SDS - POLYETHYLENEGLYCOL ELECTROPHORESIS: A POSSIBLE ALTERNATIVE TO SDS - POLYACRYLAMIDE GEL ELECTROPHORESIS

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1. Introduction

The effectiveness of SDS-polyacrylamide gel electrophoresis is usually attributed to the existence of defined pore sizes in a matrix of polymer chains linked together by *N,N'*-methylene-bis-acrylamide. It can be shown that polyethyleneglycol, soaked in cellulose acetate foils, is also capable of discriminating between particles of different molecular weights. Hence, covalent links between the polymer chains are no prerequisite for the separating effect.

2. Materials and methods

Equipment: A conventional electrophoretic apparatus for freely suspended 25 X 145 mm cellulose acetate foils (stretching devices obtained from Boskamp, Hersel, Germany) and a constant voltage power supply were used. The electrode vessels were filled with a solution of 10% (w/v) polyethyleneglycol 1% (w/v) sodium dodecylsulphate and 1 mM sodium azide (for prevention of bacterial growth). Sometimes 25% (v/v) glycerol was included. Chemicals and proteins: Polyethyleneglycol with mol. wt. ranging from 35 000 to 40 000 (PEG 40 000), 2-mercaptoethanol, iodoacetamide, sodium dodecylsulphate (SDS), and protein standards were purchased from Serva (Heidelberg, Germany); polyethyleneglycol with molecular weight of 4000 (PEG 4000) was a product of Schuchardt (München, Germany); other chemicals were from Merck (Darmstadt, Germany).

Preparation of samples: The proteins were dissolved by heating at 100°C for 5 min in a solution of 2% SDS 10% mercaptoethanol 2 mM NaN_3 and 8 M urea. In some cases the denatured proteins were further reacted with iodoacetiamide. An excess of iodoacetamide (50 mg per 100 μl sample) was dissolved by shaking under N_2-atmosphere, and after 1 h reaction at room temperature, was removed by dialysis against a 100-fold volume of 8 M urea 2% SDS 2 mM NaN_3 15 mM mercaptoethanol. Prior to use the protein mixtures were diluted with an equal volume of a solution containing twice the required PEG concentration. When glycerol was used, the PEG solution contained 50% glycerol.

Staining and evaluation: After electrophoresis the cellulose acetate foils were stained for protein in a solution containing 0.1% amidoblack 10B 7% acetic acid and 40–80% methanol (or ethanol). Foils were first destained with 7% acetic acid and finally with hot 7% acetic acid. Subsequently the wet foils were pressed between object slides and were inserted in a Zeiss-photometer equipped with a scanning device. The optical density was measured at 570 nm using an aperture of 0.5 X 4 mm.

3. Results

In the presence of high PEG concentrations, cellulose acetate foils tend to dry up during electrophoresis. Evaporation was reduced either by including 25% glycerol in all solutions, or by using a stream of moist air to humidify all parts of the electrophoresis chamber. Salts and buffers, except SDS and azide, were omitted from the electrophoresis solution, since their presence enhanced heat production and

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evaporation, and did not significantly affect the results.

In a continuous buffer system, the diameter of the sample spot is critical for the resolving power of an electrophoretic separation. Throughout this work a staple-like sample applicator (Boskamp, Hersel, Germany) was used. In contrast to the common practice, samples were applied directly to the dry foils, which were subsequently floated on the electrophoresis solution. If samples were applied to wet foils, the sample spots tended to leak out. After insertion of the foils in the apparatus, 10 min were allowed for the sample spot to equilibrate with the surrounding fluid.

Four commercially available proteins having known subunit mol. wts. [1,2] were mixed and denatured in SDS for electrophoresis: bovine hemoglobin (15 500), β-lactoglobulin (18 400), ovalbumin (43 000), and bovine serum albumin (68 000). Parallel runs were made in 1%, 3% and 10% PEG 40 000. Densitometer tracings of these foils are shown in the lower part of fig.1a. The four peptides are clearly separated in the presence of 10% and 3% PEG, but are only partially separated in 1% PEG. However, the separations of hemoglobin and lactoglobulin, and of ovalbumin and serum albumin are satisfactory even in 1% PEG. The log mol. wt gradients are shown in the upper part of fig.1a. Although in 1% PEG the curve is S-shaped, it approaches linearity with rising PEG concentrations.

In order to test the resolving power of 10% PEG more rigorously, three further proteins were included, i.e. horse myoglobin (17 200), rabbit aldolase (40 000), and bovine catalase (58 000). In this case, carboxamidomethylation with iodoacetamide was necessary to obtain optimal resolution. Only ovalbumin and catalase were not separated completely (lower part of fig.1b). Although the log mol. wt. gradient shows deviations from linearity (upper part of fig.1b), mol. wt. differences of more than 15% can be detected in the range of the peptide lengths tested.

In contrast to PEG 40 000, the low mol. wt. homologue PEG 4000 has nearly no separating effect, except for the leading and tailing shoulders visible in fig.1c. Similarly, the glycerol included in the electrophoresis solutions used in fig.1c has no effect on peptide separation.

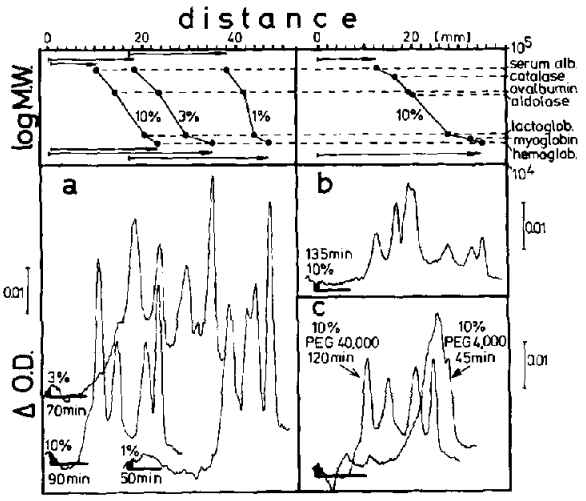

Fig.1. SDS-polyethyleneglycol electrophoresis on cellulose acetate. Upper part: log mol. wt. gradients, inferred from the peak positions of the corresponding peptides; the travelling distances of the slowest and the fastest components are indicated by arrows. (Lower part) (a) Electrophoresis of 4 proteins in 4 M urea 1% SDS 1 mM NaN_3, PEG 40 000 concentrations as indicated; 200 V; less than 5 μg per protein; (b) solution as in (a), 7 protein reacted with iodoacetamide; less than 3 μg per protein; 200 V; (c) solution as in (a), however containing 25% glycerol; mol. wts. of PEG as indicated; 300 V.

4. Discussion

As shown in the figures, PEG 40 000 influences the mobility of SDS-complexed peptides as a function of mol. wt.: the longer the peptide, the greater the frictional force on its motion. By analogy with polyacrylamide gels, this constraint may result from the formation of a fluid PEG network which is stabilized by the entangled arms of the polymer molecules. The stability of pores in such a network will be a function of the polymer length. Therefore, the short chains of PEG 4000 are ineffective. The fact that in 1% PEG 40 000 the log mol. wt. gradient seems to be S-shaped, with its point of inflection near the mol. wt. of PEG itself, may indicate some more specific interaction between polymer and peptides.

The resolving power of SDS-PEG electrophoresis is inferior to SDS-polyacrylamide gel electrophoresis. Nevertheless, certain advantages may compensate for the loss of resolving power in those cases where only a few components are to be separated. For example,

samples may easily be eluted from the foils for scintillation counting. The PEG method should also be tested in carrier-free electrophoresis for analytical (moving boundary electrophoresis) and preparative (continuous flow electrophoresis) work, since the cellulose acetate itself is likely to be dispensible in achieving the separation.

References

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[2] Weber, K., Pringle, J. R. and Osborn, M. (1972) in: Methods in Enzymology (Colowick and Kaplan, eds.), Vol. XXVI, pp. 3–27, Academic Press, New York and London.