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Simple Starch-Gel Electrophoresis of Gliadin Proteins Using the LKB-Multiphor System

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Einfache Stärkeelektrophorese der Gliadinproteine mit Hilfe des LKB Mulitphor-Systems

Zusammenfassung. Es wurde eine einfache Modifikation der horizontalen Stärkeelektrophorese der Gliadinproteine mit Hilfe des LKB Mulitphor-Systems entwickelt. Das Dünnschicht-Stärkegel wurde mit Hilfe der handelsüblichen Gelform vorbereitet, wodurch die ganze Prozedur einfacher wurde. Die Empfindlichkeit ist mit der Originalmethode vergleichbar. Unsere Modifikation erweitert die Anwendungsmöglichkeiten des LKB Multiphor-Systems.

Summary. A simple modification of the horizontal starch-gel electrophoresis of gliadin proteins with the use of LKB Multiphor System has been developed. The thin-layer starch gel is prepared by using the original gel-moulding cassette and the procedure on the whole is easier. The sensitivity is comparable with that of the original method. Our modification moreover widens the field of application of the LKB Multiphor System.

Abbreviations: PAGE – polyacrylamide-gel electrophoresis, SGE – starch-gel electrophoresis, SDS – sodium dodecyl sulphate, REM – relative electrophoretic mobility.

Introductions

Gliadins and other cereal prolamins (zein, hordein and secalin) are very soluble in 50–70 per cent ethanol, urea or SDS. The pattern of gliadin bands can be used for identifying wheat varieties or for distinguishing the various cereals in flour or in food preparations.

The separation of gliadin proteins has been performed with the use of starch [1–3] and polyacrylamide [4–7] gel electrophoresis as well as by electrofocusing in the latter medium [8, 9]. The original SGE method of Woychik [1] has been repeatedly modified. Acrylamide does not polymerize in aluminium lactate buffer of low pH value and low ionic strength if the common catalysts are used. The catalytic system of Jordan [10] or Narayan [11] should be applied under these conditions. On the whole, gliadin separation with the use of PAGE is more laborious and requires more experience.

We introduce a simple method of horizontal SGE of gliadin proteins with the use of a commercial apparatus LKB-Multiphor. The method is based on the SGE modification described by Šašek [3, 13]. A slightly modified commercial gel moulding cassette can be used. The gel need not be sliced before staining and its manipulation, including drying and documentation, is easier.

Materials and Methods

1 Gel preparation

Eight g of starch hydrolysed for electrophoresis (ELFAMYL-Czechoslovakia) is suspended to 72 ml of 0.85-m*M*-Al lactate buffer with 3-*M*-urea to prepare a gel layer (265 × 120 × 1 mm). The suspension is mixed by continous swirling whilst it is heated over a naked flame. The heating is continued until the mixture becomes thick and viscous, then for about 20 sec after thickening. Before pouring the mixture into the gel-moulding cassette, mounted as shown in Fig. 1, gas is removed by vacuum application. The gel is ready for use after a 3-day stabilization at 4 °C.

2 Electrolytes

Gel buffer (0.85-m*M*-Al lactate, 3-*M*-urea): 0.25 g of aluminium lactate (SPOFA-Czechoslovakia) and 180 g of urea are dissolved in distilled water and the pH value is adjusted to 3.1 with concentrated lactic acid.

Tank buffer (2.6-m*M*-Al lactate, 1-*M*-urea): 0.75 g aluminium lactate and 60 g urea are dissolved in distilled water and the pH value is adjusted to 3.1. Usually 5 ml of 90 per cent lactic acid is sufficient. Both buffers are diluted with distilled water to the final volume of 1 litre.

3 Sample preparation and application

Ethanolic extracts of 0.15 g of crushed grain, 0.10 g of flour or 0.02 g of lyophilized gliadin fractions were made by suspension in 1 ml of 65% ethanol. The mixture was left overnight and then centrifuged for 10 min at 5,000 g to remove undissolved material. These samples are applied to strips (3 × 10 mm) of Whatman No. 4 chromatographic paper. The quantity of sample may be increased by repeated application with subsequent drying of the strip using a hair dryer. Methylene green (5 µl of 0.1% solution in 70% ethanol) is used to mark the front of the run.

The gel-containing cassette is laid on a level pad. The rubber gasket and then the plastic foil cover are carefully removed. The cooling plate is wetted with a few ml of a 0.1% Triton X-100 solution to ensure a good thermal contact. A thin glass plate with the gel is placed on the cooling plate without trapping air. Six to eight slits for application of the sample strips are produced in a row approximately

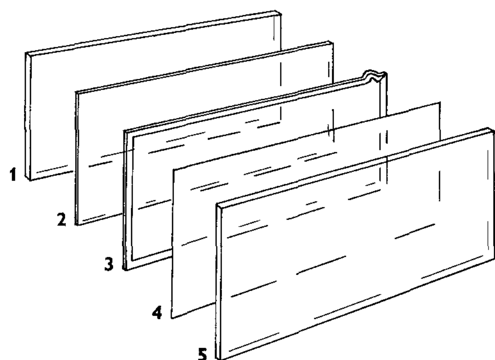

Fig. 1. Schematic representation of gel-moulding cassette for starch-gel electrophoresis. – *1,5* = glass plates 125 × 260 mm, 3 mm thick, *2* = glass plate 125 × 260 mm, 1 mm thick, *3* = rubber gasket, *4* = plastic foil 125 × 260 mm with hydrophilic side facing to the gel

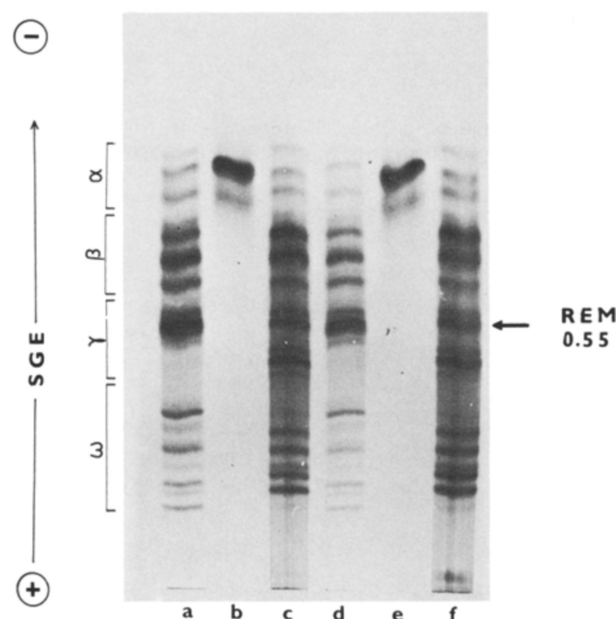

Fig. 2 a–f. Starch-gel electrophoresis of ethanol extract of wheat grains (Mironovskaja 808): **a, d.** – commercial gliadin preparation Mann Res. Labs. USA: **c, f.** – α-gliadin separated after the method of Charbonnier [12]: **b, e.** – Conditions of the run are given in Material and Methods

30 mm from the anodic side of the gel with a razor blade. Paper bridges made of 4 layers of Whatman No. 4 paper are used to contact the gel with the tank buffer.

4 Electrophoresis

The sample is concentrated for 30 min at 300 V. Thereafter the paper strips are removed and after 5 min the current is increased to approximately 700 V to achieve a field strength of 15–16 V/cm. The run is carried out for 5 h at a constant temperature of 25 °C (Multitemp-LKB). The front of the run is indicated by the migration of the methylene green.

Table 1. Relative amounts and the REM-values of α-, β-, γ- and ω-gliadins

Group	REM values[a]	%[a]	REM values[b]
Alpha	0.77–0.97	9–16	0.77–0.92
Beta	0.62–0.76	30–41	0.60–0.76
Gamma	0.49–0.60	18–30	0.50–0.59
Omega	0.15–0.48	20–35	0.15–0.49

[a] The reported method
[b] The original method of Landry [2]

5 Staining and drying

Staining and fixation is performed overnight in the staining kit using 400 ml of 0.05% nigrosine in 7% acetic acid. The excess dye is removed by 40% methanol, in which the gel is soaked for 1–2 days. The destaining solution may be regenerated with the use of decolourizing carbon (Carbosorb-SEVAC, Czechoslovakia).

To prevent cracking, the gel is immersed in a solution of 15% glycerol and 3% acetic acid for 30 min. A sheet of celophane is wetted in this solution and the gel layer is covered without any remaining air bubbles, and dried at room temperature for 1 to 2 days.

6 Densitometric scanning

The gels were evaluated by means of an intergrating densitometer Vitatron TLD-100 ($\lambda = 675$ nm; slit 2.5 × 0.25 mm) using linear scan at 2 cm/min.

Results

In Fig. 2 the electrophoretic pattern of gliadin from wheat grain *Triticum aestivum* (Mironovskaja 808) is compared, with a commercial product of gliadin (Mann Res. Labs. USA) and with α-gliadin prepared by the method of Charbonnier [12].

Approximately 25 gliadin bands can be distinguished in the samples of raw gliadin. These fractions can be divided into four groups (α-, β-, γ-, and ω-gliadins). The most intense band of the γ-gliadins with a REM value of 0.55 is used as a standard (according to Šašek; 13). Relative amounts of individual gliadin groups and their REM values found by our method and the original SGE method of Landry [2] are compared in Table 1.

Densitometric scans of the raw gliadin proteins from wheat grains and of the purified α-gliadin fraction are given in Figs. 3 and 4.

Discussion

Starch gel electrophoresis has been used for a number of years to identify wheat varieties and to distinguish other cereals. Considering that the original method is difficult and requieres some experience with gel preparation and manipulation, PAGE and its various modifications have been recommended for the same purpose. The differences among these modifications in-

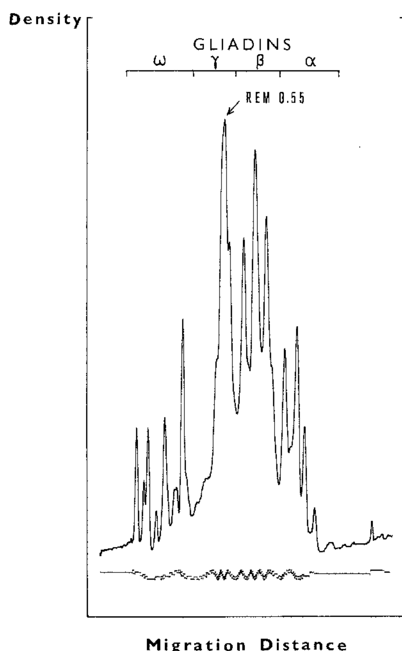

Fig. 3. Density plot of grain extract of gliadins separated by SGE on Multiphor LKB. The most intense band of γ-gliadins with a REM value of 0.55 was used as a standard [13].

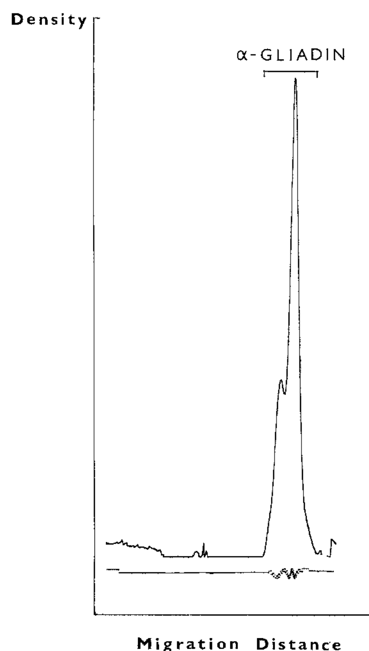

Fig. 4. Density plot of starch-gel electrophoresis of α-gliadin isolated on SP-Sephadex C 50 according to Charbonnier [12]

clude the composition of catalysts and buffers as well as urea concentration. Bushuk [4] prefers hydrogen peroxide in agreement with Jordan [10], whilst Lauriere and Mosse [7] use a catalytic system according to Narayan [11]. Sodium and aluminium lactate are the most frequently used buffers for SGE as well as PAGE of gliadins.

The separation is better with aluminium lactate [14], but the other substance is more easily available. The disadvantages of PAGE involve more difficult storage of the stained gels, due to the diffusion of dye from the colored protein bands, as well as the more complicated procedures of gel dehydration.

The reported modification retains the separation quality of the original SGE method [3], but it is simpler and easier to perform. It is not necessary to slice the gel before staining and drying as well as storage are easy. The densitometric evaluation of the gel layer dried on a glass plate is another advantage. The use of LKB Multitemp ensures a standard temperature, providing a better reproducibility compared to the method according to Autran [15] and others [1–3]. The method presents also another use of the LKB Multiphor System for horizontal electrophoresis.

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