Gluten Proteins with High Affinity to Flour Lipids[1]

U. ZAWISTOWSKA,[2] F. BEKES,[3] and W. BUSHUK[2]

ABSTRACT

Gluten proteins were fractionated by pH precipitation (method A) and ammonium sulfate precipitation (method B) to determine primarily if the association of lipids with the gliadin fraction prepared by method A results from mutual solubility of the lipids and the proteins in 70% aqueous ethanol. The distributions of lipids and proteins among fractions obtained by the two methods were different. For method A, 56% of the gluten lipid was associated with the gliadin fraction and 44% with the glutenin fraction. Almost all of the polar lipid in gliadin A, which contained 75% of glycolipid of the original gluten, coeluted with fraction I when gliadin A was fractionated by gel filtration chromatography on Sephadex G-200. For method B, acetic acid-soluble gluten lipid was distributed almost entirely between two fractions, the first precipitate, P_1 and the supernatant, S. Most of the polar lipid of P_1 was associated with its ethanol-soluble subfraction, P_1S. The two fractions of method B, P_1S and S, seemed to have a particularly strong affinity for polar lipid; they contained 69% of the polar lipid present in the acetic acid-soluble gluten that was fractionated. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and solubility characteristics showed that fraction P_1S of method B and fraction I of gliadin A contained common protein components that appear to be responsible for binding polar lipid. The results suggest an intrinsic affinity of specific gluten proteins for specific lipids. The presence of the lipids in gliadin is not influenced entirely by use of the ethanol as solvent, because both gliadin subfractions P_1S and I contained similar lipids.

Cereal Chem 62(4): 284–289

Values for lipid contents of gliadin and glutenin protein fractions of flour are highly variable. Most of the flour (Chung and Tsen 1975) and gluten (Olcott and Mecham 1947) lipid is associated with the glutenin fraction. On the other hand, Ponte et al (1967) found most gluten lipid to be associated with gliadin extracted with 70% ethanol solution. Hoseney et al (1970) reported that glutenin obtained by pH precipitation in 70% ethanol contains less lipid than that obtained by ultracentrifugation. Recent results from our laboratory (Bekes et al 1983a, 1983b) showed that gluten lipids are approximately equally divided between the gliadin and glutenin fractions. This article presents further information on some preparative factors that affect lipid distribution among gluten protein fractions, with emphasis on the potential involvement of ethanol on the association of lipid with specific gluten proteins.

MATERIALS AND METHODS

Chemicals

Reference proteins, Coomassie Brilliant Blue R250 and 3-dimethylaminopropionitrile were obtained from Sigma Company (St. Louis, MO). Acrylamide, bisacrylamide, and sodium dodecyl sulfate (SDS) were of electrophoretic purity and were obtained from BioRad (Richmond, CA). All other chemicals were of analytical reagent grade.

Preparation of Gluten

Flour was milled from Canadian hard red spring wheat cv. Neepawa on a Buhler experimental mill. Flour yield was 72%, and its protein (N × 5.7) and ash contents were 12.2% and 0.43% (dry matter) determined by approved AACC methods (AACC 1983). Dough was mixed in air in a farinograph mixer at 62.9% absorption to peak dough development (6 min). Gluten was washed by hand in a stream of tap water until the wash water was clear and was freeze-dried, ground by mortar and pestle, and stored at 4°C.

Fractionation of Gluten

Two methods were used to fractionate gluten (Fig. 1). The pH precipitation method (A) of Orth and Bushuk (1973) and the ammonium sulfate precipitation method (B) of Wasik and Bushuk (1974). Method A gave three fractions referred to as glutenin A, gliadin A, and residue A. Method B resulted in three precipitates (P_1, P_2, and P_3) and one supernatant (S).

Additionally, 50-mg portions of fractions P_1 and P_2 from method B were extracted with 5 ml of 70% ethanol, producing two soluble (P_1S and P_2S) and two insoluble (P_1R and P_2R) subfractions.

Fractionation of Gliadin

Gliadin A was fractionated by gel-filtration chromatography on Sephadex G-200 with AU (0.1 *M* acetic acid and 3 *M* urea) solvent (Khan and Bushuk 1979). The column size was 100 cm with a 5-cm diameter, flow rate was 9 ml/hr, and 3-ml fractions were collected. Protein content of the eluate was estimated by absorbance at 280 nm on a Hitachi model 100-40 spectrophotometer.

Molecular weights were estimated from elution volumes according to Andrews' method (1964) using γ-globulin (160 kD), bovine serum albumin (66 kD), egg albumin (45 kD), trypsinogen (24 kD), and lysozyme (14.3 kD) as standards.

Lipid Analysis

n-Hexane-soluble lipids (free lipid, FL) and water-saturated *n*-butanol-soluble lipids (bound lipid, BL) were extracted sequentially (Bekes et al 1983a). As before, the sum of FL and BL was taken as total lipid.

Sample size for lipid extraction varied depending on the amount of material available (10-g flour, 1-g gluten, and 0.2-g gluten fractions). Lipid content was calculated from the weight of residue remaining after evaporation of the solvent under vacuum at 40°C.

Total lipid was fractionated by silicic acid column chromatography into neutral lipids (NL, eluted with chloroform), glycolipids (eluted with acetone), and phospholipids (eluted with methanol) (Kates 1972). Polar lipid (PL) was taken as the sum of glycolipids and phospholipids.

Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) was performed by the procedure of Bushuk and Zillman (1978) except that running time was decreased from 5 hr to 2 hr.

SDS-PAGE was done on an LKB 2001 Vertical Electrophoresis Unit using a modified Laemmli (1970) method. Total gel size was 14 cm (wide) by 16 cm by 0.15 cm thick. The lower 12-cm section was a gradient gel, and the upper a stacking gel. The gradient section contained 0.375 *M* Tris-HCl, pH 8.8, 0.1% SDS, and gradients of acrylamide (6.81–11.68%), bisacrylamide (0.19–0.32%), and glycerol (1–4.5%). This section was polymerized by addition of 60 μl of 3-dimethylaminopropionitrile and 90 μl of 5% ammonium persulfate per 20 ml of gel solution. The stacking gel comprised

[1]Contribution 625; financial assistance from the Natural Sciences and Engineering Research Council of Canada.
[2]Department of Plant Science, University of Manitoba, Winnipeg, Canada.
[3]Present address: Department of Biochemistry and Food Technology, Technical University of Budapest, Budapest 1111, Hungary.

3.89% acrylamide, 0.11% bisacrylamide, 0.1% SDS, 0.125*M* Tris-HCl buffer at pH 6.8 and was polymerized by addition of 35 μl of 3-dimethylaminopropionitrile and 70 μl of 5% ammonium persulfate per 10 ml of gel solution. Electrode compartments were filled with 0.025*M* Tris plus 0.192*M* glycine buffer at pH 8.3, containing 0.1% SDS. The gels were run for 3 hr 10 min at a constant current of 30 mA.

Molecular weights in SDS-PAGE were estimated using the following reference proteins: β-galactosidase (116 kD), phosphorylase B (97.4 kD), bovine albumin (66 kD), egg albumin (45 kD), glyceraldehyde-3-phosphate dehydrogenase (36 kD), β-lactoglobulin (18.4 kD), and lysozyme (14.3 kD).

For separation under nonreducing conditions, proteins were dissolved in 62.5 mM Tris-HCl buffer, pH 6.8, containing 2% SDS and 10% glycerol. For separation under reducing conditions, the buffer also contained 5% 2-mercaptoethanol (ME). The sample solutions were heated for 1.5 min in a boiling water bath to ensure complete reduction and unfolding of molecules and were cooled before application to the gel.

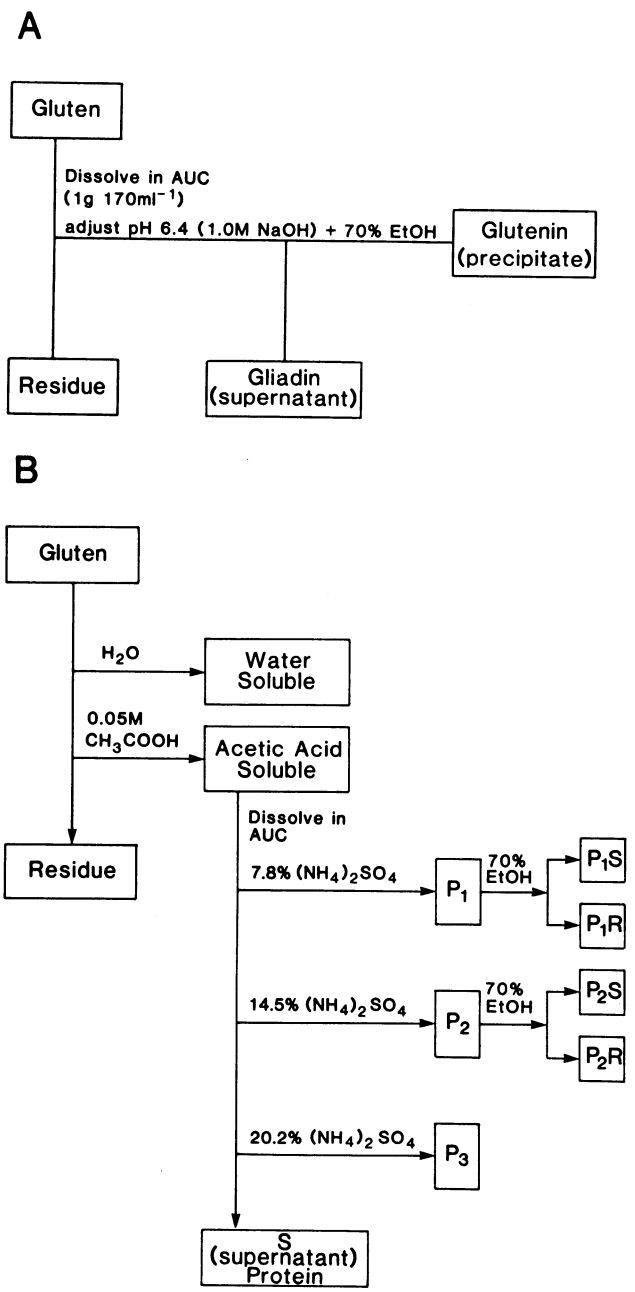

Fig. 1. Fractionation of gluten proteins by **A**, pH precipitation and **B**, ammonium sulfate precipitation.

Amino Acid Analysis

Amino acid analyses were performed on a Beckman Model 121 analyzer. Samples were hydrolyzed in 6*N* HCl for 24 hr at 110°C under vacuum. Cysteine, cystine, and tryptophan were not determined. Values for aspartic and glutamic acids include asparagine and glutamine, respectively.

TABLE I
Protein and Lipid Contents of Flour and Gluten

Constituent	Flour			Gluten[a]		
Protein (%, N × 5.7, db)	12.2			78.5		
Lipid (mg/100 g of flour, db)	Lipid			Lipid		
	Free	Bound	Total	Free	Bound	Total
Neutral	660	310	970	29	334	363
Glycolipid	90	250	340	3	283	286
Phospholipid	50	160	210	0	209	209
Polar[b]	140	410	550	3	492	495
Total	800	720	1520	32	826	858

[a]Gluten comprised 11% of flour, dry matter basis.
[b]Polar lipid = glycolipid plus phospholipid.

TABLE II
Dry Matter and Lipid Distribution in Gluten Fractions Prepared by pH Precipitation

Fraction	Dry Matter (%)	Lipid (mg/100 g flour, db)				
		Neutral	Polar[a]	Total	Glyco-lipid	Phospho-lipid
Gliadin	44.6 (56)[b]	184	255	439	213	42
Glutenin	37.0 (44)	197	166	363	25	141
Residue	18.4	0	0	0	0	0

[a]Polar lipid = glycolipid plus phospholipid.
[b]Percentage of gluten lipid shown in parenthesis.

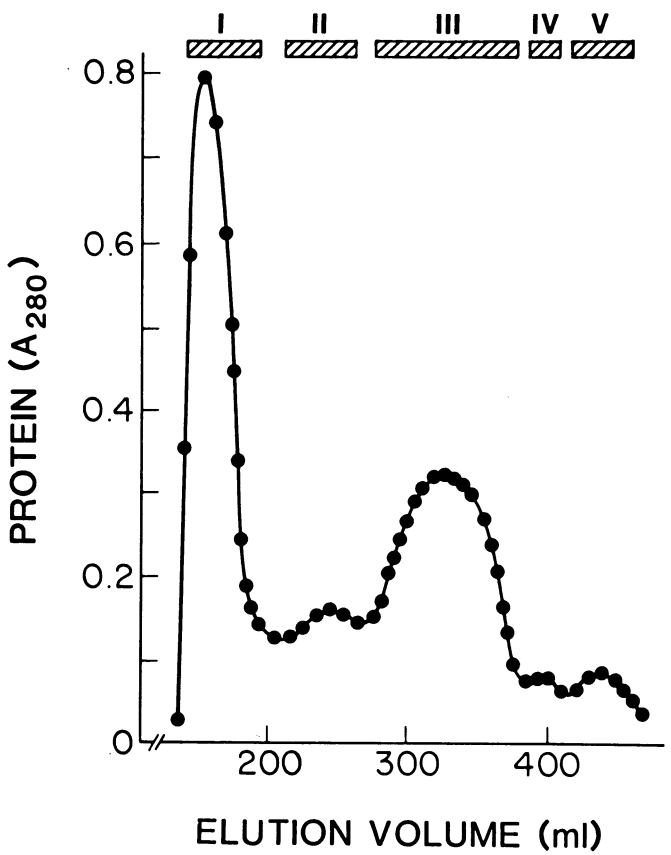

Fig. 2. Elution profile of gliadin A on Sephadex G-200. Fractions I–V were collected, as indicated by bars on top.

RESULTS AND DISCUSSION

Lipid Content of Flour and Gluten

Comparison of total lipid values for flour and gluten (Table I) showed that 43.5% of flour lipid was lost during gluten washing. These results are similar to those of Bourdet and Feillet (1967) for four hard wheat flours. FL:BL ratios for flour (1.11) and gluten (0.04) indicate that most FL remaining in gluten was transformed into BL, probably during dough making. This result is similar to those reported by Chiu and Pomeranz (1966) and Mecham (1978). The divergent NL:PL ratios for flour (1.76) and gluten (0.73) and lipid recoveries indicate that gluten washing removed considerably more neutral than polar lipid.

Gluten Fractionation by Method A

Dry material and lipid distributions for gliadin A, glutenin A, and residue A fractions are given in Table II. The calculated gliadin:glutenin ratio (1.21) is very similar to that (1.13) obtained by Hoseney et al (1969) for gliadin and glutenin separated from gluten dissolved in 0.005 *N* lactic acid. Gliadin A contained 56%, and glutenin A, 44%, of total gluten lipids. Residue A contained less than 5% of gluten protein and no lipid. Total lipid recovery in the three fractions was 93%. Lipids associated with the gliadin A and glutenin A were quite different; more than half (58%) of lipid associated with the gliadin was polar whereas 54% of the lipid associated with glutenin was nonpolar.

Gliadin Fractionation

Fractionation of gliadin A by chromatography on Sephadex G-200 yielded five fractions (Fig. 2), having approximate molecular weights greater than 200,000 (fraction I), 75,000 (fraction II), 27,500 (fraction III), 21,000 (fraction IV), and 15,000 (fraction V). In accordance with published nomenclature (Beckwith et al 1966, Bietz and Wall 1972), fraction I will be referred to as high molecular weight (HMW) gliadin, even though it is an aggregate that includes small amounts of low molecular weight (LMW) gliadin components and traces of albumins and globulins (Bekes et al 1983a). Fraction III will be referred to as LMW gliadin.

Gluten Fractionation by Method B

Gluten used for method B fractionation (Table III) contained very little water-soluble material (which contained no lipid). Approximately two-thirds of the gluten was soluble in 0.05 *M* acetic acid. This soluble fraction contained 7.9% lipid (dry matter basis), of which slightly more than half was polar.

TABLE III
Dry Matter and Lipid Content of Gluten Fractions Prepared by Ammonium Sulfate Precipitation

Fraction	Dry Matter (%)	Lipid (mg/100 g of flour, db)				
		Neutral	Polar	Total[a]	Glyco-lipid	Phospho-lipid
Gluten fraction						
Water soluble	0.7	0	0	0	0	0
HAc soluble	65.8	266	303	569	221	82
Residue	33.4	nd[b]	nd	174	nd	nd
Ammonium sulfate fraction of HAc-soluble gluten						
P_1	51.8	153	147	300	91	56
P_2	34.4	24	12	36	9	3
P_3	1.6	nd	nd	nd	nd	nd
S	12.2	76	116	192	105	11
Subfaction of ammonium sulfate fractions						
P_1S	43.6	50	94	144	48	46
P_1R	56.4	98	50	148	41	9
P_2S	98.2	nd	nd	nd	nd	nd
P_2R	1.8	nd	nd	nd	nd	nd

[a] Residue total was determined by extraction with water saturated *n*-butanol. Polar lipids = glycolipids plus phospholipids.
[b] Not determined.

The residue remaining after acetic acid extraction contained 4.7% lipid. The distribution of gluten lipid between the two fractions was 76.6% in the acetic acid-soluble and 23.4% in the insoluble residue. Lipid recovery in the two fractions was 86.6%. We have no explanation for the relatively high loss of lipid during this fractionation.

Of the lipid in the acetic acid-soluble fraction (fractionated in method B), 56.8% was associated with fraction P_1, 6.8% with fraction P_2, and 36.4% with fraction S (assuming that fraction P_3, which was too small to analyze, contained negligible lipid). Because only 92.8% of the lipid was recovered in the three fractions that were analyzed, P_3 probably contained some lipid.

Lipids of the three major fractions obtained by method B differed markedly in composition. The NL:PL ratios for P_1, P_2, and S fractions were 1.04, 2.00, and 0.66, respectively. Lipid distribution results agree generally with the results of Frazier et al (1981), who investigated binding of added triglycerides during dough mixing. They found that 55–72% of added triglycerides (neutral lipid) was associated with P_1 and the remainder with the supernatant fraction.

Fractions P_1 and P_2 differed substantially in solubility in 70% ethanol (Table III); about 50% of P_1 and essentially all of P_2 were soluble. The soluble portion of P_1 (P_1S) contained 49% of the lipid of P_1, of which about two-thirds (65.3%) was PL.

On the basis of PAGE results, Wasik and Bushuk (1974) concluded that the protein in P_1 is glutenin. However, our present results indicate that, on the basis of solubility in 70% ethanol, approximately half of the protein in P_1 would be classified as gliadin. On the other hand, essentially all of P_2 protein appears to be gliadin, as reported by Wasik and Bushuk (1974).

SDS-PAGE and PAGE Results

The SDS-PAGE pattern for unreduced fraction P_1 (not shown) was typical of patterns for unreduced glutenin; most of the protein did not enter the gel, there was considerable streaking, and only a few faint bands could be seen in the stained gel.

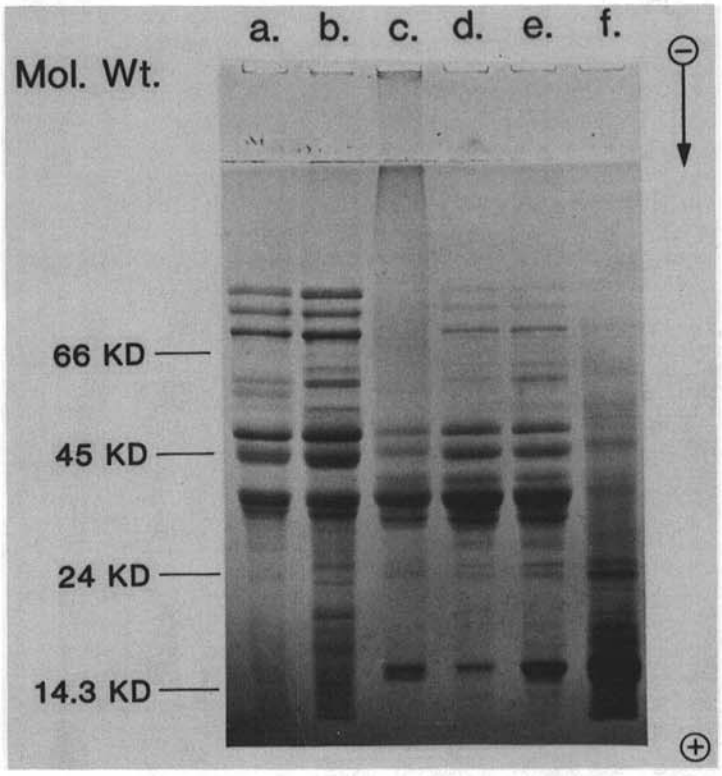

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of reduced gluten fractions: **a,** P_1 from ammonium sulfate precipitation; **b,** glutenin A; **c,** gliadin A; and **d, e,** and **f,** P_2, P_3, and S from ammonium sulfate precipitation.

SDS-PAGE pattern of reduced P_1 (Fig. 3a) and of reduced glutenin A (Fig. 3b) are quite similar and agree, in general, with the results of Wasik and Bushuk (1974), which showed that protein in P_1 was glutenin. However, the higher resolution of our SDS-PAGE technique showed several minor differences between P_1 and glutenin A. The pattern for glutenin A contained more LMW subunits (<34.7 kD), and the pattern for P_1 showed a more intense 59 kD band and band 63.2 kD was absent. The significance of these differences was not investigated. However, the absent band could be the labile glutenin constituent discussed by Bietz et al (1975).

SDS-PAGE patterns of reduced P_2 (Fig. 3d), P_3 (Fig. 3e), and gliadin A (Fig. 3c) were similar except that those for P_2 and P_3 contained several additional bands above 45 kD. For gliadin A, however, some protein did not enter the gel and presumably caused streaking. This material probably represents HMW proteins in gliadin A that have a strong tendency to aggregate. No similar material was present at the application point for fractions P_2 and P_3. Obviously, fractionation of gluten proteins by the two procedures used in the present study is different.

The SDS-PAGE pattern of reduced fraction S (Fig. 3f) showed that it contained two major bands, 14.5 and 15.3 kD, plus numerous fainter bands with molecular weights above 16 kD. We presume that the higher molecular weight bands are contaminants from the fractions that are precipitated.

One or both of the major subunits of fraction S (Fig. 3f) were also present in P_2 (Fig. 3d), P_3 (Fig. 3e), gliadin A (Fig. 3c), and fraction I from gel filtration chromatography of gliadin A (Fig. 4f). We reported earlier (Bekes et al 1983a) that the subunit composition, by SDS-PAGE, of unreduced fraction I of gliadin from untreated flour is qualitatively similar to that of total gliadin, but the LMW subunits were absent in fraction I of gliadin from totally defatted flour and gluten. The concurrent appearance of subunits of fraction S and lipid in fraction I was taken as evidence that lipid is involved in aggregation of the components of this fraction with the HMW gliadin components to form fraction I aggregates. Further work is needed to fully characterize these aggregates.

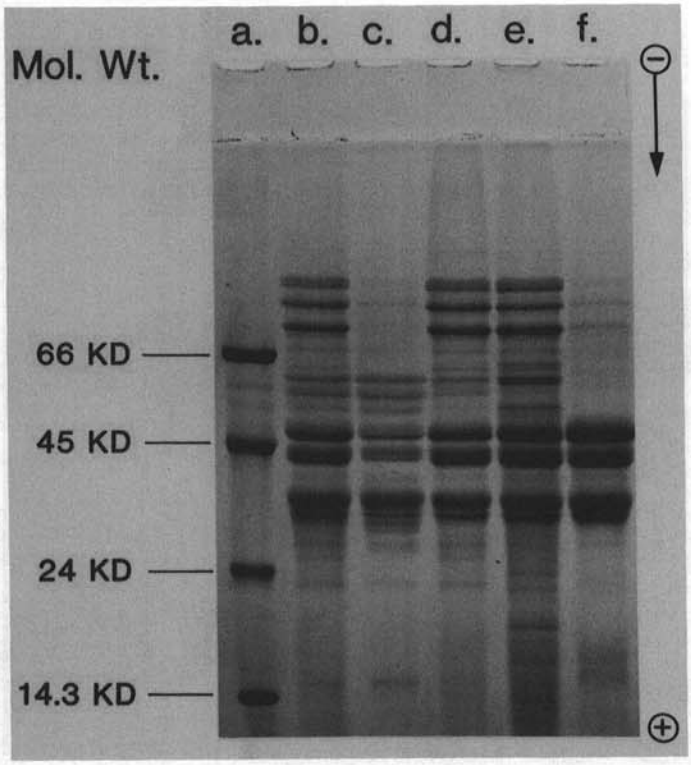

Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of reduced proteins: **a,** reference proteins; **b,** P_1; **c,** ethanol soluble subfraction (P_1S) of P_1; **d,** ethanol insoluble subfraction (P_1R) of P_1; **e,** glutenin A; and **f,** fraction I of gliadin A.

The SDS-PAGE electrophoregram of unreduced P_1R (not shown) was similar to that of unreduced fraction P_1. Reduced P_1R (Fig. 4d), reduced glutenin A (Fig. 4e), reduced fraction I of gliadin A (Fig. 4f), and reduced P_1S (Fig. 4c) were similar with respect to the main subunits, but there were also some important differences. HMW (>66 kD) subunits in P_1S and fraction I are much less abundant than in P_1R and glutenin A. Also, 45–66 kD polypeptides varied extensively among these fractions; fraction I especially had few polypeptides in this molecular weight region.

SDS-PAGE of fraction S (Fig. 5a), fraction IV (Fig. 5b), and fraction V (Fig. 5c) showed that all three contained two main LMW subunits of very similar molecular weights. In terms of the two subunits, fraction V appeared to be the purest. Fractions IV and S contained, in addition, numerous other bands. Fractions IV and V are generally considered to be albumin and globulin contaminants of gliadin extracts (Bietz and Wall 1972, McMaster 1982).

PAGE analysis of fractions S (Fig. 6a) and V (Fig. 6c) gave electrophoregrams with six bands characterized by almost the same relative mobilities for the two fractions. Accordingly, based on two different electrophoretic techniques, fraction S and V appear to comprise the same major components. Fraction IV (Fig. 6b) and gliadin A (Fig. 6d) also contained the six bands, but they were of lower intensity. In addition, they contained numerous other bands. These results indicate that gliadin A from untreated flour contained some fraction S polypeptides. Some of the fraction S polypeptides aggregate with HMW gliadins to form fraction I aggregates, whereas the rest elute in fractions IV and V during the gel chromatography.

Amino Acid Composition

Amino acid compositions of the various fractions were

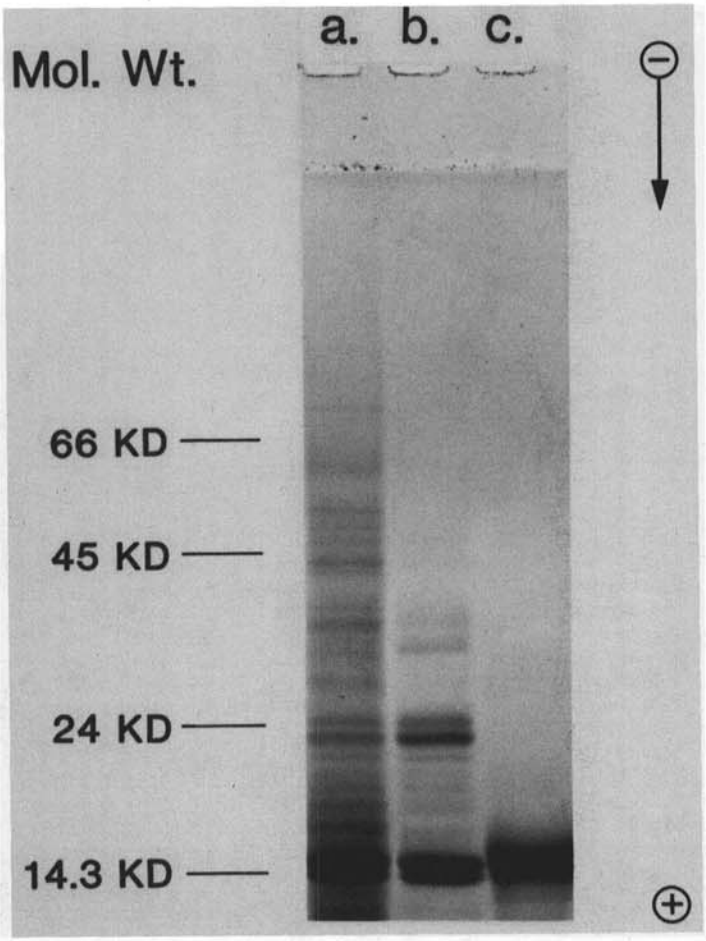

Fig. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of reduced gluten fractions: **a,** fraction S; and **b** and **c,** fractions IV and V of gliadin A.

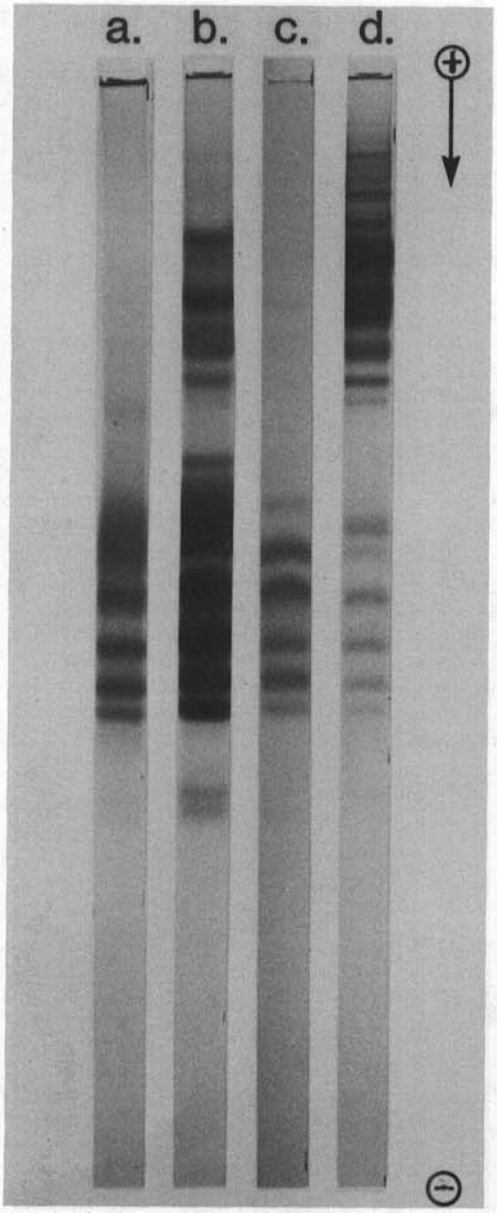

Fig. 6. Polyacrylamide gel electrophoresis patterns of gluten fractions: **a,** fraction S; **b** and **c,** fractions IV and V of gliadin A, respectively; and **d,** gliadin A.

determined to obtain additional evidence on their similarities or differences (Table IV).

Composition of P_1 is typical for gluten proteins. Comparison of its composition with reported values for glutenin and gliadin does not allow classification of P_1 as being strictly gliadin or glutenin. This is consistent with the solubility data.

The composition of P_2 is close to that of Osborne gliadin reported by Wu and Dimler (1963), but it shows slightly lower contents of hydrophobic amino acids (isoleucine and valine) and higher contents of serine and glycine. Amino acid composition of P_2 is very similar to that of gliadin A. It also resembles fraction III of gliadin A (Bekes et al 1983a; gliadin A in present article is equivalent to gliadin F in reference). This fraction, separated from gliadin A on Sephadex G-200 column, contained mainly α-, β-, and γ-gliadins according to PAGE results. The lower content of amino acids such as arginine, threonine, serine and glycine in fraction III, compared with gliadin A and fraction P_2, can be explained by the presence of relatively high levels of these amino acids in fractions IV and V.

The amino acid composition of fraction S was distinctly different from those of P_1, P_2, and P_3. S contained two times less glutamic acid and proline, about 3.5 times more aspartic acid and lysine, and about 2.5 times more alanine and arginine than P_1. Very similar results were obtained by Frazier et al (1981). Their partially purified supernatant fraction (named "ligolin") contained 2.6 and 2.2 times less glutamic acid and proline, respectively, whereas the levels of aspartic acid, alanine, lysine, and arginine were, on the average, three times higher than the values for their fraction P_1. So, both fraction S and ligolin appear to be similar in that they contain much higher levels of basic amino acids (lysine and arginine), alanine and aspartic acid, and much lower levels of glutamic acid and proline than P_1. But they differ quite substantially in proportion of some other amino acids, which is surprising, because both fractions were prepared by the same method. Moreover, dry matter distributions in fractions P_1, P_2, and P_3 and S obtained in this study and those obtained by Frazier et al (1981) were very similar (51.8, 34.4, 1.6, and 12.2, respectively, in our study versus 50.1, 37.1, 0, and 12.8 in their study). Also, amino acid compositions of P_1 fractions from our study and theirs are very close (Table IV). The differences in compositions of fraction S and ligolin are attributed to a higher degree of purification of ligolin, before amino acid analysis.

The composition of fraction V is similar to that of fraction S. Fractions IV and V also have some similarities in composition. In agreement with SDS-PAGE results, the amino acid composition of fraction IV showed the effect of the presence of a small quantity of LMW gliadin components.

TABLE IV
Amino Acid Composition of Gluten Fractions[a]

Amino Acid	P_1	P_2	P_3	S	V[b]	IV[b]	III[b]	GLI[b]	GLI[c]	GLU[c]	P_1[d]	Ligolin[d]
Aspartic acid[e]	2.2 (19.3)	2.7 (23.6)	4.5 (39.5)	7.4 (64.4)	6.9	4.1	2.5	2.7	2.4	2.7	2.8	8.6
Threonine	2.6 (23.1)	2.4 (21.2)	3.4 (29.1)	4.7 (40.7)	5.5	5.4	1.8	2.3	2.2	3.1	3.2	5.5
Serine	6.2 (54.7)	5.8 (50.4)	6.4 (55.1)	6.9 (60.7)	6.2	7.3	4.9	5.7	4.6	6.0	7.1	7.5
Glutamic acid[f]	37.2 (329)	36.8 (322)	28.3 (246)	19.3 (168)	16.8	28.9	41.4	37.9	38.0	33.1	35.7	13.4
Proline	16.6 (147)	17.0 (149)	12.9 (112)	8.5 (74.1)	12.0	11.3	17.0	17.2	17.8	13.6	14.4	6.5
Glycine	6.7 (59.4)	4.7 (41.0)	7.7 (66.7)	8.7 (75.5)	7.9	4.7	2.5	3.4	3.0	9.3	6.1	10.6
Alanine	3.0 (26.6)	3.2 (28.4)	5.2 (44.9)	7.3 (63.9)	6.4	5.6	2.7	3.1	3.0	4.1	3.5	9.2
Valine	3.8 (33.5)	4.4 (38.3)	5.3 (45.9)	6.8 (59.3)	7.9	6.9	4.2	4.4	5.2	4.9	4.0	6.6
Methionine	1.0 (8.9)	1.2 (10.1)	1.5 (13.0)	1.9 (16.4)	2.8	2.9	1.4	1.4	1.4	1.4	1.2	0
Isoleucine	3.3 (29.0)	3.8 (32.8)	3.8 (32.6)	4.5 (39.0)	3.8	5.1	4.3	4.0	4.4	3.3	3.3	4.2
Leucine	6.6 (58.3)	7.2 (63.1)	7.8 (67.4)	8.0 (69.3)	8.8	6.9	6.9	7.1	7.4	6.8	6.7	8.7
Tyrosine	2.2 (19.6)	2.2 (19.3)	2.7 (23.5)	2.4 (21.3)	4.0	1.6	1.7	1.8	1.9	3.0	2.4	3.0
Phenylalanine	4.3 (38.4)	4.3 (37.8)	3.7 (32.3)	2.8 (24.8)	1.9	1.0	5.5	4.7	4.6	3.2	4.3	4.0
Lysine	1.0 (8.8)	0.8 (6.9)	1.9 (16.8)	3.8 (33.6)	2.2	1.7	0.6	0.8	0.6	1.6	1.3	4.5
Histidine	1.5 (13.2)	1.7 (15.2)	1.9 (17.2)	2.6 (22.9)	1.4	3.7	1.5	1.7	1.8	1.6	1.6	1.8
Arginine	1.8 (15.9)	1.8 (16.1)	2.9 (25.4)	4.4 (38.2)	5.7	3.0	1.5	1.9	1.8	2.4	2.4	6.0

[a] Mole percent of the total protein; values in parentheses are moles per 10^5 protein. Cysteine and tryptophan not determined.
[b] Bekes et al (1983a).
[c] Wu and Dimler (1963). GLI = gliadin, GLU = glutenin.
[d] Frazier et al (1981).
[e] Includes asparagine.
[f] Includes glutamine.

Although some minor differences were noted, in general the amino acid compositions for clearly identified fractions agree with published data and are consistent with the observed differences in solubility and electrophoretic properties.

Interrelationship of Gluten Fractions Relative to Association with Lipid

Because of poor solubility and chemical similarity of gluten proteins, only a few of its components have been isolated in relatively pure form to permit valid comparisons. Nevertheless, it is possible to demonstrate useful interrelationships of partially purified fractions such as those examined in the present study.

Fractions P_2 and III both contained LMW gliadins. They are similar in amino acid composition and lipid content and composition. P_2 contained 1.4% lipid, of which 67% was NL, and as shown previously (Bekes et al 1983a), fraction III, which was 32% by weight of gliadin, contained 1.3% lipid, of which 67% was NL.

The sum of fractions P_1S and S appears to be equivalent to fraction I (aggregate containing mainly HMW gliadin components). The sum of P_1S and S contained 47% of the gluten lipid, of which 60% was PL; fraction I, which was 47% by weight of gliadin, contained 39% of the gluten lipid with an average PL content of 63%. SDS-PAGE patterns of P_1S and fraction I are not identical, but their major subunits appear to be the same. Both fractions are soluble in 70% ethanol solution, and both have very similar lipid composition.

SDS-PAGE and PAGE results both indicate that the proteins of fractions S and V are identical with respect to their major components. Fraction S, however, contained considerable lipid, whereas fraction V had no lipid. This difference is attributed to the difference in methods of preparation. Fraction IV, on the other hand, contained some additional protein components.

In general, protein-lipid complexes, which depend on a variety of secondary molecular forces, are not very stable (Gurr 1980). The actual stability depends strongly on solvent type and concentration, presence of salt ions, pH, temperature, etc. Lipid-protein interactions in dough are considered to contribute to the breadmaking potential of flour (Bekes et al 1983a, Chung and Pomeranz 1981, MacRitchie 1981). Recent evidence suggests that the interactions depend on the specific nature of the interacting lipids and proteins (Bekes et al 1983a). Polar lipids would be expected to interact most strongly with proteins having amino acids with polarizable or charged amino acids on their surface. On the other hand, NL would interact hydrophobically with apolar amino acids on protein surfaces. Results of our study showed that the PL of flour (especially glycolipids) bind preferentially to the fraction I gliadin proteins, whereas NL associate more strongly with glutenins and LMW gliadins. Differences in contribution of lipids to dough functionality can result from the fact that they bind to different gluten proteins.

Our study also showed that different methods of gluten fractionation can distribute proteins differently among fractions that are sometimes considered to be the same or similar. For example, HMW gliadin components are separated in the glutenin fraction when the fractionation is based on solubility. In our study, HMW gliadin components were found in both gliadin A and fraction P_1 (considered previously to be glutenin).

Although protein conformations and resulting interactions with protein and nonprotein constituents may vary with the solvent, our results suggest an intrinsic affinity of specific gluten proteins for specific lipids. We, therefore, conclude that, because both gliadin fractions P_1S and I contained similar lipids, the presence of these lipids was not influenced entirely by the use of ethanol as solvent. Finally, our results demonstrate that lipids associated with gluten fractions can only be compared if the fractions were prepared by the same procedures.

LITERATURE CITED

ANDREWS, P. 1964. Estimation of the molecular weight of proteins by Sephadex gel-filtration. Biochem. J. 91:222.

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC. Methods 08-01, 46-12, and 44-15, approved April 1961. The Association: St. Paul, MN.

BECKWITH, A. C., NIELSEN, H. C., WALL, J. S., and HUEBNER, F. R. 1966. Isolation and characterization of high-molecular weight protein from wheat gliadin. Cereal Chem. 43:14.

BEKES, F., ZAWISTOWSKA, U., and BUSHUK, W. 1983a. Protein-lipid complexes in the gliadin fraction. Cereal Chem. 60:371.

BEKES, F., ZAWISTOWSKA, U., and BUSHUK, W. 1983b. Lipid mediated aggregation of gliadin. Cereal Chem. 60:379.

BIETZ, J. A., and WALL, J. S. 1972. Wheat gluten subunits: Molecular weights determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cereal Chem. 49:416.

BIETZ, J. A., SHEPHERD, K. W., and WALL, J. S. 1975. Single-kernel analysis of glutenin: Use in wheat genetics and breeding. Cereal Chem. 52:513.

BOURDET, A., and FEILLET, P. 1967. Distribution of phosphorus compounds in the protein fractions of various types of wheat flours. Cereal Chem. 44:457.

BUSHUK, W., and ZILLMAN, R. R. 1978. Wheat cultivar identification by gliadin electrophoregrams. I. Apparatus, method and nomenclature. Can. J. Plant Sci. 58:505.

CHIU, C. M., and POMERANZ, Y. 1966. Changes in extractibility of lipids during breadmaking. J. Food Sci. 31:753.

CHUNG, O. K., and TSEN, C. C. 1975. Distribution of lipids in acid-soluble protein components as affected by dough-mixing and surfactants. Cereal Chem. 52:823.

CHUNG, O. K., and POMERANZ, Y. 1981. Recent research on wheat lipids. Bakers Dig. 55:38.

FRAZIER, P. J., DANIELS, N. W. R., and RUSSELL EGGITT, P. W. 1981. Lipid-protein interactions during dough development. J. Sci. Food Agric. 32:877.

GURR, M. J. 1980. Lipids as components of macromolecules. Page 178 in: Lipid Biochemistry: An Introduction. Great Britain at the University Press, Cambridge.

HOSENEY, R. C., FINNEY, K. F., POMERANZ, Y., and SHOGREN, M. D. 1969. Functional (breadmaking) and biochemical properties of wheat flour components. IV. Gluten protein fractionation by solubilizing in 70% ethyl alcohol and in dilute lactic acid. Cereal Chem. 46:495.

HOSENEY, R. C., FINNEY, K. F., and POMERANZ, Y. 1970. Functional (breadmaking) and biochemical properties of wheat flour components. VI. Gliadin-lipid-glutenin interaction in wheat gluten. Cereal Chem. 47:135.

KATES, M. 1972. Techniques of lipidology. Isolation, analysis and identification of lipids. Page 398 in: Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 3. T. S. Work and E. Work, eds. North-Holland Publ. Co., Amsterdam.

KHAN, K., and BUSHUK, W. 1979. Studies of glutenin. XIII. Gel filtration, isoelectric focusing, and amino acid composition studies. Cereal Chem. 56:505.

LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680.

MacRITCHIE, F. 1981. Flour lipids: Theoretical aspects and functional properties. Cereal Chem. 58:156.

McMASTER, G. J. 1982. Association of carbohydrate and protein in wheat gluten. Ph.D. thesis. University of Manitoba, Winnipeg.

MECHAM, D. K. 1978. Lipids. Page 424 in: Wheat Chemistry and Technology. Y. Pomeranz, ed. Am. Assoc. Cereal Chem., St. Paul, MN.

OLCOTT, H. S., and MECHAM, D. K. 1947. Characterization of wheat gluten. I. Protein-lipid complex formation during doughing of flours. Lipoprotein nature of the glutenin fraction. Cereal Chem. 24:407.

ORTH, R. A., and BUSHUK, W. 1973. Studies of glutenin. I. Comparison of preparative methods. Cereal Chem. 50:106.

PONTE, J. G., Jr., DeSTEFANIS, V. A., and COTTON, R. H. 1967. Studies of gluten lipids. I. Distribution of lipids in gluten fractions separated by solubility in 70% ethanol. Cereal Chem. 44:427.

WASIK, R. J., and BUSHUK, W. 1974. Studies of glutenin. V. Note on additional preparative methods. Cereal Chem. 51:112.

WU, Y. V., and DIMLER, R. J. 1963. Hydrogen ion equilibria of wheat glutenin and gliadin. Arch. Biochem. Biophys. 103:310.

[Received November 29, 1983. Revision received September 17, 1984. Accepted February 28, 1985.]