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A SIMPLIFIED SDS-GEL ELECTROPHORESIS PROCEDURE WITH IMPROVED RESOLUTION OF CHROMOSOMAL PROTEINS

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Abstract—1. The simplified SDS-electrophoresis procedure reported here allows application of total chromatin on the gel.

2. The chromosomal proteins are extracted directly on the gel, without prior removal of nucleic acids.

3. Either histones or nonhistones can be resolved completely on gels with this procedure.

INTRODUCTION

Since Shapiro *et al.* (1967) first reported that the separation of proteins by polyacrylamide-gel electrophoresis in the presence of the ionic detergent sodium dodecyl sulfate (SDS) is dependent upon the molecular weights of the polypeptide chains, this system has been a useful tool for the separation and identification of proteins and their subunits. And, with appropriate modifications, it is used to study the structure of enzymes, to determine the molecular weights of specific polypeptides (Panyim & Chalkley, 1971), and to compare proteins from cells, tissues and organisms in various stages of either normal or abnormal development. Further, SDS-gel electrophoresis is also used for the accurate determination of the molecular weights for a wide variety of proteins (Weber & Osborn, 1969).

In this paper we will describe a simplified electrophoresis system for the separation of histones and nonhistones on the same gel. This system allows the direct application of total chromatin to polyacrylamide gels and provides very sharp resolution of all histone and nonhistone proteins.

MATERIALS AND METHODS

Chemicals and buffers

Reagent grade $NaH_2PO_4.H_2O$, Na_2HPO_4, urea, glacial acetic acid, and ammonium persulfate were obtained from J. T. Baker Chemical Co. Acrylamide (for electrophoresis), bisacrylamide (methylenebisacrylamide), and TEMED (N,N,N'N'-tetramethylenediamine) were purchased from Eastman, and 2-mercaptoethanol from Calbiochem. SDS (sodium dodecyl sulfate, 95%) was obtained from SIGMA. Amido Black 10 B (electrophoresis purity reagent) was obtained from BioRad Laboratories. The solutions used for the "gel mixture" were A: 40% acrylamide and 3.0% bisacrylamide in deionized water: B: 9.6 M urea, 180 mM $NaH_2PO_4.H_2O$, 150 mM Na_2HPO_4 and 0.16% SDS (this solution has a non-adjusted pH 7.0–7.1): C: 3.2 ml TEMED in 100 ml deionised water (prepared fresh every 3–4 weeks): D: ammonium persulfate, prepared just before use at a concentration of 0.32% in deionized water. Solutions A and B were filtered through Whatman No. 1 filter paper before use, and, together with solution C, were stored in dark bottles at room temperature (22–24°C). Electrophoresis was performed using a single tray-buffer consisting of 31.3 mM $NaH_2PO_4.H_2O$, 66.3 mM Na_2HPO_4, and 0.1% SDS, pH 7.0–7.1, and conductivity of 10.3 to 10.4 mmho (conductivity was measured with a Radiometer Conductivity Meter, type CDM, Copenhagen, Denmark).

Chromatin, before being applied onto the gels was incubated in "incubation buffer" for about 5 hr at 37°C or overnight at room temperature, with gentle agitation. The incubation buffer consisted of 6.0 M urea, 1.0% 2-mercaptoethanol, 10.0 mM $NaH_2PO_4.H_2O$ and 1.0% SDS, adjusted to pH 7.0 with 1.0 N NaOH. Occasionally, 1 vol of concentrated chromatin solution was mixed with 2 vol of appropriate concentration of incubation buffer (9.0 M urea, 1.5% 2-mercaptoethanol, 1.5% SDS, and 15.0 mM $NaH_2PO_4.H_2O$, pH 7.0), and incubation proceeded as above.

Preparation of gels and gel electrophoresis

The gels were prepared by mixing solutions A, B, C and D in a ratio of 4:10:0.5:1.5 which yields a gel mixture consisting of 10.0% (w/v) acrylamide, 0.75% (w/v) bisacrylamide, 0.1% (w/v) SDS, 6.0 M urea, 109.4 mM $NaH_2PO_4.H_2O$, 93.8 mM Na_2HPO_4, 0.1% (v/v) TEMED, and 0.03% (w/v) ammonium persulfate. A volume of 1.5 ml of the gel mixture was pipetted into clean glass tubes of 5.0 mm I.D. A little deionized water (2–4 mm) were overlayed and the gels were allowed to polymerize 45–60 min.

For resolution of histones, 10–20 μl of incubation buffer containing 15–20 μg of chromatin-protein were placed on top of each gel, and electrophoresis was run for 12 hr at 6 mA per gel. For resolution of nonhistones, 50–100 μg of chromatin-protein in 50–100 μl incubation buffer were placed on top of each gel and electrophoresis was run for about 20 hr at 6 mA per gel (the time of electrophoresis was dependent on the tissue from which chromatin was prepared, i.e. number and molecular weights of nonhistones). The gels were removed from tubes using low air-pressure and stained at room temperature overnight in 0.05% or for 2 hr in 0.7% (w/v) Amido Black solution containing 10% glacial acetic acid and 40% ethanol. They were then destained by diffusion in 10% acetic acid–40% ethanol solution. The gels were scanned on a Gilford Linear Transport connected to a Gilford recording spectrophotometer, model 2000, at a scan rate of 0.5 cm/min.

The light absorbance by the protein bands, on the gel, was monitored at 570 nm, and traced on paper at a chart speed of 10 cm/min. The five major histone fractions from the calf tissues were identified according to the nomenclature proposed at the CIBA Foundation Symposium (1975).

Preparation of chromatins

Calf thymus and liver were taken from recently slaughtered animals (H. W. Stapf, Inc., Baltimore, MD) and frozen at −20°C until use. The thymus chromatin was prepared by the method reported elsewhere (Angerer & Moudrianakis, 1972), whereas the liver chromatin was prepared by a slightly modified method, used by Anderson (1971) for the preparation of chromatin from chicken embryo liver.

RESULTS AND DISCUSSION

Electrophoresis of reduced-SDS complexes on 5.0–20.0% polyacrylamide SDS gels at pH 7.0 and 10.0 was reported by Hamana & Iwai (1974). The highest resolution of histones was observed by them using 15.0% gels at pH 10.0, was 5–6 bands. Recently it was reported (Hayashi *et al.*, 1974) that with acrylamide concentrations between 11.0–14.0%, all five histones were resolved with the best resolution being obtained with 12.5% acrylamide. In the present study it was found that at a constant time (10 hr) of electrophoresis all five histones were resolved in 8.0–14.0% acrylamide gels, the highest resolution being obtained on a 10.0% gel. At acrylamide concentrations lower than 10.0%, all five histone fractions were visible but the histone bands were diffuse with poor resolution of the H1-subfractions. At acrylamide concentrations higher than 10.0% the histone bands, although sharp and well separated, were too close to be scanned. Thus, at acrylamide concentrations lower or higher than 10.0%, the histone fractions were not adequately resolved upon scanning of the gel.

It is known (Crambach & Rodbard, 1971) that the protein-SDS complex shape contributes to its speed of migration in a long-fiber gel. For globular proteins the [retardation coefficient]$^{1/2}$, is related linearly to molecular radius and proportionally to [molecular weight]$^{1/3}$, whereas for rodlike (Reynolds & Tanford, 1970) or ellipsoidal (Collins & Haller, 1973) proteins the retardation coefficient is related linearly to polypeptide chain molecular weight. Thus, it is assumed that the SDS-complexes of all H1-subspecies are rodlike, in agreement with the observations on SDS-gel chromatography (Fish *et al.*, 1970). The heterogeneity of the H1 in the SDS electrophoresis can be attributed either to the different molecular subfractions or to subfractions which are similar in molecular weight but different in the degree of modification or minor substitution of the residues (Teller *et al.*, 1965).

The similarity of the banding pattern and degree of resolution of the histones from each of the calf tissues indicated that this method may be applicable to many tissue types (manuscript in preparation). What is more important is the fact that histones need not be salt- or acid-extracted in order to be studied by means of electrophoresis. Total or partially extracted chromatin can be applied directly onto the gel. The resulting patterns, as seen by photography or gel scanning (Figs 1 and 2), are identical to those of isolated histones (Fig. 3). This yields the advantage of avoiding the possible loss of any histone(s) during extraction. Similarly, this electrophoresis system can be used successfully for the resolution of the nonhistone complement of chromatin without involving any extraction which might result in either loss or changes, structural or biochemical, of the proteins. Since nonhistone chromosomal proteins have molecular weights much larger than those of histones, it was found that the electrophoresis running period required for a complete profile of nonhistones on the gel should not be shorter than 20 hr with identical experimental conditions (i.e. gel constitution and dimensions, electrophoresis tray-buffer, and amperage per gel). However, this period can be adjusted depending on the number and molecular weights of the nonhistones under study.

The experimental approach used, included the following working steps: The gels of one set were loaded with appropriate volumes of chromatin material whose histone and nonhistone complements were to be studied. Electrophoresis was allowed to run 12 hr at 6 mA per gel; the gels loaded for histone studies were removed and the gels for nonhistone studies were allowed to run for an additional period of 10 hr, adjusting the current properly (at 7 mA per gel). The presence of high molar urea in the incubation buffer and gel mixture prevents histone-nonhistone aggregations, as confirmed by analyses of artificial mixtures (Naito & Sonnenbichler, 1972). However, the presence of urea results in a reduction of the gel porosity (Swank & Munkres, 1971) followed by an increase of the amperage across the gels during electrophoresis at constant voltage. Consequently, ohmic heating causes curvature or diffusion of bands leading to limited band resolution upon scanning. This disadvantage can be overcome either by using a power supply of constant current (amperage) or by inserting a resistor of 1.5–2.0 KOhm between the conventional power supply and the electrophoresis apparatus.

This electrophoresis system which yields good resolution of chromosomal protein complexes is very convenient for the following reasons: (1) It resolves completely histone H1 into its main subfractions; (2) Histone H2B is completely separated from histones H3 and H2A, thus allowing better resolution on scanning profiles; (3) Each histone band can be easily cut and removed for further studies; (4) A complete spectrum of histones and nonhistones can be obtained by their direct extraction from chromatin on the gel, without prior puridication; and, (5) This system reduces the working time by precluding the removal of nucleic acids from chromatin preparations and purification of histone and nonhistone complements.

In our hands, this method has also proven to be entirely applicable to the identification and characterization of chromatin fragments obtained upon fractionation of mechanically sheared calf thymus material (Pantazis, 1977) or chromatin from human leukemic cells (manuscript in preparation).

SUMMARY

The simplified SDS-gel electrophoresis system described above produces resolution of histones and nonhistones on polyacrylamide gels with a minimum of sample preparation. Chromosomal proteins can be

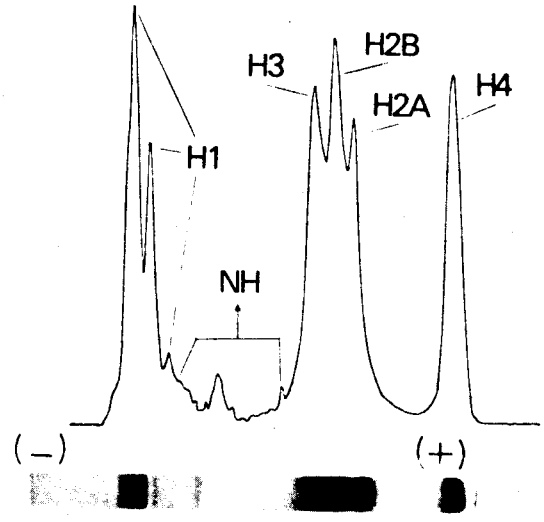

Fig. 1. SDS-gel electrophoresis of total chromatin-protein from calf thymus. Total chromatin was placed on top of the gel followed by electrophoresis as described in "Materials and Methods".

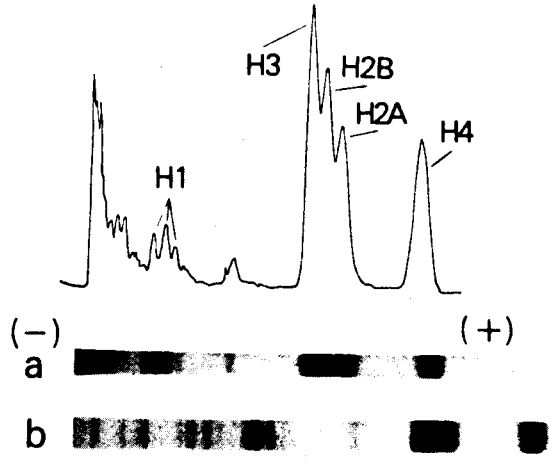

Fig. 2. SDS-gel electrophoresis of total chromosomal protein from calf liver. Gel (a) was allowed to run for histone, whereas gel (b) for nonhistone resolution. Both gels were identical and run in the same electrophoresis buffer, but gel (b) was loaded with more chromatin and allowed to run longer than gel (a) as described in "Materials and Methods" (Preparation of gels and gel electrophoresis).

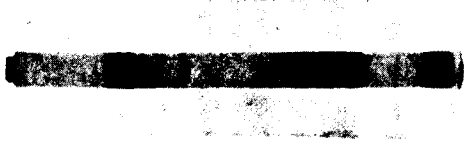

Fig. 3. SDS-gel electrophoresis of total histone prepared from calf thymus by acid treatment (0.25 N HCl).

extracted from chromatin which is already on the gel. The procedure allows the total chromatin to be applied to the gel without a preliminary step to remove nucleic acids, thus minimizing the potential for protein loss. The system works at neutral pH and the single tray-buffer used has a conductivity of 10.3–10.4 mmho.

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