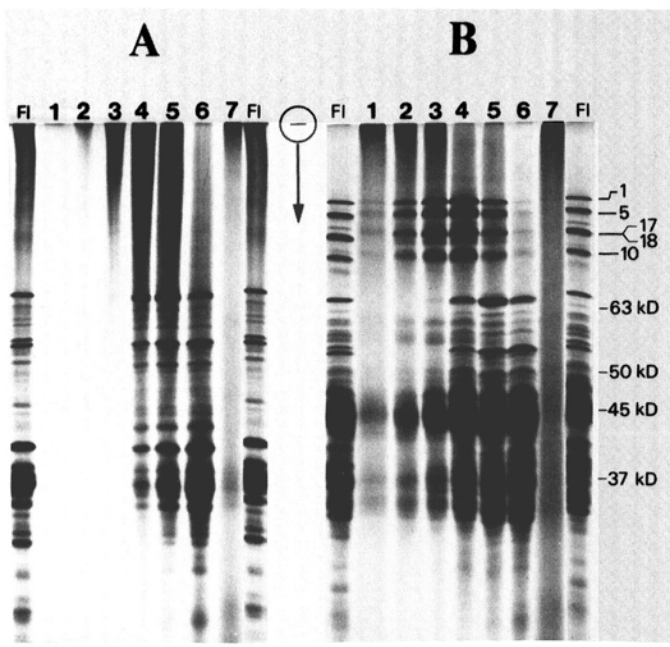

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of fractions 1–7 from gel filtration of A1 (contains all five high molecular weight glutenin subunits) and extract of A1 flour (Fl). **A,** unreduced. **B,** reduced with 5% 2-mercaptoethanol in extracting buffer.

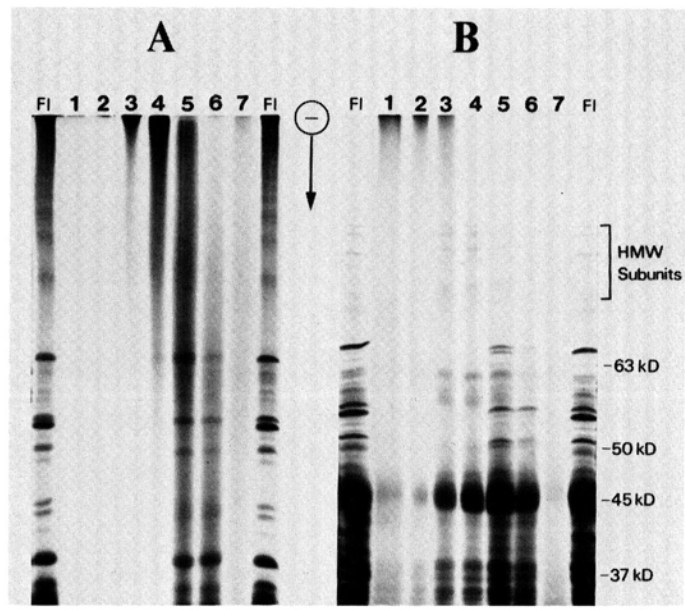

Fig. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of fractions 1–7 from gel filtration of A8 (no high molecular weight [HMW] glutenin subunits) and extract of A8 flour (Fl). **A,** unreduced. **B,** reduced with 5% 2-mercaptoethanol in extracting buffer.

glutenin subunits were present in the Osborne gliadin fraction (Beckwith et al 1966). These polymers have also been called HMW gliadin (Beckwith et al 1966, Bietz and Wall 1973, Bietz and Wall 1980, Huebner and Bietz 1991), aggregated gliadin (Shewry et al 1983), and LMW glutenin (Nielson et al 1968, Kanazawa and Yonezawa 1973, Autran et al 1987, Feillet et al 1989). Until now, the reports that the Osborne glutenin contains polymeric molecules composed of only LMW glutenin subunits have been speculative. The evidence presented here confirms the existence of polymeric glutenin molecules composed of only LMW subunits, as well as the existence of those composed of both HMW and LMW subunits.

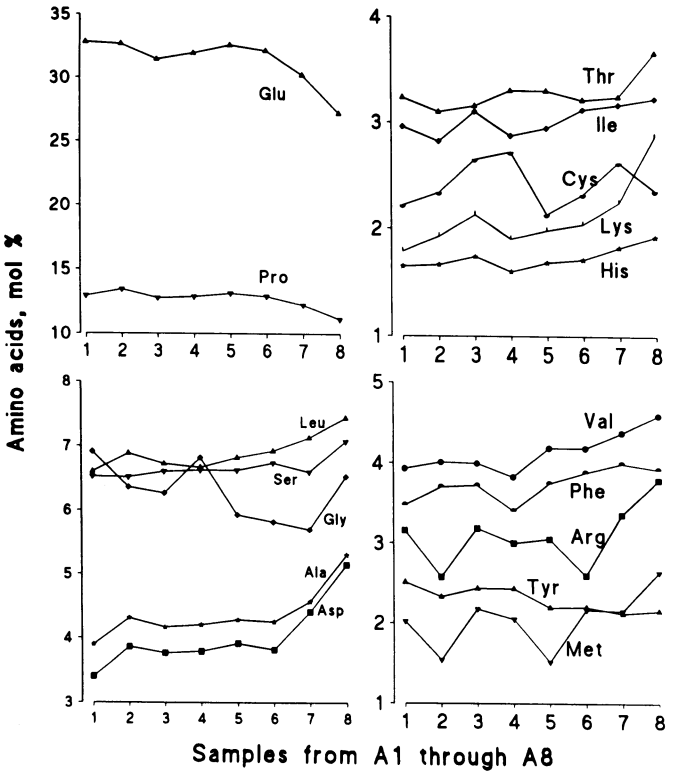

Fig. 6. Content variation of amino acids from glutenin fractions of Glu-1 null wheat lines A1–A8.

Amino Acid Composition

The amino acid composition of the glutenin preparations of the *Glu-1* null lines showed a considerable variation with number and type of HMW subunits (Fig. 6). In general, the content (mol% of total amino acids) of the glutamic acid plus glutamine (Glu) and proline decreased gradually with decreasing number of HMW subunits. Glu decreased in molar percentage from 33% for A1 to 27% for A8, and proline from 13% for A1 to 11% for A8. Tyrosine content decreased also, although to a lesser extent, from 2.5% for A1 to 2.2% for A8.

Several amino acids increased with deletion of HMW subunits. Most significant increases were obtained for alanine (from 3.9% for A1 to 5.3% for A8), aspartic acid (from 3.4% for A1 to 5.2% for A8), and lysine (from 1.8% for A1 to 2.9% for A8).

The amino acid composition (Table II) of the glutenin from A8, the line with no HMW subunits, is generally in agreement with the published composition of the LMW subunits (e.g., gel-filtration peak 4, Belitz et al 1987). Furthermore, it should be noted that the amino acid composition of A8 glutenin is significantly different from that of aqueous ethanol-soluble subunits of reduced glutenin (fraction 2, Bietz and Wall 1973). Also, the composition of A8 glutenin is different from that of the HMW gliadin (fraction 4, Bietz and Wall 1973). Tatham et al (1987) also reported the amino acid composition of HMW gliadins (aggregated gliadins). The Glu and proline contents in their study were higher (~35 and 16%, respectively) than those of A8 obtained in this study, and the glycine content was lower (~3.5%).

Polymeric Glutenin

Gel-filtration chromatography and SDS-PAGE results showed that polymeric glutenin can also be formed from LMW subunits. Furthermore, the molecular weight distribution of polymeric glutenin seems to be independent of the type of glutenin polymers present. For example, the gel-filtration profile for wheat A1, which should contain all different types of glutenin polymers, was essentially the same as the profile for A8, which contains polymers of LMW subunits only. (Compare curves A1 and A8 in Fig. 2.)

The existence of different types of glutenin polymer is consistent with the fact that the HMW subunits form only 10% of the total flour protein, whereas the LMW subunits form about 40% (Halford et al 1992). Our results for wheat line A8 (LMW subunits only) showed that the glutenin formed 32% of the flour protein. Recent studies in our laboratory and in others (Gao 1992, Khan

TABLE II
Amino Acid Composition (mol %) of A8 Glutenin Compared with Those of Relevant Fractions in Literature

Amino Acid	A8[a]	Glutenin		Peaks[b]		Glutenin Fractions[c]				Gliadin[d]
		1	2	3	4	1	2	3	4	
Asp	5.2	4.1	2.8	2.7	4.5	3.2	1.4	0.9	2.0	2.7
Thr	3.7	4.8	3.2	2.7	3.6	3.2	2.4	2.6	2.6	3.1
Ser	7.1	8.2	6.5	7.6	6.8	6.6	7.2	7.1	7.0	5.9
Glu	27.2	20.9	32.3	35.3	29.2	32.7	39.4	41.6	37.5	32.8
Pro	11.1	10.5	12.4	13.6	11.6	11.0	15.0	15.4	15.1	13.5
Gly	6.5	10.9	12.8	4.2	5.6	12.9	3.3	7.4	4.9	9.2
Ala	5.3	5.1	4.1	3.7	5.3	4.1	2.4	2.0	2.7	4.0
Val	4.6	7.3	4.2	4.9	5.8	3.5	4.3	3.1	4.0	1.4
Met	1.6	1.5	1.1	1.6	1.7	0.8	1.3	0.9	1.3	4.8
Ile	3.2	5.8	2.8	4.4	4.9	2.3	3.7	2.9	3.4	3.3
Leu	7.4	6.7	5.9	7.8	7.8	6.1	7.5	5.6	7.4	6.7
Tyr	2.2	3.7	3.8	1.7	2.1	4.2	1.5	2.6	1.7	2.7
Phe	3.9	2.7	2.3	4.2	3.9	2.5	4.5	3.9	4.1	3.2
His	1.9	1.6	1.4	1.6	1.6	1.3	1.3	2.3[e]	1.4	1.5
Lys	2.9	2.4	1.6	1.3	1.9	2.8[e]	2.7[e]	0.4	1.0	1.4
Arg	3.8	3.8	2.8	2.7	3.7	2.7	2.2	1.3	2.6	2.2
Cys	2.4	...	...	...	...	0.0	0.0	0.0	1.3	1.4

[a] Glutenin fraction by the Osborne procedure (Chen and Bushuk 1970) from the completely *Glu-1* null (A8) flour.
[b] Major glutenin subunit peaks fractionated by gel filtration in the order of elution of the reduced Osborne glutenin by Belitz et al (1987).
[c] Bietz and Wall (1973). The glutenin fractions: 1, Ethanol insoluble *S*-aminoethyl derivatives of reduced glutenin (AE-glutenin); 2, ethanol-soluble AE-glutenin; 3, 44-kDa AE-glutenin fraction from 2 after reduction; 4, high molecular weight gliadin.
[d] Bietz and Wall (1973), the purified gliadin fraction.
[e] Data including AE-Cys.

and Huckle 1992, Khan et al 1992) have shown that three types of polymeric glutenin molecules are present in wheats with the normal complement of five HMW subunits together with all of the LMW subunits. On the basis of the known number of sulfhydryl groups in the HMW subunits (Shewry et al 1992), it would not be possible (without presuming a highly branched structure) to accommodate all of the LMW subunits on a linear backbone as required by the model of Graveland et al (1985). This restriction does not apply to other reported models for polymeric glutenin (Gao et al 1992).

CONCLUSION

The results obtained in this study showed that the Osborne glutenin fraction comprises three different types of glutenin polymers: 1) LMW subunits only, 2) HMW subunits only, and 3) both HMW and LMW glutenin subunits. The type of glutenin polymers present does not seem to affect the molecular weight distribution as shown by gel-filtration chromatography. For the set of wheat lines used in this study, the variation in bread-making potential appears to be due to variation in the amount of glutenin (i.e., gliadin-to-glutenin ratio) and to variation in chemical properties (i.e., amino acid composition). Further studies are needed to reconcile these results with our earlier results (Orth and Bushuk 1972), which showed that, in normal wheat cultivars, bread-making quality (loaf volume) was significantly correlated with the proportion of acetic acid-insoluble glutenin in the Osborne fraction.

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