Separation and Characterization of Wheat Protein Fractions by High-Performance Capillary Electrophoresis[1]

GEORGE LOOKHART[2] and SCOTT BEAN[3]

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ABSTRACT

Wheat protein fractions, separated by the Osborne Solvent Fractionation Procedure, were characterized by high-performance capillary electrophoresis (HPCE). Each fraction was separated on a 27-cm fused-glass capillary (20 μm, i.d.) using 0.1*M* phosphate buffer (pH 2.5) containing hydroxypropylmethylcellulose, a polymer additive, at 45°C and 22 kV constant voltage. Albumins and globulins migrated in the first 4 min, whereas gliadins and glutenins migrated after 4 min. Individual α, β, γ, and ω gliadin proteins, which were collected from reversed-phase high-performance liquid chromatography (RP-HPLC) separations, were also separated by HPCE. Combined results of this study and our previous studies provide a catalog of individual gliadin information from HPCE, HPLC, acid-polyacrylamide gel electrophoresis (A-PAGE) and sodium dodecyl sulfate (SDS)-PAGE, relating class, relative molecular size, hydrophobicity, relative charge, and separation times of each gliadin subclass by HPLC and HPCE. The main advantages of HPCE are: 1) the complementing of other electrophoretic and chromatographic protein separation methods, and 2) safety because no toxic acrylamides and only minute amounts of organic solvents and buffers are used.

The baking quality of wheat flour is primarily dependent on the quantity and quality of the flour proteins (Finney 1943). As most of the proteins in flour are gluten, at least one gluten component should relate 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 comprise about half of the gluten and the endosperm storage proteins 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). Glutenins are the other major component of gluten and endosperm storage proteins. Numerous studies (Bietz et al 1975; Payne et al 1981, 1984; Shewry et al 1989; Graybosch et al 1990; Dong et al 1991) over the past 20 years have established that allelic variation among high molecular weight glutenin subunits (HMW-GS) is a major contributor to the mixing and baking quality of bread wheats. Breadmaking quality has also been related to allelic variation among low molecular weight glutenins (Gupta and Shepherd 1987).

High-performance capillary electrophoresis (HPCE) recently has been used to identify wheat cultivars by characterizing gliadin protein patterns (Werner et al 1994, Bietz and Schmalzreid 1995, Lookhart and Bean 1995a). Gliadins were previously divided into four classes: α, β, γ, and ω (Jones et al 1959), based on electrophoretic migration. Lookhart and Albers (1988) were the first to analyze each gliadin HPLC peak (25 peaks) of one cultivar, and relate each peak to acid polyacrylamide gel electrophoresis (A-PAGE) and sodium dodecyl sulfate (SDS)-PAGE patterns. Some individual proteins that migrate within the range of these classes have also been identified by reversed-phase high-performance liquid chromatography (RP-HPLC) of gliadins extracted from genetic stocks (Bietz and Burnouf 1985). The HPLC assignments as to gliadin class elution order by Lookhart and Albers (1988) and Bietz and Burnouf (1985) were both made by inference to the assignments of Jones et al (1959) and were in agreement. HPCE, which separates mainly by the same principles (combination of size and charge) as A-PAGE but has the automation and convenience of RP-HPLC, also provides rapid high-resolution separations of gliadins (Lookhart and Bean 1995a). To take full advantage of the high resolution of HPCE, knowing the order of migration for the respective proteins could prove useful in the study of gliadins and other components and their relationships to quality.

Werner et al (1994) analyzed one representative protein from each gliadin class by HPCE using an aluminum-lactate buffer (pH 3.1) in conjunction with a charge reversal coating (MicroCoat) to determine the migration order of the respective gliadin classes. The MicroCoat system separated proteins against the electroosmotic flow (EOF), causing the fastest migrating proteins to appear last on the electrophoregram. The phosphate buffer separation system described for rapid differentiation of wheat cultivars (Lookhart and Bean 1995a) was much faster than that of Werner et al (1994).

This article thus reports, for the first time, HPCE separations of the classical Osborne wheat protein fractions (albumins, globulins, gliadins, and glutenins) and of several proteins from each of the four classes of gliadins using a low pH phosphate buffer system. Results from the HPCE analyses of gliadins were related to RP-HPLC, A-PAGE, and SDS-PAGE analyses (Lookhart and Albers 1988) of the same proteins.

MATERIALS AND METHODS

Wheat Flour Samples

The hard red winter (HRW) wheat Shawnee was obtained from the Hard Winter Wheat Quality Laboratory in Manhattan, KS. It was grown in 1981 at Manhattan, KS; it had a protein content of 18.1%. Flour was produced by a Brabender Senior experimental mill.

Osborne Fractionation

Albumin extractions. Shawnee flour (100 mg) was sequentially extracted by the Osborne procedure (Osborne 1907) with modifications. The flour was extracted with deionized water (500 μl) for 30 min, vortexing for 1 min, every 10 min. The mixture was centrifuged for 5 min at 2,000 rpm on an Eppendorf 5415C minicentrifuge with an 18-place rotor (F-45-18-11). The supernatant

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[2]Research chemist, USDA/ARS, U.S. Grain Marketing Research Laboratory, 1515 College Ave., Manhattan, KS 66502. E-mail: george@crunch.usgmrl.ksu.edu

[3]Research assistant, Department of Grain Science and Industry, Kansas State University, Manhattan.

(albumin extract) was decanted and saved as ALB-1. The centrifugate was vortexed with 400 µl of deionized water for 1 min, then set for 5 min and centrifuged as before. The decanted supernatant was saved as ALB-2. The centrifugate was vortexed again for 1 min, then set for 5 min with 400 µl of deionized water and centrifuged. The supernatant was poured off and saved as ALB-3.

Globulin extraction. The pellet from ALB-3 was then extracted with an aqueous solution of 0.5*N* NaCl (400 µl) for 30 min, vortexed for 1 min, at 10 min intervals. The salt solution mixture was centrifuged for 5 min at 2,000 rpm on the mini-centrifuge. The supernatant (globulin extract) was decanted and saved as GLOB-1. The centrifugate was vortexed with 400 µl of 0.5*M* NaCl for 1 min, then set for 5 min and centrifuged as before. The supernatant was saved as GLOB-2, and the centrifugate was vortexed with another 400 µl of 0.5*M* NaCl for 1 min, then set for 5 min and recentrifuged. The supernatant was saved as GLOB-3, and the centrifugate was vortexed with deionized water (400 µl) for 1 min, then set for 5 min, centrifuged, and the supernate discarded. This additional wash was made with water to reduce the effect of the salt in the centrifugate for the extraction of gliadins in the following steps.

Gliadin extraction. The water-washed centrifugate from GLOB-3 was extracted with 70% aqueous ethanol (400 µl) for 30 min, vortexed for 1 min every 10 min, and centrifuged for 5 min at 2,000 rpm on the mini-centrifuge. The supernatant (gliadin extract) was decanted and saved as GLI-1. The centrifugate was vortexed with 400 µl of 70% ethanol for 1 min, then set for 5 min and centrifuged as before. The supernatant was saved as GLI-2 and the centrifugate was vortexed with 400 µl of 70% ethanol for 1 min, then set for 5 min and centrifuged. The supernate was poured off and saved as GLI-3.

Glutenin extraction. Glutenins were extracted from the gliadin centrifugate (pellet) with 400 µl of 50% 1-propanol + 1% dithiothreitol (DTT) for 30 min, vortexed for 1 min every 10 min. After centrifugation for 5 min at 2,000 rpm on the mini-centrifuge, the supernatant (glutenin extract) was decanted and saved as GLU-1.

Glutenin precipitation. An aliquot (300 µl) of the glutenin extract (GLU-1) was removed and combined with 75 µl of 100% 1-propanol + 1% DTT. This mixture was vortexed and stored at 4°C for 60 min to precipitate the HMW-GS (Marchylo et al 1988) and aid in purifying the subunits. The solution was centrifuged, decanted, and the precipitated glutenins (GLU-P) were resuspended (30-60 min were needed) with 100 µl of a solution containing 50% 1-propanol in 0.082*M* Tris-HCl (pH 7.5) containing 2*M* urea + 1% DTT (Marchylo et al 1988).

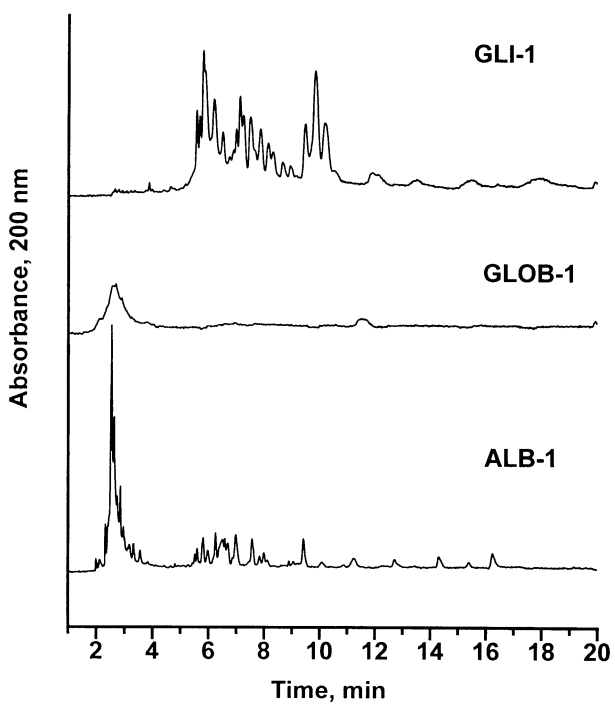

Fig. 1. High-performance capillary electrophoresis (HPCE) patterns of wheat albumins (ALB-1), globulins (GLOB-1), and gliadins (GLI-1) of hard red winter cultivar Shawnee. Protein fractions extracted consecutively with water, 0.5*N* NaCl, and 70% ethanol. Pressure-injection (20 sec, ≈1.2 nl for albumin and globulin extracts; 4 sec, ≈0.25 nl, for gliadin extracts) into a 27-cm (20 cm to detector) 20 µm i.d. capillary. Separation at 45°C and 22 kV during 10 min using 0.1*M* (pH 2.5) phosphate buffer containing a polymeric additive (hydroxypropylmethylcellulose). Detection by UV absorbance at 200 nm.

Non-Osborne Fractionation

Gliadin extraction. To utilize the previous comparison among HPLC, A-PAGE and SDS-PAGE (Lookhart and Albers 1988), gliadins were also extracted from Shawnee flour (250 mg) with 70% ethanol (750 µl) for 1 hr at room temperature. Samples were filtered through a 0.45-µm filter (Lida Mfg. Corp., Kenosha, WI) before analysis. Extracts were analyzed within 24 hr.

HPLC

A Hewlett-Packard 1090 liquid chromatograph equipped with a Vydac C18 reversed-phase column was used to analyze 10-µl aliquots of the 70% ethanol (gliadin) extract of Shawnee flour. The HPLC procedure and column (Vydac RP column 218TP54 at 1 ml/min) were the same as used previously (Lookhart and Albers 1988) for Shawnee gliadins. A new Vydac RP column 218TP54 was also used to check the loss of resolution of the old column. The column used in 1988 was used to ensure the same separation mode (order of elution), even though the resolution had declined since 1988. Gliadins were eluted by a multistep linear gradient at 65°C, starting at 25% CH_3CN and 75% water each containing 0.1% trifluoroacetic acid (TFA). The CH_3CN concentration increased to 30% at 5 min, to 40% at 55 min, to 100% at 56 min, and then returned to the initial condition (25% CH_3CN) at 58 min. The total runtime from injection to injection was 68 min, including a 10-min reequilibration step between runs.

Gliadins were separated by HPLC from three separate extractions. Each peak was manually collected three to five times for each extract. Collected fractions of each peak were combined and lyophilized overnight and then redissolved in 100 µl of 70% ethanol. A Hewlett-Packard 1040A diode-array detector was used to detect the eluted components at 210 nm (0.500 absorbance units full scale). A data point was stored every 640 msec on a Hewlett-Packard (9000-300) computer for subsequent integration, replotting, and comparison. All samples were analyzed within four days of extraction.

A-PAGE and SDS-PAGE

A-PAGE and SDS-PAGE methods and migration data on Shawnee gliadins were taken from Lookhart and Albers (1988).

Capillary Electrophoresis

A Beckman P/ACE 2100 was used for all HPCE analysis. All separations were performed at 22 kV and 45°C in 20-µm i.d. uncoated fused-silica capillaries (Polymicro Technologies) that were 27 cm in length (20-cm separation distance) (Lookhart and Bean 1995a). Capillary cleaning procedures were as previously described (Lookhart and Bean 1995b). All samples and reagents were filtered through 0.45-µm filters (Lida Mfg.). The HPCE buffer was 0.1*M* (pH 2.5) phosphate buffer with a polymeric additive, hydroxypropylmethylcellulose (HPMC), (BioRad 148-5011, Hercules, CA).

HPCE injection times varied for samples due to the relative amounts of proteins and the viscosities of the extractant. Pressure injection times for the albumins and globulins were 20 sec (1.2 nl) each. Pressure injection times for the gliadins were 4 sec (0.25 nl). Pressure injection times for the glutenins (GLU-1) and precipitated

HMW-GS were 10 sec (0.63 nl) each. Only nanoliters of samples were injected and only microliters of buffers were used per sample. Proteins were detected by UV absorbance at 200 nm. Beckman Gold software accumulated the absorption data at a sampling rate of 5 Hz and produced ASCII files for post analysis and presentation by ORIGIN software (MicroCal Software, Northampton, MA). Total analysis time including pre- and post conditioning was 26 min.

RESULTS AND DISCUSSION

HPCE of Protein Fraction Extracts

The HPCE patterns of the first albumin (ALB-1), first globulin (GLOB-1), and first gliadin (GLI-1) extract are shown in Figure 1. In ALB-1, all the major peaks eluted between 1 and 4 min. Peaks steadily decreased in height from ALB-1 to ALB-3. The only peak found in the ALB-3 extract-rinse was at 4 min (data not shown). That peak was water-soluble, absorbed at 200 nm, was extracted from the flour as its amount declined with the number of extractions, and it was not found in the solvent blank.

The GLOB-1 HPCE pattern (also shown in Fig. 1) exhibited large poorly resolved peaks migrating between 1 and 3 min. The broad peaks may be due to the lack of stacking because the globulins were extracted with 0.5*N* salt and the buffer was 0.1*M* phosphate. Stacking occurs when the injected sample has lower ionic strength than the buffer, causing sample resistance and field strength to increase in the sample plug, which in turn causes the ions to migrate rapidly and sharpen and concentrate at the leading zone of the higher ionic strength buffer (Hames and Rickwood 1981). A small peak occurred at 4 min in GLOB-1 that was nearly nonexistent in GLOB-2 (data not shown) and was absent in GLOB-3 (extraction rinses not shown). This 4-min broad doublet

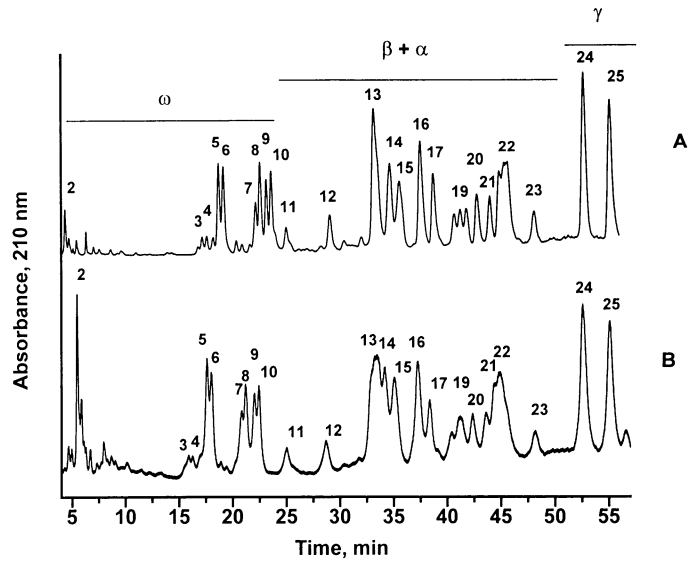

Fig. 3. High-performance liquid chromatography (HPLC) pattern of wheat gliadins extracted with 70% ethanol by the non-Osborne fractionation method from the hard red winter cultivar Shawnee. Gliadin ethanol extracts (10 µl) analyzed using the HPLC procedure and column (Vydac RP column 218TP54 at 1 ml/min) as previously reported by Lookhart and Albers (1988) for Shawnee gliadins. Elution conditions involved a multistep linear gradient at 65°C; starting at 25% CH_3CN and 75% water, each containing 0.1% trifluoroacetic acid (TFA), increasing CH_3CN concentration to 30% at 5 min, then to 40% at 55 min, to 100% at 56 min, and then returned to 25% CH_3CN at 58 min. Detection by UV absorbance at 210 nm.

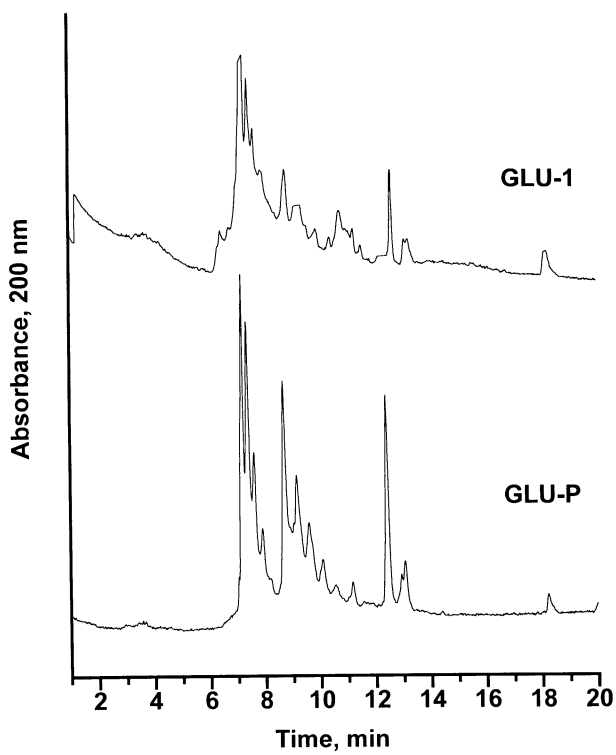

Fig. 2. High-performance capillary electrophoresis (HPCE) patterns of wheat glutenins and precipitated glutenins from the hard red winter cultivar Shawnee. Glutenins (GLU-1) extracted from the pellet, following gliadin extractions with 50% 1-propanol plus 1% dithiothreitol (DTT). Glutenin subunits were then precipitated (GLU-P) by adding 1-propanol, centrifuging, decanting the supernatant, and resolubilizing the precipitate in 50% 1-propanol plus 1% DTT. Extracts pressure-injected (10 sec each, ≈0.63 nl). Other conditions as in Fig. 1.

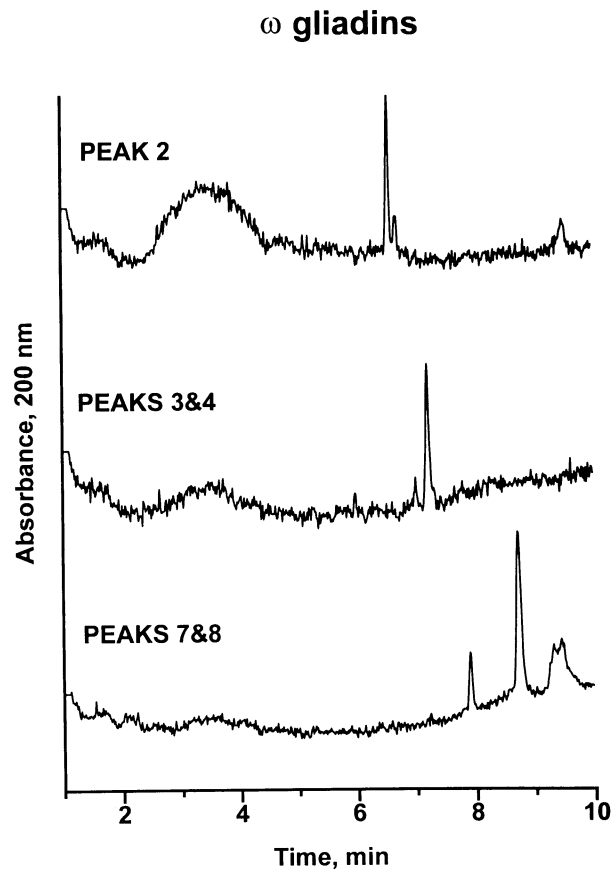

Fig. 4. High-performance capillary electrophoresis (HPCE) patterns of Shawnee gliadin high-performance liquid chromatography (HPLC) peaks 2, 3&4, and 7&8 collected from HPLC separation in Fig. 3. Gliadin peaks pressure-injected for 4 sec (≈0.25 nl). Other HPCE conditions as in Fig. 1.

peak did not appear to be the same as the single sharp peak in the albumin extracts at 4 min. HPCE migration positions for albumin and globulin extracts agreed with those recently reported (Shomer et al, *in press*).

The GLI-1 HPCE pattern in Figure 1 exhibited major peaks between 4 and 8 min. Minor peaks also occurred from 1 to 3 min; however, unlike the globulins, they were fairly sharp. Expansion of the 1–3 min range of GLI-1 also showed that those peaks differed from the typical albumins (ALB-1, Fig. 1) (data not shown). The GLI-1 pattern had its largest peak at 6 min and showed good resolution of all major peaks. The GLI-2 pattern (data not shown) appeared identical to GLI-1, except the intensities were about one fifth that of GLI-1. The GLI-3 pattern showed essentially no peaks (data not shown).

The area under the peaks of the HPCE patterns of the albumin extracts ALB-1, ALB-2, and ALB-3; the globulin extracts GLOB-1, GLOB-2, and GLOB-3; and the gliadin extracts GLI-1, GLI-2, and GLI-3 were determined and used to calculate the relative efficiencies of extraction or the relative amount of proteins in each extraction. In the consecutive extraction method used in this study, 71% of the albumins were extracted into the first water rinse (indicated by the relative percent of total albumin area). Another 26% were found in the second rinse, and only 3% were found in the third rinse.

The first salt extraction picked up 97% of extractable globulins, leaving only 3% for the second extract, and none for the third. The first ethanol extraction picked up 80% of the gliadins, leaving 20% in the second extraction, and less than 1% in the third.

HPCE patterns of the glutenin extract (GLU-1) and the precipitated glutenins (GLU-P), which migrated in the 4–9 min region (similar to the gliadins), are shown in Figure 2. The HPCE pattern of GLU-P exhibited peaks similar to the GLU-1 pattern, but were sharper and better resolved. It is of interest that the major peaks in the GLU-1 extract pattern were also the major peaks in the GLU-P pattern. HPCE analyses of the supernatant of GLU-P found small amounts of early migrating components (4–5 min), but none that coincided with the major peaks in GLU-P (data not shown). SDS-PAGE of the GLU-P fraction showed lower molecular weight components were present along with the HMW-GS (data not shown). The propanol precipitation of GLU-1 was used to help concentrate the subunits and reduce the background noise. SDS-PAGE analysis of similar extracts showed small amounts of low molecular weight glutenins present (data not shown), as well as ω-gliadins (Marchylo et al 1988). These additional proteins were probably the additional peaks seen in the GLU-P extract. Identification of each HMW-GS (2*, 5, 6, 8, 10) by this technique was not possible, but research in this area continues.

Identifying HPCE Gliadin Peaks.

Shawnee gliadins separated by HPLC using a new column (3A) and an old column (3B) are shown in Figure 3 for comparison. The gliadin HPLC patterns provided by both columns were similar and indicated that a loss of resolution had occurred. However, the proteins were still separated in roughly the same positions and order. The center (middle half) of each numbered HPLC peak was collected separately from three replicate analyses using the old column; they were combined if identical and analyzed by HPCE.

Migration times of 6.8–10 min were found in the HPCE patterns of HPLC collected peaks 2, 3&4, and 7&8 (Fig. 4). Those HPLC peaks were previously determined to be ω-gliadins by Lookhart and Albers (1988). HPLC collected peaks 5&6 and 9&10 are not shown, but they migrated in the same time range

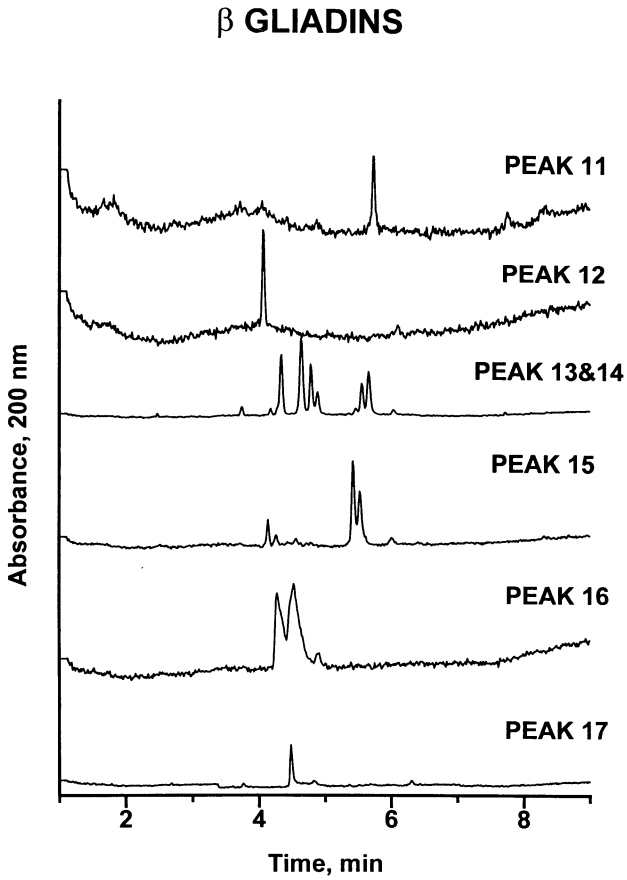

Fig. 5. High-performance capillary electrophoresis (HPCE) patterns of Shawnee gliadin high-performance liquid chromatography (HPLC) peaks 11, 12, 13&14, 15, 16, and 17 collected from HPLC separation in Fig. 3. Gliadin peaks pressure-injected for 4 sec (≈0.25 nl). Other HPCE conditions as in Fig. 1.

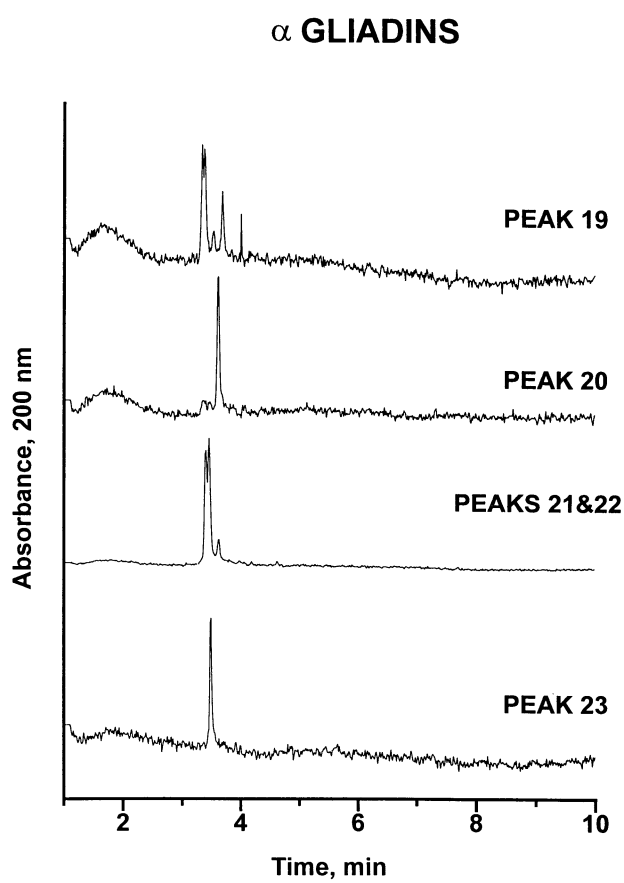

Fig. 6. High-performance capillary electrophoresis (HPCE) patterns of Shawnee gliadin high-performance liquid chromatography (HPLC) peaks 19, 20, 21&22, and 23 collected from HPLC separation in Fig. 3. Gliadin peaks pressure-injected for 4 sec (≈0.25 nl). Other HPCE conditions as in Fig. 1.

(6.8–10 min). The HPCE patterns of the HPLC collected peaks 11, 12, 13&14, 15, 16, and 17 are shown in Figure 5. They are ß-gliadins according to the nomenclature of Jones et al (1959) and the characterization of Lookhart and Albers (1988). They have HPCE migration times of 4–6 min. HPLC peaks 13&14, 15, and 16 were separated into multiple peaks by HPCE. The HPCE conditions provided better resolution for these ß-gliadins and, therefore, complemented RP-HPLC in characterizing gliadins. A-PAGE patterns of those collected peaks also showed multiple proteins from each HPLC peak (Lookhart and Albers 1988). Therefore, it was not just the old HPLC column that contributed to the resolution differences noted.

Figure 6 shows the HPCE patterns of the HPLC collected peaks 19, 20, 21&22, and 23. They were classified as α-gliadins (Lookhart and Albers 1988) with HPCE migration times of 3–4 min. HPLC peak 19 was separated into multiple peaks by HPCE, again showing the complementary nature of the methods. HPCE patterns for HPLC collected peaks 24 and 25 exhibited migration times of 5–6 min (Fig. 7). They were classified as γ-gliadins by Lookhart and Albers (1988). Note that HPCE analyses of HPLC peaks 24 and 25 produced single peaks, whereas SDS-PAGE of those HPLC peaks that were collected and then reduced, showed the individual γ-gliadins plus the HMW-GS (2*, 5, 6, 8, 10) for Shawnee (Lookhart and Albers 1988).

Overall, the migration order of gliadins was the same in HPCE as in A-PAGE (Lookhart and Albers 1988). These HPCE assignments also agree with those of single purified gliadins (HPCE) as determined by Werner et al (1994) and with the genetic assignments (HPLC) by Bietz and Burnouf (1985). The α-gliadins move the fastest and, therefore, migrate the earliest (3–4 min). These are followed by the β-gliadins (4–6 min), γ-gliadins (5–6 min), and ω-gliadins (6.8–10 min). There is some overlap between β-gliadins and γ-gliadins. This may be because open breaks in A-PAGE patterns were used to subjectively assign gliadin classes (Jones et al 1959). Sequence analysis (Bietz et al 1977, Bietz 1982) later showed that this nomenclature, although useful for some purposes, does not show true interrelationships among proteins of these classes.

The HPLC peak numbers, previously related to the classical gliadin A-PAGE nomenclature and SDS-PAGE molecular weights by Lookhart and Albers (1988) were compared with HPCE migration times (Table I). Thus, typical HPCE, HPLC, and A-PAGE mobilities and SDS-PAGE molecular weights are now available for each gliadin class; gliadin types may reasonably be assigned from mobilities in any of these separation modes. The ω-gliadins exhibited slightly slower migration times in the presence of the other gliadins. Comparison of the HPLC pattern of the Shawnee gliadin extract with the HPCE pattern of the same extract, labeled with the gliadin classification and HPLC peak numbers, are shown in Figure 8.

CONCLUSION

HPCE of wheat proteins is fast, reliable, highly resolving, and automatable. It is complementary to HPLC, A-PAGE and SDS-PAGE, and it can provide a means to characterize and understand the building blocks of wheat quality. The results presented in this

TABLE I
High-Performance Capillary Electrophoresis (HPCE) and High-Performance Liquid Chromatography (HPLC) Comparisons for Gliadin Protein Separations of Shawnee Flour

Gliadin Type	A-PAGE[a] Mobility (Rm)	SDS-PAGE[a] Molecular Size (kDa)	Separation Time (min)[b] HPLC	HPCE
α	73–85	35–38	38–48	3–4
β	47–70	37–43	22–38	4–6
γ	43–47	37–46	48–56	5–6
ω	12–40	48–63	6–22	6.8–10

[a] Data from Lookhart and Albers (1988). A-PAGE and SDS-PAGE = acid and sodium dodecyl sulfate polyacrylamide gel electrophoresis, respectively.
[b] Specific to the conditions used in this study.

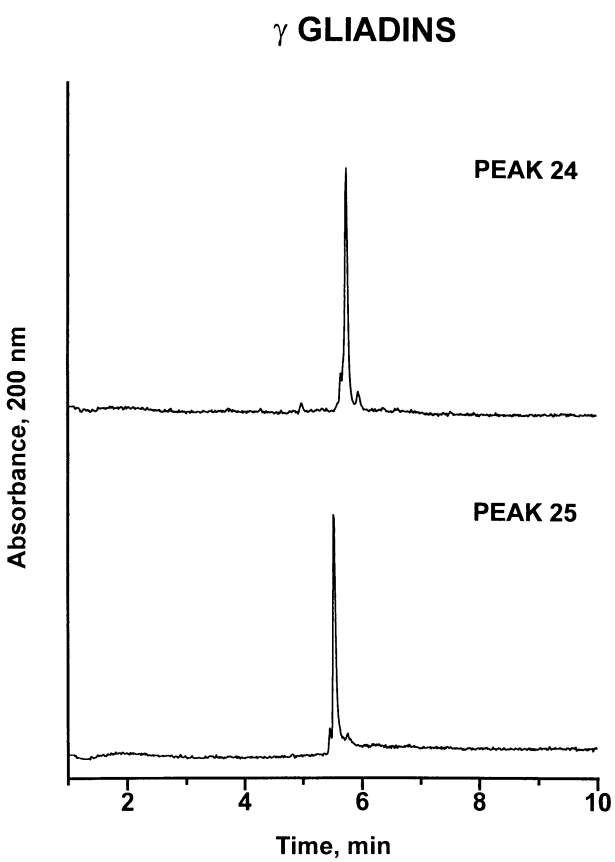

Fig. 7. High-performance capillary electrophoresis (HPCE) patterns of Shawnee gliadin high-performance liquid chromatography (HPLC) peaks 24 and 25 collected from HPLC separation in Fig. 3. Gliadin peaks pressure-injected for 4 sec (≈0.25 nl). Other HPCE conditions as in Fig. 1.

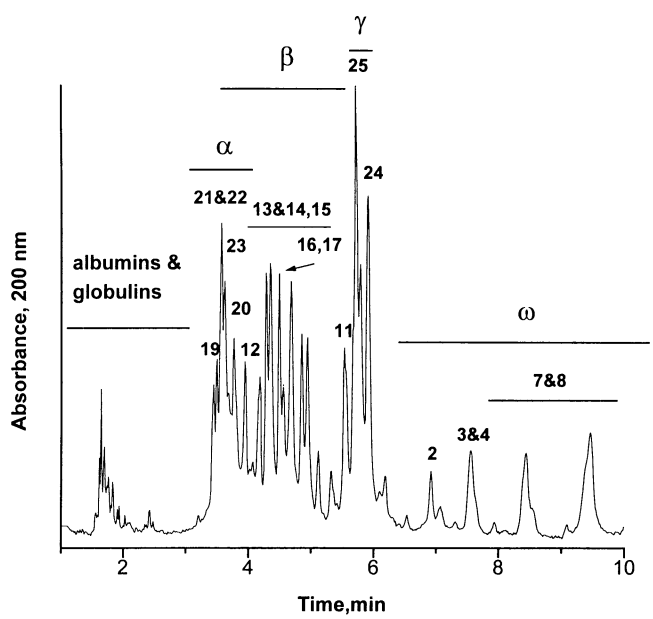

Fig. 8. High-performance capillary electrophoresis (HPCE) pattern of Shawnee gliadins, extracted with 70% ethanol, by the non-Osborne fractionation method. Gliadins peaks are labeled with respective gliadin class, high-performance liquid chromatography peak number. Regions are line coded. HPCE gliadin pattern produced using the procedures of Fig. 4.

article show the complementary nature of HPCE with HPLC and, therefore, also A-PAGE. Most single HPLC peaks were resolved into single HPCE peaks, but in HPLC, peaks 13&14, 15, 16, and 19, multiple HPCE peaks were found. Multiple bands were also found in most HPLC peaks originally detected by A-PAGE (Lookhart and Albers 1988).

This report extends the work of Werner et al (1994) where a single purified gliadin of each class was analyzed to identify migration order. The order reported was opposite what is reported here, as a charge reversal approach was used to separate the gliadins. The present procedure was faster and resolved the proteins better than the method of Werner et al (1994). Thus, HPCE is complementary to the gliadin HPLC results reported by Bietz and Burnouf (1985) and by Lookhart and Albers (1988). Taken together, the techniques allow genetic assignments of bands (Bietz and Burnouf 1985) and an understanding of the size and charge relationships of gluten proteins published by Lookhart and Albers (1988).

HPCE is faster than the HPLC method used here (however, the total analysis time is similar to the total analysis time reported by the fast HPLC method of Lookhart et al 1991) and provides automated and quantitative analyses of individual protein samples that are faster than the other electrophoretic methods described in this article. HPCE is more expensive than other electrophoretic methods, but it is much safer, requiring very small amounts of organic solvents and buffers and no toxic acrylamides!

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