AMINO ACID COMPOSITION AND SUBUNIT STRUCTURE OF RYE GLIADIN PROTEINS FRACTIONATED BY GEL FILTRATION[1]

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ABSTRACT

Gliadins of rye (*Secale cereale* cv. Prolific) were separated by gel filtration on Sephadex G-100 into four distinct groups with apparent molecular weights (mol wt) of greater than 100,000 and of 44,000, 27,000, and 10,000. These fractions contained 61, 29, 7, and 3% by weight of the total recovered protein, respectively. Each fraction had a distinct amino acid composition with the highest mol wt fraction having high glutamic acid and proline contents, whereas the two lowest mol wt fractions had amino acid compositions resembling albumins and globulins. Polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulfate before and after reduction was carried out on each fraction to determine subunit structure. The highest mol wt fraction gave five bands, all with apparent mol wt between 150,000 and 300,000, but after reduction of disulfide bonds only one band of mol wt 110,000 was present. The fractions of mol wt 44,000 and 27,000 from gel filtration each gave a single band with mol wt 42,000. The low-mol wt fraction had a single band of mol wt 10,000.

The important contribution of gliadins to the viscoelastic properties of wheat gluten has prompted a number of studies concerning the separation and characterization of these 70% ethanol-soluble proteins. However, the large number of gliadin components (1) having similar physical and chemical properties (2–7) has made these studies difficult. Rye (*S. cereale*), in contrast to tetra- and hexaploid wheats, is a diploid and might be expected to have fewer

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gliadin components. Electrophoretic studies of gliadin proteins from wheat and rye support this conclusion (8–10). Available evidence also suggests that the gliadins of wheat and rye are similar in structure. Wheat and rye gliadins have similar electrophoretic mobilities (8–10) while immunological (11) and peptide studies (7) suggest similar amino acid sequences. These results are consistent with the hypothesis, based on cytogenetic evidence, that the rye genome is derived from the same ancient diploid progenitor as are the diploid progenitors of the three genomes of hexaploid wheat (12).

Our studies were initiated because of the lack of available data concerning rye endosperm proteins. In this paper we report studies on the subunit structure and amino acid composition of rye gliadins fractionated by gel filtration.

MATERIALS AND METHODS

Fractionation of Rye Endosperm Proteins

Seed stocks of rye (*Secale cereale* cv. Prolific) were obtained from the Department of Plant Science, University of Manitoba, and milled on a Buhler experimental mill. Duplicate samples (10 g) were fractionated into water-soluble (albumins), salt-soluble (globulins), alcohol-soluble (gliadins), acid-soluble (soluble glutenins), and insoluble residue (insoluble glutenins) proteins by the modified Osborne procedure of Chen and Bushuk (13).

Determination of Protein Content

Protein content was determined by the micro-Kjeldahl method using $N \times 5.7$ as the conversion factor.

Gel Filtration of Rye Gliadins

Approximately 250 g of Sephadex G-100 (40–120 μ) was swollen in excess water and then equilibrated with several changes of the strongly dissociating solvent AUC (AUC: 0.1M acetic acid, 3M urea, 0.01M cetyltrimethyl ammonium bromide (14)). Dissolved gases were removed by heating the gel under vacuum. The hot gel slurry was then poured into a K 100/100 column (Pharmacia) and allowed to settle for 12 hr. The gel was packed by downward flow (150 ml/hr) for 12 hr followed by upward flow (140 ml/hr) for 12 hr. The final gel volume was 4.5 l.

Protein samples of approximately 1 g were dissolved in 50 ml of AUC and eluted by upward flow at rates of 140 ml/hr. The effluent was monitored at 280 nm with an Isco model UA_2 ultraviolet analyzer and appropriate peaks were collected. Each fraction was then dialyzed for 5 days against water and lyophilized. Molecular weights (mol wt) were estimated by calibration of the column with proteins of known mol wt as recommended by Whitaker (15). Recoveries of nitrogen from the column ranged from 80 to 85%. Each fraction was rechromatographed on a 2.5×40-cm column of Sephadex G-100 with AUC.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

SDS 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).

Amino Acid Analysis

Amino acid analysis was carried out on a Beckman Model 121 amino acid analyzer by the method of Spackman *et al.* (18). Protein samples (8 mg) were dissolved in 4 ml of triple distilled 6*N* HCl, frozen, and evacuated. Hydrolysis was carried out for 24 hr at 110°C. The hydrolysates were dried under vacuum over sodium hydroxide and then dissolved in pH 2.2 citrate buffer (0.1*M*).

RESULTS AND DISCUSSION

Solubility Distribution of Rye Endosperm Proteins

The distribution on the basis of solubility of the rye endosperm proteins was similar to that reported by Chen and Bushuk (13). The Osborne fractions of rye endosperm contained 29, 8, 17, 15, and 31% of the total recovered proteins for the water-, salt-, alcohol-, acid-soluble, and residue proteins, respectively. Wheat endosperm proteins, by comparison, contain much lower proportions of water-soluble proteins and a much higher proportion of alcohol-soluble proteins (19).

Amino Acid Composition of Rye Endosperm Solubility Classes

The amino acid compositions of proteins in the different solubility classes showed major differences (Table I). The albumins and globulins contained more of the ionic amino acids (basic and acidic) and less of the polar amino acids due to lower glutamine content (glutamic acid is mainly in the amide form in the intact protein) than did the gliadins and glutenins. Each solubility class could be distinguished on the basis of the content of six amino acids: lysine, arginine, glutamic acid, proline, glycine, and alanine. For example, gliadins could be distinguished from soluble glutenins by their lower content of lysine, glycine, and alanine and higher content of glutamic acid and proline. The amino acid compositions of the various rye protein fractions were similar to published data for wheat proteins (20).

TABLE I
Amino Acid Composition of Rye (cv. Prolific) Endosperm Proteins (mol %)[a]

Amino Acid	Albumins	Globulins	Gliadins	Glutenins
Lysine	2.1	4.3	0.7	2.0
Histidine	1.5	2.1	1.3	1.3
Arginine	2.6	4.8	1.5	1.7
Aspartic acid	4.1	7.5	2.1	2.7
Threonine	3.2	4.0	2.0	2.5
Serine	5.1	5.5	4.9	5.3
Glutamic acid	27.7	16.4	36.7	33.7
Proline	16.6	6.5	20.3	16.6
Glycine	4.3	8.1	2.3	7.2
Alanine	4.5	7.4	2.5	3.4
Valine	10.5	14.0	9.6	9.4
Methionine	1.3	1.7	1.1	1.0
Isoleucine	3.5	3.9	3.2	2.3
Leucine	6.6	7.6	5.9	5.2
Tyrosine	1.6	2.2	1.0	2.3
Phenylalanine	4.6	3.5	4.6	3.4

[a]Cystine and tryptophan not determined.

Gel Filtration of Rye Gliadins

The elution profile obtained by chromatography on Sephadex G-100 of the gliadin proteins of rye is shown in Fig. 1. Four fractions were isolated with average mol wt of greater than 100,000 (F_1) and of 44,000 (F_2), 27,000 (F_3), and 10,000 (F_4). Fractions F_1 (61%) and F_2 (29%) accounted for 90% of the total gliadin protein, whereas fractions F_3 and F_4 accounted for 7 and 3%, respectively. Rechromatography of fractions F_1, F_2, F_3, and F_4 gave single peaks with values of Ve/Vo identical to the corresponding original fraction.

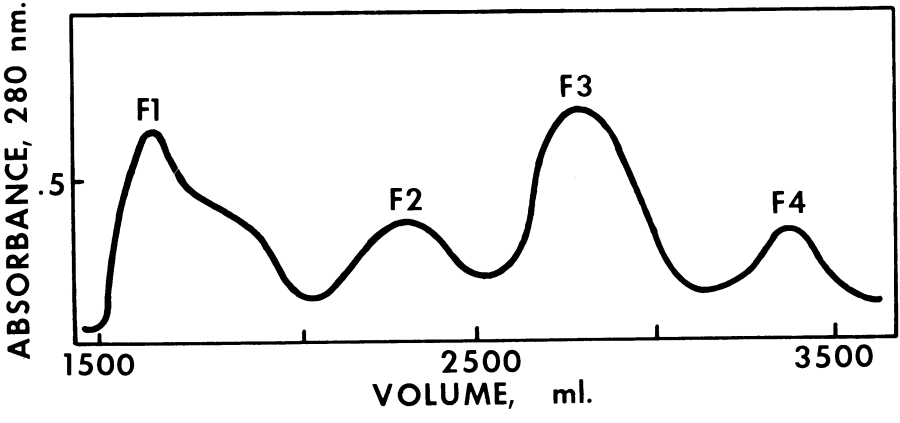

Fig. 1. Elution profile of rye (*S. cereale* cv. Prolific) gliadins on Sephadex G-100 in AUC (0.1 *M* acetic acid, 3 *M* urea, 0.1 *M* cetyltrimethyl ammonium bromide). (Vo = 1600 ml with blue dextrin).

TABLE II
Amino Acid Composition of Rye (cv. Prolific) Gliadin Fractions from Sephadex G-100 Chromatography in AUC (mol %)[a]

Amino Acid	F_1	F_2	F_3	F_4
Lysine	0.5	0.7	2.2	2.8
Histidine	1.1	1.2	1.6	1.7
Arginine	0.8	1.4	2.1	2.6
Aspartic acid	1.4	2.3	3.8	5.1
Threonine	1.4	2.3	3.8	3.8
Serine	4.7	4.5	5.5	7.6
Glutamic acid	41.4	35.9	25.3	24.6
Proline	20.8	20.0	14.4	11.2
Glycine	1.5	2.3	3.8	8.4
Alanine	2.0	2.1	3.6	4.7
Valine	10.1	9.7	13.2	10.0
Methionine	0.8	1.1	1.4	1.5
Isoleucine	2.3	4.0	5.3	3.9
Leucine	4.6	6.2	7.7	6.6
Tyrosine	1.0	0.9	1.3	2.0
Phenylalanine	4.6	5.1	4.4	3.5

[a]Cystine and tryptophan not determined.

Amino Acid Composition of Rye Gliadin Fractions

The amino acid compositions of the four gliadin fractions are shown in Table II. The high-mol wt fraction (F_1) had very high contents of proline and glutamic acid while the second fraction (F_2) had a similar high proline content but less glutamic acid. The low-mol wt fractions (F_3 and F_4) had amino acid compositions similar to the albumin fraction. There was a definite trend toward an increase in proline and glutamic acid and a decrease in the basic amino acids, aspartic acid, glycine, and alanine as mol wt increased.

The unique amino acid composition of the high-mol wt fraction indicates that these proteins are not produced through association of the lower mol wt gliadins in fractions F_2, F_3, or F_4. Neither can this mol wt gliadin fraction be considered to be glutenin-like since glutenins generally contain higher relative proportions of glycine and lower contents of proline and glutamic acid (20,21).

SDS-Polyacrylamide Gel Electrophoresis of Rye Gliadin Fractions

To determine the subunit structure of the isolated rye gliadin proteins, fractions isolated by gel filtration were subjected to electrophoresis on polyacrylamide gel in the presence of SDS. The lower mol wt fractions (F_2 and F_3) each showed only a single band (faint contaminating bands disappeared on rechromatography) for both the reduced and nonreduced preparations (Fig. 2), indicating that these proteins are probably single-chain polypeptides with no interchain disulfide bonding. In each case, a mol wt of approximately 42,000 daltons was calculated.

Although the apparent mol wt of fraction F_2 and F_3 subunits were identical, these fractions differed widely in amino acid composition and therefore do not appear to represent similar polypeptide chains. The mol wt of fraction F_2 gliadins determined by SDS electrophoresis agreed with the values obtained by gel filtration (mol wt 44,000). However, the mol wt of fraction F_3 gliadins was much lower by gel filtration (mol wt 27,000) than by SDS-gel electrophoresis (mol wt 42,000). There is no obvious reason for the discrepancy in the two mol wt values determined for F_3. However, it may be of interest that this fraction makes a large contribution to the chromatographic area (Fig. 1) but accounts for only 7% of the protein on a Kjeldahl nitrogen basis. This high absorption may indicate binding of phenolic compounds to the protein which might cause an apparent reduction in mol wt by Sephadex chromatography through association with the Sephadex gel.

The SDS-gel pattern for the nonreduced high-mol wt rye gliadin fraction (F_1) is shown in Fig. 2. At least five major bands could be identified in the high-mol wt region (mol wt 150–300,000) of the gel. After reduction of disulfide bonds with β-mercaptoethanol, the high-mol wt gliadin fraction gave a single band with a mol wt of approximately 110,000. The multiplicity of high-mol wt bands in the nonreduced pattern may have arisen in two ways. The high-mol wt gliadins in rye may be produced through interchain disulfide bonding between polypeptide chains with mol wt of approximately 110,000. Alternately, there may be conformational isomers due to different sites of disulfide bonding within the same polypeptide chains which may determine the mobility of the SDS-protein complex. It has been shown that intact disulfide bonds reduce SDS binding to proteins and thus decrease the mobility of the complex (22).

SDS-Polyacrylamide Gel Electrophoresis of Gliadins from Other Rye Varieties

The results reported concern the rye cultivar, Prolific. SDS electrophoresis of the gliadins of three additional varieties (Argentine, Explorer, and Apizaco) was carried out. All three varieties gave patterns for both reduced and nonreduced gliadins which were identical to that of Prolific.

GENERAL DISCUSSION

SDS-polyacrylamide gel electrophoretic studies by Bietz and Wall (23) have shown that the majority of gliadin proteins in hard red winter wheats are single-chain polypeptide chains with subunit mol wt of 11,400, 36,500, 44,200, 29,300, and 78,000. The high-mol wt wheat gliadins were composed of disulfide-linked polypeptide chains with subunit mol wt of 44,200 and 36,500. In contrast, the majority of high-mol wt gliadins in rye were composed of one or more subunits with mol wt near 110,000. These high-mol wt subunits may also lack the ability to form interchain disulfide bonds.

Lower mol wt rye gliadins appeared to consist of at least three distinct groups on the basis of gel filtration and amino acid analysis. One fraction (F_2), accounting for the majority of this protein, had properties similar to wheat gliadin. The amino acid composition of this rye gliadin fraction was similar to the major wheat gliadin fraction (fraction 3) separated by gel filtration (24) and had a subunit mol wt (mol wt 42,000) similar to the major subunits (mol wt 36,000 and

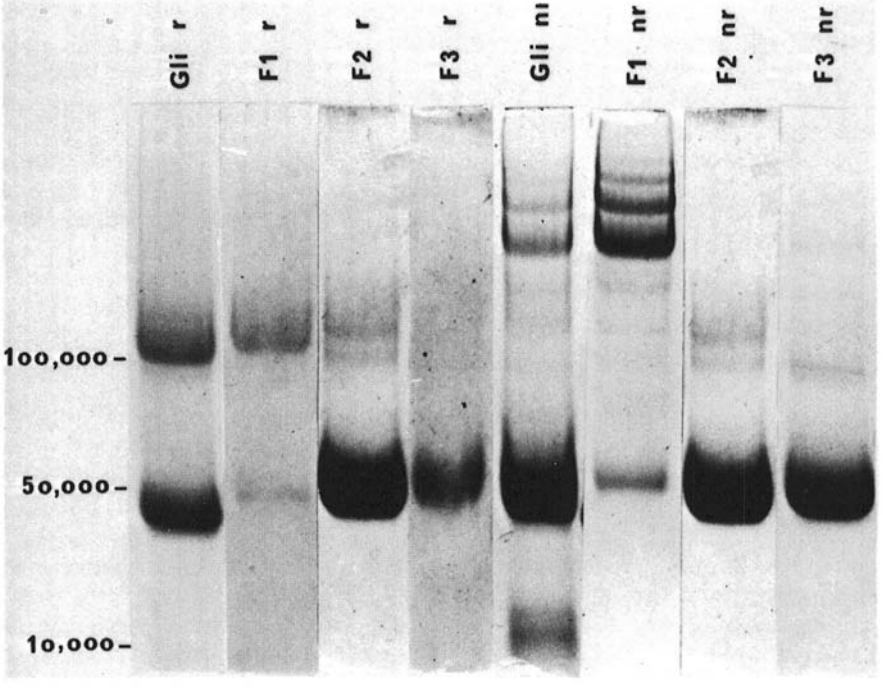

Fig. 2. SDS-polyacrylamide gel pattern of reduced (r) and nonreduced (nr) rye gliadin fractions from Sephadex G-100 chromatography.

44,000) in wheat gliadin as determined by SDS-gel electrophoresis (23). The dissimilarity in chemical and physical properties between the other three rye gliadin fractions and wheat gliadins would seem to suggest that fraction 2 rye gliadins may be almost totally responsible for previously reported similarities between rye and wheat gliadins with respect to peptide (7) and immunological studies (11).

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