"TWO-STAGE" GEL ELECTROPHORESIS: APPLICATION TO THE SEPARATION OF BOVINE THYROID STIMULATING HORMONE SUB-UNITS AND CALF THYMUS HISTONES

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SUMMARY: Sodium dodecylsulfate (SDS) can be removed from protein by gel electrophoresis. This principle is useful for separating protein bands which are close to each other in SDS gel electrophoresis. We accomplished this by "two-stage" gel electrophoresis. In this system, SDS gel electrophoresis was carried out as the first step. Gel electrophoresis was then continued (after replacing the buffer) without SDS. SDS was then eluted from the gel into the lower buffer during the second stage. Separation of the subunits was significantly improved relative to simple SDS gel electrophoresis.

INTRODUCTION

During recovery of separated proteins after simple SDS gel electrophoresis, Weber and Kuter (1) and Lenard (2) used an anion exchange resin to remove the SDS. In gel electrophoresis, the proteins move slower than the buffer "front" indicated by the tracking dye. If the upper buffer system is changed to one without detergent, the second buffer front passes the proteins. As the second buffer front passes the proteins, it removes SDS and changes the mobility of the proteins. As electrophoresis continues, the protein components move according to their charge as well as their hydrodynamic properties. A second time dimension is thus achieved, analogous to the second spatial dimension in two dimensional chromatography, which leads to improved separation of the proteins in the case of TSH subunits and calf thymus histones.

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Abbreviations: b-TSH, bovine thyroid stimulating hormone; SDS, sodium dodecyl sulfate; DOC, sodium deoxycholate.

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MATERIALS

Bovine TSH was extracted and purified as described by Condliffe (5). TSH was dissociated by treatment at pH 2.5, 45° C for 1 hour as described by Sorimachi and Condliffe (6). Calf thymus histones were generously supplied by Dr. K. Hamana of the Institute of Endocrinology of Gunma University.

SDS was purchased from BDH Chemicals, Poole, England. [^{35}S] SDS was purchased from New England Nuclear. Other chemicals were commercial products of reagent grade.

METHODS

TSH (300 µg were dissolved in 100 µl of 0.01 M sodium phosphate buffer at pH 7.0) was treated with [^{35}S] SDS (15 µg dissolved in 50 µl of water) at room temperature for 30 min. before gel electrophoresis. 50 µl were applied to the gel and electrophoresis was carried out at pH 9.5 and 2mA per tube in the absence of SDS, as reported by Liao et al (7). The gel concentration was 7.5%. The gel was cut in 0.2 to 0.3 cm sections and the radioactivity in each segment was counted in Aquasol after standing overnight at 37° C. This method allowed total recovery of the radioactivity.

SDS gel electrophoresis was carried out as reported by Weber and Osborn (2). For TSH and histones, 10% and 15% gels, respectively, were used. The current was 8mA per tube in each case. The gel size was 0.6 x 10 cm.

In the two-stage gel electrophoresis, the first stage in SDS was carried out for 2 hours. The electrophoretic buffer was changed to the buffer without SDS, and the electrophoresis was continued. Removing SDS from the buffer did not affect the current.

In order to extract the TSH subunits after gel electrophoresis, the part of the gel which contained protein was cut out, and placed in a glass electrophoresis tube. Buffer solution (0.012 M sodium glycinate at pH 9.5) was poured on the piece of gel, and the end of the tube was covered with a dialysis membrane. Electrophoresis was then carried out at 2mA per tube for 3-4 hours. The protein moved from the gel into the buffer solution which was then recovered and freeze dried.

RESULTS AND DISCUSSION

TSH was treated with [^{35}S] SDS. Figure 1 shows the gel electrophoretic pattern of TSH, and the distribution of [^{35}S] SDS. Over 90% of the counts were associated with the tracking dye (band 2) whereas there were no counts in the position of the TSH. Even if TSH was pre-treated with a higher concentration of SDS (1.0%), the electrophoretic pattern was almost the same as that of the native sample. In the latter case, when the gel was soaked in 0.1 M potassium phosphate after electro-

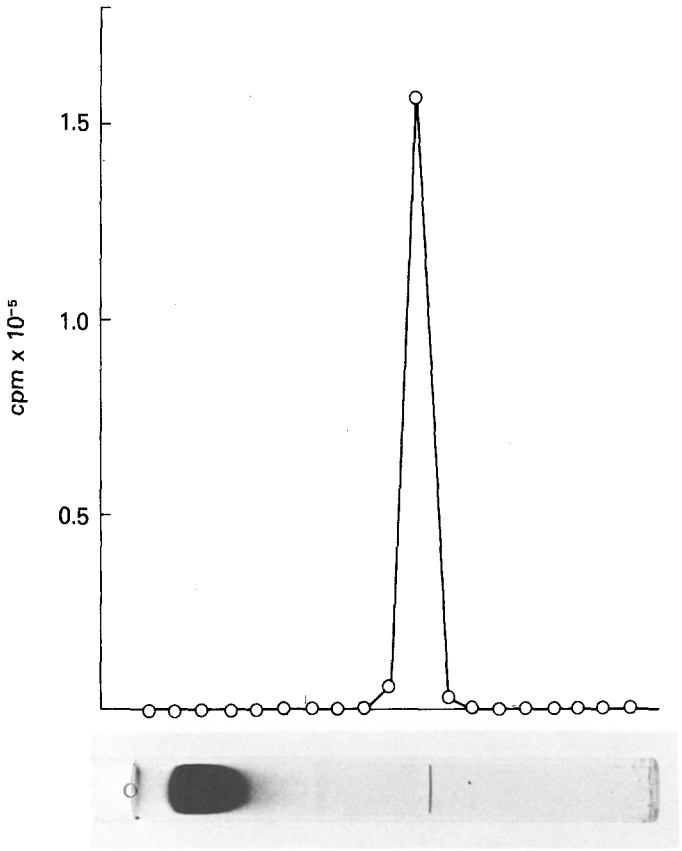

Figure 1. Electrophoresis of the b-TSH[^{35}S] SDS complex at pH 9.5, 0.012 M sodium glycinate without SDS.

phoresis, the precipitate of potassium dodecyl sulfate appeared at the tracking dye position

When sodium deoxycholate (DOC) was used instead of SDS, DOC was also removed from the protein by the gel electrophoresis. In order to see the distribution of DOC in the gel, the gel was soaked in the destaining solution to precipitate deoxycholic acid. Even if 1.0% DOC was used for the pretreatment, a precpitate was never seen at the protein band, but was observed only at the tracking dye position.

Even if gel electrophoresis is conducted at the same pH as the isoelectric point of the protein, an SDS protein complex will move

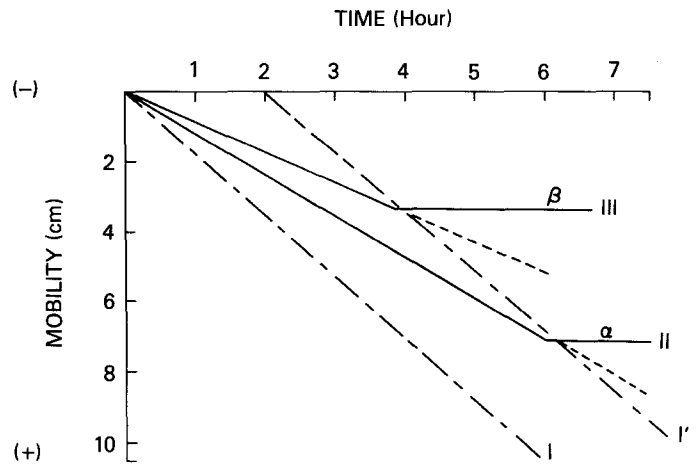

Figure 2. Theoretical relationship between the electrophoretic time and mobility of the components. This scheme represents the behavior of the subunits of TSH. I represents the position of tracking dye at the front of the first stage buffer containing SDS. I' represents the position of the front of the second stage buffer without SDS.

II and III represent the positions of the α and β subunits of b-TSH. The first stage of electrophoresis was carried out for 2 hours, followed by the second stage without SDS. The area above and to the right of the dashed line I' represents the second buffer without SDS. The area below and to the left of the dashed line I' represents the first buffer containing SDS. The dashed line I represents the position of the first buffer as indicated by the tracking dye.

towards the anode due to its negative charge, whereas the protein alone will not move in the absence of SDS. The distance moved is proportional to the hydrodynamic volume of the SDS-protein complex. When SDS is removed from the electrode buffer, the mobility of the protein does not change until it is passed by the second buffer front without SDS. When this occurs, the mobility of the protein depends on the net charge as well as hydrodynamic volume of the protein. For TSH at pH 7, when the second buffer without SDS overtakes the complex the SDS is removed from the protein and the protein, now at its isoelectric point, stops moving. Figure 2 shows the hypothetical behavior of the subunits of TSH in such During the time periods shown, positon of the β subunit did not change (Gel #II, III and IV), because it was already by-passed by the second

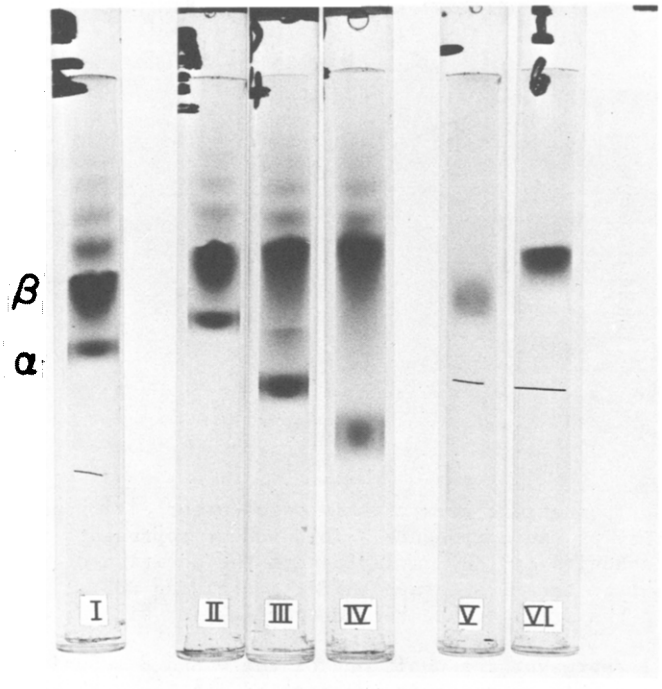

Figure 3. Electrophoretic patterns: Gel #1 shows b-TSH pretreated at pH 2.5, 45° C for 1 hour in SDS gel electrophoresis at pH 7.0, 8mA/tube, for 4 hours. Gel #II, III, and IV were obtained from the two-stage gel electrophoresis system using acid-treated TSH. Normal SDS gel electrophoresis was carried out for the first 2 hours, and then electrophoresis was continued after removal of SDS from the buffer solution. Gel #II, III, and IV show the patterns after 1.5, 2.5 and 3.5 hours, respectively, after changing the buffer solution. Gel #V and VI represent purified, recovered α and β subunits of b-TSH rerun in SDS gel electrophoresis.

buffer front, but the α subunit continued to move. The experimental behavior of the subunits in the two-stage gel electrophoresis was consistent with the theoretical predictions detailed in Figure 2. In order to prepare each subunit of TSH, several gels equivalent to gel #IV were cut and the protein was extracted electrophoretically from the gel pieces. Thus gel #V and VI represent purified α and β subunits of TSH, respectively, examined by the standard SDS system. The mobility of each a two-stage gel electrophoresis system. In SDS gel electrophoresis, the relationship between the time of electrophoresis and the positions or

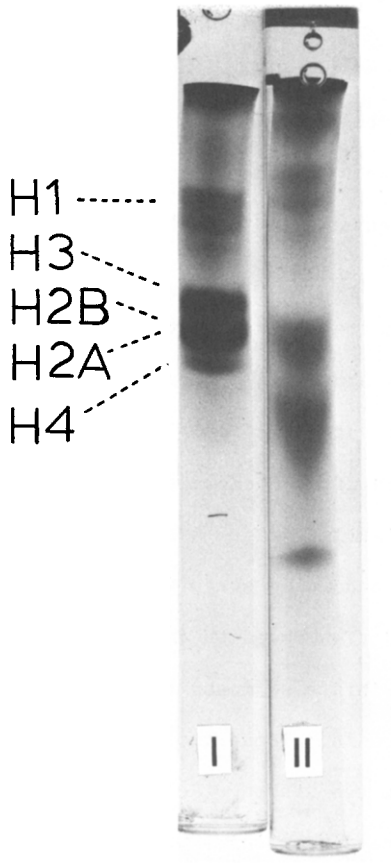

Figure 4. Gel electrophoresis patterns of calf thymus histones. Gel #I was obtained from SDS gel electrophoresis at pH 7.0, 8mA/tube for 4.5 hours. Gel #II was obtained from the two-stage method. SDS gel electrophoresis was carried out for first 2 hours and then the buffer was changed and electrophoresis was continued for 5.5 hours.

migration distances of protein or the tracking dye is linear. After removing SDS from the electrode buffer the β subunit of TSH is first to encounter the second buffer front and stops, but the α subunit continues to move until it encounters the second buffer front. Therefore, an improved separation is obtained as shown in Figure 2.

Figure 3 shows the electrophoretic patterns that are obtained. subunit in SDS gel electrophoresis was the same as found previously (6). This procedure can be conveniently used to prepare the purified subunits

from 20-100 μg quantities of b-TSH. It can be scaled up for larger quantities. Liao and Pierce, (7) used long gel columns (300 x 2.5 cm) of Sephadex G-100 to separate the subunits from each other. Ion exchange chromatography has been used by Kourides et al. (18). These columns require milligram quantities of TSH and take much longer to separate the sub-units.

The two-stage gel electrophoresis system was also applied to calf thymus histones. Figure 4 shows the electrophoretic patterns of these histones. Gel #I shows the pattern after SDS gel electrophoresis for 4.5 hours. Gel #II was obtained after the two-stage gel system was applied. The SDS gel electrophoresis (1st stage) was carried out for 2 hours; then the second gel electrophoresis stage without SDS was carried out for 5.5 hours. The H1 fraction moved to the cathode after encountering the second buffer front. This also means that SDS was removed from the protein by gel electrophoresis. These two experiments, using TSH and histones, suggest that the two-stage gel electrophoresis system is generally useful for separating proteins which are close to each other in SDS gel electrophoresis.

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