ADVANTAGES AND DISADVANTAGES OF SILVER PROTEIN STAINING IN POLYACRYLAMIDE GEL

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UDC 547.96:543.545+616-008.
939.6-074:543.545

KEY WORDS: proteins; electrophoresis; staining; silver impregnation; sensitivity; selectivity.

Electrophoresis is one of the most widely used methods in modern biochemistry, molecular biology, immunology, and other biological disciplines. Polyacrylamide gel (PAG) electrophoresis is widely used to study the properties of single proteins and also to analyze the protein composition of complex biological mixtures.

PAG electrophoresis is constantly being improved. New versions of the method with high resolving power, such as gradient electrophoresis [9], two-dimensional electrophoresis [8], electrophoresis in gel blocks of various shapes [2], and so on, have been created. Meanwhile methods of detection of proteins in PAG are being developed. New staining methods are being introduced, the quality of the dyes is being improved, and fundamentally new methods for detection of proteins after electrophoresis are being suggested.

An important place among the new methods of protein staining in PAG is occupied by staining with silver salts [3, 6, 7]. There are several modifications of the silver staining method. Possibly the simplest, yet a highly effective version, of the method is that developed by Heukeshoven and Dernik [4]. This paper gives the results of a comparative analysis of the sensitivity of the traditional method of staining proteins in PAG with "Serva blue G-250" (an analog of Coomassie bright blue G-250) and of staining with $AgNO_3$ [4].

EXPERIMENTAL METHOD

A set of marker proteins (SDS-6H) from Sigma (USA), including (with molecular weights in kilodaltons) myosin (205), β-galactosidase (116), phosphorylase b (97.4), bovine serum albumin (66), ovalbumin (45), and carbonic anhydrase (29). Single marker proteins also were used; bovine serum albumin (from Koch-Light, England), ovalbumin (Sigma), trypsinogen (24, from Sigma), and ribonuclease (12.7, from Boehringer, West Germany). A weighed sample of a mixture of marker proteins (0.4 mg) was dissolved in 1 ml of 0.4 M Tris-HCl buffer, pH 6.8, and weighed samples of the single marker proteins were mixed, in a weight of 0.5 mg of each,

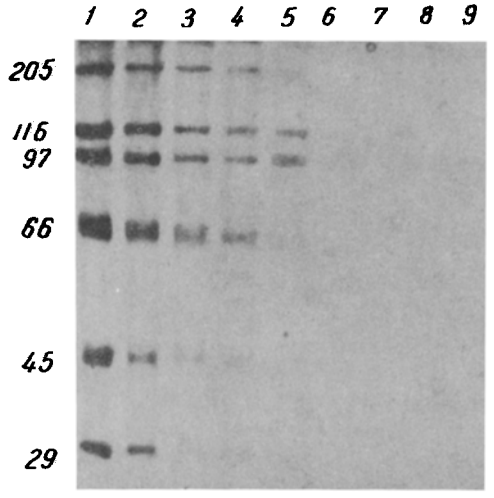

Fig. 1. Determination of limit of sensitivity of protein staining in PAG with Serva blue G-250; results of electrophoresis of a series of successive dilutions of a commercial mixture of marker proteins. Molecular weight of marker proteins indicated on left (in kilodaltons). Original concentration of each protein (lane 1) 666 ng. Lane 2) 333 ng; 3) 166 ng; 4) 83 ng; 5) 41.5 ng; 6) 21 ng; 7) 10.5 ng; 8) 5.2 ng; 9) 2.6 ng.

Laboratory of Immunology of Oncogenic Virses, All-Union Oncologic Science Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Trapeznikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 11, pp. 620-623, November, 1986. Original article submitted December 17, 1985.

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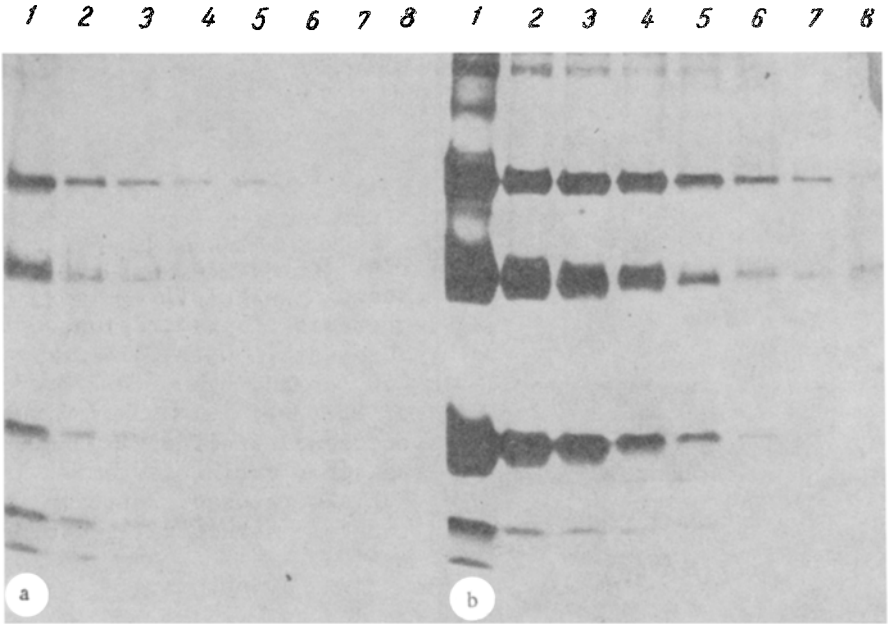

Fig. 2. Comparative sensitivity of protein staining in PAG with 0.1% Serva blue G-250 (a) and $AgNO_3$ (b). A mixture of the following marker proteins (with molecular weight in kilodaltons) was used: bovine serum albumin (66), ovalbumin (45), trypsinogen (24), and ribonuclease (12.7). Original concentration of each protein in the lane was not more than 570 ng (lane 1); lane 2) 285 ng; 3) 142 ng; 4) 71 ng; 5) 35.5 ng; 6) 18 ng; 7) 9 ng; 8) 4.5 ng.

and dissolved in 4 ml of the same buffer. Consecutive serial dilutions were prepared in siliconized test tubes, and 0.4 M Tris-HCl, pH 6.8, was used for dilution. Samples measuring 10 µl were treated with 3% sodium dodecylsulfate (SDS) and 2% β-mercaptoethanol, and incubated for 2 min at 100°C and applied to PAG.

Preparations of retroviruses were obtained from the USA under the terms of the Soviet-American Collaboration in Oncovirology. Before electrophoresis the preparations were treated in the same way as the mixtures of marker proteins. Preparations of the following retroviruses were used: Rauscher murine leukemia virus (R-MuLV), Mason–Pfizer monkey virus (M-PMV), and endogenous feline virus RD-114.

Electrophoresis was carried out in an apparatus for ventrical electrophoresis, in plates produced by the Kiju Kalur laboratory (Estonian SSR). Gradient (7.5-15%) PAG with 0.1% SDS was used, and the thickness of the gel was 1 mm. Electrophoresis was performed in Laemmli's buffer system [5] with a constant current of 15 mA applied to the plate. After electrophoresis the gels were stained with 0.1% Serva blue G-250 (Serva, West Germany) in solution containing methanol, acetic acid, and deionized water (1:1:8). Staining continued for 1 h at 37°C and 23 h at 20°C. Background staining was washed out with the same solution but without the dye. After the background had been washed out the gel was photographed, SDS was removed with a mixture of 30% ethanol and 10% acetic acid for 12-16 h at room temperature, with the solution changed twice, after which the gel was stained with 0.1% $AgNO_3$ by the method in [4]. The stain was developed with 3% Na_2CO_3, containing 0.02% formaldehyde. The development procedure took 15-20 min. The background stain was removed with Farmer's photographic reducer for 10-15 sec. After thorough washing with running water the procedure of silver staining was repeated. After staining the gel can be kept for a long time in 10% ethanol.

EXPERIMENTAL RESULTS

According to data given by different workers, from 5 to 0.5 µg of protein can be detected by staining PAG with dyes of the Coomassie type [1, 3]. The first task of the investigation was therefore to determine the precise sensitivity of protein staining in PAG with Serva blue G-250. To investigate this problem a commercial mixture of marker proteins from Sigma (USA) was used.

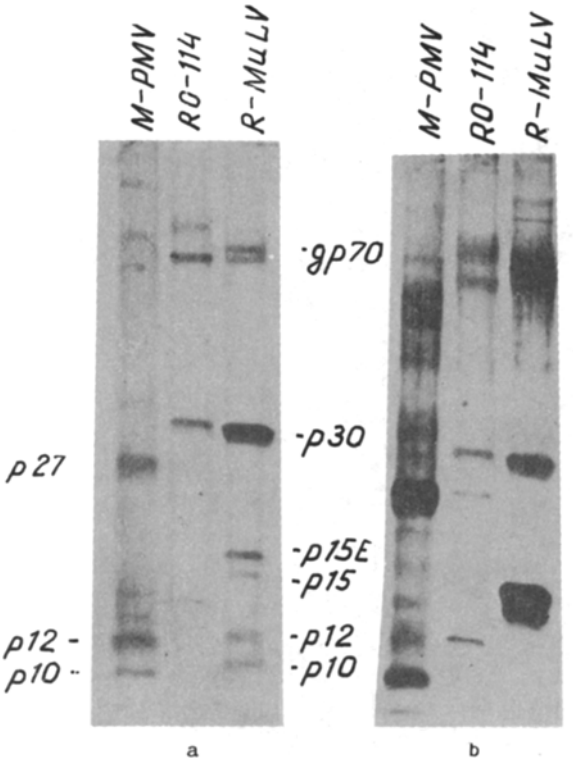

Fig. 3. Example of selective protein staining with silver ions. PAG electrophoresis of preparations of retroviruses stain with 0.1% Serva blue G-250 (a) and $AgNO_3$ (b). Preparations of Mason–Pfizer monkey virus (M-PMV), endogenous feline virus (RD-114), and Rauscher murine leukemia virus (R-MuLV) were used. Position of virus-specific structural proteins indicated.

The results of electrophoresis of a series of consecutive dilutions of a mixture of marker proteins are shown in Fig. 1. The original concentration of each protein (lane 1) was 666 ng. In each successive lane the protein concentration was reduced by half: lane 2 333 ng, lane 3 166 ng, lane 4 83 ng, and so on. All six marker proteins were clearly revealed in a concentration of 83 ng (lane 4). In lane 5 (protein concentration 41.5 mg) only two markers could be found, namely β-galactosidase and phosphorylase B, and the remaining proteins (because of their greater dispersion) could only be guessed by examination of the gel. Thus the sensitivity of the protein staining method with Serva blue G-250 in a PAG gradient 1 mm thick was 80 mg, or in the absence of any appreciable dispersion, 40 ng.

The next stage of the work was to compare the sensitivity of protein staining in PAG with Serva blue and $AgNO_3$. Electrophoresis of marker proteins (bovine serum albumin, avalbumin, trypsinogen, ribonuclease) after staining with Serva blue (a) and $AgNO_3$ (b) is illustrated in Fig. 2. The original concentration of each protein was not more than 570 ng (lane 1). Staining with Serva blue revealed in this case not less than 71 ng protein (lane 4), but bovine serum albumin could be clearly detected in a concentration of 35.5 ng (lane 5). After staining twice with $AgNO_3$, bovine serum albumin and ovalbumin were most effectively stained with silver, and trypsinogen rather less well. The concentration of the first two proteins was 4.5 ng (lane 8). In some experiments with silver staining proteins present in concentrations of not more than 2 ng could be seen, in particular β-galactosidase and phosphorylase b from the set of marker proteins obtained from Sigma. Meanwhile ribonuclease could be detected after staining with silver in a concentration of only 35.5 ng (lane 5), i.e., the method is only twice as sensitive as staining with Serva blue. The general effectiveness of silver staining by the method in [4] is superior to staining with Serva blue by not more than 18-30 times, but not by 2 orders of magnitude, as was reported previously [3, 4]. Silver staining takes place selectively, and the decisive factor influencing the effectiveness of silver staining is the amino-acid composition of the protein molecule. It has been shown, in particular, that leucine does not bind silver ions, but that histidine interacts most effectively with silver [4], and that is why ribonuclease, which has only four histidine residues, stains badly with silver. Silver staining is most effectively observed in the case of proteins which are not clearly distinguishable after staining with Serva blue (concentration from 50 to 18 ng). In protein concentrations of over 200 ng silver staining, in our opinion, has little to commend it, for the bands are so large that, if two or more proteins are close together, they may overlap one another.

The selectivity of silver staining could be most clearly demonstrated by staining the gels after electrophoresis of virus proteins (Fig. 3a, b). By staining with Serva blue,

traditional structural proteins of the retroviruses were revealed in corresponding quantitative portions (Fig. 3a). For instance, p27 of M-PMV, p30 of RD-114, and p30 of R-MuLV, the principal internal porteins of these retroviruses, stained most brightly. This same gel, after staining with silver, is illustrated in Fig. 3b. The intensity of staining of p27 and p10 of M-PMV, of p12 of RD-114, and of p15E, p15, and gp70 of R-MuLV was increased. However, the intensity of staining of p10 of M-PMV, p30 of RD-114, and p12 and p10 of R-MuLV was sharply reduced. Other disparities also were observed between the results of original staining and of staining with silver, further evidence of the selectivity of binding of silver ions with proteins.

The combined use of two stains — Serva blue and Amido black 10B — did not lead to increased sensitivity of staining. Moreover, the brightness of the protein bands was reduced, indicating competition between the dyes for the binding sites on the protein.

The data given above are evidence of the higher sensitivity (relative to the generally accepted level) of the method of protein staining with dyes of the Coomassie G-250 type, which for most proteins is not more than 70-80 ng. On the whole, however, silver staining is 18-30 times more effective for many proteins, but silver staining takes place selectively. Some proteins stain weakly with silver, whereas others may not be revealed by it at all. All these facts indicate that in order to obtain reliable results in the study of unknown protein or of complex protein mixtures, combined staining of the gel is essential.

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RAPID METHOD OF VISUALIZING ENDOTHELIUM IN UNFIXED TISSUE

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UDC 611.13/.14-018.74-086.16

KEY WORDS: endothelium; endocardium; thioflavine; silver impregnation; rapid method.

After it had been shown that the endothelium influences the response of vessels to certain drugs [3, 4] research began into its role in the regulation of vascular tone. However, interpretation of the physiological parameters of the vessel wall is largely determined by the integrity of the endothelial lining, and this raises the question of a simple and rapid method of detecting endothelial cells. Several methods are used nowadays to verify integrity of the endothelium. The physiological method consists essentially of recording responses of vessels to substances releasing endothelial relaxation factors (acetylcholine, calcium ionophore A 23187, etc.). The method is simple but does not allow the state of the endothelium to be observed directly. Certain species differences also may exist in the physiological response of the vessels [5]. It is often necessary to resort to the use of film ("Häutchen") preparations impregnated with silver [2, 7]. The two-dimensional film prepara-

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 11, pp. 623-625, November, 1986. Original article submitted January 13, 1986.