PROPERTIES OF WHEAT GLIADINS SEPARATED BY GEL FILTRATION[1]

K. R. PRESTON[2] and W. WOODBURY, Department of Plant Science, University of Manitoba, Winnipeg, Canada R3T 2N2

ABSTRACT

Gliadins isolated from three hexaploid and one tetraploid wheat variety were fractionated on Sephadex G-100 in a dissociating solvent. Four fractions of average molecular weights (mol wt) greater than 100,000 and of 44,000, 27,000, and 10,000 were obtained from each variety. Significant varietal variation in the mol wt distribution of fractions was found. The first three fractions of each variety had amino acid compositions similar to whole gliadin but the fourth fraction had an amino acid content similar to wheat albumin. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis showed that the hexaploid varieties had major subunits of mol wt 36,000, 40,000, and 50,000 and minor subunits of mol wt 10,000, 53,000, 78,000, 82,000, 88,000, 108,000, 120,000, and 130,000. The tetraploid variety lacked minor subunits of mol wt 88,000, 120,000, and 130,000. Comparison of reduced and nonreduced fractions indicated that the three lower mol wt fractions were composed of single-chain proteins. The nonreduced high-mol wt fractions gave a large number of bands of apparent mol wt 150,000–300,000, but upon reduction gave three subunits of mol wt 40,000, 50,000, and 53,000.

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The gliadin proteins of wheat flour have been shown to consist of a number of different-sized components ranging in molecular weight (mol wt) from 10,000 to over 100,000 (1–4). They are mainly single polypeptide chains stabilized by intrachain disulfide bonding (5,6). Bietz and Wall (6) have shown by electrophoresis in the presence of sodium dodecyl sulfate (SDS) that reduced gliadins contain polypeptide chains with mol wt of 11,400, 36,500, 44,200, 69,300, and 78,100. In contrast to the wide variation in mol wt, various gliadin components have similar amino acid compositions (7–11) and sequences (12).

Studies in our laboratory (13) have shown that alcohol-soluble (gliadins) proteins of rye could be fractionated by gel filtration with AUC [0.1*M* acetic

[1]Contribution No. 369 from the Department of Plant Science, The University of Manitoba.

[2]Present address: The Canadian Grain Commission, Grain Research Laboratory, 1404 - 303 Main Street, Winnipeg, Canada R3C 3G9.

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acid, 3*M* urea, and 0.01*M* cetyl-trimethyl ammonium bromide (3)] into four distinct fractions. Each fraction had a distinct amino acid composition and subunit structure. In the present paper, we have extended this study to the gliadin proteins isolated from different varieties of wheat. Quantitative differences in gliadin fractions isolated by gel filtration, as well as the amino acid composition and subunit structures of each fraction, have been studied.

MATERIALS AND METHODS

Fractionation of Wheat Endosperm Proteins

Three hexaploid wheat varieties, Manitou (hard red spring), Pembina (hard red spring), and Talbot (soft white winter), and one tetraploid variety, Stewart 63 (amber durum), were milled on a Buhler experimental mill. Gliadins were extracted from the resulting flours by the modified Osborne procedure of Chen and Bushuk (14) and accounted for 38.1, 34.8, 31.2, and 40.0% of the total flour nitrogen, respectively, for Manitou, Talbot, Pembina, and Stewart 63. Protein contents of the gliadin preparations (N × 5.7) were 73.0, 67.5, 77.5, and 65.7%, respectively.

Gel Filtration of Gliadins

Wheat gliadins (1 g in 50 ml AUC) were fractionated on a K 100/100 column (Pharmacia) containing approximately 4.5 l. of Sephadex G-100 swollen with AUC as previously described (13). Appropriate fractions were collected and exhaustively dialyzed against water and lyophilized. Protein contents (N × 5.7) were determined by the micro-Kjeldahl procedure. Rechromatography of fractions was performed on a 2.5 × 40-cm column of Sephadex G-100 with AUC. Molecular weights were determined according to the method of Whitaker (15) using γ-globulin (mol wt = 160,000), bovine serum albumin (mol wt = 132,000 for dimer, 66,000 for monomer), ovalbumin (mol wt = 46,000), myoglobin (mol wt = 16,900), cytochrome C (mol wt = 12,700) and N-ethyl-maleimide (mol wt = 125).

SDS Polyacrylamide Gel Electrophoresis

Proteins (10 mg/ml) were incubated overnight in pH 7.3 phosphate buffer (0.015*M*) at 45°C containing 1% SDS. For reduction of disulfide bonds, 1% 2-mercaptoethanol was included.

SDS gel electrophoresis was performed in a 5% polyacrylamide gel with pH 7.3 phosphate buffer containing 0.1% SDS according to the method of Orth and Bushuk (16). Gels were stained in Coomassie Brilliant Blue R250 by the method of Koenig *et al.* (17). Molecular weights were determined by running reduced protein of known mol wt in gels parallel to the samples. Standard proteins used (following reduction with 2-mercaptoethanol) included those used for gel filtration (see above) and chymotrypsinogen (mol wt = 25,700).

Amino Acid Analysis

Amino acid analysis was carried out as previously described (13), with a precision of ±4% for duplicates. Nitrogen recoveries ranged from 85 to 100%.

RESULTS AND DISCUSSION

Gel Filtration of Wheat Gliadins

Gel filtration profiles of each variety of wheat gliadin obtained by chromatography in the strongly dissociating solvent AUC are shown in Fig. 1. Four gliadin fractions were isolated from each variety with approximate mol wt of greater than 100,000 (F_1), 44,000 (F_2), 27,000 (F_3), and 10,000 (F_4). The range of mol wt, as determined by gel filtration, for each fraction was greater than 70,000 (F_1), 33,000 to 70,000 (F_2), 20,000 to 33,000 (F_3), and less than 20,000 (F_4). There was little variation in calculated mol wt (V_e/V_o) between corresponding fractions of different varieties. Rechromatography of the three lower mol wt fractions (F_{2-4}) gave single peaks with values of V_e/V_o corresponding to the original fraction. The high-mol wt fraction (F_1) gave a large peak with a V_e/V_o value corresponding to the original fraction and a much smaller peak with a V_e/V_o value corresponding to F_2. The presence of the small peak was shown to be due to contaminating protein from F_2 by rechromatography and SDS electrophoresis of appropriately selected portions of F_1 from the Sephadex column.

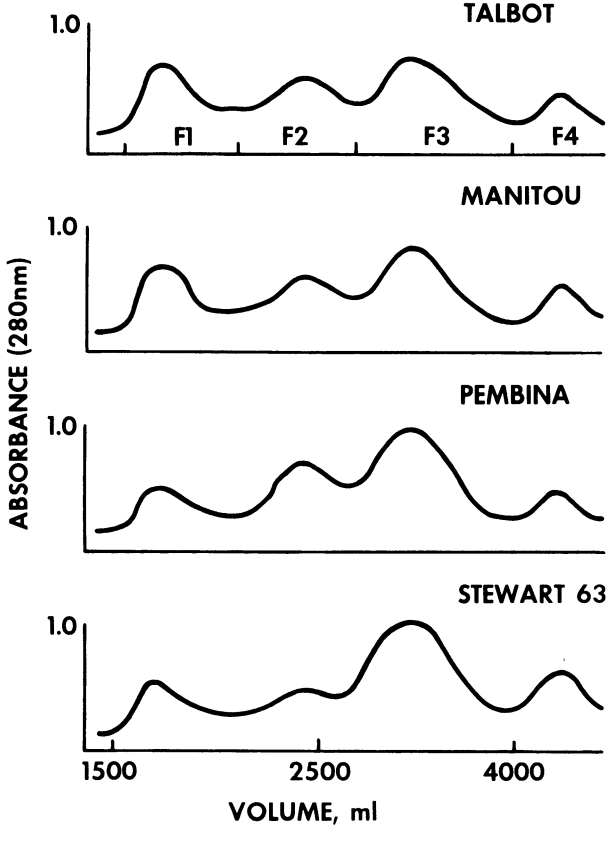

Fig. 1. Elution profile of wheat gliadins on Sephadex G-100 with AUC.

Varietal differences in the protein distribution of the gliadin fractions separated by gel filtration were apparent (Table I). Stewart 63, an amber durum, and Pembina, a hard red spring (HRS) variety, had relatively low proportions of high-mol wt gliadins (F_1), whereas Manitou, a HRS variety, and Talbot, a soft white winter (SWW) variety, had relatively high proportions of high-mol wt gliadins. In the case of Talbot, high-mol wt gliadins accounted for the highest proportion of recovered nitrogen. Fraction 2 gliadins accounted for 6 to 27% of the recovered nitrogen with Stewart 63 having the lowest proportion and Pembina the highest. All four varieties contained high relative proportions of fraction 3 gliadins with Stewart 63 having the highest (63%) and Talbot (33%) the lowest. This fraction (F_3), with the exception of Talbot, accounted for the highest proportion of gliadin proteins. With the exception of Stewart 63 (19%), the low-mol wt fraction (F_4) accounted for less than 10% of the total recovered nitrogen.

Nitrogen recoveries from the column varied from 75 to 85% following dialysis of fractions. Some of this loss may be due to proteolysis and to the presence of nonprotein dialyzable nitrogen. However, nitrogen losses due to dialysis, especially in the case of the low-mol wt fraction (F_4), may have occurred. Thus the actual distribution of the low-mol wt fractions (Table I) may be somewhat higher. The possibility that cetyl trimethyl ammonium bromide (CTAB), a nitrogen-containing detergent present in the eluting buffer, might have affected nitrogen recoveries was discounted. SDS gel electrophoresis of isolated gliadin fractions showed no band corresponding to CTAB (R_f = 1.0 relative to dye band). In contrast, dilute solutions of CTAB or whole gliadin dissolved in AUC gave intense bands, following staining, corresponding to the detergent.

Amino Acid Composition of Wheat Gliadin Fractions

The amino acid compositions of the gliadins of the four wheat varieties are shown in Table II. The whole gliadins had characteristically low contents of basic amino acids and high contents of glutamic acid and proline. High contents of ammonia in the hydrolysates indicated that most of the glutamic acid present was in the form of glutamine. There was little varietal variation in amino acid composition between varieties. Even the absence of the D-genome in the durum variety, Stewart 63, did not have any effect on amino acid composition. This result is in agreement with Dronzek *et al.* (18) who found that the gliadins of an extracted tetraploid (AABB) had a similar amino acid composition when compared to its parent hexaploid (AABBDD).

The first three gliadin fractions (Table III) from each variety had amino acid

TABLE I
Protein Distribution of Wheat Gliadin Fractions from Sephadex G-100 Chromatography[a]

Variety	F_1	F_2	F_3	F_4
Pembina	17	27	51	5
Manitou	37	17	41	5
Talbot	43	15	33	9
Stewart 63	12	6	63	19

[a]Data based on per cent of recovered nitrogen of duplicate runs.

compositions similar to whole gliadin (Table II). The Pembina fractions had amino acid compositions almost identical to those of Manitou and, therefore, are not included in Table III. Differences between equivalent fractions among the four varieties were small. Within each variety, the amino acid composition of the first three gliadin fractions was also similar. Fraction 1 gliadins tended to have

TABLE II
Amino Acid Compositions of Gliadins (mol %)[a]

Amino Acid	Pembina	Manitou	Talbot	Stewart 63
Lysine	0.5	0.6	0.7	0.5
Histidine	1.6	1.4	1.4	1.6
Arginine	1.5	1.3	1.6	1.6
Aspartic acid	2.5	2.5	2.6	2.7
Threonine	2.1	2.0	2.2	1.9
Serine	5.5	4.7	5.3	4.7
Glutamic acid	40.0	40.0	38.8	39.7
Proline	17.5	18.5	17.5	17.6
Glycine	2.9	2.6	3.3	2.6
Alanine	2.9	2.7	3.0	3.0
Valine	4.0	4.0	4.2	4.1
Methionine	1.1	0.9	1.2	1.2
Isoleucine	3.8	3.9	3.8	4.1
Leucine	7.2	7.0	7.4	7.3
Tyrosine	1.9	1.7	2.1	2.1
Phenylalanine	4.9	5.3	4.8	4.9

[a]Tryptophan and cystine not determined.

TABLE III
Amino Acid Composition of Gliadin Fractions (mol %)[a]

	Fraction 1			Fraction 2			Fraction 3		
Amino Acid	M[b]	T[b]	S[b]	M	T	S	M	T	S
Lysine	0.5	0.7	0.7	0.5	0.7	0.5	0.5	0.5	0.4
Histidine	1.6	1.7	1.4	1.2	1.2	1.4	1.5	1.7	1.4
Arginine	1.8	2.0	1.5	1.1	1.3	1.2	1.5	1.5	1.4
Aspartic acid	1.7	1.6	1.7	1.8	1.9	1.3	3.0	2.7	2.6
Threonine	2.8	2.7	2.6	1.9	2.2	2.0	1.8	1.8	1.9
Serine	6.3	6.6	6.9	4.5	5.1	4.7	4.8	4.9	4.6
Glutamic Acid	39.1	38.2	38.8	42.6	39.9	42.7	39.5	39.4	44.4
Proline	15.6	16.4	17.0	20.1	19.4	23.0	17.6	18.0	17.3
Glycine	4.5	4.9	4.2	2.4	3.0	2.4	2.5	2.7	2.3
Alanine	2.4	2.6	2.5	2.3	2.7	1.8	2.9	3.1	2.6
Valine	4.3	4.0	4.0	3.2	3.6	2.6	4.3	4.2	3.8
Methionine	1.2	1.4	1.4	0.7	1.1	0.6	0.5	1.1	0.8
Isoleucine	4.0	3.3	3.7	3.6	3.3	3.4	4.3	3.8	3.8
Leucine	7.3	7.3	7.0	6.2	6.8	5.9	7.1	7.5	6.3
Tyrosine	1.6	1.9	1.1	0.6	0.9	0.6	1.8	2.4	1.8
Phenylalanine	4.2	4.7	4.5	6.0	5.8	5.9	4.9	4.7	4.6

[a]Tryptophan and cystine not determined.
[b]M, T, and S refer to Manitou, Talbot and Stewart 63, respectively.

more serine and glycine and less proline than the other fractions. Fraction 2 gliadins had more proline and phenylalanine and less leucine and tyrosine while fraction 3 gliadins had more aspartic acid.

In contrast to the first three gliadin fractions, the low-mol wt fraction (F_4) of each variety except Talbot (Table IV) had amino acid compositions more closely related to albumin (18). The content of glutamic acid and proline was much lower in this fraction than in whole gliadin. Contents of lysine, aspartic acid, threonine, glycine, and alanine were higher. The low-mol wt gliadin fraction (F_4) of the SWW variety, Talbot, had an amino acid composition similar to whole gliadin. Other studies in our laboratories involving polyacrylamide gel electrophoresis of the low-mol wt fraction indicated the presence of components with mobilities similar to both gliadins and albumins (unpublished data). The presence of albumins have previously been reported in gliadin preparations (6,8).

SDS Electrophoresis of Wheat Gliadins

The SDS polyacrylamide gel patterns of reduced and nonreduced whole gliadin proteins are shown in Fig. 2 and calculated mol wt for these bands in Table V. Molecular weights determined from gel patterns were reproducible to ±500 for comparative purposes, although the actual mol wt of these polypeptides are probably only accurate to ±10%, due to the limitations of the method (19). The reduced gliadin patterns of the three hexaploid varieties (Talbot, Manitou, and Pembina) had three major subunits with mol wt of approximately 36,000, 40,000, and 50,000 and minor subunits of mol wt 10,000, 53,000, 78,000, 82,000, 88,000, 108,000, 120,000, and 130,000. The tetraploid variety (Stewart 63) lacked minor subunits of mol wt 88,000, 120,000, and 130,000. Previous studies have shown that SDS complexes of reduced and nonreduced single-chained proteins have similar mobilities (6,20). The nonreduced gliadin patterns (Fig. 2) had three major bands in the 40,000 to 55,000 mol wt region of the gel, indicating that these

TABLE IV
Amino Acid Composition of the Low-Molecular-Weight Gliadin Fraction (mol %)[a]

Amino Acid	Pembina	Manitou	Talbot	Stewart 63
Lysine	3.6	2.6	0.9	1.6
Histidine	1.5	1.6	1.6	1.6
Arginine	1.4	1.8	1.7	2.3
Aspartic Acid	4.8	4.8	3.2	4.8
Threonine	3.6	3.7	2.1	3.4
Serine	7.4	8.4	5.3	5.3
Glutamic Acid	28.2	31.2	38.0	31.4
Proline	14.4	12.5	17.0	14.2
Glycine	6.5	6.9	3.1	4.7
Alanine	4.6	5.3	3.5	6.0
Valine	4.0	4.4	4.2	5.1
Methionine	1.1	0.9	1.1	1.2
Isoleucine	3.6	4.3	3.8	4.2
Leucine	8.1	6.9	7.5	7.0
Tyrosine	2.1	1.9	2.3	2.5
Phenylalanine	5.1	2.8	4.6	3.8

[a]Tryptophan and cystine not determined.

proteins were single-chain polypeptides (subunits of mol wt of 36,000, 40,000, and 50,000). Bands were also present in hexaploid varieties corresponding approximately to subunits of mol wt 78,000, 82,000, 88,000, 108,000, 120,000, and 130,000, indicating the presence of a number of minor single polypeptide chain gliadin proteins. The tetraploid durum variety lacked bands corresponding to subunit mol wt of 88,000, 120,000, and 130,000.

The nonreduced SDS gel patterns of each variety also had at least ten minor bands in the high mol wt region (mol wt >150,000) which, upon reduction, disappeared. These results indicated the presence of a number of high-mol wt gliadin proteins consisting of interdisulfide linked polypeptide chains. Similar results were obtained by Bietz and Wall (6) for high-mol wt gliadins of hard red winter wheats. The low staining intensity of the high-mol wt gliadin protein bands compared to bands present in the mol wt 40,000 to 55,000 region of the gel appeared to contradict the relatively high proportion (see Table I) of high-mol wt gliadin obtained by gel filtration. The most plausible explanation of these results may have been the prevention of an extended structure for these proteins under the influence of SDS due to the presence of interchain disulfide bonds, resulting in less efficient binding of the protein stain. Visual comparison of SDS gel patterns of reduced and nonreduced high-mol wt gliadins run at similar protein concentrations supported this conclusion. Staining intensities of the reduced patterns were much more intense than the corresponding nonreduced patterns.

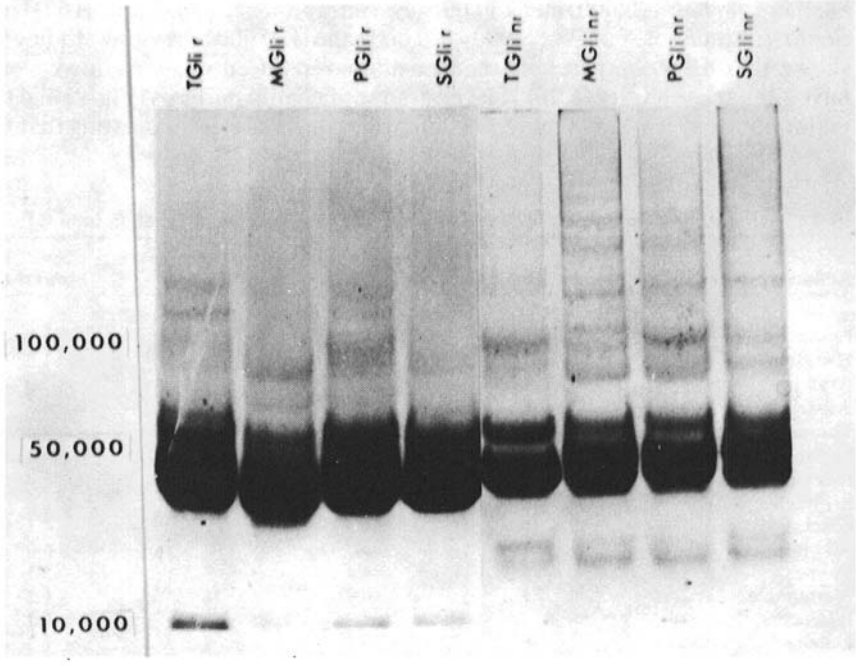

Fig. 2. SDS polyacrylamide gel patterns of reduced (r) and nonreduced (nr) wheat gliadins. Letters T, M, P, and S refer to Talbot, Manitou, Pembina, and Stewart 63.

SDS Electrophoresis of Wheat Gliadin Fractions from Sephadex G-100

The SDS gel patterns of the high-mol wt gliadins (F_1) from the hexaploid varieties isolated by gel filtration had a large number of bands in the high-mol wt (mol wt >150,000) region of the gel as well as bands corresponding to reduced subunits of mol wt 78,000, 82,000, 88,000, 108,000, 120,000, and 130,000 (Table V). Upon reduction, the high-mol wt gliadin bands (mol wt >150,000) disappeared and were replaced by major subunits of mol wt 40,000, 50,000, and 53,000. Thus the high-mol wt gliadins appear to be built up of smaller polypeptide chains of mol wt 40,000, 50,000, and 53,000 through interchain disulfide bonds. The major subunits of the high-mol wt gliadins found in these varieties are similar to values of 36,500 and 44,200 reported by Bietz and Wall (6), considering the inherent errors in the calculation of mol wt by this method (19).

The fraction 2 gliadins for both the hexaploid and tetraploid varieties gave major subunits of mol wt 40,000 and 50,000. The fraction 3 gliadins had major subunits of mol wt 36,000 and 40,000. The nonreduced fraction 2 and fraction 3 gliadins had bands with mobilities similar to the corresponding reduced fractions. Thus both these fractions contain proteins that are single-chain polypeptides. Fraction 2 gliadins from both the hexaploid and tetraploid varieties also had minor subunits with mol wt of 78,000 and 82,000, while the hexaploids had an additional minor subunit of mol wt 88,000. These minor subunits disappeared when fraction 2 gliadins were rechromatographed on Sephadex G-100.

Attempts to characterize the low-mol wt (F_4) gliadins by SDS gel electrophoresis were unsuccessful due to streaking. However, the reduced whole gliadins of each variety had a subunit of mol wt 10,000 absent in the other three gliadin fractions. This subunit is probably the only subunit present in the fraction 4 gliadins.

GENERAL DISCUSSION

A number of studies have previously utilized the AUC solvent of Meredith and Wren (3) in the extraction and separation of wheat proteins by gel filtration

TABLE V
Molecular Weights of Gliadin Subunits Determined by SDS-Gel Electrophoresis (MW × 10^3)

Gliadin Fraction	Major Subunits		Minor Subunits	
	Hexaploid	Tetraploid	Hexaploid	Tetraploid
Whole gliadin	36, 40, 50	36, 40, 50	10, 53, 78, 82, 88, 108, 120, 130	10, 53, 78, 82, 108
Fraction 1	40, 50	40, 50	53, 78, 82, 88, 108, 120, 130	53, 78, 82, 108
Fraction 2	40, 50	40, 50	78, 82, 88	78, 82
Fraction 3	36, 40	36, 40		
Fraction 4	10	10		

(3,21,22,23). The advantage of this solvent compared to previously utilized solvents (24–27) appears to be its ability to minimize protein-protein and gel-protein interactions (3). In the present study, four gliadin fractions could be isolated from each wheat variety studied following Sephadex G-100 chromatography in AUC. Corresponding fractions from each variety had similar average mol wt but significant varietal differences in the proportion of protein in each fraction occurred (Table I). Two of the gliadin fractions (mol wt of 27,000 and 44,000) had calculated mol wt similar to those reported by Meredith and Wren (3) (mol wt of 30,000 and 42,000) following Sephadex G-200 chromatography of wheat flour extracts with AUC. The other two gliadin fractions with calculated mol wt of 10,000 and of greater than 100,000 obtained in the present study had mol wt similar to previously isolated gliadin fractions (25,27).

Previous studies (28–30) have shown quantitative varietal variations in gliadin components by electrophoresis but, to the authors' knowledge, no previous studies have utilized gel filtration to demonstrate varietal differences in the distribution of gliadin fractions varying in mol wt. The present results indicate that significant varietal differences in the mol wt distribution of gliadin components exist. These differences may be important in determining varietal variations in mixing and baking characteristics and deserve further consideration. Studies have recently shown that the addition of gliadin fractions varying in mol wt to a synthetic dough system containing starch and glutenin can, in fact, have significant effects on mixing properties (31).

Although only four varieties from three different wheat classes (two HRS, one SWW, and one amber durum) were studied, the similarity in their SDS gel patterns suggests that gliadins from different classes of wheat all have polypeptide chains of similar mol wt. Both the hexaploid (AABBDD) and tetraploid (AABB) varieties had major gliadin subunits of mol wt 36,000, 40,000, and 50,000 and minor subunits of mol wt 10,000, 53,000, 78,000, 82,000, and 108,000. The hexaploid varieties had additional minor subunits of mol wt 88,000, 120,000, and 130,000. These additional minor subunits are probably controlled by genes on the D-genome. Electrophoretic studies of compensating nullisomic-tetrasomic stocks of Chinese spring wheat and of Canthatch (AABBDD)-Tetracanthatch (AABB) have shown that the D-genome controls several slow moving gluten proteins (32,33).

The wheat gliadin subunits identified by SDS gel electrophoresis represent reduced and denatured polypeptide chains. In contrast, the structures of intact gliadin proteins are stabilized by disulfide bonding. The high-mol wt gliadin fraction (F_1) isolated by gel filtration consists mainly of disulfide cross-linked polypeptide chains of mol wt 40,000, 50,000, and 53,000 while the low-mol wt fractions consist mainly of single polypeptide chains stabilized by intrachain disulfide bonding. At the present time, it is not known if the subunits of high-mol wt gliadins are similar to the subunits in the low-mol wt fractions. However, similarities in subunit mol wt and amino acid compositions found in the present study would suggest that subunits present in the high-mol wt gliadin fractions (or vice-versa) may have been evolved from those present in the lower mol wt fractions by amino acid mutations which increased interchain disulfide bonding. Ewart (34) has presented a similar argument for the origin of glutenins.

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