A SIMPLE METHOD OF CHOOSING THE SAMPLE VOLUME FOR ULTRAMICROELECTROPHORESIS OF PROTEINS

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Optimal values for the quantity of proteins in samples for ultramicroelectrophoretic fractionation in polyacrylamide gel and capillary tubes of different diameters enabling clear and highly reproducible results to be obtained by electrophoresis were established experimentally. A simple method of calculating the necessary sample volumes for protein solutions of different concentrations and capillary tubes of different diameters by means of a table and formula is suggested.

KEY WORDS: ultramicroelectrophoresis; proteins; isozymes.

Every year more and more research workers use the technique of ultramicroelectrophoresis (UME) in polyacrylamide gel [1-6]. However, the conditions for obtaining stable and high-quality results of electrophoresis have been inadequately studied. For example, the choice of optimal concentration and volume of the protein solution for testing is still made empirically.

The object of this investigation was to study the effect of different protein concentrations on the quality of the results obtained by UME.

EXPERIMENTAL METHOD

UME was carried out by Neuhoff's method [7]. Calibrated glass capillary tubes 0.4, 0.32, 0.2, and 0.1 mm in diameter and from 14 to 54 mm in length, depending on the volume of the sample, were used as containers for the gels. The capillary tubes were filled with measured quantities of solutions of the gels and the specimens on a special apparatus. The duration of UME was 20-30 min. The current during electrophoresis was lowered from 60 μA (at the time of switching on) to 6-10 μA (at the end of UME). The voltage was kept constant at 60-70 V throughout electrophoresis. After electrophoresis the gels were expelled with a stilet from the capillary tubes into a 0.5% solution of Amido Black 10B in 7.5% acetic acid. The protein was stained for 10 min, after which the specimen was washed with 7.5% acetic acid (20 min). To determine the lactate dehydrogenase isozymes, malate dehydrogenase, and acid phosphatase the gels were placed in the corresponding mixtures. The resulting chromatograms were examined in a densitometer and photographed. Human blood serum diluted with 20% sucrose was used as the specimen. The protein concentration was determined refractometrically or by direct spectrophotometry at 210 nm.

EXPERIMENTAL RESULTS

The optimal dilution of proteins for a layer of specimen 2 mm high was obtained with a protein solution in a concentration of 6 mg/ml for all the capillary tubes used in the experiments (Fig. 1). The absolute quantities of protein in the sample calculated in Table 1 were stable values for each diameter of the capillary tube. It will be clear from Table 1 that with a decrease in the diameter of the capillary tube the optimal

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TABLE 1. Optimal Protein Content of Sample for Capillary Tubes of Different Diameters

Diameter of capillary tube (mm)	Optimal protein content (μg)	Specific protein concn. (μg/mm²)	Relative sensitivity of method
0,40	1,507±0,0125	12	1,0
0,32	0,960±0,0080	12	1,6
0,20	0,377±0,0030	12	4,0
0,10	0,094±0,0008	12	16,0

TABLE 2. Optimal Volumes of Samples Depending on Protein Concentration and Diameter of Capillary Tubes

Diam. of capillary tube (mm)	Volume of layer of specimen in capillary tube (μl)											
	C	H	C	H	C	H	C	H	C	H	C	H
	0,2	60	0,4	30	0,75	16	1	12	3	4	6	2
0,10	0,4710		0,2355		0,1256		0,0942		0,0314		0,0157	
0,20	0,8840		0,9420		0,5024		0,3768		0,1256		0,0628	
0,32	4,800		2,400		1,280		0,960		0,320		0,160	
0,40	7,5360		3,7680		2,0096		1,5072		0,5024		0.2512	

Legend: C) protein concentration (in mg/ml), H) height of layer of specimen in capillary tube (in mm).

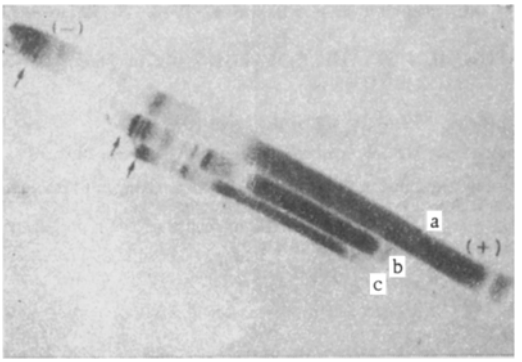

Fig. 1. Ultramicroelectrophoresis of proteins in polyacrylamide gels with diameters of 0.40 (a), 0.32 (b), and 0.20 (c) mm. Arrows mark boundaries between concentrating and fractionating gels.

quantity of protein in the sample also decreased. However, the calculation of the total protein content in each type of capillary tube per unit area of cross section (or per unit diameter) gave a value which was the same for all capillary tubes studied (12 $\mu g/mm^2$). On the basis of this relationship an empirical formula was deduced in which the product of the height of the layer of sample (in mm) and the protein concentration (in mg/ml) is a constant, equal to 12 for capillary tubes of whatever diameter. By using this formula it is easy to determine the optimal height of the layer of the specimen for a solution of any concentration regardless of the diameter of the capillary tube. It follows from this formula that in tests on protein solutions of low concentrations the volume of the specimen must be increased. Experimental verification showed that, for example, with a sixfold decrease in the protein concentration the best results were obtained when the volumes of the samples were increased sixfold. Table 2, for choosing optimal volume of the specimen depending on the concentration of the protein solution and the diameter of the capillary tube used, was compiled on the basis of the experiments and calculations.

Work with specimens containing different concentrations of protein in the sample (blood serum, homogenates of cultures of adult human fibroblasts and embryonic human fibroblasts and hepatocytes) completely confirmed the validity of the results of the calculations given in Tables 1 and 2.

To determine the isozyme spectra of lactate dehydrogenase, malate dehydrogenase, and acid phosphatase in experiments by this method a specific protein concentration in the sample from 5 to 7 times less than for separation of the protein fractions was required.

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