Correlations Between Reversed-Phase High-Performance Liquid Chromatography and Acid- and Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoretic Data on Prolamins from Wheat Sister Lines Differing Widely in Baking Quality[1]

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ABSTRACT

Gliadins extracted from closely related (sister) wheat lines that vary widely in breadmaking quality were separated by reversed-phase high performance liquid chromatography (RP-HPLC). Each RP-HPLC peak was collected and analyzed by lactic acid-polyacrylamdide gel electrophoresis (A-PAGE) and sodium dodecyl sulfate (SDS)-PAGE. RP-HPLC separates proteins on the basis of hydrophobicity, a surface-related phenomenon. A-PAGE separates on the basis of size and charge. The combination of all this information on one sample allows correlation of data among methods. However, of greater importance are the differences found between the good baking quality sister line and the poor baking quality line. Three differences were found in the gliadins by both HPLC and A-PAGE. One of those differences was an ω-gliadin found only in the good quality line, whereas the other two differences were β-gliadins found only in the poor quality line. Molecular weights, hydrophobicity, elution order, and gliadin type for each peak are reported.

The baking quality of flour and bread products made from it are primarily dependent on the quantity and quality of the protein components in the flour (Finney 1943). Because over half of the protein in flour is gluten, at least one of the components in gluten should be related to breadmaking quality. In fact, two of the more important flour quality factors, mixing time and loaf volume, are related to one or more gluten components (Finney et al 1982).

Gliadins constitute about one-half of the gluten and of the endosperm storage protein of wheat. They have been used to identify wheat varieties (Wrigley and Baxter 1974, Bushuk and Zillman 1978, Jones et al 1982, Lookhart et al 1982) and are known to correlate with breadmaking quality (Finney et al, 1982).

In this study, gliadins from closely related wheat lines differing greatly in breadmaking quality were analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC) and lactic acid- and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (A-PAGE and SDS-PAGE). Both lines were selected from the variety Ottawa. They are agronomically and morphologically very similar but differ strongly in their baking quality parameters. Because they are very similar in everything but baking quality, it seemed likely that any difference in their protein constituents might be related to their baking behavior. In lieu of isogenic lines differing only in baking quality, these lines are the best available to study quality characteristics with the least influence from other complicating factors.

The three methods, RP-HPLC, A-PAGE, and SDS-PAGE, separate proteins or polypeptides by different mechanisms. A-PAGE separates proteins on the basis of surface charge combined with size, and SDS-PAGE separates by apparent molecular size. RP-HPLC separates on the basis of surface hydrophobicity. Because each method uses a different separation mode, the combination of all three is at least complementary and at best synergistic. In addition, the data allow the translation or correlation of data from one protein analysis system to another.

MATERIALS AND METHODS

Wheat Varieties

L. Bolte provided flour samples of the closely related (sister)

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lines, Shawnee (C.I. 14157) and "Ottawa Selection" (KS 619042). Both lines were selected from the cultivar Ottawa in the middle 1960s and have been grown every year since for research quality evaluation. The agronomic properties of the lines are essentially identical. They differ mainly in their baking properties. Shawnee is a good baking quality line with good loaf value (1,328 ml/100 g), mixing time (4 3/8 min), and overall baking quality. Ottawa Selection is a poor baking quality line with poor loaf volume (693 cm^3) and mixing time (7/8 min), and very poor overall baking quality (Menkovska et al 1987). The laboratory shorthand identifiers for these lines were 402 for Shawnee and 406 for Ottawa Selection.

TABLE I
High-Performance Liquid Chromatography Gradient Program[a]

Time	0	5	55	56	57	58
%A[b]	75	70	60	0	0	75
%B[c]	25	30	40	100	100	25

[a] C_8 SynChrom reversed-phase column (25 cm × 4.1 mm) at 65°C.
[b] H_2O + 0.1% trifluoroacetic acid.
[c] CH_3CN + 0.1% trifluoroacetic acid.

Gliadin Extractions

Flour samples (250 mg) were extracted with 750 ml of 70% ethanol by the method of Lookhart et al (1982).

Electrophoresis

The A-PAGE procedure was that of Lookhart et al (1986), which used a Hoefer SE600 vertical gel system (160 × 180 × 3.0 mm). It is a fast procedure utilizing 500 V, 2 hr, 20°C, and a 6% polyacrylamide gel.

The SDS-PAGE procedure was a combination of that of Laemmli (1970) and that of the Hoefer manual (1983). A Hoefer SE 600 system (160 × 180 × 1.5 mm) utilizing a 20% separation gel was run at 12°C for 5 hr at 30 mA. Bio-Rad molecular weight markers were analyzed on each gel, and apparent molecular weights were calculated from the slope of the semilog plots of molecular weight versus band position.

HPLC

The HPLC procedure used was a modification of that reported by Lookhart et al (1986). A C_8 SynChrom (SynChrom Inc., Linden, IN) reversed-phase column (250 × 4.1 mm) was used at 65°C. The solvent pumping system was a Varian Associates 5060

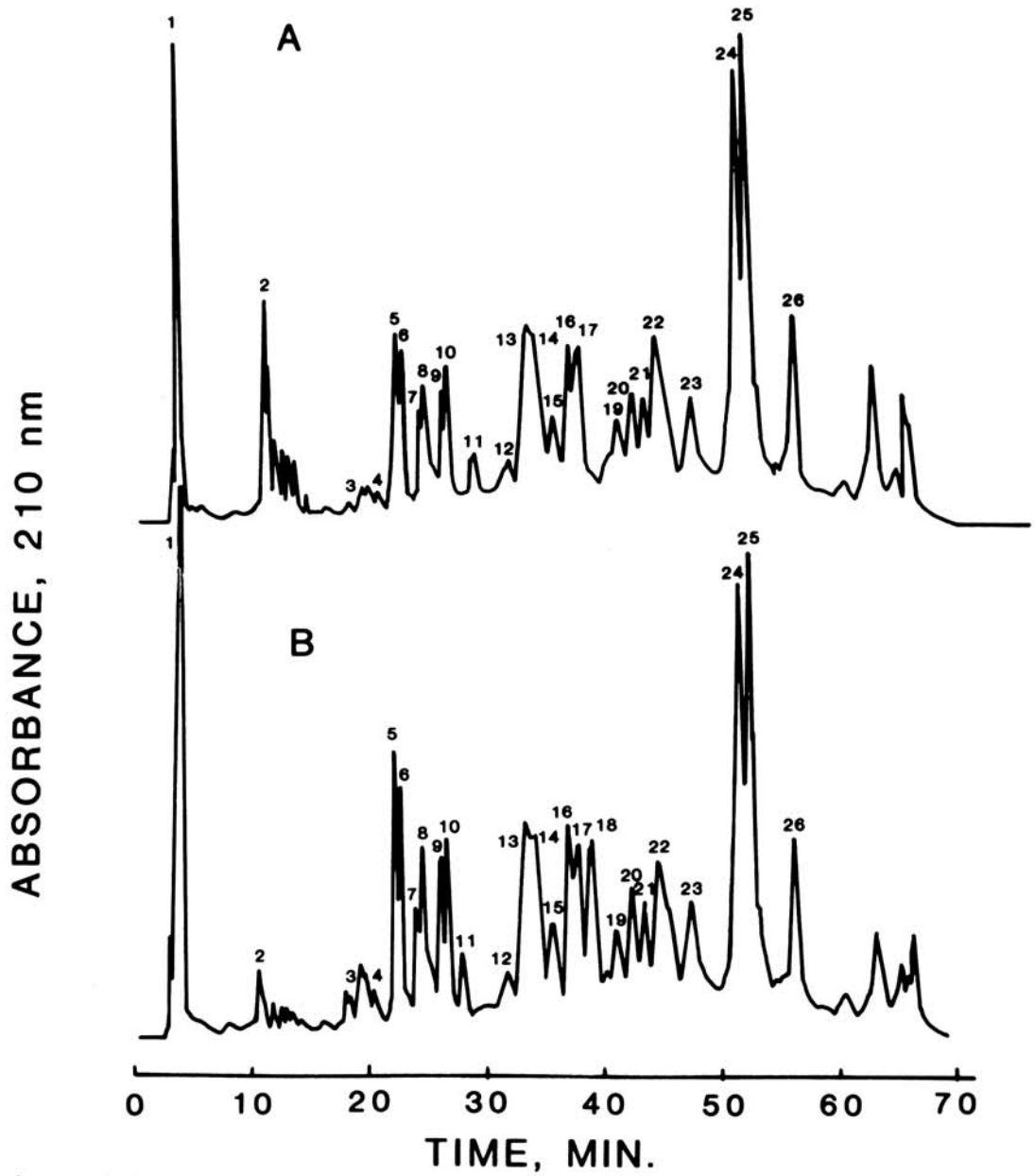

Fig. 1. High-performance liquid chromatography patterns of gliadins extracted from sister lines 402 (**A**) and 406 (**B**). The small numbers indicate the peaks that were collected individually. The gradient and column used were those described in Table I.

ternary system. The flow rate was 0.8 ml/min, and a multistep linear gradient (Table I) utilized water (solvent A) and acetonitrile (solvent B), each containing 0.1% trifluoroacetic acid.

Injections (20 μl) were made every 75 min by a Waters Associates WISP autosampler. Detection was at 210 nm for optimum sensitivity using a Tracor Instruments Corporation model 970 ultraviolet-visible spectrophotometer.

Each individual peak from each wheat line extract was collected in separate vials from each of the five separate injections. HPLC analyses were done in at least triplicate to assure reproducibility. Peaks from each line with identical elution characteristics (time and height) were combined and freeze-dried. One fraction was analyzed by A-PAGE, one by SDS-PAGE, and the third fraction was saved for amino acid analysis.

RESULTS

HPLC

The chromatograms for the prolamin extracts of lines 402 (Fig. 1A) and 406 (Fig. 1B) grown in 1981 (16% protein) were similar, with significant differences in peaks 2, 11, and 18. Those differences were that the 402 extract gave a much larger peak 2 than the 406 extract; peak 11 of 402 eluted at about 28.5 min, whereas that of 406 eluted at nearly 30 min; and peak 18 was present in the 406 extract and completely absent from 402.

Peak 1 from both lines eluted at the void volume peak, with all other nonretained materials. When peak 1 was collected and reinjected, a chromatogram nearly identical to the original was produced. Less absorbance (protein) was seen, of course, but the major pattern difference was that peaks 24, 25, and 26 had greatly decreased intensities. The fact that material from all of the peaks was present in the void volume implies that only a portion of the material injected interacted with the column. Since peaks 24–26 bind most strongly (presumably have the greatest hydrophobicity), they exhibited the smallest peaks relatively in the chromatogram of the reinjected sample (data not shown).

Electrophoresis

Gliadin extracts from lines 402 and 406 grown in the years 1972, 1975, 1976, 1980, and 1981 are shown in Figure 2. The patterns of each line were consistent from year to year. Three band differences were found between the two lines and are marked with arrows. The 402 line has one band (e) not found in 406, and the 406 line has two bands (u and ab) not found in 402. The bands were lettered to facilitate comparison with the peaks separated by HPLC.

After combining samples of each peak obtained from five separate HPLC runs, one-third of each sample was analyzed by A-PAGE and another third by SDS-PAGE. The results are listed in Table II. Peak 1, the void volume HPLC peak, contained proteins giving all of the bands found in the original extract by A-PAGE but with quite diminished band intensities from p to af (Fig. 3). The p and q bands, which were darkly stained in the original A-PAGE pattern, were relatively much lighter in the peak 1 collection; therefore, they must have interacted more strongly

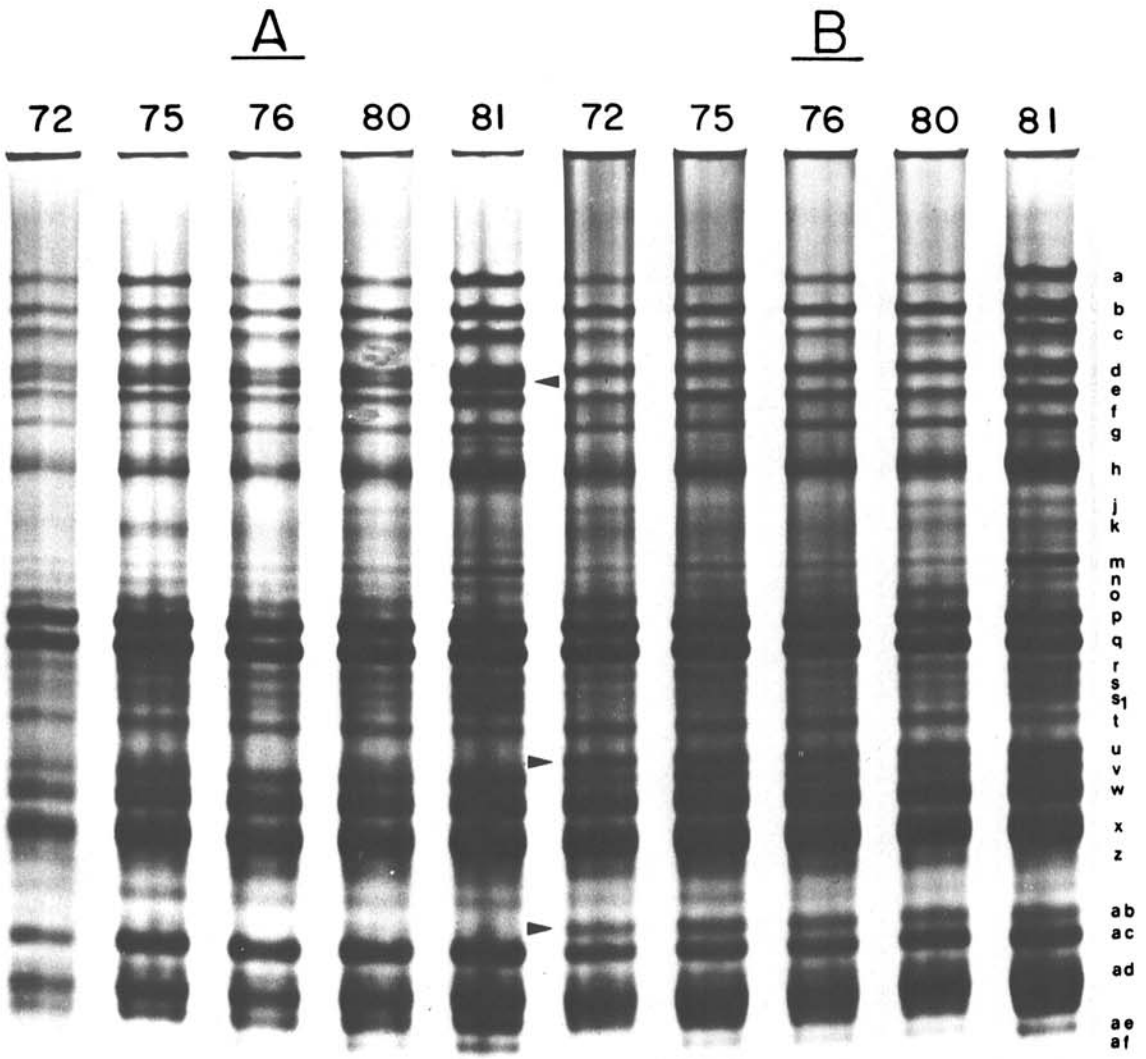

Fig. 2. Lactic acid-polyacrylamide gel electrophoretic patterns of gliadins extracted from sister lines 402 and 406. Samples were extracted and analyzed from wheats grown in five separate years: 1972 (**72**), 1975 (**75**), 1976 (**76**), 1980 (**80**), and 1981 (**81**). The arrows indicate differences in the patterns. The small letters indicate band identifications used for correlating with high-performance liquid chromatography peaks.

with the C_8 column than any of the other proteins. All of the gliadin types (α, β, γ, and ω) described by Jones et al (1959) were present in the peak 1 fraction of 406 and their apparent molecular weights ranged from 31 to 86 kilodaltons (kDa). Only the ω-gliadins are readily seen in the peak 1 fraction of 402.

The majority of proteins eluted in peaks 2 through 10 were ω-gliadins as defined by their A-PAGE mobilities (Jones et al 1959). The band letters in bold print in Table II denote the most intensely stained band of that fraction. Therefore, in the electrophoregram of peak 2, which eluted at 27% acetonitrile with a relative mobility of 29.8, the most darkly staining band of which was h in both 402 and 406 (Fig 3 IA and B). The h band exhibited an apparent molecular weight of 63 kDa. Band e, in the electrophoregram of peak 2 from 402 (Fig. 3 IA) was not present in the same fraction from 406 (Fig. 3 IB). Therefore, the difference (by HPLC) in peak 2 is also manifested as a difference in A-PAGE (band e). The A-PAGE pattern of peak 3 from both lines had two bands of equal intensity with relative mobilities of 15.2 (b) and 17.5 (c).

Their SDS-PAGE patterns showed both proteins had molecular weights of 48 kDa. The electrophoretic band found in peak 4 of line 402 (Fig. 3 IA) had a relative mobility of 29.8, and thus was an h band, with an apparent molecular weight of 48 kDa. Evidently, at least two proteins (eluting in peaks 2 and 4) with relative mobilities of 29.8 (h) were not separated by A-PAGE but were by HPLC and SDS-PAGE. The SDS-PAGE pattern of peak 4 (406) did not show any darkly stained bands (data not shown), but the A-PAGE pattern did show the h band (Fig. 3 IB).

Peaks 5 and 6 (Fig. 3 IA and B) contained predominantly electrophoretic bands b and a, respectively, both having apparent molecular weights of around 60 kDa. Electrophoregrams of peaks 7 and 8 (Fig. 3 IA and B) exhibited predominantly bands g and f, respectively, whereas those of peaks 9 (Fig. 3 IA and B) and 10 (Fig. 3 IIA and B) contained mostly bands d and c, respectively. Since the electrophoregrams of one of the HPLC paired peaks showed one darkly stained band and one light band while in the other one of the pair the intensities were reversed, the HPLC method and/or the collection procedure did not completely separate the protein peaks. The molecular weights of the four proteins (bands c, d, f, and g) were all in the range of 50 to 55 kDa. The A-PAGE patterns of HPLC peaks 3 through 10 were the same from both 402 and 406. Only in peak 2 of 402 were extraneous bands (e, i, and j) found that were not present in the corresponding peak of 406.

Bands with mobilities ascribed to β-gliadins (Jones et al 1959) were found in the A-PAGE analysis of the HPLC peaks 11 through 18 (Fig. 3 IIA and B), representing proteins with molecular weights ranging from 37 to 43 kDa, and eluting from the C_8 column at acetonitrile concentrations between 33.5 and 35.5%. Line 402 lacked the proteins found in 406 peaks 11 and 18, an important difference. The lack of bands in peak 11 of 402 was due to the fact that those proteins were not typical gliadins. They must either have very high native charge or no native charge. In either case they would not be seen in the β region. Since peak 18 was not present in 402, no sample was collected for analysis.

Because numerous proteins (not overlapping) were found in the HPLC peaks 11, 12, and 14, the HPLC separation in this region appears poor. The d, f, and g bands in peak 11 are ω-gliadins by electrophoretic mobility and are mixed in with the β-gliadins. The q protein(s) in peak 12 are γ-gliadins and the ad and ae proteins in peaks 14 and 18, respectively, are α-gliadins. It is apparent that the β-gliadins are the group least resolved by HPLC.

On the other hand, two very important proteins (present only in the poor baking quality line) were found in the electrophoregrams of peaks 11 and 18 from line 406 (Fig. 3 IIB). Peak 11 contained predominantly band ab and peak 18 had mostly the band u protein, in agreement with the original (Fig. 2) electrophoregrams of 402 and 406. The β-gliadins appear to be resolved better by A-PAGE than by HPLC, as shown by the large number of electrophoretic

TABLE II
Correlations of Gliadin Proteins Separated by High-Performance Liquid Chromatography (HPLC) and Analyzed by Acid (A)- and Sodium Dodecyl Sulfate (SDS)-Polyacrylamide Gel Electrophoresis (PAGE)

HPLC Peak No.	A-PAGE R_m	Band Positions[a] 402	Band Positions[a] 406	Gliadin Type	% CH_3CN (range)	SDS-PAGE[b] 402	SDS-PAGE[b] 406
1	12–85	c–n	a–ag	All	25.0	31–48	31–86
2	29.8	e,**h**,i,j	**h**	ω	27.0	63	63
3	15.2, 17.5	b,c	b,c	ω	↑	48	48
4	29.8	**h**	**h**	ω		48	48
5	15.2	1,**b**	1,**b**	ω		61	59
6	12.0	**a**,b	**a**,b	ω		60	60
7	26.2	f,g	f,**g**	ω		55	55
8	23.2	**f**,g	**f**,g	ω		53	51
9	21.5	c,**d**	c,**d**	ω	↓	53	51
10	17.5	**c**,d	**c**,d	ω	33.0	52	50
11	70.0		d,f,g,p, v,w,y,**ab**	β,ω	33.5 ↑	40	40
12	47.0	**q**,v,w, z,aa	**q**,s,s_1, v,w,z,aa	β,α		43	42
13	58.5	**v**,y	**v**,y	β		43	42
14	48.7, 58.5	**r**,**v**,w, z	**r**,**v**,w,z ad	β,α		38,42	36,39
15	50.5	**s**,s_1	**s**,s_1	β		41	41
16	64.8	**y**	**y**	β		38	37
17	60.8	**w**,x	**w**,x	β	↓	39	38
18	55.4		**u**,ae	β,α	35.5		39
19	85		af,ag	α	36.0	36,37	35,36
20	73.0	**ac**	**ac**	α	↑	36	35
21	73.0	**ac**	**ac**,ad	α		37	36
22	77.1	**ad**	**ad**	α	↓	35	36
23	77.1	**ad**	**ad**	α	37.5	38	37
24	47.0	p,**q**	p,**q**	γ	38.0	37	39
25	43.3	**p**,q	**p**,q	γ		44	46*[c]
26	47.0	**q**,x	**q**,x	γ,β	39.0	43	43

[a] Band letter in bold type indicates most intensely stained band.
[b] Molecular weight $\times 10^{-3}$.
[c] Indicates significant difference in staining intensity.

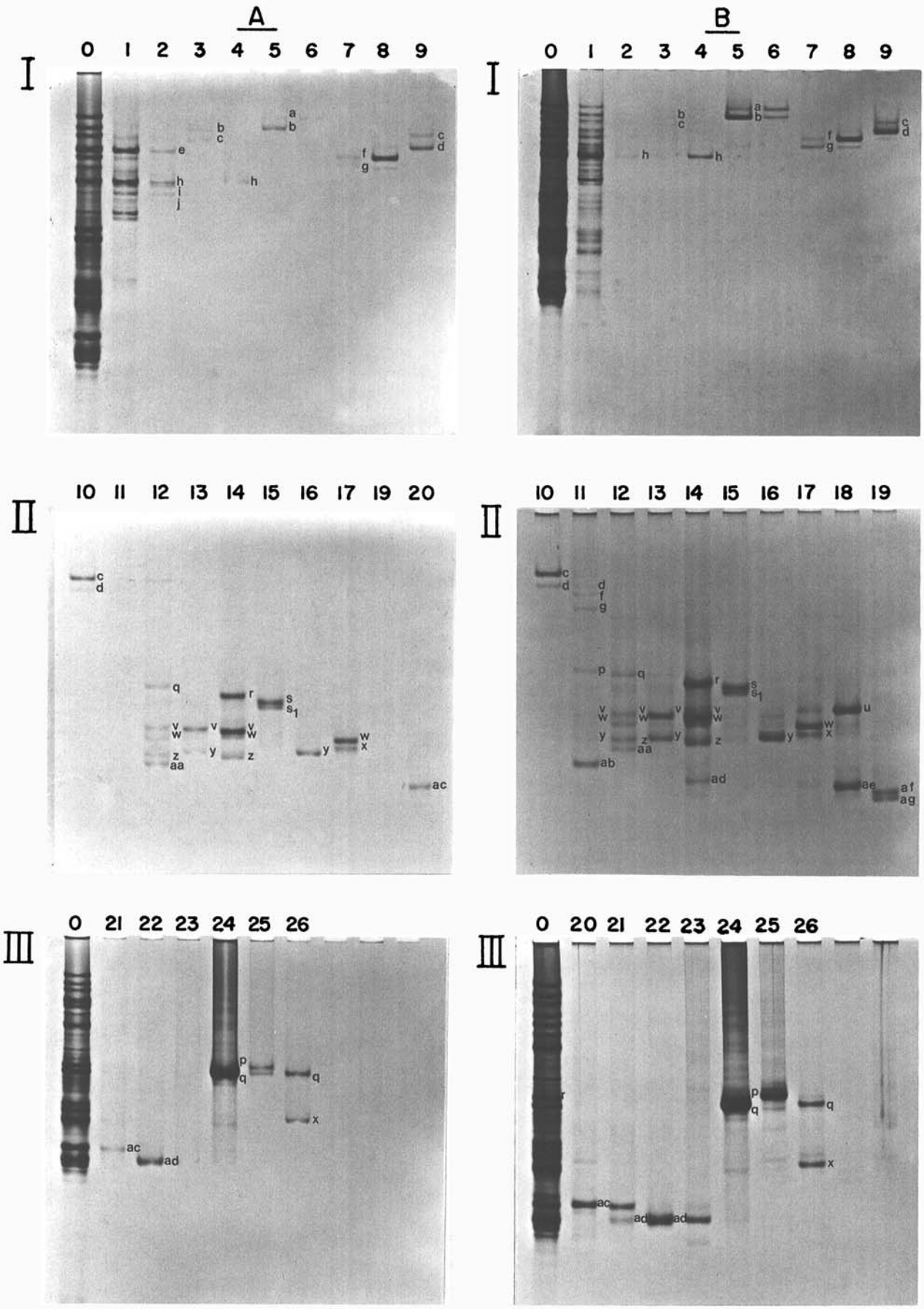

Fig. 3. Lactic acid-polyacrylamide gel electrophoretic patterns of gliadins separated by high-performance liquid chromatography (HPLC), fractions shown in Fig. 1. Lanes are numbered to correspond with HPLC peaks 1 through 26, for lines 402 (**A**) and 406 (**B**). The prolamin extracts are listed as lane 0. The small letters designate the band assignments as shown in Fig. 2 and Table II.

TABLE III
General Correlations Among Gliadin Protein Characteristics

	General Correlations					
	Gliadins				ab	u
Methods[a]	α	β	γ	ω	PK 11	PK 18
HPLC, $[CH_3CN]$[b]	36.0–37.5	33.5–35.5	38–39	27–33	33.5	35.5
A-PAGE, R_m	73–85	47–70	43–47	12–40	70	55.4
SDS-PAGE, kDa	35–38	37–43	37–46	48–63	40	39.0

[a] HPLC = High-performance liquid chromatography, A-PAGE and SDS-PAGE = acid- and sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
[b] Concentration of acetonitrile needed to elute proteins from C_8 column under conditions in Table I.

bands resolved from each HPLC peak (Fig. 3).

Bands found in the A-PAGE analyses of the HPLC peaks 19 (Fig. 3 IIA and B) to 23 (Fig. 3 IIIA and B) had mobilities that characterized them as containing α-gliadin proteins (Jones et al 1959). They were eluted with 36.0–37.5% acetonitrile and had molecular weights of 35–38 kDa. Peak 19 of 402 gave no A-PAGE bands, but two dark bands with molecular weights similar to those found in peak 19 of 406 were found by SDS-PAGE. A similar effect was noted previously for peak 11 of 402. The α-gliadins appear to be resolved better by HPLC than by A-PAGE, since peaks 20 and 21 both contain the ac protein(s), and peaks 22 and 23 both contain protein(s) ad. Molecular weights of the proteins in those four peaks are the same within experimental error (+10%). Therefore, only the surface hydrophobicities of those proteins show differences.

The A-PAGE bands p and q, corresponding to γ-gliadins (Jones et al 1959), were found in the HPLC peaks 24–26. They eluted from the HPLC column at acetonitrile concentrations between 38 and 39%. Lines 402 and 406 gave identical A-PAGE and HPLC results for peaks 24–26. The q proteins eluted in peaks 24 and 26 (Fig. 3 IIIA and B) did, however, have different molecular sizes. The apparent molecular weight of the q band in peak 24 was 38 kDa, that from peak 26 was 43 kDa. The p bands (peak 25) from 402 and 406 (Fig. 3 IIIA and B) differed from each other in the staining intensity of their SDS-PAGE bands even though their molecular weights were the same, indicating that differences must exist in the surface properties (amino acid composition or position) that allows for differences in binding of the Coomassie blue stain. A β-gliadin band x is also present in HPLC peak 26 from both 402 and 406 (Fig. 3 IIIA and B). The elution of the β-gliadin x with the γ-gliadin q in peak 26 shows some degree of hydrophobic similarity between those gliadin types.

Only bands k, l, m, n, o, and t were not found during A-PAGE analysis of the HPLC peaks. They may be present in one of the small HPLC peaks not collected between peaks 2 and 3 (for k–o) or between 11 and 12 (for t). It may be significant that those proteins were not found during analysis of the HPLC peaks. It is possible that they do not elute from the HPLC column even though electrophoresis characterizes them in the ω to β regions.

CONCLUSIONS

Table III correlates the information obtained from the three methods (HPLC, A-PAGE, and SDS-PAGE) and allows a comparison of information among the methods. The relative hydrophobicity (acetonitrile concentration needed for elution) of each gliadin type (α, β, γ, and ω) is compared to their A-PAGE relative mobility values and apparent molecular weights. The hydrophobicity was least for the ω-gliadins and increased through the series β-, α-, and γ-gliadins. The molecular sizes of the α-, β-, and γ-gliadins overlapped, but differences in their surface properties allowed separation by HPLC and A-PAGE. The R_f and molecular size data are consistent in that the smaller molecules moved through the gel faster.

Interestingly, the gliadins were separated into four distinct groups by A-PAGE (molecular size combined with charge) and nearly so by HPLC (surface hydrophobicity) but the α-, β-, and γ-gliadins showed some overlap in terms of their apparent molecular weights by SDS-PAGE. That implies that the surface properties (charge and hydrophobicity) were different for each gliadin type, and the molecular sizes, at least for the α-, β-, and γ-gliadins, were very similar. These separations and correlations point out the usefulness of independent methods.

The bands ab and u, which were unique to the poor quality line, were briefly looked for in other poor baking quality wheats without success. This points out the necessity of repeating this work with true isogenic lines, so that any differences can be related to quality.

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