

Purification and Characterization of the Glutenin Subunits of *Triticum tauschii*, Progenitor of the D Genome in Hexaploid Bread Wheat

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ABSTRACT

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N-terminal amino acid sequences and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) molecular weights have been determined for high-performance liquid chromatography (HPLC)-purified high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits (GS) of *Triticum tauschii* ssp. *strangulata*, contributor of the D genome to hexaploid bread wheat. The use of three different extraction procedures resulted in similar glutenin preparations. On the basis of N-terminal sequences, the same types of glutenin subunits that have been reported in bread and durum wheats (HMW-GS of both

the x and y types and LMW-GS of the LMW-s, LMW-m, α -, and γ -types) were found in *T. tauschii*. However, the HMW-GS in *T. tauschii* were in greater proportion relative to LMW-GS when compared to reported values for a bread and durum wheat. Our results support the likelihood that differences in the proportions of the various subunits contributed by the A, B, and D genomes, rather than qualitative differences in the types of subunits, are responsible for the major differences in quality characteristics between bread wheat and durum wheat.

Bread wheat (*Triticum aestivum* L.) is a hexaploid plant in which three different genomes, designated A, B, and D, have become combined in an additive fashion through the rare, but naturally occurring, mechanism of polyploid formation. Vegetative tissues of bread wheat, which contain duplicate sets of chromosomes, have the genomes AABBDD (hence, hexaploid). The closest extant progenitors of the A, B, and D genomes of hexaploid bread wheat appear to be *T. urartu*, *T. speltoides*, and *T. tauschii* (Morris and Sears 1967, Kihara 1975, Bushuk and Kerber 1978, Dvorak and Zhang 1990, Daud and Gustafson 1996). Each of the three different genomes that have become additively combined in hexaploid bread wheat is likely to have contributed different properties to hexaploid wheat. Each, for example, will have contributed a somewhat different complement of gluten proteins (Kasarda et al 1984), which make the single most important contribution to breadmaking quality of any of the wheat endosperm components. An understanding of the nature of these protein differences associated with the A, B, and D genomes and the extent to which they represent qualitative differences in the types of protein components, as opposed to quantitative differences in proportions of the protein components, should be helpful in understanding the molecular basis of breadmaking quality, including the genetic and environmental contributions to variation in quality. In addition, the information may make possible more efficient incorporation of specific quality characteristics when genetic material from wild species is introduced to polyploid wheat.

In this initial study, we have tried to provide insight into the molecular basis for differences contributed by the various genomes of hexaploid wheat by purifying and characterizing the glutenin subunits of *T. tauschii* ssp. *strangulata*, the presumed D-genome contributor (Bushuk and Kerber 1978, Kasarda et al 1984). Purification of glutenin subunits was by solubility fractionation followed by reverse-phase high-performance liquid chromatography (RP-HPLC). Characterization consisted mainly of N-terminal amino acid sequencing and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

MATERIALS AND METHODS

T. tauschii Grain and Flour

The HPLC-purified glutenin components were prepared from seeds or flour of *T. tauschii* ssp. *strangulata*, obtained from the University of California, Riverside, collection (accession no. G1276) and increased at the University of California, Davis. The endosperm fraction (flour) was obtained by milling with a Quadrumat Jr. Mill (C.W. Brabender, South Hackensack, NJ).

Glutenin Preparation

Three different methods of glutenin preparation were compared. These included the methods of Singh et al (1991) and Burnouf and Bietz (1989), which were both based on gliadin-depleted residues, and an acetic acid-soluble glutenin preparation (briefly described in Tao et al [1989]). When the method of Singh et al (1991) was used to remove gliadins before glutenin extraction, several minor modifications were incorporated into the procedure: extractions were made using 2-propanol instead of 1-propanol, gliadin was extracted three times instead of twice, 50 mg instead of 20 mg of flour were extracted per milliliter of propanol, centrifugation was at 14,000 \times g instead of 10,000 \times g, and extractions and reduction were at 65°C. The essentially gliadin-free pellet was suspended in 1 mL of a solvent prepared by mixing equal volumes of 2-propanol and 0.2M Tris-HCl (pH 8.1). Dithiothreitol (DTT) (4 mg) was added, the pellet was vortexed, and reduction was made at 65°C for 30 min. During that interval, the pellet was vortexed several times. The pH was lowered by the addition of 50 μ L of 88% formic acid, and the suspension was centrifuged for 15 min at 14,000 \times g in an Eppendorf centrifuge (Brinkman Instruments, Westbury, NY). In preparation for HPLC, the glutenin-containing supernatant solution was transferred to a clean Eppendorf tube and recentrifuged. The clarified solution was diluted 1:1 with 0.1% aqueous trifluoroacetic acid (TFA), centrifuged at 22,000 \times g for 15 min at 15°C, and loaded into the HPLC injection loop. A similar procedure was followed for seeds, except that extraction was performed in 2-mL screw cap tubes, and the extraction volume was increased to 1.8 mL.

The method of Burnouf and Bietz (1989) was used with minor modification: a second 70% ethanol wash to remove any residual dimethyl sulfoxide (DMSO) following DMSO gliadin and starch removal was incorporated into the procedure. Following the final centrifugation, the 70% ethanol was decanted and the pellet was dried under vacuum before reduction and pyridylethylation.

To obtain the acetic acid-soluble glutenin fraction, the acid-solubilized gluten protein was precipitated by the addition of NaCl.

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The precipitate was then fractionated by gel-permeation chromatography. Early eluting fractions that contained glutenins, but not ω -gliadins (by SDS-PAGE), were pooled and freeze-dried (Tao et al 1989). Before HPLC, the proteins were reduced and alkylated with 4-vinylpyridine.

RP-HPLC

Purification of solubility fractions was done using either a SpectraPhysics or a Beckman System Gold HPLC apparatus. Eluted proteins were monitored at either 215 or 230 nm. The column was a Vydac C-4 tp214 (5 μ m particle size, 30 nm pore size) analytical column (4.6 \times 250 mm). Columns were protected by a 2- μ m inline removable stainless steel frit (Valco Instruments Co., Houston, TX). Organic solvents for column elution were of HPLC grade. Acetonitrile (MeCN) was obtained from Fisher Scientific (Pittsburgh, PA); 2-propanol was obtained from Burdick and Jackson (Muskegon, MI). Trifluoroacetic acid (TFA) was obtained from Pierce (Rockford, IL). Solvents for elution of the column, both aqueous and organic, contained 0.1% (v/v) TFA. The limit solvent was 0.1% TFA in 6.75:2.25:1 MeCN, 2-propanol, and H₂O solution (Tarr and Crabb 1983, Vensel et al 1989). Resolution was enhanced with shallow gradients (0.32% per minute). The column was operated at a flow rate of 1 mL/min. Initial conditions were 70% of the aqueous solvent and 30% of the limit solvent. For all separations, initial conditions were held constant for 5 min before starting the gradient. Gradient conditions for each chromatograph are indicated in the accompanying figure legends. Individual peaks were collected in disposable plastic tubes and dried in a Speed Vac SC100 (Savant Instruments, Hicksville, NY).

Reduction and Alkylation

Reduction and alkylation of individual glutenin protein peaks prepared by the method of Singh et al (1991) were made by the following approach. Reaction medium (0.5 mL of 6M guanidine hydrochloride, 0.1M in Tris-HCl, pH 8.2) was added to each tube. A weighed amount of DTT (~4 mg) was added. The sample was vortexed, and reduction was allowed to continue for 30 min at 65°C. The tube was cooled to room temperature and 1.4 equivalents of alkylating reagent to each equivalent of reductant thiol was obtained by using two parts of 4-vinylpyridine (in microliters) to one part of DTT (in milligrams) for each fraction (Tarr 1986). Alkylation was terminated by acidification with 100 μ L of 88% formic acid. The reduced and pyridylethylated glutenin components were then immediately purified by RP-HPLC as previously described. Reduction and alkylation of the residues prepared by the method of Burnouf and Bietz (1989) were made in 8M urea instead of guanidine hydrochloride.

SDS-PAGE

Purified protein components prepared by RP-HPLC were analyzed by SDS-PAGE as previously described (Kasarda et al 1988).

Edman Sequencing

Sequencing was done with an automatic protein sequencer (model 477A, Applied Biosystems, Foster City, CA) equipped with an online 120A HPLC system for identification of phenylthiohydantoin (PTH)-amino acids. The sample of protein or peptide to be sequenced was dissolved in 20 μ L of 70% formic acid, immediately applied to a preconditioned Biobrene-coated glass fiber filter (Applied Biosystems), and dried. A standard cleavage time of 5 min at 48°C was used for the high molecular weight (HMW) glutenin subunits (GS). The proline-rich and low molecular weight (LMW)-GS were sequenced using a cleavage time of 10 min at 53°C (Vensel and Kasarda 1991). Sequence determination was made by visual inspection of the chromatograms and by examination of the data as analyzed by the data analysis software (version 1.61, Applied Biosystems).

RESULTS AND DISCUSSION

Glutenin Preparations

Comparison of the different procedures for glutenin preparation indicated that the procedures of Burnouf and Bietz (1989) and Singh et al (1991) resulted in very similar RP-HPLC patterns, both qualitatively and quantitatively, for the reduced subunits of these two residue preparations. HMW-GS eluted in the approximate range 35–45 min, whereas LMW-GS eluted in the approximate range 55–95 min (Fig. 1A). When recovery of LMW-GS was expressed as part of total glutenin subunit recovery (HMW-GS + LMW-GS) on the basis of chromatogram peak areas (monitored at 215 nm), it was found that LMW-GS made up ~45% of the total glutenin subunits from the two procedures. The insoluble residues presumably correspond mainly to very high molecular weight glutenin polymers; some of the lower molecular weight range polymers having been extracted with the gliadins. In contrast, the acetic acid-soluble glutenin, which would tend to favor the lower molecular weight range of glutenin polymers, was ~65% LMW-GS. It is likely that the HMW-GS tend to promote the formation of large, insoluble polymers more than do LMW-GS. Despite the higher ratios of HMW-GS to LMW-GS in the preparations resulting from the procedures of Burnouf and Bietz (1989) and Singh et al (1991) as compared with the acetic acid-soluble glutenin prepa-

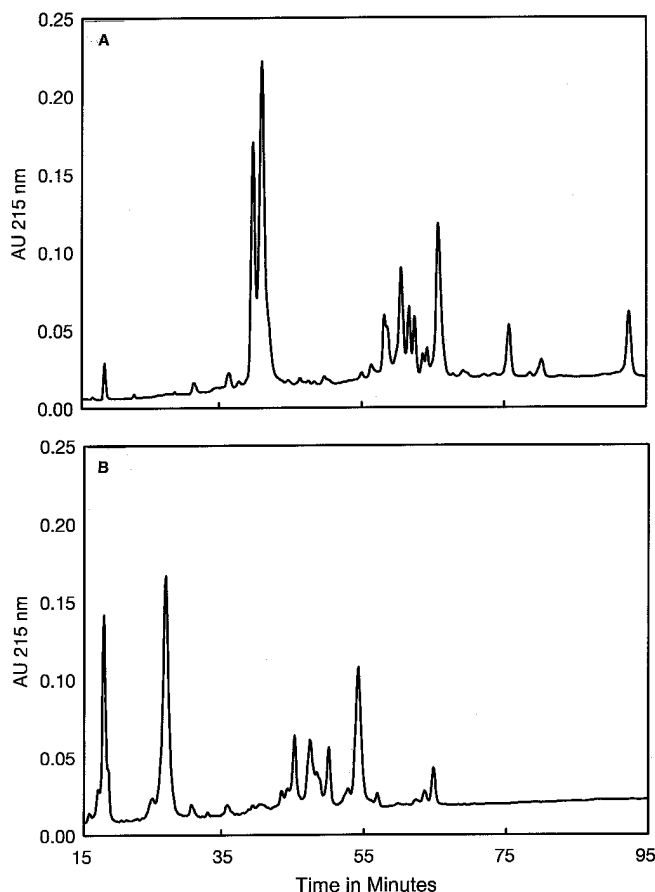


Fig. 1. Reversed-phase high-performance liquid chromatography (RP-HPLC) elution profile of the reduced (A) and the reduced and alkylated (B) *Triticum tauschii* ssp. *strangulata* residue glutenins extracted from endosperm by the method of Singh et al (1991). A 100- μ L portion of an extract (2 mL), from 100 mg of flour, was acidified with 20 μ L of 88% formic acid and diluted to 100 μ L with 0.1% trifluoroacetic acid (TFA) and water for injection onto the column. Column was operated at a flow rate of 1 mL/min; initial conditions were 70% of the aqueous solvent and 30% of the limit solvent. A linear gradient was initiated 5 min after injection, extending from the initial conditions to 65% of the limit solvent in 110 min. AU = absorbance units.

ration, the types of subunits, on the basis of the RP-HPLC chromatograms, were qualitatively the same for all three procedures. This was supported by N-terminal sequence investigations done on selected fractions obtained from these different extraction conditions. The method of Singh et al (1991) was chosen to prepare starting material for sequencing studies because of its relative simplicity.

Reduction vs. Reduction and Alkylation

Analytical RP-HPLC chromatograms of the separation of the glutenin proteins from *T. tauschii* ssp. *strangulata*, prepared by the method of Singh et al (1991) are shown in Figure 1. The chromatogram in Figure 1A shows the HPLC elution profile of the reduced glutenin proteins, while that in Figure 1B shows the HPLC elution position of the reduced glutenin proteins following alkylation. Alkylation with 4-vinylpyridine caused the proteins to elute considerably earlier (become more hydrophilic at low pH where the pyridine moiety would be positively charged) and appeared to alter the resolution of some components. The analytical chromatograms (Fig. 1) suggested that more components could be resolved by a two-dimensional strategy in which separation of the reduced proteins (1st dimension) was followed by RP-HPLC separation of reduced-alkylated proteins (2nd dimension). This approach was employed for preparative work. The preparative HPLC chromatogram (Fig. 2; upper left hand panel) was quite similar to the analytical chromatogram of Figure 1A, which was expected as the same column and conditions were used for both. The only difference between the two separations was the considerably larger sample loading used for the preparative chroma-

togram. Chromatograms shown in Figure 2 of proteins corresponding to selected major peaks indicate that, after alkylation and HPLC separation, these fractions yielded mainly single peaks, with the exception of Peaks 1 and 4. As far as the ability of RP-HPLC to resolve the components was concerned, reduction alone gave the better separation, with almost all peaks resolved except for Peaks 1, 4, and 12 (Fig. 2). However, if material had been reduced and alkylated and then fractionated in a single step, elution times indicated that a number of components (in particular Peaks 2 and 4, 5 and 6, and 9 and 10) would have been poorly resolved.

SDS-PAGE

Fourteen peaks or peak fractions from the reduced-only, preparative-level fractionation of the residue fraction by RP-HPLC (Fig. 2; upper left hand panel) were chosen for SDS-PAGE examination (Fig. 3). The electrophorograms of Figure 3 indicated that some overlapping of protein components occurred from fraction to fraction, but usually there was a predominant component in each peak. The earlier eluting HMW-GS had an apparent molecular weight of 85,000 and the later eluting HMW-GS had an apparent molecular weight of 115,000, on the basis of SDS-PAGE calibration with globular protein standards. LMW-GS subunits, based on their mobility in SDS-PAGE, are usually divided into two groups: B and C. The B group corresponds to proteins ranging in MW from 40,000 to 50,000, and the C group corresponds to proteins ranging in MW from 30,000 to 40,000. The first LMW-GS fractions to elute (Peaks 2–4) corresponded mainly to B group components; each contained one major band and a few minor bands. Peaks 5–12 appeared to

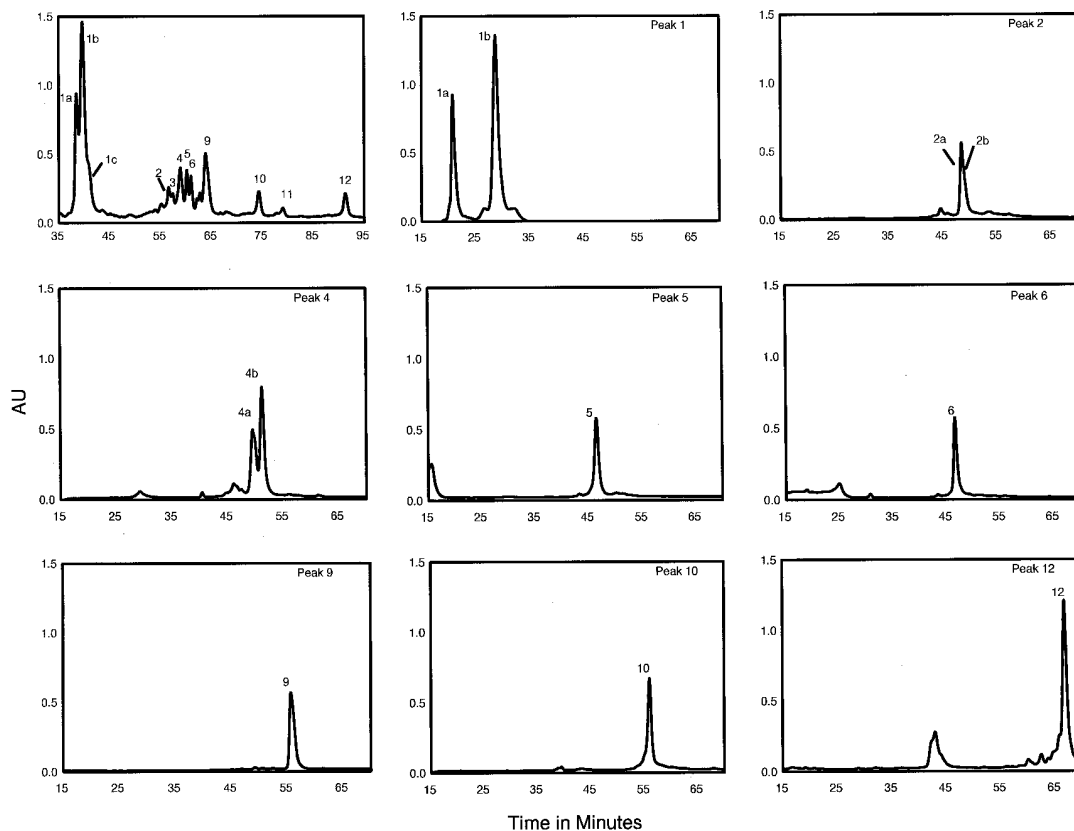


Fig. 2. Preparative reversed-phase high-performance liquid chromatography (RP-HPLC) separation of the reduced (upper left panel) and selected reduced and alkylated glutenin components (Peaks 1–12) from a residue glutenin fraction of endosperm of *Triticum tauschii* ssp. *strangulata* prepared by the method of Singh et al (1991). Gradient conditions for the initial separation of the reduced components as described in Fig. 1. Analytical column was used also for the “preparative” separations of the glutenin subunits. The entire 2-mL extract from 100 mg of flour was acidified with 100 μ L of 88% formic acid and diluted to 4.5 mL with 0.1% trifluoroacetic acid (TFA) and water before injection onto the column. Reduction and pyridylethylation of selected peaks are described in text. Detector wavelength was 230 nm for the preparative separation. Detector wavelength was 230 nm for Peaks 1 and 9. All other separations were monitored at 215 nm. For separation of reduced and alkylated proteins, 5 min after each injection, a gradient with the same slope as in Fig. 1 was begun. Gradient was 30–52% of the limit solvent over a 70-min period. AU = absorbance units.

contain mainly C group components that gave predominantly single bands in SDS-PAGE.

N-Terminal Sequence Analysis

Twelve fractions corresponding to RP-HPLC peaks or fractions of peaks were selected for N-terminal amino acid sequencing. The results of the protein sequencing indicated that most of the samples were fairly pure and yielded one strongly predominant N-terminal sequence. These sequences are shown in Figure 4. The three earliest eluting components were HMW glutenin subunits by sequence, whereas the remaining components were LMW glutenin subunits. The combined initial sequencing yield indicated a total of 1,020 pmol from peaks corresponding to HMW-GS proteins and 1,548 pmol from peaks corresponding to LMW-GS protein sequences. The combined sequencing yield gives an idea of the relative molar ratio of the two classes of proteins in the separation shown in Figure 2. The sequencing yields also indicate that

100 mg of flour yields ~2.5 nmol of protein. About 15–20 seeds would be required to obtain a similar amount of protein.

The first-to-elute HMW-GS (peak fraction 1a; Fig. 2) was a γ -type subunit, based on sequence classification (Shewry et al 1984, 1989) and on MW as determined by SDS-PAGE. By the same criteria, the second-to-elute and most abundant HMW-GS (peak fraction 1b) was of the α -type, as was peak fraction 1c, a minor shoulder on peak fraction 1b. The remainder of the sequences corresponded to LMW-GS, which were divided into two groups on the basis of N-terminal sequences: 1) subunits with sequences corresponding to LMW-m and LMW-s types, and 2) subunits with sequences similar to those of α - and γ -type gliadins. The designations LMW-s and LMW-m are based on the nomenclature of Lew et al (1992), where m and s refer to methionine and serine, the first N-terminal amino acid of the respective sequences.

The α - and γ -type glutenins had C-type mobility in SDS-PAGE, whereas the LMW-m and the LMW-s types had mainly B-type

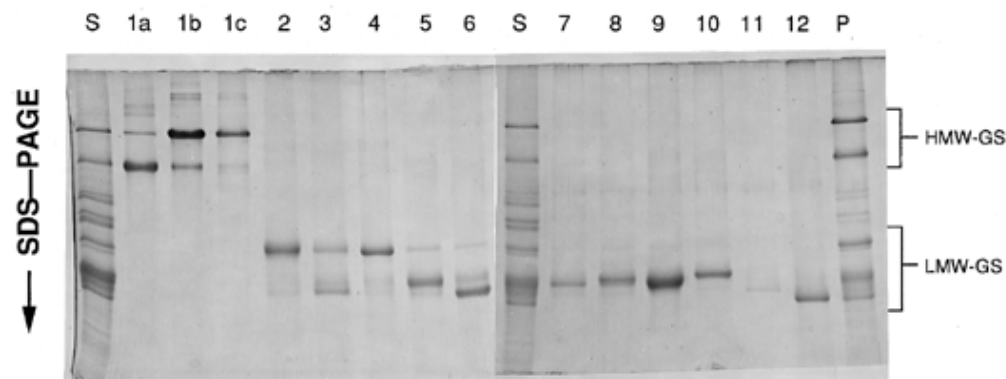


Fig. 3. One-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of selected reduced fractions from the reversed-phase high-performance liquid chromatography (RP-HPLC) separation from Fig. 2 (upper left-hand panel). Numbers refer to peaks or peak fractions indicated in Fig. 2. S = whole seed extract (gliadin + glutenin); P = residue glutenin preparation from seeds (Singh et al [1991]). Peaks 7 and 8 not shown in Fig. 2 but they correspond to two small peaks between 6 and 9. HMW-GS = high molecular weight glutenin subunits; LMW-GS = low molecular weight glutenin subunits.

N-terminal amino acid sequence and recovery for selected <i>Triticum tauschii</i> glutenin proteins								
Fraction #	Sequence Type	picoMoles in Fraction	N-terminal amino acid sequences					
			1	5	10	15	20	25
1a	HMW- γ	422	E G E A S R Q L Q C E R E L Q E S S L E A - Q - - V V -					
1b	HMW- α	587	E G E A S E Q L Q C Q R E L Q E L Q - R - L K A C Q -					
1c	HMW- α	11	- G E A S E Q L Q C Q R E L Q -					
2a	LMW-s	339	S H I P G L E K P S Q Q Q P L P L Q Q T L S -					
2b	LMW-s	27	S - I P G L E K P -					
4a	LMW-m	33	M E T S C I P G L E K P -					
	LMW-s	25	S - I P G L E K P S Q -					
4b	LMW-m	73	M E T S R I P G L E K P -					
5	α -type	306	V R V P V P Q L Q P Q N P S Q Q Q P Q E Q V P -					
6	α -type	463	V R V P V P Q L Q P Q N P S Q Q Q P Q K Q V P L -					
9	LMW-m	64	M E T S C I P G L - R					
10	γ -type	102	N I Q V D P S G Q V Q W L Q - Q - V P Q Q Q					
11	γ -type	6	N M Q V D P S S Q V -					
12	γ -type	110	N M Q V D P G Y Q V Q W P Q Q Q P F P Q P Q Q P F C Q Q P -					

Fig. 4. N-terminal amino acid sequence recovery for selected *Triticum tauschii* ssp. *strangulata* proteins (peaks) keyed to Figs. 2 and 3. Sequence type listed adjacent to each fraction number. Amino acids: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine. HMW- γ and HMW- α = N-terminal sequences characteristic of high molecular weight glutenin subunits of γ and α types, respectively. LMW-s and LMW-m = N-terminal sequences of low molecular weight glutenin subunits types beginning with serine and methionine, respectively. α -type and γ -type = N-terminal sequences of LMW-GS types corresponding to α - and γ -type gliadins.

mobility, with the exception of the Peak 9 protein, which was apparently LMW-m type although the yield was low in comparison with the area of the peak (Fig. 2) when compared with the yield from other peaks—indicating the possibility of some N-terminal blockage of the component (or components) of Peak 9.

The most abundant LMW-GS sequence was of the α -type, found for proteins of Peaks 5 and 6. The combined sequencing yield for the material in these two peaks was 769 pmol. The finding that the α -type glutenin subunits predominated in the LMW-GS of *T. tauschii* was surprising in that α -types were minor in previous studies of bread and durum wheats (Lew et al 1992, Masci et al 1995), although Masci et al (1995) found that the α -type was relatively high in proportion (not predominant) in a durum wheat having the poor quality LMW-1 protein complement. The next most abundant sequence type in our accession of *T. tauschii* was the LMW-s type at 390 pmol, the γ -type at 218 pmol, and finally the LMW-m type at 170 pmol. On a percentage basis (mole/mole) of sequencible proteins, the HMW-GS made up 40% of the glutenin fraction; the α -type subunits made up 30%; the combined LMW-s and LMW-m subunits (mostly LMW-s) made up 20%; and the γ -type subunits made up 10%.

The predominance of the α -gliadin type sequences might not be typical of all types of *T. tauschii* as considerable variation has been reported for the storage proteins of different accessions of *T. tauschii* (Lagudah and Halloran 1988, William et al 1993). To examine this possibility additional accessions of *T. tauschii* would need to be analyzed, but the accession we used was not simply a random choice. We chose it for two reasons: 1) it was subspecies *strangulata*, which seems likely to be the progenitor of the D genome, having α -gliadins and ω -gliadins with acid-gel patterns that are in better agreement with known D-genome contributed gliadins in bread wheats than the other subspecies, *eusqarrosa* (which is, for example, on the basis of acid gel electrophoresis, completely lacking in α -gliadins); and 2) the two-dimensional gliadin pattern of this accession (D. Lafiandra and D. Kasarda, unpublished results) as determined by the method of Lafiandra and Kasarda (1985) showed that it was extremely close to the D-genome coded gliadins of the bread wheat cultivar Cheyenne (Lafiandra et al 1984). Differences in *tauschii* types, for example, accessions from ssp. *eusqarrosa*, might not be highly meaningful in regard to the D genome of hexaploid wheats, and careful selection of other types should be made for any future studies.

The protein in Peak 5 had a typical α -gliadin type sequence with glutamic acid (E) at position 20, whereas the slightly more abundant α -type subunit of the Peak 6 fraction had a lysine (K) at position 20. This latter amino acid at position 20 has not been found in sequences derived from clones of α -gliadin components that have a typical gliadin complement of six cysteines. Lysine does, however, appear in the A735 sequence of Okita et al (1985), which has seven cysteine residues, six of which are homologous to the usual six cysteines of α -gliadins. Müller and Wieser (1995) have provided evidence for a specific arrangement of disulfide bonds in α -type gliadins. It seems likely that a protein corresponding to clone A735 would form three normal intramolecular disulfide bonds, leaving one cysteine available for intermolecular disulfide bond formation. The extra cysteine would enable the protein to function as a chain terminator during glutenin subunit polymerization (Lew et al 1992). Although the Peak 5 material had glutamic acid at position 20, which is typical of normal α -type gliadins, it is likely that the protein of Peak 5 represents a glutenin type subunit with at least one free sulfhydryl group available for intermolecular disulfide bond formation after intramolecular bonds have formed.

The second most abundant LMW-GS type sequence (22%) was that of the LMW-s type (Lew et al 1992) found for proteins in Peaks 2 and 4. Because of the asymmetrical nature of Peak 2, both the leading and trailing edges of the peak were sequenced, these are designated 2a and 2b respectively. However, only LMW-s type

glutenin sequences were found (Fig. 4). In contrast to the results of others (Tao and Kasarda 1989, Lew et al 1992, Masci et al 1995) obtained for bread and durum wheats, the LMW-s sequence type was not the major sequence found for LMW-GS of *T. tauschii* proteins.

Fourteen percent of the LMW-GS components of *T. tauschii* ssp. *strangulata* had γ -type sequences. Peak 12 had a characteristic γ -gliadin sequence, but with cysteine present at position 26. The latter is similar to the γ -glutenin sequence reported by Lew et al (1992) and Tao and Kasarda (1989). It is likely that this subunit corresponds to the clone (pW1020) described by Scheets and Hedgcoth (1988) and the glutenin component described by D'Ovidio et al (1995), which have nine cysteine residues in contrast to the usual eight found in monomeric γ -gliadins. The ninth cysteine residue at position 26, unlike the homologous set of eight cysteine residues involved in intramolecular bonding in the γ -gliadins (Köhler et al 1993), would be expected to remain free to form an intermolecular bridge with another glutenin subunit. We were not able to extend the sequences of the γ -type glutenins of Peaks 10 and 11 sufficiently to see whether they also had a cysteine residue at position 26, but each of these components must have at least one cysteine available for formation of intermolecular disulfide bonds in order for them to be incorporated into glutenin.

The least abundant glutenin subunit type, by N-terminal sequencing, was the LMW-m type subunit (found in Peaks 4a, 4b, and 9). The LMW-m type sequences seemed to be of two types: those in Peaks 4a and 9 with a cysteine residue at position five as typically found in sequences based on DNA clones (Cassidy and Dvorak 1991); and that present in Peak 4b that had an arginine residue at position four. It has been proposed (Lew et al 1992) that the cysteine at position five is involved in glutenin polymer formation. The presence of a LMW-m type sequence without a cysteine residue at position 5 indicates that there may be a cysteine (or cysteines) available at some other position in the N-terminal half of the molecule for formation of intermolecular disulfide bonds, such as the C^{b*} cysteine described by Köhler et al (1993) and Keck et al (1995). This would be in addition to the C^x cysteine in the C-terminal half of the molecule also described by Köhler et al (1993) and Keck et al (1995). Absence of the C^{b*} cysteine would very likely make the LMW-m type a chain terminator (Lew et al 1992). Because of the indication that some of the protein of Peak 9 was blocked, we may be underestimating the amount of LMW-m type glutenin subunits.

The α -type and γ -type sequences were present in similar proportion in the glutenin from all three extraction procedures tested. HPLC analysis of the gliadin-containing supernatant solution (data not shown) revealed that over 90% of the gliadins were removed in the first of the three extraction steps of the Singh et al (1991) procedure. The α -type and γ -type components were present in large proportion following reduction of the gliadin-depleted residue protein. That these proteins were not extracted until reducing agent was added supports the likelihood that they were covalently linked to other glutenin proteins through disulfide bonds. Earlier studies (Lew et al 1992, Masci et al 1995) have also provided evidence that α -type and γ -type components of glutenin are not contaminants.

Glutenin Subunits of *T. tauschii* and Quality Differences

We have found that all three of the procedures we used to prepare glutenin resulted in preparations containing essentially the same types of glutenin subunits on the basis of HPLC chromatograms and N-terminal amino acid sequences. There were no qualitative differences, and the assemblage of subunits was the same as that found in glutenin preparations from bread wheat (Tao et al 1989, Lew et al 1992) and durum wheat (Masci et al 1995). Accordingly, it appears likely that differences between durum wheat cultivars with only the A and B genomes and bread wheat

cultivars having the D genome in addition to the A and B genomes result mainly from differences in the proportions of the various glutenin subunits rather than from components unique to one or the other species.

The proportion of the HMW-GS in residue glutenin from *T. tauschii* ssp. *strangulata* was much greater than had been found for the equivalent fractions of two lines from the tetraploid durum wheat cultivar Lira (see Figs. 1 and 2 of Masci et al 1995) and almost as large as the total percentage of HMW-GS in some bread wheats (Gupta and MacRitchie 1994). The HMW-GS made up ~40% of our preparations on the basis of sequencing yield (molar basis) and ~50% on the basis of UV absorption in the HPLC chromatograms (weight basis). Although there is considerable variability in the properties of durum wheat cultivars, doughs from durum wheats generally do not have as much mixing strength and viscoelasticity as do doughs from bread wheat. This may have resulted from a strong contribution of HMW-GS by the D genome progenitor—tending to increase the proportion relative to LMW-GS, partly by the addition of two more genes to the system, and partly because the amount of protein resulting from this addition is almost always relatively substantial, particularly for the Dx genes. However, one reviewer has pointed out that the possibility that the relatively large amount of HMW-GS in the residue of *T. tauschii* might result from the presence of large amounts of α -type and γ -type glutenin subunits in the glutenin from this species. If these are chain terminators, they would promote the formation of small, readily extracted polymers (particularly for the LMW-GS fraction), leaving an enriched proportion of HMW-GS in the residue fraction. Thus, the large amount of HMW-GS in the residue fraction of *T. tauschii* might not represent a greater amount of HMW-GS in the total protein from this species. These speculations provide some interesting possibilities for further investigations, including analysis of the protein types and proportions in the progenitors of the A and B genomes.

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LITERATURE CITED

BURNOUF, T., and BIETZ, J. A. 1989. Rapid purification of wheat glutenin for reversed-phase high-performance liquid chromatography: Comparison of dimethyl sulfoxide with traditional solvents. *Cereal Chem.* 66:121-127.

BUSHUK, W., and KERBER, E. R. 1978. The role of *Triticum carthlicum* in the origin of bread wheat based on gliadin electrophorograms. *Can. J. Plant Sci.* 58:1019-1024.

CASSIDY, B. G., and DVORAK, J. 1991. The molecular characterization of a low molecular weight glutenin protein from *Triticum durum*. *Theor. Appl. Genet.* 81:653-660.

DAUD, H. M., and GUSTAFSON, J. P. 1996. Molecular evidence for *Triticum speltoides* as B-genome progenitor of wheat (*Triticum aestivum*). *Genome* 39:543-548.

D'OVIDIO, R., SIMEONE, M., MASCI, S., PORCEDDU, E., and KASARDA, D. D. 1995. Nucleotide sequence of a γ -type glutenin gene from a durum wheat: Correlation with a γ -type glutenin subunit from the same biotype. *Cereal Chem.* 72:443-449.

DVORAK, J., and ZHANG, H.-B. 1990. Variation in repeated nucleotide sequences sheds light on the phylogeny of the wheat B and G genomes. *Proc. Natl. Acad. Sci. USA* 87:9640-9644.

GUPTA, R. B., and MACRITCHIE, F. 1994. Allelic variation at glutenin subunit and gliadin loci, *Glu-1*, *Glu-3*, and *Gli-1* of common wheats. II. Biochemical basis of the allelic effects on dough properties. *J.*

Cereal Sci. 19:19-29.

KASARDA, D. D., LAFIANDRA, D., MORRIS, R., and SHEWRY, P. R. 1984. Genetic relationships of wheat gliadin proteins. *Kulturpflanze* 32:S33-S52.

KASARDA, D. D., TAO, H. P., EVANS, P. K., ADALSTEINS, A. E., and YUEN, S. W. 1988. Sequencing of protein from a single spot of a 2-D gel pattern: N-terminal sequence of a major wheat LMW glutenin subunit. *J. Exp. Botany* 39:889-906.

KECK, B., KÖHLER, P., and WIESER, H. 1995. Disulphide bonds in wheat gluten: Cystine peptides derived from gluten proteins following peptic and thermolytic digestion. *Z. Lebensm. Unters. Forsch.* 200:432-439.

KIHARA, H. 1975. Origin of cultivated plants with special reference to wheat. *Seiken Ziho* (Report Kihara Inst. Biol. Res.) No. 45-26.

KÖHLER, P., BELITZ, H.-D., and WIESER, H. 1993. Disulfide bonds in wheat gluten: Further cystine peptides from high molecular weight (HMW) and low molecular weight (LMW) subunits of glutenin and from γ -gliadins. *Z. Lebensm. Unters. Forsch.* 196:239-247.

LAFIANDRA, D., and KASARDA, D. D. 1985. One- and two-dimensional (two-pH) polyacrylamide gel electrophoresis in a single gel: Separation of wheat proteins. *Cereal Chem.* 62:314-319.

LAFIANDRA, D., KASARDA, D. D., and MORRIS, R. 1984. Chromosomal assignments of genes coding for the wheat gliadin protein components of the cultivars 'Cheyenne' and 'Chinese Spring' by two-dimensional (two-pH) electrophoresis. *Theor. Appl. Genet.* 68:531-539.

LAGUDAH, E. S., and HALLORAN, G. M. 1988. Phylogenetic relationships of *Triticum tauschii* the D genome donor to hexaploid wheat. 1. Variation in HMW subunits of glutenin and gliadins. *Theor. Appl. Genet.* 75:592-598.

LEW, E. J.-L., KUZMICKY, D. D., and KASARDA, D. D. 1992. Characterization of low molecular weight glutenin subunits by reversed-phase high-performance liquid chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and N-terminal amino acid sequencing. *Cereal Chem.* 69:508-515.

MASCI, S., LEW, E. J.-L., LAFIANDRA, D., PORCEDDU, E., and KASARDA, D. D. 1995. Characterization of low molecular weight glutenin subunits in durum wheat by reversed-phase high-performance liquid chromatography and N-terminal sequencing. *Cereal Chem.* 72:100-104.

MÜLLER, S., and WIESER, H. 1995. The location of disulfide bonds in α -type gliadins. *J. Cereal Sci.* 22:21-27.

MORRIS, R., and SEARS, E. R. 1967. The cytogenetics of wheat and its relatives. Pages 19-87 in: *Wheat and Wheat Improvement*. K. S. Quisenberry and L. P. Reitz, eds. *Am. Soc. Agron.*: Madison, WI.

OKITA, T. W., CHEESBROUGH, V., and REEVES, C. D. 1985. Evolution and heterogeneity of the α - β -type and γ -type gliadin DNA sequences. *J. Biol. Chem.* 260:8203-8213.

SCHEETS, K., and HEDGCOTH, C. 1988. Nucleotide sequence of a γ gliadin gene: Comparisons with other gliadin sequences show the structure of γ gliadin genes and the general primary structure of γ gliadins. *Plant Sci.* 57:141-150.

SHEWRY, P. R., HALFORD, N. G., and TATHAM, A. S. 1989. The high molecular weight subunits of wheat, barley and rye: Genetics, molecular biology, chemistry and role in wheat glutenin structure and functionality. *Oxf. Surv. Plant Mol. Cell Biol.* 6:163-219.

SHEWRY, P. R., FIELD, J. M., FAULKS, A. J., PARMAR, S., MIFLIN, J., DIETLER, M. D., LEW, E. J.-L., and KASARDA, D. D. 1984. The purification and N-terminal amino acid sequence analysis of the high molecular weight glutenin polypeptides of wheat. *Biochem. Biophys. Acta* 788:23-34.

SINGH, N. K., SHEPHERD, K. W., and CORNISH, G. B. 1991. A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. *J. Cereal Sci.* 14:203-208.

TAO, H. P., and KASARDA, D. D. 1989. Two-dimensional gel mapping and N-terminal sequencing of LMW-glutenin subunits. *J. Exp. Bot.* 40:1015-1020.

TAO, H. P., CORNELL, D. G., and KASARDA, D. D. 1989. Surface and optical properties of wheat glutenin monolayers. *J. Cereal Sci.* 10:5-18.

TARR, G. E. 1986. Manual Edman sequencing system. Pages 155-194 in: *Methods of Protein Microcharacterization: A Practical Handbook*. J. E. Shively, ed. Humana Press: Clifton, NJ.

TARR, G. E., and CRABB, J. W. 1983. Reverse-phase high-performance liquid chromatography of hydrophobic proteins and fragments thereof.

Anal. Biochem. 131:99-107.

VENSEL, W. H., and KASARDA, D. D. 1991. Effect of cleavage conditions on Edman degradation of proline-rich proteins: Application to wheat storage proteins. Pages 181-190 in: Techniques in Protein Chemistry. II. J. Villafranca, ed. Academic Press: New York.

VENSEL, W. H., LAFIANDRA, D., and KASARDA, D. D. 1989. The

Effect of an organic eluent modifier and pH on the separation of wheat-storage proteins: Application to the purification of γ -gliadins of *Triticum monococcum* L. Chromatographia 28:133-138.

WILLIAM, M. D. H. M., PEÑA, R. J., and MUJEEB-KAZI, A. 1993. Seed protein and isozyme variations in *Triticum tauschii* (*Aegilops squarrosa*). Theor. Appl. Genet. 87:257-263.

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