Thin-layer acrylamide gel electrophoresis

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SYNOPSIS A thin-layer acrylamide gel electrophoresis technique suitable for analysis of serum or urine proteins is described in detail. The method gives sharp differentiation of protein fractions and is particularly suitable for the analysis of biological solutions containing proteins in low concentration.

This paper describes a two-phase acrylamide gel electrophoresis technique based on the disc electrophoresis methods described by Davis (1964), Ornstein (1964), and Williams and Reisfield (1964). It is suitable for analysis of as many as 14 serum protein samples at a time with an electrophoretic running time of three hours. The procedure is more economical of materials and simpler than both vertical slab (Raymond, 1964) and disc acrylamide gel electrophoreses. In addition, because of the thinness of the gel used, staining and subsequent background clearing can be achieved rapidly.

Materials and Methods

APPARATUS

1 A water-cooled Laurell type electrophoresis apparatus with accessories for thin-layer agar gel electrophoresis². Accessories consist of (a) a rectangular glass plate of $8 \times 4\frac{1}{4} \times \frac{1}{8}$ in.,

(b) a rectangular plastic slot forming plate of $8 \times 4\frac{1}{4} \times \frac{1}{8}$ in., and (c) a three-sided plastic frame of $\frac{1}{8}$ in. thickness and $\frac{1}{2}$ in. width which when sandwiched between the glass and plastic plates forms a gel space $7 \times 3\frac{1}{4} \times \frac{1}{8}$ in.

2 Pasteur pipettes drawn out to fine points.

REAGENTS

1 Acrylamide (Cyanogum-41 from American Cyanamide Co.), 10% solution in distilled water. Following filtration this solution can be stored at 4°C.

2 TEMED-N, N, N¹, N¹, Tetramethylethylenediamine (Eastman Organic Chemicals).

3 Buffer solutions used included the following:
Running gel (pH 8.9): Tris, 12g (1 M); glycine, 3g (0.4 M); TEMED, 0.3 ml; and distilled water is added to make 100 ml.

Spacer gel (pH 6.7): Tris, 1.2g (0.1 M); glycine, 0.3g (0.04 M); TEMED, 0.6 ml; dissolved in 75 ml of distilled water and adjusted to pH 6.7 with orthophosphoric acid. Final volume is made up to 100 ml with distilled water.

Electrode buffer (pH 8.3)

Stock solution is made up of Tris, 431g (1.8 M); Na₂ EDTA, 37g (0.05 M); boric acid, 220g (1.8 M); and distilled water is added to make final volume of 2 litres.

The working solution is made up of stock solution diluted 20 times before use. The buffers are stored at 4°C.

4 Ammonium persulphate—1% solution in distilled water.

5 Bromophenol blue crystals.

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²Apparatus with accessories obtainable from Metaloglass Inc., Boston, Mass., USA.

Procedure and Results

PREPARATION OF GEL

A sandwich of the glass plate, plastic frame, and the slot forming plate is made so that the teeth of the slot forming plate lie near the open end of the gel forming space. The three components, held together by bulldog paper clamps, are then supported in a vertical position with the open end uppermost. Running gel, made of 21 ml of 10% acrylamide solution, 3 ml of buffer, 1.5 ml of 1% ammon. persulph., and 4.5 ml of distilled water, is stirred vigorously in a small beaker, using a magnetic stirrer for two minutes, and immediately poured into the gel forming space so as to fill it to 0.3 cm below the line of the slot forming teeth. Distilled water is then layered very gently on top of the gel so as to eliminate meniscus formation. The frame is then left undisturbed for 15 minutes to allow polymerization to take place. The water is removed and the inside is dried with narrow strips of Whatman 3MM filter paper. Spacer gel, made of 9 ml of 10% acrylamide solution, 3 ml of buffer, 2.0 ml of 1% ammon. persulph., and 16.0 ml of distilled water, is stirred for two minutes and poured on top of the running gel so as to fill the remaining space. Following polymerization (15-20 min), the sandwich is laid on a flat surface with the slot forming plate uppermost. The slot forming plate is then eased from the frame using a fine spatula.

Fig. 1 Serum protein patterns. Sera samples from six medical patients run on acrylamide gel and stained with amido black. Pertinent diagnoses: 1, Laennec's cirrhosis; 2, normal serum; 3, hypogammaglobulinaemia; 4 and 5, multiple myelomas; 6, post-necrotic cirrhosis.

ELECTROPHORESIS

The gel on the supporting glass plate without samples is placed on the cooled platform of the electrophoretic tank and run for 15 minutes to allow equilibration to take place between gel and electrode buffers. The electric current is turned off and the samples (to each of which a few crystals of bromophenol blue have been added to act as a marker) are placed in the application slots with fine drawn out Pasteur pipettes. A current of three milliamperes per cm width is applied for three hours (precise time of running depends on the migration of bromophenol blue dye attached to albumin). Circulating tap water is used for cooling the electrophoresis tank.

STAINING

Serum proteins

The gel is stained with 1% amido black in 10% acetic acid for one hour. Background destaining of the gel is effected by continuous washing (using a magnetic stirrer) in 7% acetic acid eight to 12 hours (overnight). A permanent record of the protein patterns can be made by direct scanning of each in a densitometer (eg, Chromoscan, Joyce and Loebel Co.) using transmitted light. As an alternative, a high quality black and white photograph of the gel (Polaroid MP camera and 4 × 5 in. type 52 film) can be scanned using reflected light. The results obtained using either of these methods is for all practical purposes identical. The latter procedure has the advantage that a permanent photographic record is available for future reference.

Haptoglobin phenotype

The gel, after electrophoresis, is flooded with 5% (w/v) orthotolidine in 90% acetic acid for 10 minutes and then washed with 1 vol% hydrogen peroxide. Within two to three minutes haptoglobin phenotype patterns appear which reach a point of maximum clarity within 10 to 15 minutes. A photographic record taken at this time can be used for quantitating the various fractions.

Results and Discussion

Figures 1 and 2 show the quality of the patterns obtained using the thin-layer acrylamide gel electrophoresis technique. The patterns are highly reproducible and give more detailed fractionation of serum proteins than is obtainable by use of paper, cellulose acetate, agarose, or starch gel electrophoresis. The improvement is mainly due to the employment of a two-phase system. This allows protein fractions in low concentration to aggregate at the inter-gel boundary (Ornstein, 1964) inducing separation of protein into fine compacted linear bands.

Fig. 2 Serum haptoglobin phenotype patterns. Eight mg Hb% was added to each sample before electrophoresis on acrylamide gel. Phenotypes (left to right) 1-1 (unsaturated), 2-2, and 2-1. The fast moving band in each instance corresponds to the position of ferrihaemalbumin. The intermediate band is free haemoglobin.

This method is very suitable for routine analysis of sera samples in a clinical laboratory setting and can be used as an alternative method to disc electrophoresis (Davis, 1964) for research purposes (Hawiger, Niewiarowski, Gurewich, and Thomas, 1970). This technique has been found to be more sensitive than starch gel for typing haptoglobin phenotypes in the presence of profound hypohaptoglobinaemia (Nandi, Lewis, Jick, Slone, Shapiro, and Siskind, 1970).

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