A Rapid Method for Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis Evaluation

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Sodium Dodecyl Sulfate Polyacrylamide Gel 전기영동 결과의 경제적인 평가 방법

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ABSTRACT: We propose a method for rapid evaluation and permanent record of sodium dodecyl sulfate polyacrylamide gel electrophoretic runs. This method is based on the photocopy process, rather than on photography and requires no extensive or expensive investment. Comparison of a print obtained through this method and a 35 mm photography indicated, on a balanced gel, equal sensitivity.

KEY WORDS □ SDS-PAGE, Gel evaluation, Electrophoresis, Technique, Molecular biology

The development of the SDS-PAGE technique for protein electrophoresis has been associated with the development of several evaluation methods involving various stains (Maizel, 1966; Meyer, 1965; Chrambach *et al.*, 1967). These methods do not give measurable results directly because of the wet and elastic nature of the gel and, therefore, suitable photographic reproduction is usually needed for further analysis. Amongst the methods most frequently used in this case are Polaroid (for rapid evaluation) and standard 35 mm films (for permanent record or publication). Both methods are considered of equal sensitivity for gel evaluation. These photographic techniques are unwiedly and expensive. This note describes a method for rapid evaluation of SDS-PAGE gels using an office photocopier. We also compared this technique with standard 35 mm photography and report on their relative sensitivities.

MATERIAL AND METHODS

Bovine serum albumin fraction V (BSA) was chosen for its solubility in water, molecular weight (66.2 Kdal.) and reproductible electrophoretic mobility. Various amounts (0.2, 0.4, 0.5, 1, 2, 4, 8 and 10 μg) of BSA were used for comparison with 35 mm photography. The upper loads are to show if overload affects visibility of neighboring faint bands (dimeric and trimeric forms of BSA). The lower loads are to determinate band detectability at very low protein concentration. Electrophoresis

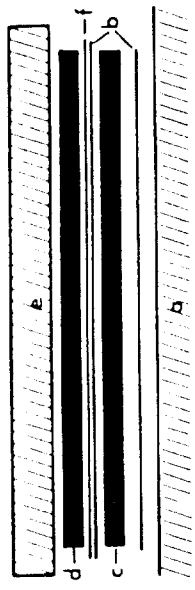

Fig. 1. *Experimental apparatus consisting of the photocopier (a), Saran Wrap (b), gel (c), glass plate (d), copier's cover (e) and white background paper (f).*

time was 3 hours at 50 milliampers with a 9% acrylamide gel (Laemmli, 1970; Maizel, 1971). Protein bands were visualized as follows:(-i-) 60 min. in 10 volumes of staining solution (7% acetic acid, 50% methanol, 0.02% G-250 Coomassie Brilliant Blue (G-250 CBB)) with weak agitation; and (-ii-) repeated washing in 20 volumes of destaining solution (7% acetic acid, 5% methanol) (Laemmli, 1970). Photocopying was done as follows: the gel was first blotted dry with ordinary absorbent paper, then wrapped in Saran Wrap, laid on top of an office copier and covered with a glass plate to expel air bubbles (Fig. 1). Various yellow, red and orange acetate filters were tried (inlaid between the copier and the Saran Wrap) to increase contrast, but discarded because of their excessive quenching of transmitted light, even when the copier was set to its highest light intensity. 35 mm photography was done on Kodak 100 ASA film with a 1/15 sec. exposure.

RESULTS

Analysis of Fig. 2a reveals in lanes 1 and 2, in addition to the 66.2 Kd band, two other bands of

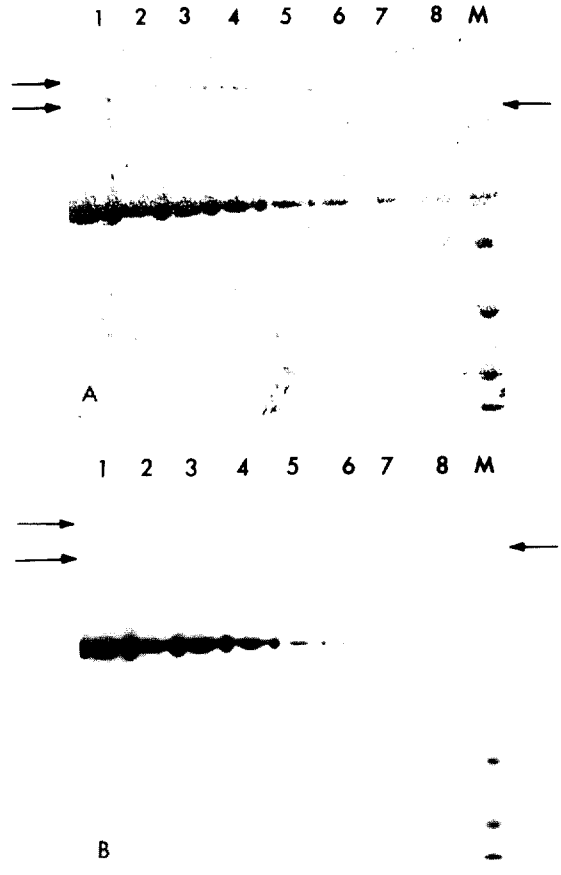

Fig. 2. *Photocopy (a) and photograph (b) of SDS-PAGE gel.*
Lanes 1 through 8 contain decreasing quantities of BSA (see text). Lane M: molecular weight standards (116, 66.2, 43, 31, 24 and 14.5 Kdal).

130 and 170 Kd, corresponding respectively to addition to the 66.2 Kd band, two other bands of 130 and 170 Kd, corresponding respectively to in the same stoechiometric ratio as the monomeric, coloration intensity can be compared between multimeric and monomeric bands (Ornstein, 1969). The lower limit of detection for this staining method being of ca 0.1 μg per band (Hames, 1981), we estimate that this is the protein concentration of the faint bands in lane 2. the 66.2 Kd band can be also seen on lane 8 (0.2 μg) of Fig. 2b (photocopy), as well as the multimeric bands of lanes 1 and 2 (0.1-0.15 μg), albeit dimly. We therefore assume 0.1-0.15 μg to be, under these conditions, the detection limit of the photocopy method. However, since a copier requires a sharply

contrasted original, care must be taken to ensure the lowest level of background stain in the gel. The difference in band intensity between lane 8 and the multimeric bands of lane 2 is higher in the photocopy than in the photography, and would seem to indicate that overloading in one band will adversely affect visibility of fainter, neighbouring bands.

DISCUSSION

This technique represents significant improvement over existing methods. It gives results the fastest and is of acceptable sensitivity. This procedure also gives nonreduced copies of the gel, thereby reducing measurement errors. Furthermore, it does not require extensive installations. Finally, the sensitivity of this technique, as well as its rapidity and low cost should be useful where many samples must be subjected to SDS-PAGE, such as after gel or ion-exchange chromatography.

적 요

SDS-PAGE 겔의 전기영동 결과를 신속히 평가하는 방법을 고안하였다. 이 방법은 photography 보다는 photocopy의 기법을 이용하므로써 쉽게 그리고 경비를 절약할 수 있었다. 35mm의 photography와 이 방법으로 얻은 print를 비교하면 선명도는 거의 동일하였다.

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