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HIGH-RESOLUTION ELECTROPHORESIS IN A NEW POLYACRYLAMIDE GEL BLOCK

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Electrophoresis is widely used in modern biochemistry and molecular biology for fractionating and analyzing macromolecules. The undisputed merits of this method are its relative simplicity, sensitivity, high resolving power, and universality.

Polyacrylamide gel (PAG) is an exceptionally convenient supporting medium for the electrophoretic separation of proteins and nucleic acids. The chief advantages of PAG are its inertia, transparency, and the possibility of obtaining a gel of assigned pore size, so that high resolution can be achieved for macromolecules of different sizes. However, in homogeneous PAG it is impossible to obtain high resolution for a mixture of different macromolecules, and also of macromolecules with very close values of molecular weight. This problem has largely been successfully solved by Slater [5, 6], who used PAG with a concentration gradient for analysis of serum proteins, and in this way the resolving power of electrophoresis of proteins in this gel was increased to 1000 daltons (over a wide range of molecular weights).

Effective separation of macromolecules in a PAG gradient is based on the gradual slowing of movement of the particles to be separated on account of narrowing of the pores in the gel, as a result of which the molecules for analysis are effectively grouped into narrow zones, for molecules "in the lead" are retarded more strongly than the "laggards" [2]. The conditions of this kinematic focusing can be described formally as follows:

$$\frac{du}{dx} < 0, \tag{1}$$

where u(x) is the velocity of movement of the test **molecules.** The velocity of movement of the separated molecules during electrophoresis can be represented as follows:

$$u = \mu E, \tag{2}$$

where μ denotes mobility and E the intensity of the electric field. It follows from equation (2) that the velocity of movement of the particle during electrophoresis is proportional to its mobility (which is basically a combination of the properties of the supporting medium and the structure of the molecule itself) and the intensity of the electric field. It will be evident that condition (1) can be satisfied, i.e., the macromolecules for separation can be gradually retarded, by changing μ for E. During electrophoresis in a traditional PAG gradient,

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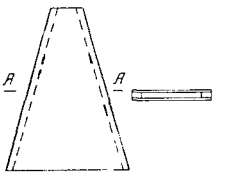
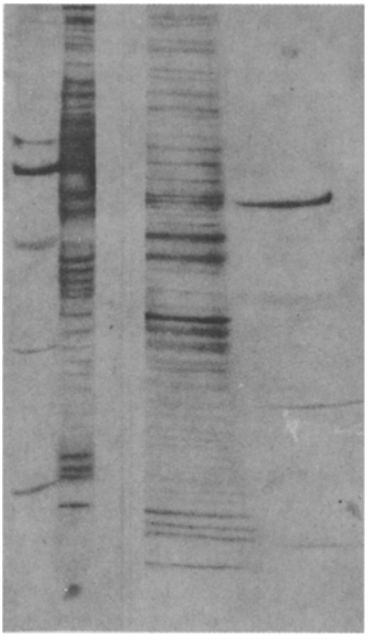


Fig. 1. Diagram of new block for obtaining **trapezoidal** polyacrylamide gel.



A B

Fig. 2. Comparative electrophoresis of cell proteins in ordinary (A) gradient PAG and trapezoidal (B) gradient PAG. Electrophoresis carried out in 7.5-15% PAG gradient with 0.1% SDS. Position of marker proteins (M) indicated: bovine serum albumin 68,000 daltons, ovalbumin 43,000 daltons, chymotrypsinogen A 25,500 daltons, ribonuclease 12,000 daltons. Gels stained with Coomassie R-250.

only a decrease in μ through narrowing of the pores in the gel is used. One other factor still remains, namely the intensity of the electric field.

Previously the writer suggested a new gel block for electrophoresis of macromolecules, whereby electrophoresis could be carried out in an electric field intensity gradient, and also in a PAG concentration gradient and field intensity gradient simultaneously [1].

The aim of this paper is to analyze the resolving power during electrophoresis of polypeptides in PAG in these new blocks.

Electrophoresis of polypeptides was carried out in a slab and in the new trapezoidal PAG blocks with a concentration gradient of 7.5-15% and with 0.1% sodium dodecylsulfate (SDS), using the buffer system suggested by Laemmli [4]. The gels were calibrated by means of a set of repeatedly recrystallized marker proteins from Serva, West Germany: bovine serum albumin with molecular weight 68,000 daltons, ovalbumin – 43,000 daltons, chymotrypsinogen A – 25,500 daltons, and ribonuclease – 12,000 daltons. The gels were stained with 0.5% Coomassie R-250 in a mixture of isopropanol–acetic acid–water (2:1:7). The gels were washed free from excess of the dye in the same solution without the dye. Densitometry of the gels after electrophoresis was carried out on a densitometer (Kipp en Zonen, Holland) at a wavelength of 575 nm.

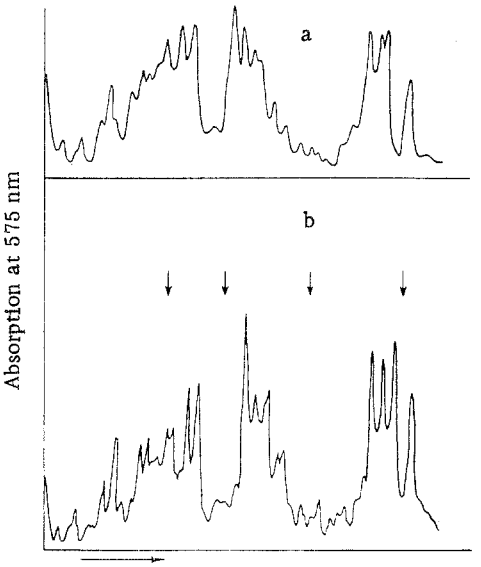


Fig. 3. Comparative densitograms obtained by scanning gels illustrated in Fig. 2: a) electrophoresis of proteins in ordinary PAG gradient, b) electrophoresis of proteins in trapezoidal block with PAG gradient. Vertical arrows indicate positions of marker proteins. Horizontal arrow — direction of electrophoresis. Scanning on densitometer at wavelength of 575 nm.

Cells of human transplantable line HEp-2 were removed mechanically from glass, washed with Hanks' solution, and centrifuged at 3000g for 10 min (the washing procedure was done twice). The washed cells were disintegrated with 1% Triton X-100, 1% sodium deoxycholate in 0.01% Tris-HCl buffer, pH 7.4, and 0.1 M NaCl for 20 min at room temperature, and by five procedures of freezing and thawing. The lysed cells were centrifuged at 10,000g for 15 min. The supernatant was used for electrophoresis. Before electrophoresis, to each sample (15 µl) was added 10 µl of a solution containing 5% SDS, 3% glycerol, and 0.05% bromphenol blue in 0.5 M Tris-HCl buffer, pH 6.8. The samples were heated to 95°C, cooled to room temperature and applied to the prepared PAG. Electrophoresis was carried out with a steady current of 10-15 mA for 6-7 h.

To increase the resolving power during electrophoresis of macromolecules in PAG, a block of gel shaped in longitudinal section like an equal-sided trapezium (Fig. 1) was suggested. Fractionation in such a block is carried out from the shorter to the longer base. Effective fractionation of macromolecules during electrophoresis is achieved by slowing the movement of the components to be separated as a result of a gradual decrease in current density in the gel block. The highest resoltuion for samples to be fractionated can be obtained by the use of a combination of such a gel with a PAG concentration gradient. Gels obtained by comparative electrophoresis of proteins from a lysate of HEp-2 cells, obtained in an ordinary PAG gradient (Fig. 2A) and in a trapezoidal PAG block with the same concentration gradient (Fig. 2B) are illustrated in Fig. 2. Differences in resolving power of the systems analyzed were observed most clearly in the range from 45,000 to 8000 daltons. In this region it was possible to distinguish polypeptides differing by 300-400 daltons (corresponding to two to four amino acids). In the trapezoidal block dispersion of the protein bands was considerably (compared with the traditional "slab") reduced over the whole range of macromolecules separated. Quantitative evaluation of the efficiency of polypeptide separation in the new gel block compared with the traditional are reflected in the densitogram obtained by scanning these tracks (Fig. 3). Comparative analysis of the densitograms shows the substantially higher resolving power of the trapezoidal block of gel and its lower dispersion.

The current level of research requires the introduction of new (or substantial modernization of old) methods of study of macromolecules. Despite the high resolving power of electrophoresis at the present time it cannot always satisfy the research worker. The creation of systems with even higher resolution is thus an urgent task.

The use of gradient PAG to study macromolecules has led to a marked increase in resolving power and also, consequently, in the general standard of research. However, preparation of gradient PAG involves definite additional expenditure, and the gradient obtained is virtually impossible to monitor visually.

An effect similar to that of the gradient gel can be obtained by using our suggested gel block shaped like an equal-sided trapezium in frontal section. In such a gel block a gradient of electric field intensity is produced during electrophoresis, the shape of which is assigned by the angle of inclination of the sides to the base. The necessary field intensity gradient that will be most effective for separating particular macromolecules can thus be selected beforehand. The electric field intensity gradient arising during electrophoresis, according to equation (2), leads to a gradual decrease in the velocity of movement of the separated macromolecules. A combination of such a block with gradient PAG can enable separation of proteins with a difference of molecular weights of 300-400 daltons, and dispersion of the individual protein bands, moreover, is considerably reduced. The new gel block (in conjunction with gradient PAG), according to preliminary data, can be used with success for comparative analysis of proteins subjected to restricted hydrolysis by Cleveland's method [3], for polypeptides with molecular weights in the region of 5000-8000 daltons can be effectively separated in this block. There are definite grounds for considering that resolution of macromolecules of as little as 100-150 daltons (i.e., one amino acid for polypeptides) can be achieved in the trapezoidal gel block in conjunction with gradient PAG (admittedly, within a rather narrow range of molecular weights).

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