Effect of Temperature and Alternate Lactate Buffer Systems on Resolution of Wheat Gliadin Proteins by Polyacrylamide Gel Electrophoresis[1]

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ABSTRACT

Polyacrylamide gel electrophoregrams of wheat gliadins at different constant gel temperatures and in sodium and aluminum lactate buffer systems were compared in terms of band resolution, band curvature, and electrophoresis time. Electrophoresis of gliadins was improved in both the sodium and aluminum lactate systems by reducing the temperature from 21° C to 7 or 10° C. No major differences in resolution or time were found between the two systems at constant voltage (290 v) and 10° C. Minor differences were noted, whereby one gel system gave better resolution on particular proteins but the other system resolved other proteins better.

Polyacrylamide gel electrophoresis (PAGE) of wheat gliadins is widely used for wheat varietal identification (see review by Wrigley et al 1982, Khan et al 1983), because most cultivars yield unique gliadin electrophoretic patterns; comparing the patterns of unidentified with those of known cultivars is usually sufficient for identification.

In most commonly used gliadin PAGE methods (Bushuk and Zillman 1978, Autran et al 1979, Lookhart et al 1982, Wrigley et al 1982, and Khan et al 1983), electrophoresis is done at 20–30° C. Tkachuk and Mellish (1980) described an electrophoresis method that used high voltage and 7° C. In an attempt to decrease band irregularities due to temperature effects, we studied the effects of lowering the electrophoresis temperature on band resolution, band curvature, and the length of time needed to complete electrophoresis. We also compared the protein separations obtained with gels prepared with and run in sodium lactate and aluminum lactate buffer systems.

MATERIALS AND METHODS

Preparation and Extraction of Wheat Samples

Wheat samples (~3.0 g) of various pure cultivars were ground and their gliadins extracted with 70% ethanol (Lookhart et al 1982).

Electrophoresis

The cultivars examined in this study were Marquis, Luke, Marfed, Pioneer S-76, Eagle, and TAM W-101. They were previously shown to be correctly identified and free of contamination by other wheat cultivars (Jones et al 1982). Electrophoresis was done in triplicate in an EC-470 (EC Corp., Clearwater, FL) vertical gel electrophoresis apparatus (180 × 120 × 6 mm) with temperature controlled at 4, 7, 10, or 21° C by a Forma Scientific 2006 water bath. The preparation of aluminum lactate PAGE gels, the electrophoresis equipment, the fixing and staining of gels, and photographic methods were described by Lookhart et al (1982). Destained gels were placed in a refrigerator at 4° C for 2 days before photographing, which makes the bands crisper. Sodium lactate (98% minimum purity) was obtained from Sigma Chemicals as a 60% syrup.

The sodium lactate buffer and gels were made by a modification of the procedure of Wrigley et al (1982). The sodium lactate gels were the same as the aluminum lactate gels, except that each 500 ml of solution contained 0.8 g of sodium lactate syrup (60%) in place of aluminum lactate. Each 2 l of electrophoresis buffer solution contained 0.8 g of sodium lactate (60% syrup) and 2.40 ml of lactic acid, giving a final pH of 3.1.

RESULTS AND DISCUSSION

A typical aluminum lactate PAGE gel prepared and run (at 21° C) by the method of Lookhart et al (1982) is shown in Figure 1. This electrophoresis method (5¼ hr at 21° C) has been used to accurately identify the 88 most commonly grown U.S. wheat cultivars (Jones et al 1982). Gliadins extracted from the cultivar, Marquis, were placed in slots 1, 4, and 8, and extracts from the cultivars, Luke, Marfed, Pioneer S-76, Eagle, and TAM W-101 were put in slots 2, 3, 5, 6, and 7, reading from left to right. Marquis is a hard red spring wheat, Luke and Marfed are white wheats, Pioneer S-76 is a soft red winter wheat, and Eagle and TAM W-101 are hard red winter wheats. These varieties have complex gliadin electrophoretic patterns and represent all of the major wheat classes except durums. All of the commonly grown durum wheats yield electrophoregrams with fewer bands (Jones et al 1982). Band resolution of a given gel was deemed acceptable if the two bands of the doublet found in the center of the Marquis standard (Bushuk and Zillman 1978, Lookhart et al 1982) electrophoregram were resolved. Two other bands ("X", Fig. 1) with mobilities slightly less than the Marquis doublet were not well resolved, and the fastest moving bands in all slots were slightly curved. (The protein in the center of a particular band tended to move slightly faster than that towards the edge.) Even so, results are repeatable enough that computerized pattern recognition can identify cultivars (Lookhart et al 1983).

The curvature across the gel among the faster moving bands was reduced and resolution was increased by reducing the gel temperature during electrophoresis and increasing the electrophoresis time. Representative gliadin electrophoregrams of six wheat cultivars (all in 6% polyacrylamide gels) after electrophoresis at 10, 7, and 4° C are shown in Figures 2, 3, and 4, respectively. Gels electrophoresed at 7 and 10° C have gliadin bands showing improved resolution, sharper definition, and less curvature than the corresponding bands of the gel run at 21° C. The lighter staining of the bands in Figure 3 (7° C) shows the actual resolution of the darker bands in Figure 2. The relative position of protein bands in the electrophoregrams of a given variety was nearly identical at 7 or 10° C and very similar to those at 21° C. To obtain similar band separations, electrophoresis had to be continued for longer periods when lower temperatures were used—thus, electrophoresis at 10, 7, and 4° C required 6-½, 7, and 8 hr, respectively. Electrophoresis at 4° C, however, had several drawbacks: The gel consistently pulled

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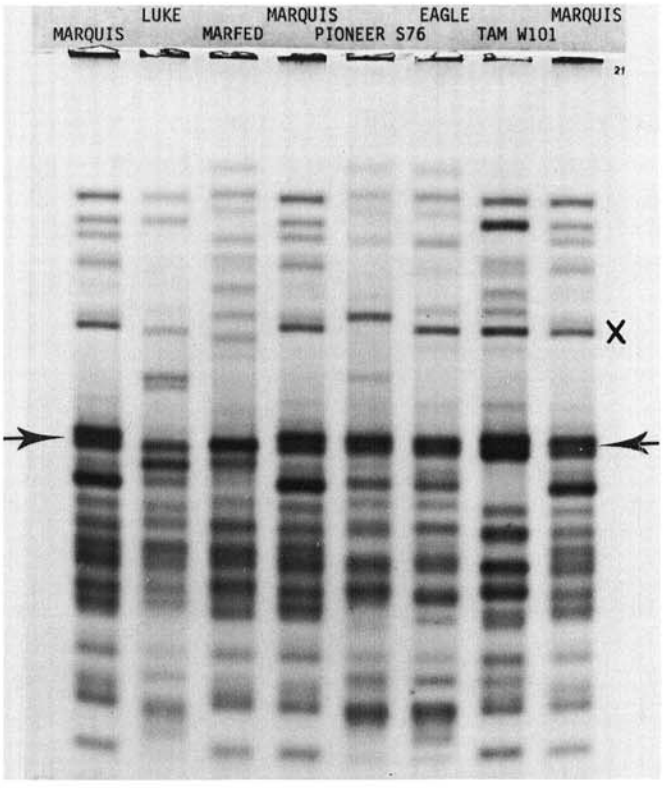

Fig. 1. Aluminum lactate PAGE of wheat gliadins run at 21° C. Arrows indicate Marquis doublet bands. "X" denotes a particular set of Marquis bands.

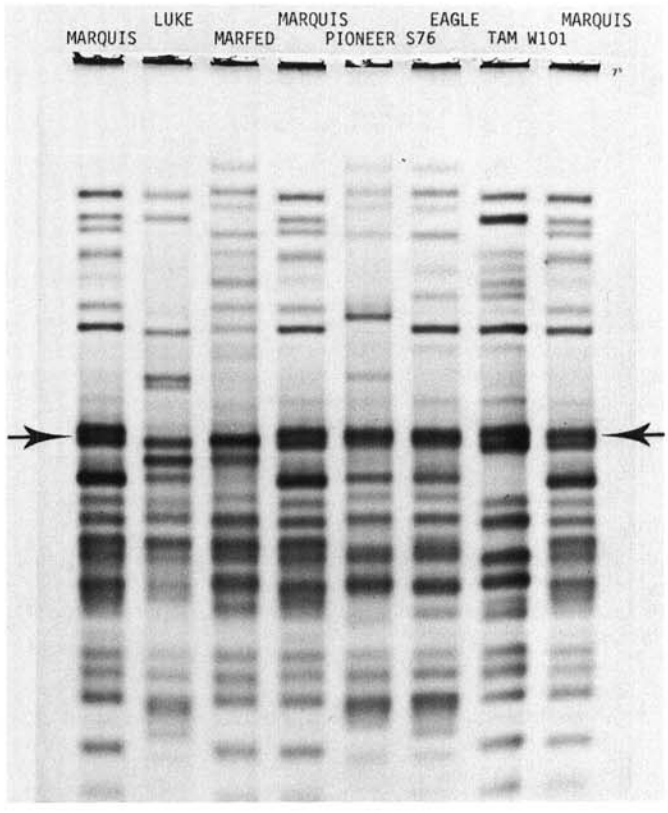

Fig. 3. Aluminum lactate PAGE of wheat gliadins run at 7° C. Arrows indicate Marquis doublet bands.

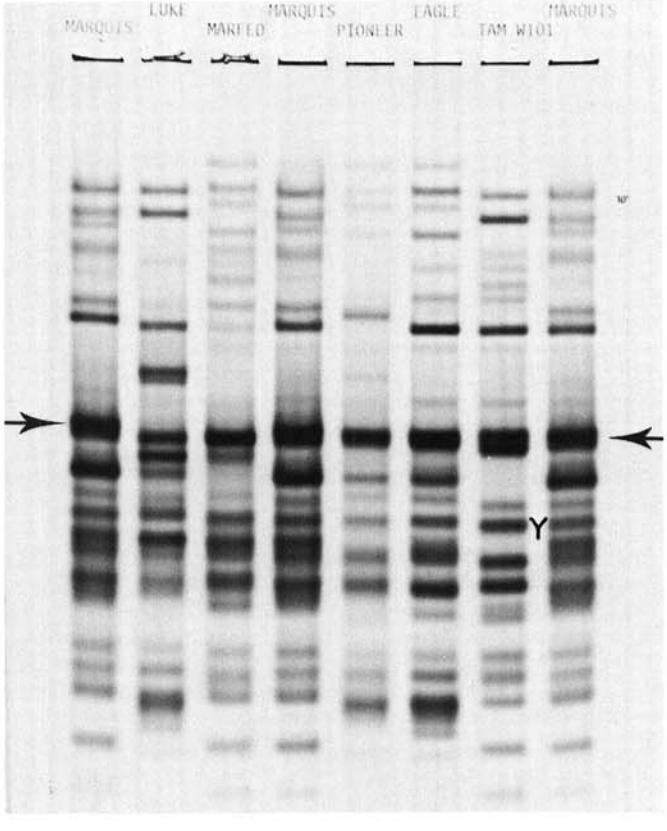

Fig. 2. Aluminum lactate PAGE of wheat gliadins run at 10° C. Arrows indicate Marquis doublet bands. "Y" denotes particular TAM W-101 bands.

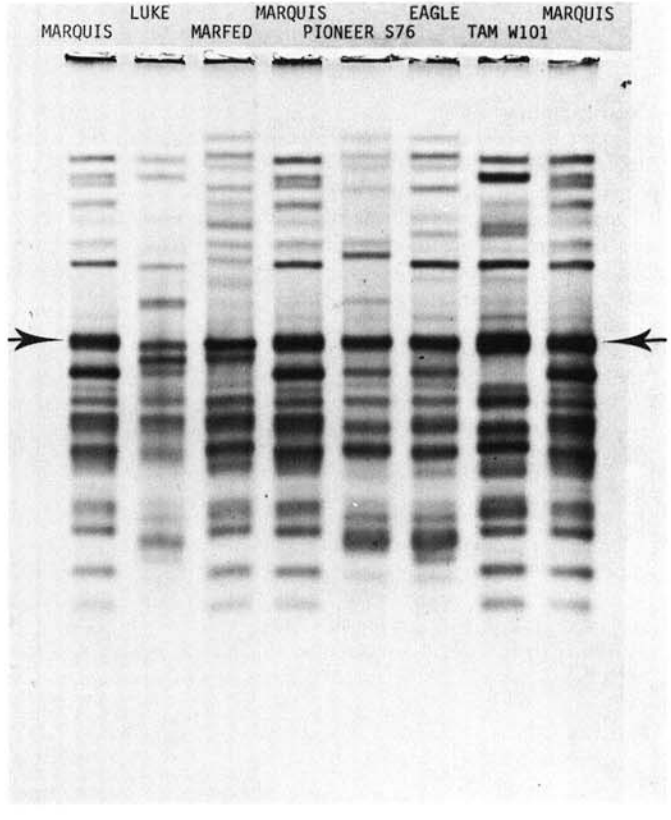

Fig. 4. Aluminum lactate PAGE of wheat gliadins run at 4° C. Arrows indicate Marquis doublet bands.

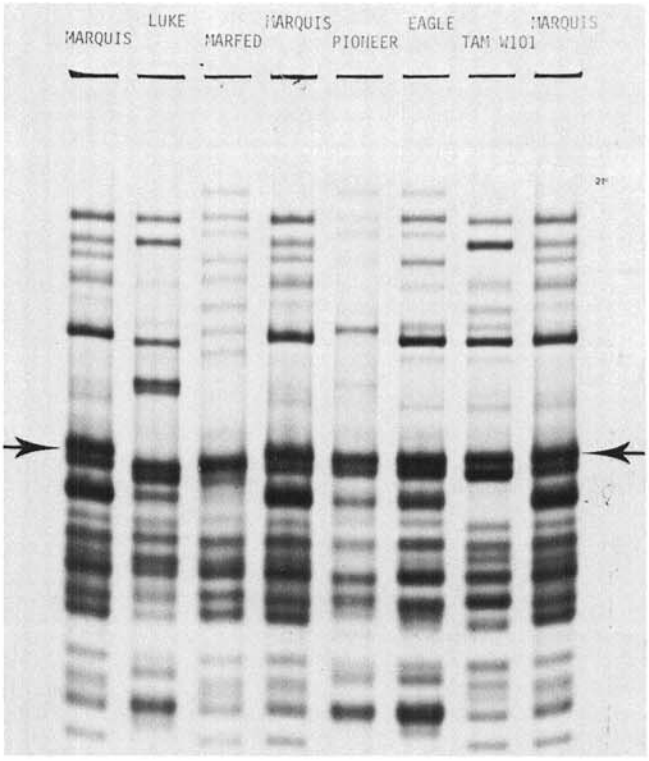

Fig. 5. Sodium lactate PAGE of wheat gliadins run at 21° C. Arrows indicate Marquis doublet bands.

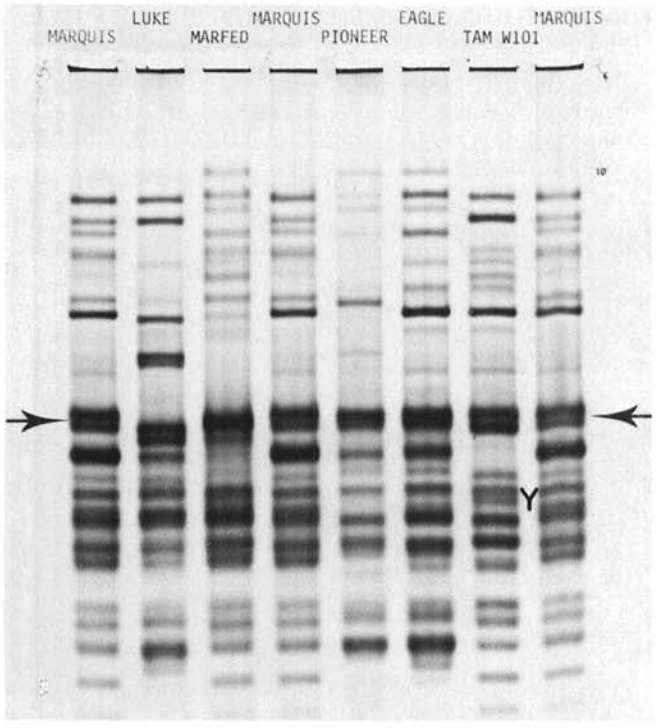

Fig. 6. Sodium lactate PAGE of wheat gliadins run at 10° C. Arrows indicate Marquis doublet bands. "Y" denotes particular TAM W-101 bands.

away from the apparatus during a run, and the time required for maximum band resolution exceeded that of a normal work day. The lower temperatures decreased the current and increased resistance. Because metals and liquids normally decrease resistance with decreasing temperature, the resistance of the gel must increase rapidly with decreasing temperature. For these reasons, electrophoresis at 4° C was not acceptable.

Visual comparison of the bands marked "X" in Figure 1 with the corresponding bands in Figures 2, 3, and 4 shows the improvement in resolution of lower temperatures for bands with lower mobility. The electrophoregrams for TAM W-101 (Figs. 2, 3, and 4) show a similar effect for the lower-mobility bands and an opposite effect for the higher-mobility bands. Gliadin bands from electrophoresis at 7 or 10° C were nearly equally resolved and sharply defined (Figs. 2 and 3, respectively). Analysis at 10° C was preferred, however, because the separation required less time and bands were as sharp and as well-resolved as at any other temperature tried.

Sodium Lactate vs Aluminum Lactate Buffers

The ability to consistently produce sharp, well-resolved protein bands in gels depends, in part, on reagent purity (Lookhart et al 1982, Khan et al 1983). One major problem has been the availability of pure "white" aluminum lactate (Lookhart et al 1982, Khan et al 1983); however, this has not been a problem during the last three years. Sodium lactate has been suggested as a suitable substitute (Wrigley et al 1982). Figures 5 and 6 are representative pictures of gliadin extracts electrophoresed on sodium lactate polyacrylamide gels at 21 and 10° C, respectively. The samples were loaded into slots, as in Figures 1–4.

At 21° C, however, aluminum lactate PAGE resolves gliadins better over the entire gel than does sodium lactate PAGE; curvature of the bands is noticeable in both systems.

At 10° C, aluminum lactate PAGE resolves protein bands in the top and lower thirds of the gel better than does the sodium lactate PAGE; in the middle third of the gel, however, both systems are almost equal. In the center of the cultivar, Luke's electrophoregram (e.g., aluminum lactate) gave better resolution of the doublet than did sodium lactate, but in TAM W-101, sodium lactate gave better resolution of the middle doublet and the Y doublet than did aluminum lactate.

Gels run at 21° C in the presence of sodium lactate showed some bending of the faster moving protein bands along the sides of the gel, but those run at 10° C did not, as was also noted for aluminum lactate gels. Resolution of the "Marquis doublet" (arrows on all figures) in sodium lactate gels at 10° C (Fig. 6) was better than that obtained in aluminum lactate gels at either 7 or 10° C (Figs. 2 or 3), but that might be a visual artifact due to staining intensities. Sodium lactate gels required less time for gliadin separations—4½ hr at 21° C (Fig. 5) and 6 hr at 10° C (Fig. 6). The sodium lactate gels consistently drew 20 milliamps more current at constant voltage (290 v), than did the corresponding aluminum lactate gels. The higher current demands lower resistance in the gel, which can be due only to the difference in the ionic strengths of the gel and buffers. Because certain proteins are better resolved by aluminum lactate and other proteins by sodium lactate, changes in relative mobilities may actually describe differences in interaction of the proteins with the sodium or aluminum lactate complexes.

In summary, for optimal results, PAGE analysis of wheat gliadins should be conducted at a temperature of 7–10° C, regardless of whether the buffer is aluminum lactate or sodium lactate.

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