

Involvement of Carbohydrates and Lipids in Aggregation of Glutenin Proteins¹

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ABSTRACT

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Glutenins were prepared from untreated flour, from flour partially defatted by *n*-butanol and then by *n*-hexane, and from flour totally defatted by water-saturated *n*-butanol. The three glutenins differed in lipid contents, but all contained similar amounts of carbohydrate that upon hydrolysis yielded almost entirely glucose. Glutenins were then reduced and alkylated and fractionated on Sephadex G-200. Of the resulting three fractions, fraction I, which eluted at the void volume, had unusually high, for gluten, contents of lysine and aspartic acid, lower contents of glutamic acid and proline, and contained all the carbohydrate and lipid originally present in the glutenin. Sodium dodecyl sulfate electrophoresis showed that fraction I contained subunits of lower molecular weight than expected from their elution volumes, indicating aggregation. Fractions II and III were

characteristic of gluten proteins rich in glutamic acid and proline. They contained no carbohydrate or lipid. After partial or total defatting, the proportion of fraction I changed, indicating that lipid contributed to the aggregation of the proteins in this fraction. Removal of carbohydrate from fraction I by digestion with amyloglucosidase produced further disaggregation. Sodium dodecyl sulfate electrophoresis of subfractions of fraction I from which the carbohydrate had been removed showed that they contained the same glutenin subunits as in the original fraction I but in different proportions. We concluded that the strong aggregative tendency of fraction I proteins of glutenin is determined mainly by protein structure, but specific lipids and carbohydrates contribute significantly to this behavior.

There is considerable evidence indicating that functional gluten, produced during dough mixing and development, involves formation of aggregates through hydrogen bonds and hydrophobic, ionic, and polar interactions (Bushuk 1985, and references cited therein), mainly involving proteins. However, recent results indicate that specific nonprotein constituents of flour (and added ingredients) are also involved. This conclusion has been drawn from observations that certain nonprotein constituents, especially lipids, are bound to flour proteins during dough mixing (Chung et al 1978), and that certain lipids (Bekes et al 1983a, Zawistowska et al 1984) and carbohydrates (McMaster and Bushuk 1983) strongly bind to specific proteins in isolated fractions. Other indirect evidence suggests that flour galactolipid can cause aggregation of isolated gliadin (Bekes et al 1983b).

In our continuing studies of the association of nonprotein constituents with proteins in gluten and dough, this article presents results on the involvement of carbohydrates and lipids in aggregation of glutenin proteins.

MATERIALS AND METHODS

Flour

Flour for the preparation of the various fractions was milled from the grain of a pure cultivar of hard red spring wheat (Nee pawa) on a Bühler Laboratory mill to 72% extraction. Its protein (N × 5.7) and ash contents were 12.2% and 0.43%, respectively, both expressed on a dry basis.

Chemicals

Reference proteins, carbohydrate standards, Coomassie Brilliant Blue R250, 2-mercaptoethanol, 3-dimethylamino-propionitrile, and *Aspergillus niger* amyloglucosidase were obtained from Sigma Chemical Company (St. Louis, MO). Acrylamide, bisacrylamide, and sodium dodecyl sulfate (SDS) were of electrophoresis grade and were obtained from Bio-Rad (Richmond, CA). All other chemicals were of analytical reagent grade.

Defatting of Flour

Two defatting procedures were used. In the first procedure, the

flour was partially defatted by extraction with *n*-butanol, followed by *n*-hexane to remove residual butanol. In the second, the flour was extracted with water-saturated *n*-butanol to remove all nonstarch lipids. All extractions were made as described by Bekes et al (1983a). Defatted flour was air dried and stored at 4°C.

Preparation of Gluten

Gluten was washed out manually under tap water from doughs mixed to optimum development in air in a farinograph, freeze-dried, ground in a mortar and pestle, and stored at 4°C.

Preparation and Fractionation of Glutenin

Glutenin was prepared from gluten by the pH precipitation procedure of Orth and Bushuk (1973) and was reduced and alkylated as reported in Friedman et al (1970).

Reduced/alkylated glutenin (250 mg) was fractionated by gel-filtration chromatography on a 100 × 5 cm column of Sephadex G-200, with AU (0.1M acetic acid and 3M urea) solvent, at 9 ml/h. Three-milliliter fractions were collected. Rechromatography of 15-mg fractions was performed on a 25 × 1 cm Sephadex G-200 column in AU solvent at 3 ml/h, collecting 1-ml fractions.

Molecular weights were estimated from elution volumes according to Andrews (1964) using γ -globulin (160,000), bovine albumin (66,000), egg albumin (45,000), trypsinogen (24,000) and lysozyme (14,300) as molecular weight standards.

Enzymatic Digestion of Carbohydrate in Glutenin

Fraction I of glutenin was incubated with amyloglucosidase in 0.05M potassium acetate buffer, pH 4.2, for 4 hr at 37°C. The ratio of enzyme to sample was 1 to 46. After hydrolysis, the mixture was centrifuged (20 min, 12,000 × g) and the clear supernatant analyzed for reducing sugar (Roybt and Whelan 1968). Glucose was used as the standard sugar.

A separate portion of the hydrolyzate was concentrated on an Amicon YM5 membrane (5,000 mol wt cutoff), equilibrated with AU solvent by overnight dialysis, and centrifuged. The clear supernatant was subsequently examined by gel-filtration chromatography.

Amyloglucosidase Activity

Amyloglucosidase activity was determined using glycogen as the substrate (Jeffrey et al 1970).

Proteolytic Activity

To ensure that amyloglucosidase had no proteolytic activity, endopeptidase activity was assayed using azocasein (Kruger 1973) and α -benzoyl-L-arginine-*p*-nitroanilide (Burger 1966). Exopeptidase activity was assayed by measuring the free amino acids

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content of the amyloglucosidase hydrolyzate before and after the reaction by ninhydrin (Mertz et al 1974).

Determination of Carbohydrate by Paper Chromatography

Samples (10 mg) were hydrolyzed at 100°C for 2 hr in 1M sulfuric acid (1 ml), cooled, neutralized with barium carbonate, and centrifuged. The pellet was washed twice with distilled water (0.5 ml each) and centrifuged, and the three supernatants were combined. Carbohydrate in the supernatant was separated by descending paper chromatography (Partridge and Westall 1948) using ethyl acetate, pyridine, and water (8:2:1) solvent; chromatograms were developed with silver nitrate (Trevelyan et al 1950). Spots were identified by comparison to the standards: α -D(+)-glucose, D(+)-galactose, maltose, maltotriose, D(+)-xylose, and D(-)-arabinose.

Other Analytical Procedures

Absorbance at 280 nm was used routinely to estimate protein content of gel filtration fractions. Glutenin peak fractions were pooled (as indicated by horizontal bars on the gel filtration profile),

dialyzed, frozen, and freeze-dried. Protein ($N \times 5.7$) content of freeze-dried glutenin fractions was determined by the micromethod of Nkonge and Ballance (1982).

For lipid determination, lipids were extracted as described by Bekes et al (1983a). Sample size varied (10 g flour, 1 g gluten, and 0.2 g gluten fractions) depending on the amount of material available. Total lipid, extracted with water-saturated *n*-butanol, was fractionated into neutral lipid (NL), glycolipid (GL), and phospholipid (PhL) by chromatography on silicic acid (Kates 1972). The sum of GL and PhL was defined as polar lipid (PL).

Total carbohydrate content of glutenin and its fractions was determined by the phenol-sulfuric acid method (Dubois et al 1956). The average coefficient of variation for carbohydrate determinations was 4%.

Amino acid compositions were determined using a Beckman model 119C automatic amino acid analyzer. Samples were hydrolyzed in 6N HCl for 16 hr at 121°C under vacuum, evaporated to dryness, dissolved in 0.2M sodium citrate buffer, pH 2.2, and filtered. The filtrate was used for analysis. Cysteine, cystine, methionine, and tryptophan were not determined. Values for aspartic and glutamic acids include asparagine and glutamine, respectively. The data reported are means of duplicates.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was done on gradient gels as described by Zawistowska et al (1985). This procedure is a modification of that of Laemmli (1970). Molecular weights were estimated from mobilities of β -galactosidase (116,000), phosphorylase B (97,400), bovine albumin (66,000), egg albumin (45,000), trypsinogen (24,000) and lysozyme (14,300).

RESULTS AND DISCUSSION

Characterization of Glutenin Preparations

Protein and carbohydrate contents and lipid content and composition of the glutenin preparations used are given in Table I. Glutenin from untreated flour contained twice as much lipid as that from partially defatted flour. Partial defatting removed practically all free lipid but only a part of bound lipid (Bekes et al 1983a). Glutenin from totally defatted flour contained no lipid.

The most abundant PL of the glutenin preparations was PhL (Table I). Determination of the distribution of PhL in flour, gluten, and the gluten fractions showed that of the 210 mg of PhL present in 100 g of flour almost all (209 mg) was present in the gluten. After fractionation of the gluten, 42 mg was in the gliadin fraction and 141 mg in the glutenin fraction. Thus, assuming 100% lipid recovery, about three-quarters of flour PhL was associated with glutenin. (Amounts of gluten, gliadin, and glutenin obtained from 100 g of nondefatted flour were: 11, 4.9, and 4.07 g, respectively.) According to our earlier data (Zawistowska et al 1984) on lipid distribution in Osborne fractions in Neepawa wheat, the major portion of flour PhL (64.5%) was associated with acetic acid soluble and acetic acid insoluble glutenin fractions. The remainder of the PhL was in the gliadin (27%) and albumin/globulin (8.5%) fractions. The fact that the gluten preparation in the present study contained almost all of the flour PhL suggests that the albumins

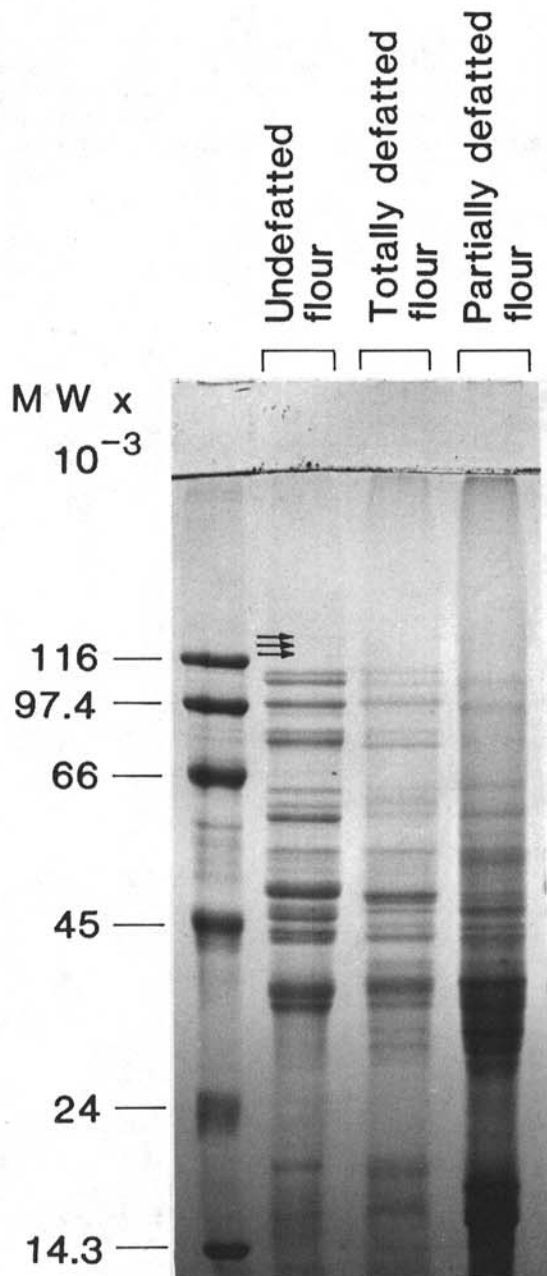


Fig. 1. SDS-PAGE patterns of the glutenin preparations. Left pattern is for molecular weight reference proteins.

TABLE I
Protein, Carbohydrate, and Lipid Contents and Composition (% db)
of Glutenin Preparations

Glutenin	Undefatted	Partially Defatted	Totally Defatted
Constituent			
Protein	84.2	90.8	94.0
Carbohydrate	6.5	4.9	6.0
Lipid			
Total (TL)	9.0	4.3	0
Neutral (NL)	4.9	2.1	0
Glycolipid (GL)	0.6	0.5	0
Phospholipid (PhL)	3.5	1.7	0
Polar ^a (PL)	4.1	2.2	0

^aPolar lipids = glycolipids plus phospholipids.

removed during gluten washing probably did not contain any PhL. Thus, we concluded that the PhL present in the Osborne albumin/globulin fraction would be associated with the globulins. This is in agreement with our unpublished results concerning the presence of globulin-like proteins with strong affinity for PL in gluten preparations.

Our lipid results for glutenin are consistent with those of Bourdet and Feillet (1967), which showed that essentially all of the flour phosphorus was associated with glutenin. The source of the PhL is still unknown, but it may originate from endoplasmic reticulum, as implied by Simmonds (1972).

Determination of the sugar composition of carbohydrate present in glutenins (Table I) indicated glucose as the only major component. This agrees with earlier results (McMaster and Bushuk 1983) for a different wheat cultivar. Although the presence of carbohydrate in glutenin preparations was reported before by many authors (Huebner et al 1974, Danno et al 1978, Khan and Bushuk 1979, Stachelberger and Schachermayer-Schilling 1982, McMaster and Bushuk 1983), only McMaster and Bushuk (1983) quantitatively determined carbohydrate composition. Presence of glucose as a major sugar component in glutenin excludes the presence of GL in glutenin preparations, suggested by Stachelberger and Schachermayer-Schilling (1982), because the main sugar present in wheat flour GL is galactose (Morrison 1978).

SDS-PAGE (Fig. 1) showed approximately 30 subunits ranging in molecular weights (mol wts) from 14,000 to 120,000 for glutenins from control, partially defatted, and defatted flours. Removal of lipid affected the intensity as well as presence of some bands. Surprisingly, patterns of glutenins from control and totally defatted flours appeared to differ less than the patterns of glutenins from control and partially defatted flours. In the pattern for gluten from partially defatted flour, the intensity of bands with molecular weights above 66,000 was noticeably lower, whereas the intensity of bands below 36,000 was higher. In the high molecular weight region, glutenin from control flour had three additional bands (see arrows, Fig. 1) not present in the other two glutenins. These bands were hardly visible in the gels stained with Coomassie Brilliant Blue but were readily detectable with silver staining (Merril et al 1981). A similar decrease in proportion of high molecular weight glutenin subunits as a consequence of defatting flour samples by petroleum ether or a mixture of chloroform-methanol (1:1, v/v) was observed by Stachelberger (1977).

The higher content (estimated from band intensity) of low molecular weight subunits in glutenin from partially defatted flour compared with glutenin from the control flour is interpreted as resulting from disaggregation of gluten aggregates, which results from removal of lipids. Conversely, the presence of high molecular weight bands in glutenin from the control flour is attributed to aggregates that are formed in the presence of free lipid. However, a

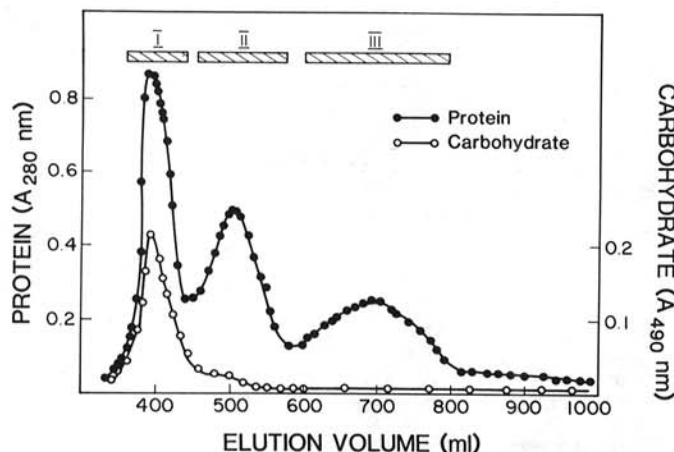


Fig. 2. Protein and carbohydrate elution profiles of glutenin from control flour on Sephadex G-200. Fractions collected (I-III) are indicated by horizontal bars.

possibility that lipid enhances the solubility of some glutenin proteins cannot be excluded. These speculations remain to be verified.

Amino acid compositions of glutenin preparations are given in Table II. The compositions of the three preparations are typical of glutenin (Orth and Bushuk 1973, Huebner et al 1974, Danno et al 1978, Khan and Bushuk 1979) with lower contents of glutamic acid and proline and higher contents of glycine and lysine compared with the compositions of gliadin (Wu and Dimler 1963, Bekes et al 1983a). However, comparison of the three glutenins showed substantive differences in proportions of some amino acids. Proportions of the hydrophobic amino acids, such as alanine, leucine, and tyrosine, were somewhat higher in glutenins from partially or totally defatted flours. Removal of lipid could decrease solubility of proteins enriched in these amino acids, causing these proteins to precipitate with glutenin, rather than remain in solution and separate with gliadin during the pH precipitation fractionation.

Another interesting change in amino acid composition that results from defatting flour before gluten extraction was in the proportion of aspartic acid and proline. Removal of total lipid did not produce any change in the two amino acids, whereas removal of free lipid and only a small part of the bound lipid caused a relative increase in aspartic acid and relative decrease in proline level. Defatting of flour appears to differentially affect flour protein solubility, and resulting amino acid compositions, so comparison of proteins extracted from untreated and defatted flour should be made with caution.

Results on Fractions of Glutenins

For additional comparison, the glutenins were reduced and alkylated, and fractionated by gel filtration chromatography (Fig.

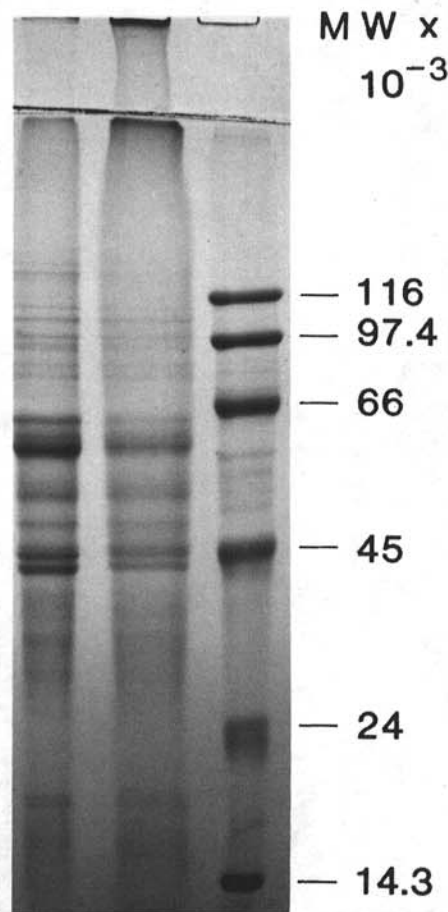


Fig. 3. SDS-PAGE patterns of subfractions of fraction I of glutenin from control flour. Lane 1, subfraction I; lane 2, subfraction III; lane 3, molecular weight reference proteins.

2). The profile for glutenin from control flour had three peaks corresponding to molecular weights of approximately 200,000 for fraction I, 125,000 for fraction II, and 63,000 for fraction III. Glutenins from partially or totally defatted flours gave profiles with peaks at approximately the same positions, but proportions of peaks changed. Fraction I (expressed on the basis of area of peak

equivalent to volume collected) was 38% for glutenin from control flour, and 32 and 30% for glutenins from partially and totally defatted flours, respectively. (Peak areas are averaged from 3-4 separations; the experimental error was $\pm 3\%$.)

A smaller proportion of fraction I glutenin was found from nitrogen determination by Huebner and Wall (1974; 23%) and Arakawa et al (1977; 10-21%) for glutenins obtained from flours differing in quality. Proportions of fraction I obtained in our study were similar to those calculated from the area of elution pattern by Arakawa and Yonezawa (1975; 31-47%) for different flours. The higher proportions of fraction I glutenin obtained from the area under the elution profile compared with the values from nitrogen determination can result from the opaque appearance of this fraction. The high opacity is probably related to the high carbohydrate content and protein aggregation. Interestingly, in the studies by Arakawa and coworkers (Arakawa and Yonezawa 1975, Arakawa et al 1977) glutenins from weak flours contained a higher proportion of fraction I than those from stronger flour. However, their studies concerned only the acetic acid soluble glutenin (which constitutes only about one-third of total glutenin), whereas our studies deal with practically all of the glutenin.

All of the carbohydrate in the glutenin fraction coeluted with fraction I (Fig. 2). Similarly, the lipid occurred only in this fraction (results not shown).

SDS-electrophoresis showed that fraction I contained lower molecular weight subunits than expected from their elution volumes, indicating aggregation (pattern identical with rechromatographed fraction I; Fig. 3). Aggregative tendency of fraction I glutenin has been discussed in several publications (Huebner and Wall 1974, Hamauzu et al 1975, Khan and Bushuk 1979) however the effect of nonprotein flour constituents on this aggregation had not been investigated.

Since the amounts of carbohydrate in the three glutenin preparations were similar (Table I), the decrease in the proportion of fraction I upon defatting can be attributed to decreased lipid content. This result indicates lipid involvement in aggregation of fraction I proteins.

Amino acid compositions of fractions obtained by gel filtration (Table III) were widely variable. All fraction I samples had significantly lower proportions of glutamic acid and proline, and higher proportions of lysine and aspartic acid than fractions II and III. This agrees with published data (Huebner et al 1974, Hamauzu et al 1975, Arakawa et al 1977, Danno et al 1978, Khan and Bushuk 1979). These results indicate that fraction I contained significant

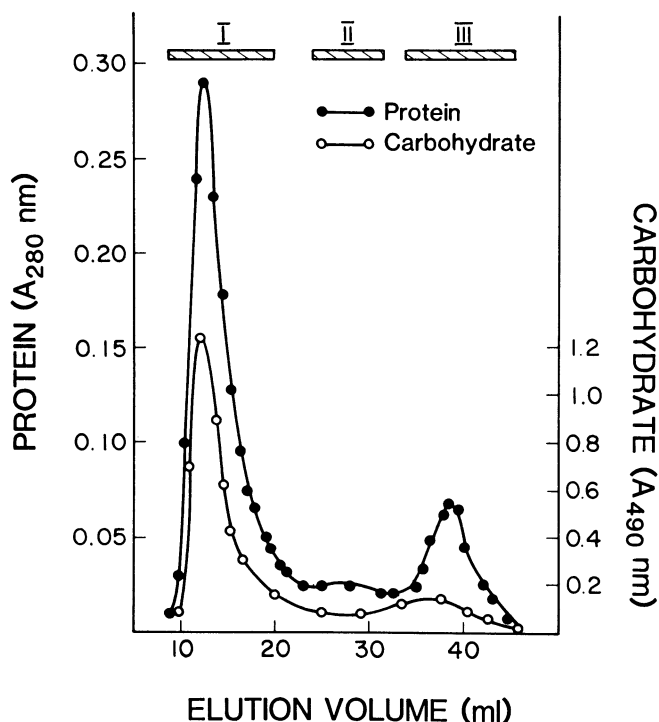


Fig. 4. Protein and carbohydrate elution profiles of fraction I of glutenin from control flour. Collected subfractions I-III are indicated by horizontal bars.

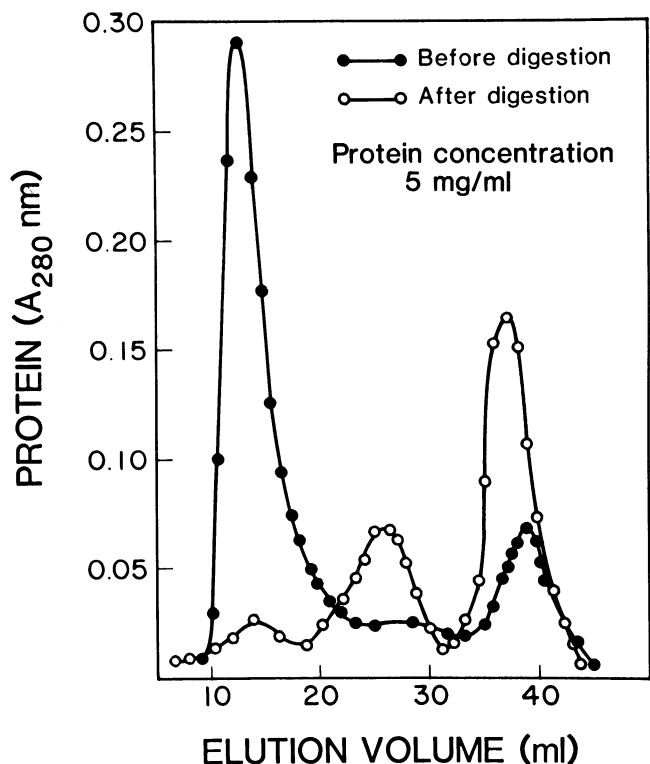


Fig. 5. Elution profiles of fraction I of glutenin from control flour (solid circles) and the same fraction after amyloglucosidase digestion (open circles).

TABLE II
Amino Acid Composition of Glutenin Preparations^a

Amino Acid	Undeffatted	Partially Defatted	Totally Defatted	Glutenin ^b	Gliadin ^c
Aspartic acid ^d	4.4 (39.1)	5.3 (46.7)	4.4 (38.4)	4.4	2.7
Threonine	3.4 (30.3)	3.3 (29.3)	3.1 (27.4)	3.0	2.3
Serine	5.5 (48.7)	5.3 (46.1)	5.4 (47.6)	6.1	5.7
Glutamic acid ^e	31.2 (275)	29.8 (260)	31.4 (276)	30.4	37.9
Proline	13.2 (116)	10.8 (94.0)	13.1 (115)	12.5	17.2
Glycine	11.1 (97.6)	8.7 (76.3)	9.9 (86.8)	9.3	3.4
Alanine	4.9 (42.9)	5.9 (51.2)	5.3 (46.6)	4.5	3.1
Valine	5.1 (44.9)	5.6 (48.9)	5.0 (43.5)	4.7	4.4
Methionine	ND ^f	ND	ND	1.4	1.4
Isoleucine	3.4 (29.5)	4.0 (35.1)	3.2 (28.1)	3.2	4.0
Leucine	6.2 (54.7)	7.7 (67.3)	7.1 (62.2)	7.0	7.1
Tyrosine	1.7 (15.1)	2.4 (21.1)	2.2 (19.2)	3.3	1.8
Phenylalanine	3.1 (27.2)	3.5 (30.4)	3.1 (26.7)	3.4	4.7
Lysine	2.3 (19.9)	2.7 (23.4)	2.4 (20.7)	2.2	0.8
Histidine	1.7 (14.8)	1.8 (16.1)	1.6 (14.3)	1.8	1.7
Arginine	2.8 (25.0)	3.1 (27.1)	2.9 (25.1)	3.0	1.9

^a Mole percent of the total protein; values in parentheses are moles per 10⁵ g protein. Cysteine, methionine, and tryptophan were not determined.

^b Orth and Bushuk 1973.

^c Bekes et al 1983.

^d Includes asparagine.

^e Includes glutamine.

^f ND = Not determined.

TABLE III
Amino Acid Composition of Fractions from Glutenin Preparations^a

Amino Acid	Undefatted			Partially Defatted			Totally Defatted		
	I	II	III	I	II	III	I	II	III
Aspartic Acid ^b	8.1 (71.2)	1.7 (14.9)	2.5 (22.3)	8.4 (74.3)	1.6 (13.7)	2.1 (18.7)	7.3 (64.0)	1.6 (13.9)	3.7 (32.7)
Threonine	4.7 (41.2)	2.7 (23.6)	2.6 (22.9)	4.2 (37.3)	2.5 (22.2)	2.4 (21.1)	3.7 (32.3)	2.6 (22.8)	3.0 (26.6)
Serine	5.8 (51.0)	5.0 (44.1)	5.5 (49.1)	5.5 (48.4)	4.9 (43.3)	5.5 (48.9)	5.6 (49.3)	5.0 (44.3)	6.2 (54.6)
Glutamic acid ^c	17.2 (151)	32.4 (284)	34.7 (308)	16.7 (147)	36.0 (316)	38.5 (340)	22.1 (193)	35.5 (312)	35.2 (312)
Proline	9.8 (86.6)	16.5 (144)	19.3 (172)	11.4 (101)	19.7 (172)	18.8 (166)	9.6 (84)	14.8 (130)	14.7 (130)
Glycine	9.1 (80.5)	18.9 (166)	4.6 (40.8)	14.0 (123)	16.6 (145)	4.1 (36.5)	11.1 (97)	18.3 (161)	5.9 (51.8)
Alanine	9.5 (83.1)	4.2 (36.5)	3.9 (34.8)	7.9 (69.2)	2.6 (23.1)	3.2 (28.0)	6.6 (58.1)	2.4 (21.5)	3.8 (33.2)
Valine	6.9 (60.7)	2.8 (24.1)	4.9 (43.5)	6.2 (54.9)	2.2 (18.9)	4.6 (40.7)	6.0 (52.7)	3.2 (28.0)	5.0 (44.4)
Isoleucine	4.2 (37.4)	1.5 (12.9)	3.8 (33.9)	3.4 (29.7)	1.4 (12.1)	3.7 (32.4)	3.5 (30.9)	2.0 (17.4)	3.8 (33.4)
Leucine	8.1 (71.7)	4.5 (39.2)	7.8 (69.1)	6.6 (57.9)	4.2 (37.0)	7.5 (65.9)	8.2 (71.3)	5.3 (46.6)	7.3 (65.0)
Tyrosine	2.1 (18.3)	4.0 (35.3)	1.1 (10.1)	1.9 (16.4)	3.1 (26.8)	0.9 (7.5)	3.4 (29.7)	3.0 (26.5)	1.8 (16.1)
Phenylalanine	3.9 (34.5)	1.4 (12.5)	3.9 (34.8)	3.7 (32.4)	1.4 (12.2)	3.9 (34.1)	3.6 (31.5)	2.0 (17.2)	3.7 (33.1)
Lysine	4.9 (43.1)	1.5 (13.5)	1.6 (14.6)	4.3 (38.2)	1.2 (10.7)	1.4 (12.0)	3.5 (30.9)	1.1 (9.4)	1.6 (14.0)
Histidine	1.8 (15.8)	0.9 (7.9)	1.4 (12.5)	1.5 (12.9)	0.8 (6.7)	1.2 (10.7)	2.0 (17.5)	1.3 (11.6)	1.8 (15.9)
Arginine	3.9 (34.1)	2.1 (18.2)	2.4 (21.1)	4.3 (38.0)	1.9 (16.4)	2.2 (19.1)	3.6 (31.2)	2.0 (17.4)	2.6 (22.8)

^a Mole percent of the total protein; values in parentheses are moles per 10⁵ g protein. Cysteine, methionine, and tryptophan were not determined.

^b Includes asparagine.

^c Includes glutamine.

amounts of albumin/globulin type proteins, consistent with the suggestion of Simmonds (1972) that glutenin contains insoluble proteins originating from the endoplasmic reticulum. In addition, the three fractions I differed somewhat in amino acid composition, indicating a slight shift in solubility of gluten proteins caused by defatting of flour.

Fraction II samples had much higher contents of glycine and lower contents of valine, isoleucine, leucine, and phenylalanine than fractions I and III. Fraction III samples had the highest contents of glutamic acid and proline and the lowest glycine content of all glutenin fractions.

Rechromatography of fractions I produced elution profiles with three peaks (Fig. 4), eluting at volumes similar to those for the peaks of original reduced/alkylated glutenins (when reduced/alkylated glutenin was chromatographed on small columns used for rechromatography studies). However, peak III was considerably larger than peak II, and ratios of peak I to II or III were much lower. All carbohydrate eluted at volumes coincident with fractions I and III.

The presence of more than one peak upon rechromatography of fraction I (Fig. 4) indicates protein disaggregation. Similar disaggregation was observed by Dalek-Zawistowska et al (1975) for glutenin extracted from flour with acetic acid.

The two major subfractions (I and III) obtained by rechromatography of fractions I gave identical SDS-PAGE subunit patterns (Fig. 3), except for some differences in band intensity. Thus, both peaks I and III (Fig. 4) contained primarily the same subunits but in different proportions and states of aggregation.

Effect of Carbohydrate Removal on Fraction I Aggregation

Because the subunit compositions and carbohydrate distributions of the two major subfractions of fraction I were quite similar, the role of carbohydrate on its aggregation was further investigated. Carbohydrates were removed with amyloglucosidase free of proteinase activity. The residual protein-lipid complex was separated from sugars and concentrated using an Amicon membrane filter. The resulting protein, presumably unaltered by the amyloglucosidase treatment, was then examined by gel-filtration chromatography and SDS-PAGE.

Gel-filtration (Fig. 5) showed a significant decrease in peak I and a concomitant increase in the size of peaks II and III after amyloglucosidase digestion, indicating that carbohydrate is involved in the aggregation of fraction I proteins. SDS-PAGE results (not shown) indicated that removal of the carbohydrate did not alter the subunit composition of subfraction I. These results clearly show that carbohydrates contribute to the strong aggregative tendency of glutenin fraction I proteins. Because fraction I from partially and totally defatted flours contained

varying amounts of subfractions I with similar subunit compositions, we conclude that the aggregative tendency of these glutenin subunits results mainly from the unique structure of the proteins, which is modified by specific flour lipids. Because such protein aggregation may contribute substantially to dough functionality, further research in this area is warranted.

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