MICROELECTROPHORESIS OF PROTEINS AND ISOZYMES IN FLAT CAPILLARIES. ANALYSIS OF ISOZYME SPECTRUM IN ISOLATED CELLS

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1. Introduction

Polyacrylamide gel electrophoresis is widely applied in modern biochemical and genetical studies for separating proteins and isozymes [1,2]. The techniques involve polymerization of the gel in tubes or in plates, both variants have their advantages.

Tubes are suitable for assaying microamounts of protein, since the resolution can be increased by changing the tube diameter. However, difficulties arise when comparing the mobility of different samples due to differences arising in the polymerization of the gels at the start in different tubes.

When plates are used the electrophorograms are easier to scan and the polymerization process in the zones of application of the samples is more standard, so that, in general, this variant of the technique is much more convenient for comparing the protein mobilities of different samples. However, since the electrophoresis takes place over a relatively large plate surface, microamounts become difficult to analyse.

In order to combine the advantages of the two variants we propose the use of flat glass capillaries (1 × 2mm, 50 × 100μ and 25 × 50μ).

In the present study this modification was employed for determining the isozymes of single cells

Abbreviation:

LDH, lactate dehydrogenase (EC 1.1.1.27).

2. Materials and methods

Ornstein's method was used for preparing polyacrylamide gel and for carrying out the electrophoresis. The 1 × 2mm tubes were filled by a syringe or micropipette with gel solution in the first variant of electrophoresis; the other two types were filled by placing a drop of gel solution on a glass slide and drawing it into the capillary held at an acute angle to the slide. For the electrophoresis the capillaries were placed in the smaller standard chambers for vertical tube electrophoresis [3]. Rat liver supernatant was obtained by centrifuging 10% homogenates in 0.14 M KCl (Centrifuge Superspeed 40, MSE) at 90,000 *g* for 30 min. The protein concentration was determined according to Kalckar [4]. Mouse kidney supernatants were obtained by centifuging 50% homogenates in distilled water at 20,000 *g* for 1 hr.

Compressed air was used to press the gel out of the 50 × 100μ and 25 × 50μ capillaries into buffer solution for staining. For this purpose the capillary was sealed into a polyethylene tube and compressed air applied at 3 – 4 atmospheres. The proteins were stained with Amido Black 10 B and Coomassie Brilliant Blue [5]. The isozymes of LDH and esterases (EC 3.1.1.1 and 3.1.1.2) were assayed by standard methods [6].

3. Results and discussion

Two variants are described to illustrate the technique.

Fig. 1,2. Spectra of *Drosophila virilis* and *Drosophila texana* esterases obtained by microelectrophoresis in polyacrylamide gel in flat capillaries (1 – 2 mm). In both cases: left, stained with α- and β-naphthyl acetate as substrate; right, with α-naphthyl propionate.

3.1. *Capillaries (1 × 2 mm).*

To determine the minimal amounts of protein detected by electrophoresis in 7.5% polyacrylamide gel, experiments were performed using rat liver supernatant. The supernatant was diluted so that the samples 3.0, 0.6 and 0.1 – 0.05 μg of protein.

The electrophoresis was carried out for 25 min at 1.5 mA per capillary (4 – 5 capillaries simultaneously) with gel buffer, pH 8.4. Staining for protein with Amido Black 10B gave positive results when the sample contained 3.0 and 0.6 μg. With Coomassie Brilliant Blue protein at amounts of 0.1 μg per sample were detected (in this case, however, only 5 – 6 fractions were observed). The results were qualitatively in good agreement with the electrophoretic data obtained earlier in standard tubes [5]. The enzymes (LDH, esterases) are also detectable at lower protein contents (for example, Drosophila larval protein). The high resolution enables the isoenzyme spectra of various small organisms to be compared (at 3 mA for 3 min).

Figs. 1 and 2 show the esterase spectra of *Drosophila virilis* and *Drosophila texana* larvae. The zymograms disclose a number of differences. The technique permits differences in the isozyme spectra of small animals in the course of ontogenesis to be detected. For instance, 20 – 30 eggs per sample are sufficient to analyse the esterases of Drosophila.

3.2. *Capillaries (50 × 100μ and 25 × 50μ).*

Experiments with these two capillary sizes were first made on model samples. Mouse liver supernatant was mixed with sucrose and different amounts were applied to the start of the flat capillary tubes under an MBS-1 microscope. For both types of capillaries a current of 4 mA per capillary was passed for 10 min. The gel strips were stained for LDH.

With samples containing 1000 pg of protein 4 LDH fractions were well distinguished (fig.3). The amounts of sample protein applicable in the analysis were compared with protein content in large neural cells.

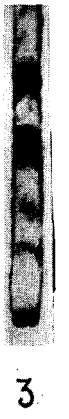

Fig. 3. Spectrum of kidney LDH. Microelectrophoresis in flat capillaries (50 × 100μ). Protein content of sample 1000 pg. Photographed under MBS-1 (USSR) microscope.

Fig.4. LDH spectrum in nerve cells of Deiter's nucleus. Microelectrophoresis in flat capillaries (25 – 50μ). Photographed under MBS-1 (USSR) microscope.

Some investigators have succeeded in separating hemoglobin and LDH of single cells rich in these substances by means of polyacrylamide gel electrophoresis in microtubes [7 – 9].

In view of this we have attempted to determine the enzyme content of single neural cells isolated from the nucleus of Deiteirs by protein electrophoresis in the flat capillaries.

The cells were destroyed in microdrops of saline containing 0.01 M pronase by stirring with a tungsten needle under visual control (MBS-1 microscope). Several sucrose crystals were added to the resulting homogenate and it was transferred by a micropipette into a gel-filled capillary. Electrophoresis was carried out at 5 mA for 3 – 5 min. The gel strips were stained for LDH.

An illustrative zymogram is shown in fig. 4. It follows that the proposed electrophoretic procedure is applicable to the determination of isozymes in single cells.

This technique may be applied in biochemical assays of the heterogeneity of cell populations and for the determination of the biochemical properties of heterozygotes at the cellular level.

3.3. *Possible application of flat capillaries for the separation of hemoglobin from erythrocytes polymerized in gel*

Following the experiments described above, we attempted to apply electrophoresis in flat capillaries to the separation of the hemoglobin constituents of single erythrocytes. For this purpose 0.02 ml of axolotl or human blood was added to a gel mixture prepared according to Raymond [10]. The capillaries (0.3 × 0.1 mm, 3 – 4 cm long) were then filled with this mixture and electrophoresis was carried out at 0.5 – 1.0 mA for 3 – 7 min (V = 1500 – 1800). As figs. 5 and 6 show, hemoglobin of single erythrocytes clearly separates into a number of fractions.

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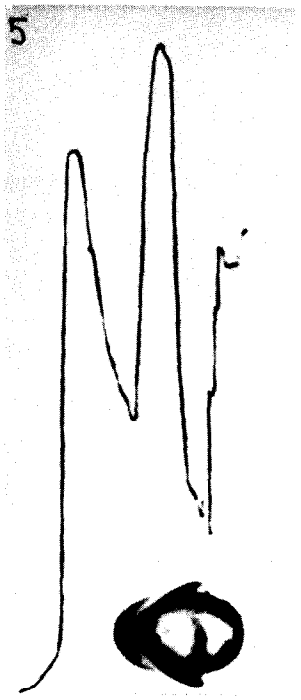

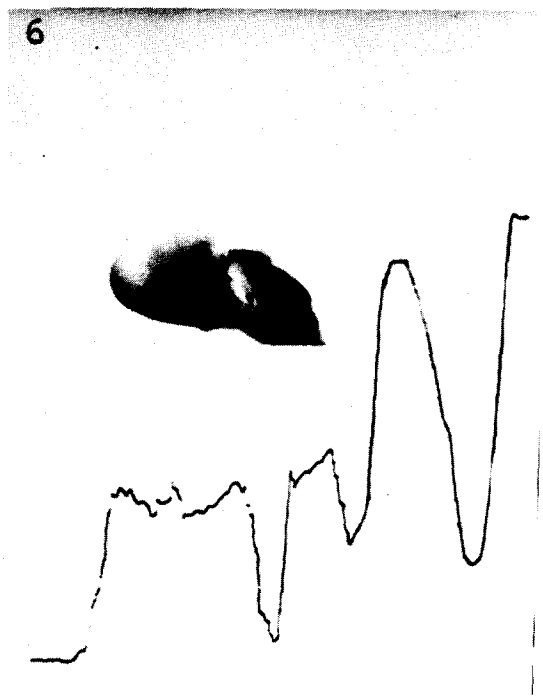

Figs.5,6. Separation of hemoglobin from single axolotl erythrocytes polymerized in polyacrylamide gel. Staining with Coomassie Brilliant Blue. Microphotographs. NU4 microscope.

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