Research Note

Wheat Varietal Identification by Capillary Electrophoresis: an Inter-Laboratory Comparison of Methods*

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(Received March 27, 1996; accepted April 22, 1996)

Capillary electrophoresis (CE) is a rapid automated method for wheat protein analysis. It is sensitive, reproducible, and gives high resolution separation of gliadins, differentiating most genotypes. Optimal CE conditions have not yet been established, however, nor methods compared between laboratories. We therefore analysed gliadins from several varieties by two methods in two laboratories using comparable CE systems. A commercial 0.1 mol/L phosphate buffer, pH 2.5, containing a linear hydrophilic polymer, was used with uncoated 27 cm silica capillaries (20 cm from inlet to detector) of 50 and 20 μm i.d. Separations with 50 μm capillaries were performed at 7 kV and required 30–40 min; those with 20 μm capillaries were performed at 22 kV, and separations took about 10 min. In each laboratory, both methods gave excellent separations, with comparable selectivity and resolution. For some analyses (especially those using 50 μm capillaries), however, elution times and operating currents varied with different batches of commercial buffers, giving unacceptable reproducibility. Thus, while CE is a useful alternative to slab gel electrophoresis or high performance liquid chromatography for wheat varietal identification, CE buffer compositions must be carefully controlled to ensure acceptable reproducibility.

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Keywords: wheat; proteins; gliadin; capillary; electrophoresis

Introduction

Capillary electrophoresis (CE) is a valuable new method for wheat protein analysis (1–5). CE is rapid, sensitive, and automated, and gives high resolution separations (6). Analyses of extracted gliadins readily differentiate genotypes, permitting varietal identification using various buffer systems (2,3,7). Protein fractions such as albumins, globulins, gliadins and glutenins can be characterized quickly and with high resolution (3). When the inside diameter (i.d.) of the capillary is reduced to 20 μm, 10 min analyses are possible (6). CE is thus a potentially valuable alternative to gel electrophoresis and reversed-phase high-performance liquid chromatography for varietal identification.

For CE to become widely used, however, good reproducibility is essential. As a test of reproducibility between laboratories, samples were analysed in two locations using the same methods. We found, as described in this note, that comparable results can be achieved, but slight differences among lots of commercial buffers can cause unacceptable variation in CE migration times.

Materials and Methods

Samples

Flours from ten hard red winter wheat varieties (81-406, 81-402, TAM 105, TAM 107, Larned, Sage, Eagle, Triumph, Triumph 64, and Triumph Improved) were obtained from a Quadrumat Senior experimental mill (Brabender Instruments, Inc., South Hackensack, NJ) (6). Gliadins were extracted from the flours with 300 m/L aqueous ethanol; gliadins so extracted were qualitatively and quantitatively similar to those extracted with 700 m/L ethanol, and gave better CE separations (2,6). Extracts were filtered (0.8 μm) before analysis. Gliadin extracts of all varieties were extensively analysed, but results for TAM 107 were representative of those for all varieties. Thus, this note shows detailed results only for the variety TAM 107.

Capillary electrophoresis

Samples were analysed at USDA-ARS laboratories in Peoria, IL and Manhattan, KS on comparable Beckman® 2100 P/ACE systems with Gold Software. Uncoated fused silica capillaries (Beckman) were 27 cm long (20 cm inlet to detector), and 50 or 20 μm inside diameter (i.d.). The buffer, from Bio-Rad®, was 0.1 mol/L phosphate, pH 2.5; this buffer contains a linear cellulosic hydrophilic polymer (hydroxypropyl methyl cellulose, HPMC). In later studies, a comparable pH 2.5, 0.1 mol/L sodium phosphate buffer [1.839 mL of concentrated phosphoric acid and 8.47 g of sodium monobasic phosphate (molecular weight 120) to 1 L], containing 0.500 g/L HPMC (Aldrich, viscosity of 20 g/L solution 4000 cp), was prepared. Samples were pressure injected for 1 s (50 μm i.d. capillary) or 4 s (20 μm i.d. capillary). Separations on 50 μm capillaries were performed at 7 kV for 40 min; analyses on 20 μm capillaries at 22 kV took 10 min. Operating voltages were determined from Ohm’s Law plots to ensure that heat dissipation was adequate. Capillary temperature was maintained at 40 °C, and proteins were detected at 200 nm. Using these conditions, an average coefficient of variation between runs of 0.35 was reported for peak elution times in the Peoria laboratory (2), and relative standard deviations of 0.1 to 0.2% were reported for peak migration times over 20 runs at the Manhattan laboratory using the pH 2.5 phosphate buffer plus 5 g/L HPMC described above and also containing 200 mL/L acetonitrile (8).

Results and Discussion

In a previous study, comparable CE separations of gliadins were achieved on 20 and 50 μm i.d. capillaries in ca. 10 and 25 min, respectively (see Fig. 2 in ref. 6). Smaller (5–10 μm i.d.) capillaries have been tried but were too easily occluded, and therefore are not used. Thus, in the present study, gliadins were analysed on 20 and 50 μm i.d. capillaries in both laboratories.

Figure 1 shows 10 min CE separations (20 μm i.d. capillaries) of proteins extracted and analysed in both laboratories by the same procedure from subsamples of the same lot of TAM 107 flour. These electrophoreograms exhibit extremely similar resolution, elution times, and peak heights. For example, elution times of corresponding peaks (especially up to 7 min) differ by an average of only ±0.030 min between electrophoreograms. Nevertheless, later-eluting peaks show greater inter-laboratory differences in elution time. CE separations of TAM 107 gliadins performed on 50 μm i.d. capillaries in the two laboratories are shown in Fig. 2. Resolution and selectivity of the overall separations were comparable, and most matching peaks were easily identified. Corresponding peaks, however, showed great and unacceptable differences, of as much as several minutes, in elution times. Such differences seemed most likely due to (a) different capillaries, (b) different lots of buffer, or (c) different instruments, even though part numbers and sources were identical.
Thus, capillaries and buffers were interchanged between laboratories, and analyses were performed to determine the source of variation shown in Fig. 2. Representative results from these analyses of TAM 107 gliadins are shown in Fig. 3. When the Peoria capillary and buffer were tested on the Manhattan instrument (Fig. 3b), results were similar to those at Peoria (Fig. 3a), showing that the two instruments yield comparable data. (Little is yet known about resolution and reproducibility of gliadin separations on other commercial CE instruments. We anticipate, however, since CE is inherently a simple method, that any instrument with suitable temperature control and voltage stability should give results comparable to those we achieved with Beckman® 2100 P/ACE systems.) Even under these conditions, however, slight differences in elution times of late-eluting peaks were apparent, suggesting that minor variation between different CE instruments may be expected. Less variation was found with small-diameter capillaries and short analysis time (Fig. 1). Substituting the Manhattan buffer for the Peoria buffer, however, led to significant decreases in retention times (compare Fig. 3c to 3b), showing that different lots of the commercial buffer used varied sufficiently in composition, pH, and/or viscosity to cause major differences in migration times. These studies also showed (compare Fig. 3c and 3d) that retention times on capillaries of the same nominal length and inner diameter may differ significantly. We found, however, that resolution and selectivity of separations of the same samples were nearly identical between laboratories, even when migration times varied due to the buffer or column.

In this study, CE separations using 50 μm i.d. capillaries (Fig. 3) differed far more between laboratories than those performed on 20 μm i.d. capillaries (Fig. 1). The reason for this is not totally apparent, but differences are larger for later migrating peaks. Heat dissipation was adequate, since voltages were determined from Ohm’s Law plots. Perhaps capillaries of certain diameters or from specific sources may be more uniform than others. (Recent unpublished studies at the Manhattan laboratory have identified another potential problem in separating gliadins by CE on 20 μm uncoated fused silica columns — the source and nature of the capillary. For reasons not yet apparent, capillaries from certain suppliers sometimes vary significantly in performance from lot to lot, some giving inferior and unacceptable resolution.) Possible factors for differences in migration times include, but are not limited to, high voltage power supply standardization, temperature of buffers and the surrounding environment, and the actual temperature within the capillary. Small differences in any of these factors could cause differences in electroelution times that would be larger for later-eluting components. Buffering capacity of the solvent might also be greater for small capillaries, giving better reproducibility. This matter needs further investigation.

In subsequent studies, we prepared and tested homemade buffers comparable to the commercial one used. Our results suggest that best results are achieved by preparing relatively large volumes of buffer using only gravimetric and volumetric procedures (not adjusting pH) to achieve maximum consistency, since even slight variation in final pH or ionic strength may cause major differences in elution times (Figs 2 and 3). Our studies also suggest that use of relatively fresh buffers is important — buffers were stored in a refrigerator, and aliquots for anodic or cathodic buffers were replaced at least daily. Alternatively, with commercial buffers, use of a single lot number is strongly recommended throughout a series of analyses. Under such conditions, acceptable inter-laboratory reproducibility can be achieved, and CE can become a widely-used, dependable method for varietal identification.

Conclusions

Our results confirm that CE gives excellent separations of wheat gliadins which can readily differentiate varieties. Analyses performed in 10 min on 20 μm i.d. capillaries had resolution equivalent to that of 40 min separations on 50 μm i.d. capillaries, and were less sensitive to differences between buffer lots or capillary columns that caused migration times to vary. Smaller capillaries exhibited superior resolution because of better heat dissipation, reducing band spreading.
Our results clearly show that batch-to-batch variation among lots of pH 2.5 0.1 mol/L phosphate buffer can cause major differences in peak elution times of gliadins. Care is required to ensure constant buffer composition. When comparable CE buffers are used, however, acceptable inter-laboratory reproducibility is achieved, confirming the validity and value of CE for wheat varietal identification. Results suggest that cultivar identification by CE, as by other methods, may best be accomplished through pattern recognition rather than on the basis of absolute electroelution times. Normalization of results through use of suitable internal standards might also increase the robustness and transferability of the method.

References


