Protein-Lipid Complexes in the Gliadin Fraction[1]

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ABSTRACT

Gliadin preparations were obtained from glutens with different lipid contents and compositions so that the speculated association in dough of certain flour lipids with gliadin proteins could be investigated. Lipid content of the glutens (and the gliadins) varied according to the nature of defatting solvent (*n*-hexane, *n*-butanol, water-saturated *n*-butanol). Gliadin preparations were fractionated into five fractions by gel filtration chromatography on Sephadex G-200. Lipid was found on fractions I and III. Fraction I (eluted in void volume) contained mainly polar lipids, and fraction III contained mainly nonpolar lipids. Gliadin preparations from completely defatted flour (or gluten) contained no carbohydrate, whereas carbohydrate and lipid contents of other gliadin preparations were directly related. Protein subunit composition of fraction I, determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis, depended on whether or not lipids were present in the gliadin preparation. Fraction I of the preparation from completely defatted flour (or gluten) contained only high (>100,000) molecular weight subunits, whereas the analogous fraction from undefatted or partially defatted flour contained, in addition, low (<40,000) molecular weight subunits. These results indicate that in the gliadin preparations from undefatted flour (or gluten), lipids (likely galactolipids) caused aggregation of low molecular weight subunits with the high molecular weight subunits.

Lipids, a minor component of wheat flour, are very important to the bread-making properties of the flour. Current knowledge on the chemistry of flour lipids and their functionality in breadmaking was recently reviewed in considerable detail (Chung and Pomeranz 1981, MacRitchie 1981).

It has been known for more than 40 years (McCaig and McCalla 1941) that the effect of flour lipids on bread-making properties originates mostly from the associations among lipids and gluten proteins that are formed during doughmaking. The binding of lipid to glutenin was so strong that Olcott and Mecham (1947) suggested the name "lipoglutenin" for the complex isolated from gluten. Several different models have been proposed to explain the nature of protein-lipid interactions in dough (Chung et al 1978). Most of the previous studies focused on the lipid component of the complexes and did not address the nature of the protein component with specific lipid-binding ability.

On the other hand, much has been published about wheat proteins found in experimental samples that were either undefatted or only partially defatted. Researchers have only speculated on the nature of the modifying effects of lipids on the results obtained (Popineau and Godon 1982). It is generally accepted that nonprotein components of wheat flour, carbohydrates, and lipids, mainly glycolipids, play an important role in the formation of gluten during doughmaking (Graveland et al 1979, Huebner and Wall 1979, Pomeranz 1971, Rohrlich et al 1963).

Previous work in our laboratory showed that the ethanol-soluble fraction of gluten (mainly gliadin) contains about 10% nonprotein material, of which 0.6% was carbohydrate and the remaining 9.4% was presumed to be lipid (Bushuk et al 1980, McMaster 1982). The objective of present study was to investigate further the nature of the nonprotein component of that fraction in the context of our study of the interactions of flour proteins with nonprotein constituents in dough.

MATERIALS AND METHODS

Flour

Flour was milled from Canadian hard red spring wheat cultivar Neepawa on a Buhler experimental mill. Flour yield was 72%, protein content was 12.2% (N × 5.7), and ash content was 0.43% (dry basis).

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Chemicals

Monosaccharides, protein molecular weight markers, and protein reagents were obtained from Sigma (St. Louis, MO), and lipid standards were obtained from Supelco (Bellefonte, PA). All other chemicals used were of analytical grade.

Sample Preparation

Flour and gluten samples with different lipid contents and compositions were prepared by extracting with *n*-hexane, *n*-butanol, and water-saturated *n*-butanol (WSB) (Fig. 1). By using the procedures shown in Fig. 1, it was possible to prepare flour and gluten samples with different lipid content and composition. The order of samples on the basis of lipid content predictable from published information is: flour, A > C > B > D and gluten, F > G > I > E > K = H = L.

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

Lipid Extraction

All extractions were performed by repeatedly slurrying with solvent in a glass flask immersed in a water bath at 40° C. An atmosphere of nitrogen was maintained over the slurry throughout

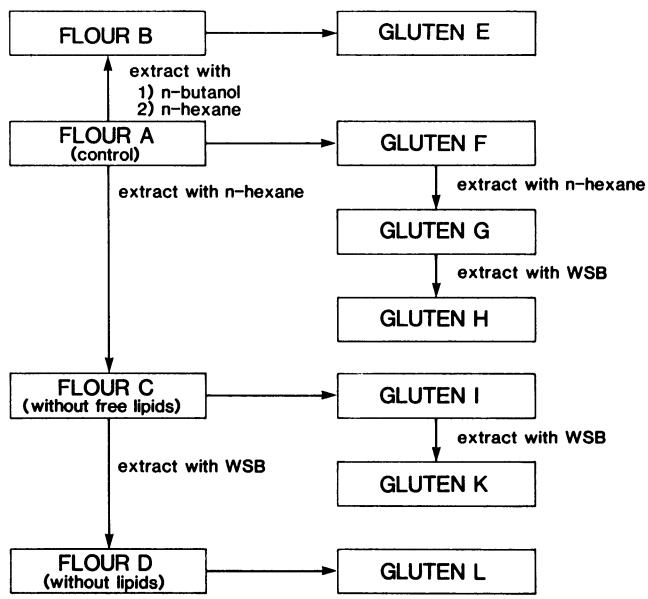

Fig. 1. Scheme of sample preparation.

the extraction. In the first stage, the ratio of solvent to sample was 12.5 to 1 (v:w). Minimum sample amounts were 300 g for flour, 100 g for glutens, 1 g for gliadins, and 0.2 g for gliadin fractions. The slurry was stirred for 12 hr, and the liquid separated by filtration, centrifugation, or both. The remaining solids were extracted a second time (for 6 hr) with the same amount of solvent.

Residual solvent was removed from the solids by aeration with nitrogen at room temperature. The filtrates were combined and freed of solvent by rotary vacuum evaporator and by aeration with dry nitrogen. The dry residues of filtrates were extracted with chloroform at 30° C. Chloroform slurries were filtered, and the chloroform removed from the filtrate by heating (40° C) under vacuum and aeration with dry nitrogen. The nonlipid residue from the chloroform extraction was dried and added to the original defatted solids. Extracted lipids were stored at −20° C under nitrogen.

Gliadin Preparation and Fractionation

Gliadin was prepared from each gluten by separating the glutenin from the AUC (0.1*M* acetic acid, 3*M* urea, and 0.01*M* cetyltrimethylammonium bromide) solution of gluten by the pH precipitation procedure of Orth and Bushuk (1973). Gliadin preparations (250 mg) were fractionated by gel filtration chromatography on a 100 × 5-cm column of Sephadex G-200, with AU (0.1*M* acetic acid and 3*M* urea) solvent. The flow rate was 9 ml/hr, and the eluate was collected in 3-ml portions. Protein content of the eluate fractions was measured at 280 nm on a Hitachi spectrophotometer, and carbohydrate content was determined by the phenol-sulfuric acid method (Dubois et al 1956). Rechromatography, where required, was performed on a 100 × 2.5-cm Sephadex G-200 column under the same conditions, except the amount of sample applied was 120 mg.

Analytical Procedures

Moisture, protein (N × 5.7), and ash contents of flour samples were determined by standard AACC methods (AACC 1969). Protein content of glutens and gluten fractions was determined by the micromethod of Nkonge and Ballance (1982).

Lipid content was calculated from the weight of the dry material remaining after the solvent was removed. Hereafter, *n*-hexane-extracted lipids will be referred to as free lipids (FL), and WSB-extracted lipids, after removal of free lipids, will be referred to as bound lipids (BL). Total lipids (TL) were calculated as the sum of FL and BL. If the amount of FL was only a trace or zero, lipid content will be reported as TL.

Free and bound lipids were fractionated by silicic acid column chromatography into three fractions: NL, eluted with chloroform; GL, eluted with acetone; and PhL, eluted with methanol using the method of Kates (1972). Polar lipids (PL) were calculated as the sum of GL and PhL.

Thin-layer chromatography (TLC) was used for further fractionation and qualitative comparison of lipids. Kieselgel 60F_{254} (precoated plates no. 5715, Merck) was the adsorbent. The developing solvents were as used by Morrison et al (1980); nonpolar lipids, to 12 cm with diethylether-toluene-ethanol-acetic acid (40:50:2:0.2), then to 18 cm with diethyl ether-*n*-hexane (6:94), glycolipids, to 15 cm with chloroform-acetone-acetic acid-water (10:90:2:3), then to 18 cm with diethyl ether-acetic acid (99:1), and phospholipids, with chloroform-methanol-ammonia (33%, w/v)-water (65:35:5:2.5). Unknowns were identified by comparison with known compounds. To facilitate identification, several different specific spray systems were used, such as Dragendorf reagent for methylated amines, ninhydrin-reagent for primary amines, Zinzadze-reagent for phosphatides, and orcyn-reagent for glycolipids (Kates 1972, Stahl 1969). For quantitative comparisons, sulfuric acid-dichromate reagent was used as a spray (Rouser et al 1967).

Carbohydrate content was determined by the phenol-sulfuric acid method of Dubois et al (1956), using galactose as the standard. Carbohydrate was determined directly on gliadin preparations and gliadin fractions and on hydrolysates of total lipids (extracted by WSB). Hydrolysis conditions were the same as those used by Chung et al (1982).

Polyacrylamide gel electrophoresis (PAGE) was performed according to Bushuk and Zillman (1978) as modified by Gobran (1981). The latter modification used sodium lactate buffer solution instead of aluminum lactate. The method of Fairbanks et al (1971) was used to stain for carbohydrate in polyacrylamide gel electrophoresis (PAGE) gels.

Sodium dodecyl sulfate-PAGE was performed according to the method of Khan and Bushuk (1977). The following were used as mol wt markers: lysozyme (14,300), trypsinogen (24,000), pepsin (34,700), ovalbumin (45,000), and bovine serum albumin (66,000). Molecular weights of gliadin subunits were estimated from two calibration curves: one for reduced and the other for unreduced proteins (Khan 1977). Reduced and alkylated mol wt markers and gliadin components were prepared by the procedure of Friedman et al (1970).

Amino acid analysis was done on a Beckman model 121 analyzer using the standard hydrolysis procedure. The data were used to calculate the average hydrophobicities and charge potentials by the procedure of Bigelow (1967).

RESULTS AND DISCUSSION

Lipid Extraction and Gluten Preparation

Extraction by *n*-butanol followed by *n*-hexane (flour B and gluten E), the procedure used in several previous studies (Bietz and Wall 1973, Khan and Bushuk 1977, McMaster 1982), removed most of the free lipid but only about half of the bound lipid. As noted by other workers, removal of the *n*-butanol from flour (or gluten) after lipid extraction with WSB was incomplete. It is extremely difficult to prepare the gluten from WSB-extracted flour. The use of this solvent, however, is attractive because it removes all of the nonstarch lipids (Chung et al 1978). The WSB-extracted flour (D) and the three glutens (H, K, and L) were completely free of nonstarch lipids, regardless of the sequence of lipid extraction and gluten washing. Glutens H, K, and L are considered to be equivalent, hence results for gluten L only will be reported.

TABLE I
Protein, Gluten, and Lipid Contents of Flour Samples (%, dry basis)[a]

Component	Flour									
	A			B			C			D
Protein	12.2			12.2			12.3			12.4
Gluten	11.0			12.0			12.1			10.8
Lipids	FL	BL	Total	FL	BL	Total	FL	BL	Total	Total
NL	0.66	0.31	0.97	trace	0.16	0.16	0	0.30	0.30	0
GL	0.09	0.25	0.34	0.01	0.18	0.19	0.02	0.25	0.27	0
PhL	0.05	0.16	0.21	trace	0.12	0.12	0.01	0.16	0.17	0
PL	0.14	0.41	0.55	0.01	0.30	0.31	0.03	0.41	0.44	0
Total	0.80	0.72	1.52	0.01	0.46	0.47	0.03	0.71	0.74	0

[a] FL = Free lipids; BL = bound lipids; NL = chloroform eluate (from silicic acid column); GL = acetone eluate; PhL = methanol eluate; PL (polar lipids) = GL + PhL.

TABLE II
Protein and Lipid Contents of Gluten Samples (%, dry basis)[a]

Component	Gluten										
	E			F			G	H	I	K	L
Protein		82.4			78.5		78.8	86.3	81.7	86.0	86.6
Lipids	FL	BL	Total	FL	BL	Total	Total	Total	Total	Total	Total
NL	0	1.02	1.02	0.26	3.04	3.30	2.93	0	1.67	0	0
GL	0.01	1.34	1.35	0.03	2.57	2.60	2.50	0	1.97	0	0
PhL	0	0.93	0	1.90	1.90	1.82	0	0	1.28	0	0
PL	0.1	2.27	2.28	0.03	4.47	4.50	4.32	0	3.25	0	0
Total	0.01	3.29	3.30	0.29	7.51	7.80	7.25	0	4.92	0	0

[a]FL = Free lipids; BL = bound lipids; NL = chloroform eluate (from silicic acid column); GL = acetone eluate; PhL = methanol eluate; PL (polar lipids) = GL + PhL.

Hexane extraction of the flour (B and C in Table I) did not remove all of the free lipids. Our recoveries are slightly lower than those of Chiu and Pomeranz (1966). TLC analysis of the hexane-soluble lipids showed that the chloroform eluate (NL) contained only nonpolar lipids, the acetone eluate (GL) contained only glycolipids, and the methanol eluate (PhL) contained phospholipids with a trace of digalactosyl diglyceride.

Lipid content and composition data of the flours (Table I) and the glutens (Table II) are in general agreement with published data. Lipid content of both sets of samples depended on the solvent used. The ratios NL/PL and FL/BL decreased in the same order as the lipid content. For flours A, C, and B, NL/PL values were 1.52, 0.74, and 0.47, respectively, and FL/BL values were 1.76, 0.68, and 0.52, respectively. For glutens F, G, I, and E, the NL/PL values were 1.33, 0.68, 0.51, and 0.45, respectively, and the FL/BL values were 0.039, 0.003, 0, and 0, respectively. Conversely, the GL/TL and PhL/TL values increased for the gluten samples in the same order (0.248 to 0.409 and 0.181 to 0.282 for GL/TL and PhL/TL, respectively).

FL (0.80) and TL (1.52) contents of the original flour (A) are in agreement with the values of 0.80 and 1.40 published by Hoseney et al (1970). However, our values for BL and BL/FL are higher, and the NL/PL value is lower than those published previously (Hoseney et al 1970).

Average protein content (82.76%) of the gluten preparations (Table II) agreed with published values for hand-washed glutens: Doguchi and Hlynka (1967), 80–86.7%; Bourdet and Feillet (1967), 85.9–91.0; and Finney (1943), 80.2%.

Lipid recoveries for flour-gluten pairs (A-F, C-I, and B-E) were compared. The recovery of TL was 56.4% for A and F, 80.4% for C and I, and 86.4% for B and E. Recovery for undefatted samples (A-F) agreed with the value calculated from the data of Hoseney et al (1970), assuming a gluten content of 12% for their flour. Recovery of TL in gluten of hard wheat flours of 57.4% was reported by Bourdet and Feillet (1967).

Comparison of recoveries of NL, GL, and PhL classes explained the significantly higher recovery of TL in the C-I and B-E flour-gluten pairs. Recovery of polar lipids was practically the same in all three pairs (90.0%, 89.4%, and 86.4%), but recovery of NL (37.4%, 67.4%, 76.7%) was inversely related to the NL content of flours. The result was: the higher the NL/PL ratio, the lower the recovery of TL. Recoveries of GL (84.1%, 88.2%, and 85.2%) and of PhL (99.5%, 91.4%, and 93.0%) were practically constant.

Composition of Gliadin Preparations

The dry material balances obtained for the gliadin preparation step (Table III) showed that the amounts of residue insoluble in AUC were similar for all preparations. Protein content of the residues varied between 18 and 20%, which represented 2–3% of the gluten protein. Residues did not contain any lipid.

The gliadin/glutenin values (Table III) showed the following trend. Glutens with higher lipid content yielded higher proportions of ethanol-soluble (gliadin) material. Compared on the basis of protein recovery (Table IV), sample F (contained lipids) contained 8% more ethanol-soluble protein than the totally defatted sample L. Although the presence of lipids apparently did not modify the solubility of gluten proteins in AUC (no more than 3% of protein

TABLE III
Dry Material Distribution (%) after pH - Precipitation and Gliadin/Glutenin Ratios of Different Glutens

Fraction	Gluten				
	E	F	G	I	L
Gliadin	39.5	44.6	43.0	42.2	37.2
Glutenin	42.2	37.0	38.7	39.5	44.5
Residue	18.3	18.4	18.3	18.3	18.3
Gliadin/glutenin	0.94	1.19	1.11	1.07	0.92

remained in the residues, regardless of lipid content), the presence of lipids did affect the distribution of ethanol-soluble proteins in the AUC-ethanol system. Chung et al (1979) investigated in detail the effect of flour lipids on protein extractability by different aqueous systems. The AUC solvent system, however, has not been investigated previously. Nevertheless, their protein-polar lipid-protein interaction model may explain our results. In the presence of lipids, some of the gluten proteins (presumably glutenin) in the form of protein-lipid complex could be soluble in 70% aqueous ethanol.

Lipid content of different gliadin preparations (Table IV) varied between 0 and 8.9%, depending on the defatting procedure applied to the flour or the gluten. The NL/PL values decreased in the same order as for the glutens from which the gliadins were obtained (F = 0.72, G = 0.62, I = 0.57, E = 0.44, and L = 0). The relative proportion of PhL of total lipid was quite constant. Therefore the change of NL:PL ratio resulted from the change of NL/TL (0.42, 0.38, 0.36, 0.32, respectively) and GL/TL (0.49, 0.52, 0.54, 0.59, respectively).

Association of lipids with gliadin and glutenin depends on the conditions used to isolate these proteins (Hoseney et al 1970). Accordingly, the lipid data for the gliadins prepared by the procedures used in the present study cannot be compared directly with previously published gliadin-lipid results. TL content of gliadin F is similar to that in the data of Hoseney et al (1970). The FL:BL ratio in our case, however, is 0.49, whereas they obtained 1.1. On the other hand, Chung and Tsen (1975) obtained 23% (33.7% of nonpolar and 5.7% of polar) of the gluten lipids in the gliadin fraction separated by column chromatography of the acid-soluble proteins from optimally mixed dough. Our analogous values were 51.1, 50.8, and 51.4%.

TLC analysis of the lipids of the gliadin fractions showed that the main components of NL were triglycerides and sterol esters with trace amounts of free fatty acids, and di- and monoglycerides. The major component of GL was digalactosyl diglyceride (more than half), and the major component of PhL was phosphatidyl ethanolamine.

Surprisingly, carbohydrate was not detected in the gliadin preparations from completely defatted gluten (H, K, L). Accordingly we compared carbohydrate content of total lipid content for the gliadin preparations used in this study. Total carbohydrate of gliadin F expressed as galactose was 1.80% (without hydrolysis); carbohydrate content of the total lipids (direct WSB extraction) were 14.3% (with hydrolysis) and 15.4%

(without hydrolysis). Calculated as percent of gliadin, these lipid results give 1.6% and 1.72%, respectively. Conditions of the analytical method used are therefore sufficiently strong to destroy the protein-lipid complex as well as to hydrolyze the bond between the carbohydrate and glycerol quantitatively. On the other hand, the recoveries obtained and the absence of carbohydrates in lipid-free gliadin preparations indicate that the source of the carbohydrate in the gliadin preparations is the glycolipids.

By PAGE and SDS-PAGE, the seven gliadin preparations examined in this study were essentially identical in protein subunit composition. However, the lipid seemed to be involved in the

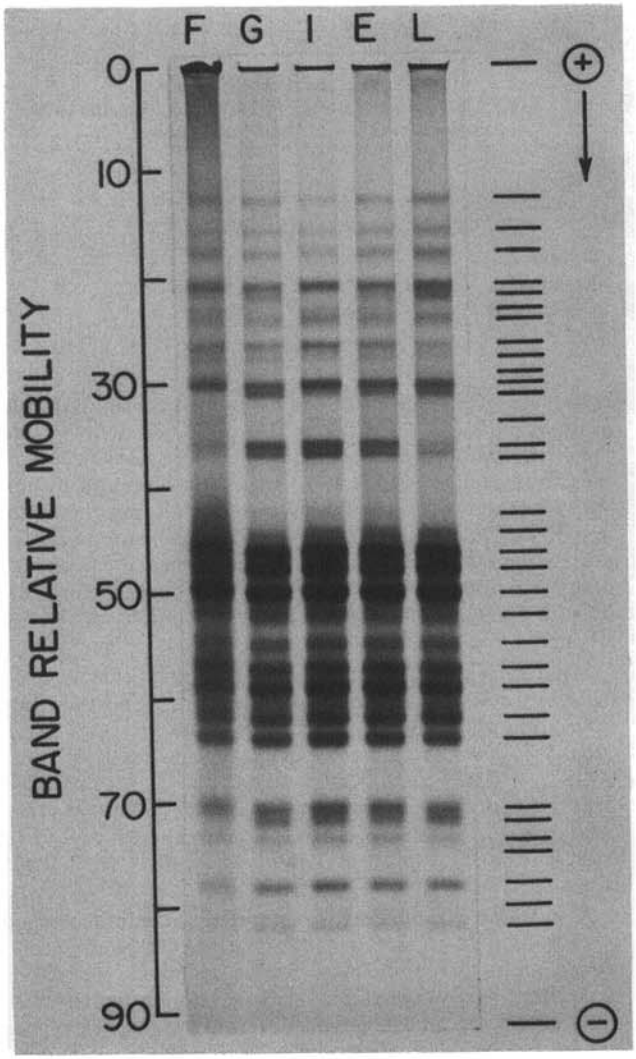

Fig. 2. Polyacrylamide gel electrophoresis patterns of gliadin preparations F, G, I, E, and L.

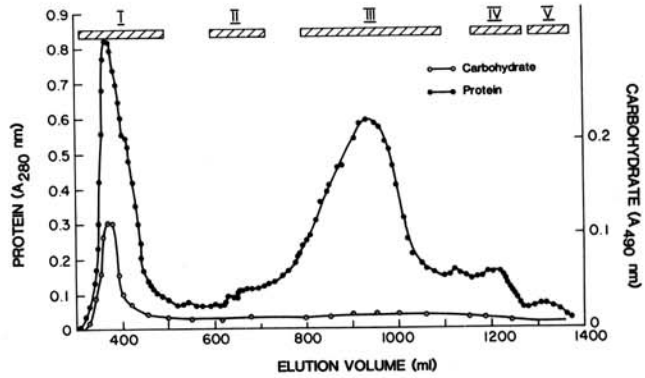

Fig. 3. Protein and carbohydrate elution profiles of gliadin E on Sephadex G-200 and fractions (I–V) that were collected.

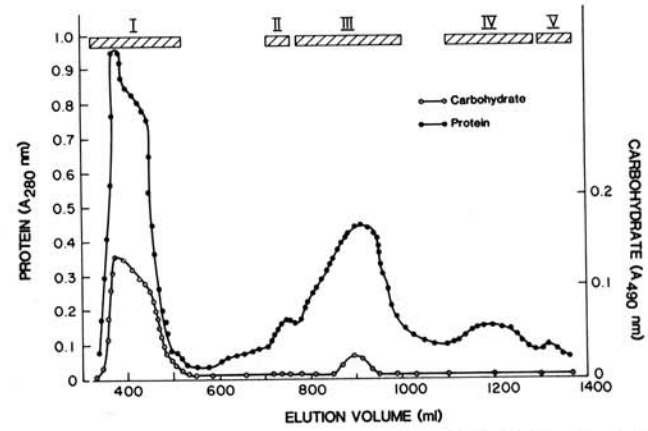

Fig. 4. Protein and carbohydrate elution profiles of gliadin F on Sephadex G-200 and fractions (I–V) that were collected.

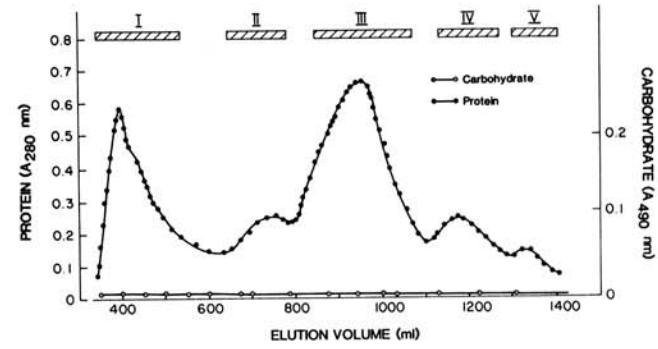

Fig. 5. Protein and carbohydrate elution profiles of gliadin L on Sephadex G-200 and fractions (I–V) that were collected.

TABLE IV
Protein, Carbohydrate, and Lipid Contents of Gliadin Preparations (%, dry basis)

Component	Gliadin								
	E	F			G	H	I	K	L
Protein	96.4	89.4			92.1	98.8	91.9	99.1	99.0
Carbohydrate	0.60	1.80			1.30	0	1.05	0	0
Lipids[a,b]	Total	FL	BL	Total	Total	Total	Total	Total	Total
NL	1.00 (38.7)	2.90	0.86	3.76 (50.8)	3.00 (44.0)	0	2.04 (51.5)	0	0
GL	1.94 (56.7)	0.03	4.32	4.35 (74.6)	4.08 (70.2)	0	3.05 (65.3)	0	0
PhL	0.35 (14.9)	0	0.84	0.84 (19.7)	0.77 (18.2)	0	0.54 (17.8)	0	0
PL	2.29 (39.6)	0.03	5.16	5.19 (51.4)	4.85 (46.3)	0	3.59 (46.6)	0	0
Total	3.29 (39.3)	2.93	6.02	8.95 (51.1)	7.85 (46.6)	0	5.63 (48.2)	0	0

[a] Gliadin lipids as a percentage of gluten lipids shown in parentheses.
[b] NL = Chloroform eluate (from silicic acid column); GL = acetone eluate; PhL = methanol eluate; PL (polar lipids) = GL + PhL.

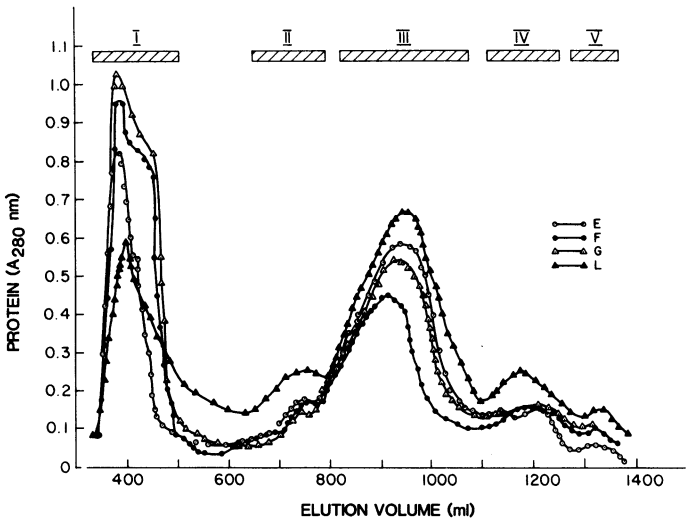

Fig. 6. Protein elution profiles of gliadins E, F, G, and L and fractions (I–V) that were collected.

TABLE V
Protein, Carbohydrate, and Lipid Contents of Gliadin Fractions (%, dry basis)

	Gliadin					
Component	**E-I**	**F-I**	**F-III**	**G-I**	**G-III**	**I-I**
Fraction weight[a]	21	47	32	41	37	35
Protein	84.5	80.2	98.5	82.7	97.8	82.4
Carbohydrate	2.8	3.5	0.1	3.0	trace	2.9
Lipids[b]						
NL	4.75	7.12	0.88	5.93	0.55	4.8
GL	8.62	9.00	0.32	8.74	0.15	8.7
PhL	1.51	1.70	0.11	1.60	0.17	1.5
PL	10.13	10.70	0.43	10.34	0.32	10.2
Total	14.88	17.82	1.31	16.27	0.87	15.1

[a] Fraction weight as a percentage of total weight of fractions recovered (after dialysis and freeze-drying of the separated fractions).
[b] NL = Chloroform eluate (from silicic acid column); GL = acetone eluate; PhL = methanol eluate; PL (polar lipids) = GL + PhL.

quality of results obtained by PAGE (Fig. 2). A relationship appears to exist between the lipid content and the amount of material that did not enter the gel. Furthermore, the sharpness of bands 46 and 48 (Zillman and Bushuk 1979) depended on the lipid content of the gliadin preparation. In the presence of lipids, the separation was poorer, probably because of lipid association with these subunits. Some differences were also observed in the low-mobility region of the electrophoregram, as indicated by difference

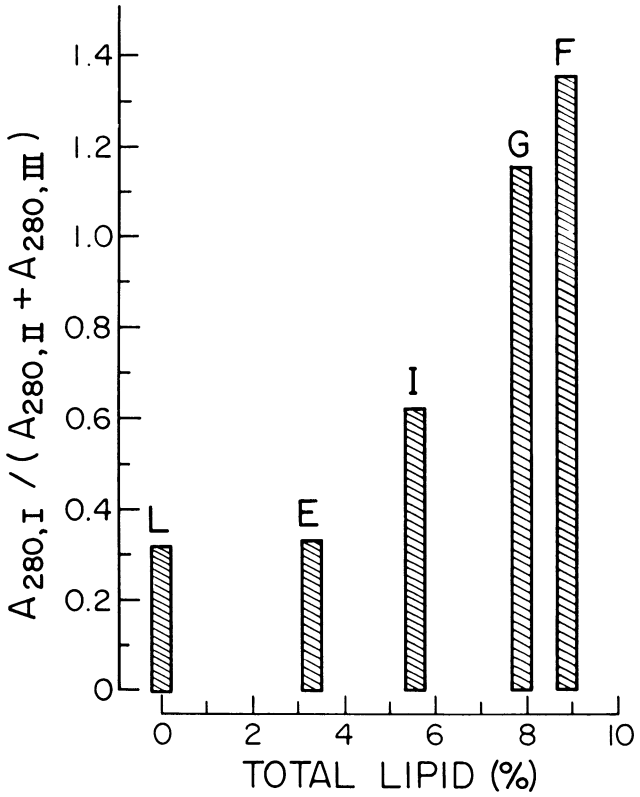

Fig. 7. The ratio of the absorbance of fraction I over fractions II plus III for different total lipid contents (percent) of gliadin preparations.

TABLE VI
Amino Acid Compositions and Derived Properties of Gliadin F and Its Fractions

		Fraction				
Amino Acid	**Gliadin F**	**F-I**	**F-II**	**F-III**	**F-IV**	**F-V**
Aspartic acid[a]	2.73	1.89	0.51	2.39	3.85	5.32
Threonine	2.11	2.07	1.47	1.51	4.48	3.83
Serine	4.58	4.63	4.27	3.56	5.33	3.74
Glutamic acid	43.32	39.25	47.95	43.46	29.74	14.42
Proline	15.31	12.98	18.83	13.92	9.10	8.05
Glycine	1.96	1.98	0.89	1.33	2.51	3.46
Alanine	2.12	1.57	0.42	1.72	3.45	3.30
Valine	3.94	3.37	0.76	3.52	5.65	5.43
Methionine	1.56	1.57	0.61	1.55	2.93	2.39
Isoleucine	4.04	3.59	1.46	3.93	4.68	2.85
Leucine	7.23	6.55	2.27	6.28	6.26	6.74
Tyrosine	2.58	1.87	1.29	2.12	1.90	4.12
Phenylalanine	6.01	5.54	10.83	6.42	1.18	1.77
Lysine	0.88	0.77	0.49	0.51	1.78	1.85
Histidine	1.98	1.66	0.52	1.69	4.04	1.31
Arginine	2.61	2.41	1.47	1.99	3.58	5.81
Ammonia	4.72	3.96	6.03	4.40	5.12	7.07
Average hydrophobicity (kcal residue^{-1})	1.064	1.036	1.225	1.102	0.898	0.728
Negative potential (moles per 100 kg)	314.90	281.21	356.12	313.68	231.20	250.64
Corrected negative potential (moles per 100 kg)	37.24	23.27	33.72	28.77	46.14	44.22
Positive potential (moles per 100 kg)	33.84	29.83	14.19	25.85	56.94	60.94
Positive to negative charge ratio	0.900	1.283	0.427	0.895	1.234	1.378

[a] Grams of amino acid per 100 g protein; cystine and tryptophan not determined.

in relative intensity of bands 35 and 38 as well as by the quality of separation of 20.5–21.5 and 30.5–31.5 doublets. Minor differences were observed in the fast-moving region (relative mobility 70–90).

Composition of Gliadin Fractions Obtained by Gel Filtration

Figures 3, 4, and 5 show the fractionation of the gliadin preparations on Sephadex G-200. Regardless of the lipid content, the elution profile of each preparation had five peaks (designated for gliadin E as E-I, E-II, etc.). Approximate mol wts of the fractions are >200,000, 75,000, 27,500, 21,000, and 15,500. Carbohydrate and lipids were detected in fraction I of all gliadin preparations except H, K, and L, and in fraction III of gliadin F and G only.

The relative amounts of fractions for the gliadin preparations were different (Fig. 6). Each fraction I of the lipid containing gliadins was turbid; that of the lipid-free (L-I) was clear. Bietz and Wall (1973) suggested that turbidity in their fraction I was the result of the presence of lipids. This can explain the increase of the absorbance of fraction I from these lipid-containing preparations, but not the decrease of the absorbance of fractions II and III. The direct relationship of the ratio of the total absorbances of fraction I to fractions II + III ($A_{280,I}/(A_{280,II} + A_{280,III})$) vs total lipid content of the gliadin preparation (Fig. 7) indicated that, in the presence of lipids, some of the low molecular weight subunits aggregated with the high molecular weight subunits.

Lipid contents and compositions of fractions I and III (Table V) were quite different. Differences in total lipids were large, and the NL:PL ratios of 0.66 and 0.57 for F-I and G-I, respectively were much lower than 2.04 and 1.71 for F-III and G-III. The major lipid component of fraction I was digalactosyl diglyceride, whereas that of fraction III was triglyceride. Lipids present in fraction III were only of those gliadin preparations from flour (or gluten) not extracted by *n*-hexane. This indicates that the lipids of F-III and G-III originated from the free lipids of the flour.

Despite differences in polar lipid content of the unfractionated gliadin (Table IV), PL values (10.13–10.70%) and GL:PhL ratios (5.14–5.71) of the fraction I were remarkably similar (Table V). The differences in the amounts of TL and NL:PL ratios (0.468–0.660) were the result of differences in the amount of NL.

Amino acid composition of gliadin F and its fractions are presented in Table VI. The table also gives calculated charge potentials and average hydrophobicity parameters. Comparing the results for F-I and F-III, the greater average hydrophobicity of the latter and the greater positive charge of the former can be matched with the different quantity and nature of its bound lipids. That is, the lipids associated with F-I are more polar (less hydrophobic) than those associated with F-III.

PAGE results for gel filtration fractions of gliadin F (Fig. 8) indicated that in F-I, the low mol wt subunits were aggregated, and most of the protein did not enter in the gel. The small amount of protein that entered the gel did not produce any distinct bands. This effect did not occur in the case of fraction I of gliadin preparations H, K, and L, all prepared from totally defatted flour or gluten. The electrophoretic patterns of the other fractions of gliadin F showed that F-II contained gliadin subunits of low mobility (bands 13–40), F-III also contained the high mobility (bands 42–93) subunits, and fractions F-IV and F-V were mainly globulins and albumins.

Figure 9 shows the gel stained for carbohydrate. For better demonstration, the gel was photographed at an angle of 45° to make the vertical surface of the sample slots more visible. The results indicate that all of the carbohydrate in the samples examined is associated with the protein that cannot enter in the gel.

SDS-PAGE patterns (Figs. 10 and 11) showed that fraction F-I contained essentially all of the subunits of total gliadin. Because of this unexpected result, this fraction was rechromatographed on

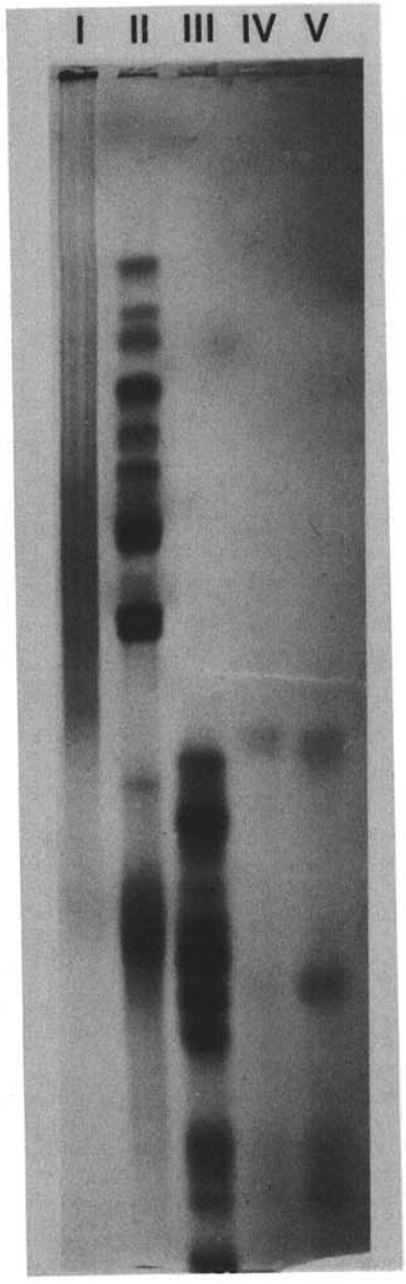

Fig. 8. Polyacrylamide gel electrophoresis patterns of gliadin F fractions I, II, III, IV, and V stained for protein.

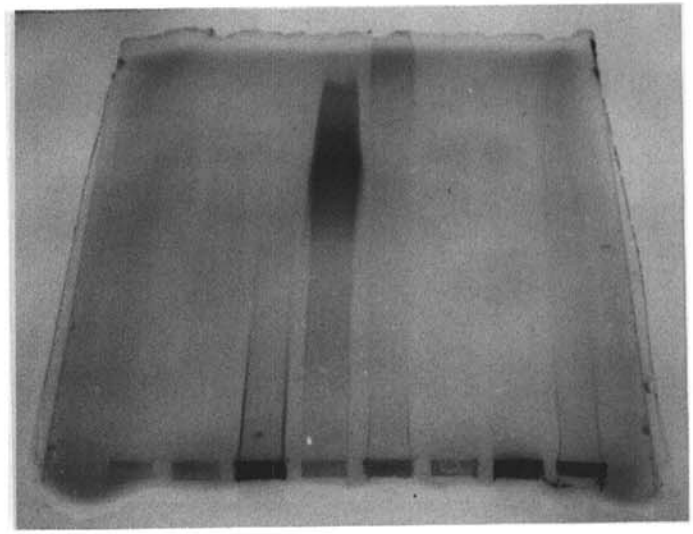

Fig. 9. Polyacrylamide gel electrophoresis patterns of fractions of gliadin F stained for carbohydrate. From left to right: F-III; F-II; F-I; fetuin (a glycoprotein); human serum β-lipoprotein; gliadin L; gliadin E; and gliadin F.

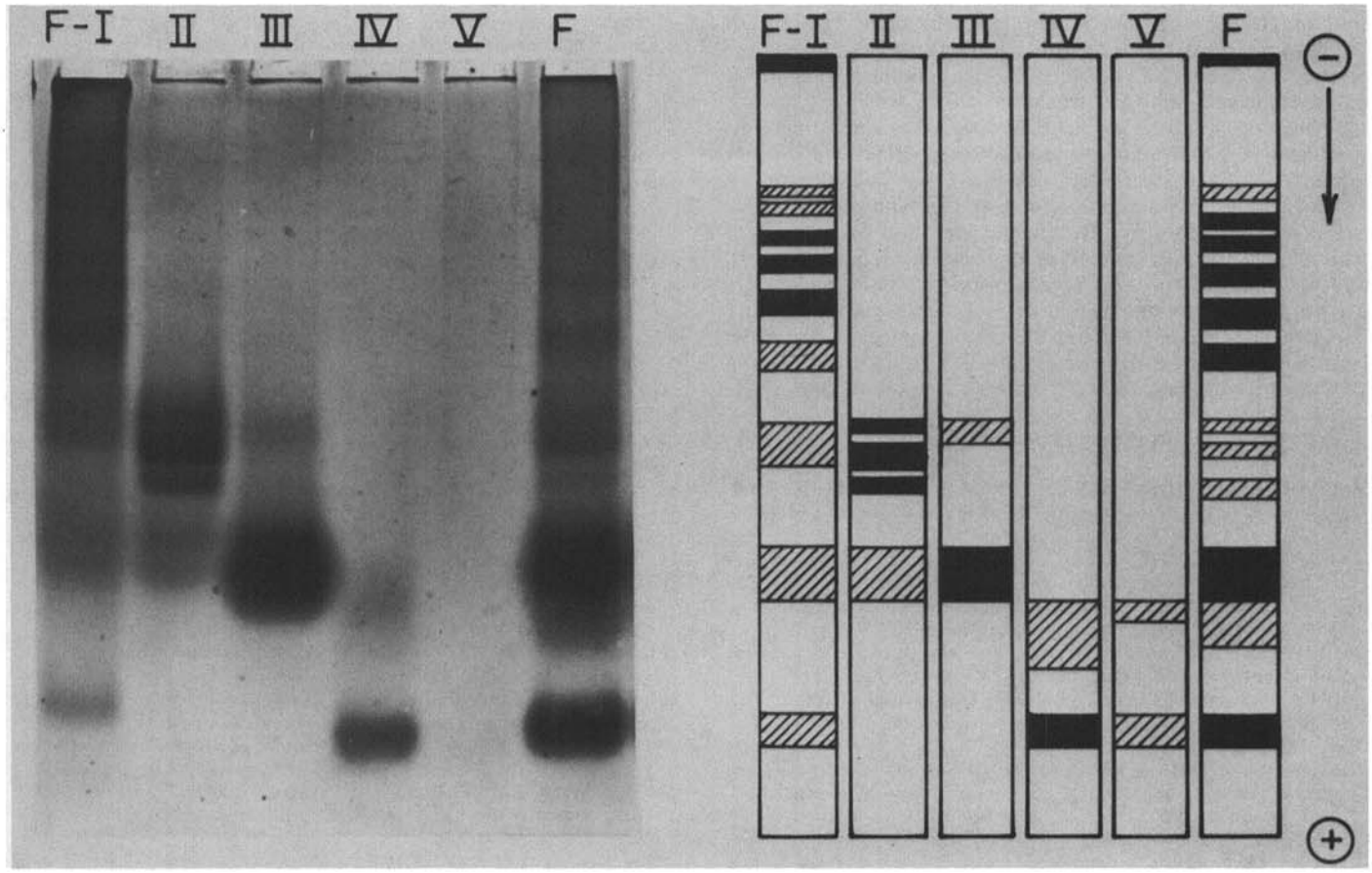

Fig. 10. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of gliadin F and its fractions.

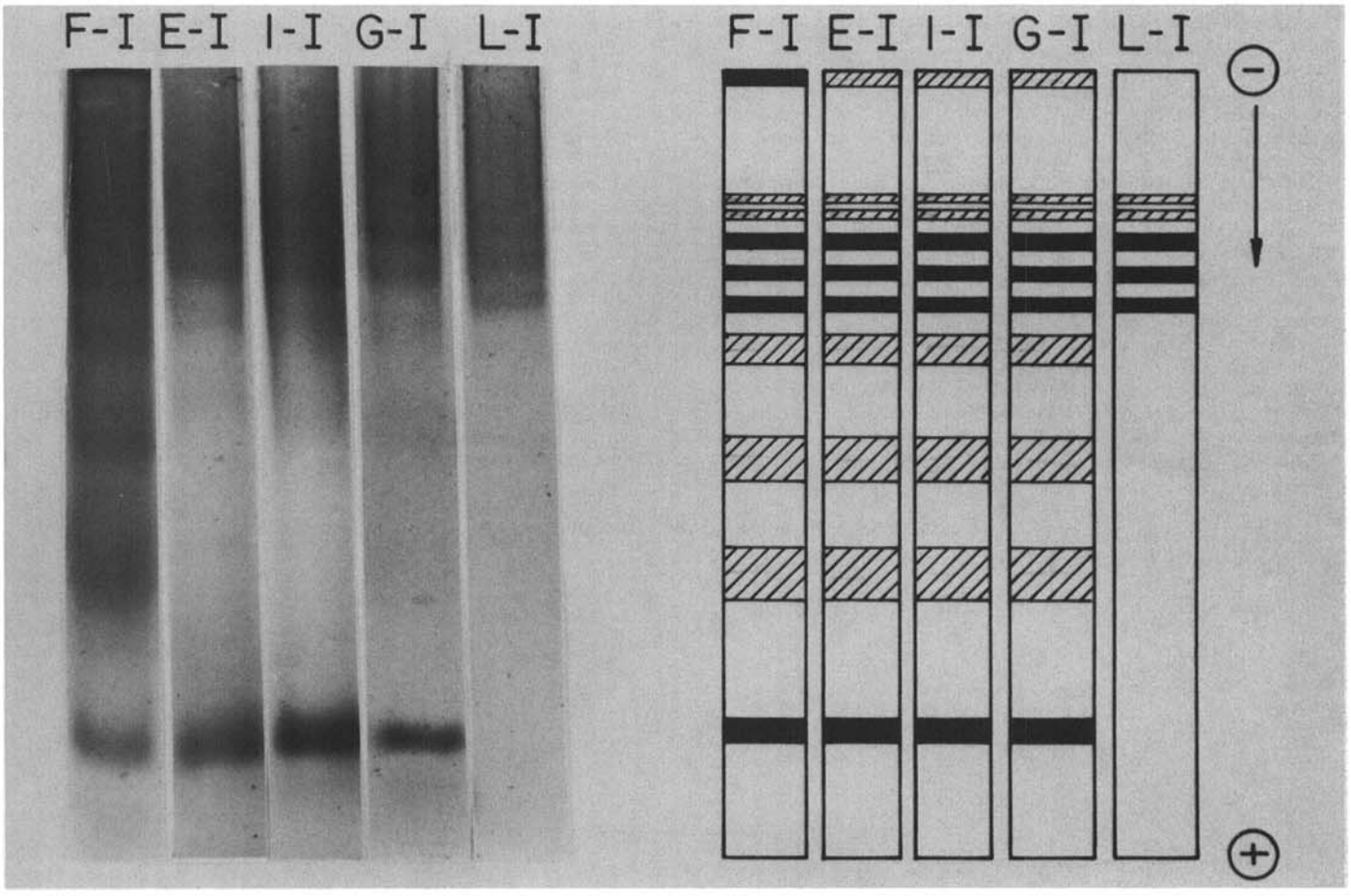

Fig. 11. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of fraction I of gliadins F, E, I, G, and L.

Sephadex G-200. Fraction I again gave the same SDS-PAGE pattern (not shown). Figure 10 shows that low mol wt subunits were present in every fraction I except L-I, which contained no lipid. L-I comprised only the high mol wt subunits.

Differences in ratios of Sephadex G-200 gliadin fractions, differences in PAGE patterns of gliadins with (F, G, I, E) and without (L) lipids, and differences in SDS-PAGE patterns of gliadin fraction I of preparations with and without lipids indicate that in the presence of lipids, low and high mol wt subunits of gliadin, as well as trace amounts of albumin and globulin proteins, can form aggregates of high apparent mol wt. The lipid, mainly galactolipid, appears to play a key role in the formation of those aggregates (Békés et al 1983), and this may be the mechanism of the already demonstrated involvement of this lipid in bread-making quality of bread flours.

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