SEPARATION AND RADIOAUTOGRAPHY OF MICROGRAM QUANTITIES OF RIBOSOMAL PROTEINS BY TWO-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS

G.A. HOWARD and R.R. TRAUT

Department of Biological Chemistry, School of Medicine, University of California, Davis, Calif. 95616, USA

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1. Introduction

Recently a two dimensional polyacrylamide slab gel electrophoresis system was described by which all of the ribosomal proteins of *E. coli* were separated into distinct spots [1]. This system and others have been used to separate eukaryotic as well as prokaryotic ribosomal proteins [2–6]. The system developed by Kaltschmidt and Wittmann [1] gives excellent resolution of both types of ribosomal proteins; moreover, standard and highly useful nomenclature of *E. coli* ribosomal proteins has been defined in terms of this system [7].

We have adopted the basic system of Kaltschmidt and Wittmann to use thinner and smaller slabs which require smaller amounts of sample, and which can be dried for radioautography of radioactive proteins. The advantages of the smaller two-dimensional slab described here are summarized as follows: Ribosomal protein required: 50 – 100 μg; total electrophoresis time: 12 hr; staining and destaining time: 3–5 hr; radioautography: readily possible; gel can be totally dried in 2–3 hr without distortion or shrinkage for accurate radioautograms; estimated cost of basic apparatus, about $ 160 (not yet commercially available).

The resolution of *E. coli* ribosomal proteins with the miniature slab gel system described here is essentially identical to that reported by Kaltschmidt and Wittmann [1]. It has also been employed to separate eukaryotic ribosomal proteins and to identify by radioautography those phosphorylated by protein kinase [8].

2. Methods and materials

2.1. First dimension by disc gel electrophoresis

Separating gel, pH 8.7 (modification of that previously reported [1]): urea, 360 g/ℓ; acrylamide, 40 g/ℓ; bisacrylamide, 1.33 g/ℓ; EDTA-Na_2, 8 g/ℓ; boric acid, 32 g/ℓ; Tris, 48.6 g/ℓ; TEMED, 0.45 ml/ℓ.

This solution is filtered and may be stored at 4° for several weeks. It is deaerated just before polymerization which is catalyzed with 5 μl of a freshly prepared 10% (wt/vol) solution of ammonium persulfate/1.0 ml of gel solution.

Running buffer, pH 8.2: EDTA-Na_2, 2.4 g/ℓ; boric acid, 4.8 g/ℓ; Tris, 7.25 g/ℓ. Acrylamide (technical grade), bis-acrylamide, and TEMED were obtained from Eastman Chemicals; EDTA-Na_2 and Tris from Sigma; and the remaining reagents from Mallinckrodt. Recrystallization of the reagents was unnecessary, as results were the same with crude or purified reagents.

Electrophoresis in the first dimension was performed in a standard disc gel electrophoresis apparatus as described by Davis [9], in which gels are run in glass tubes either 0.45 cm × 9 cm, or 0.45 cm × 12.5 cm.

Two separate methods of applying sample in the first dimension have been used to minimize the substantial loss of protein at the center-origin reported in other systems in which the sample is polymerized in a sample gel containing acrylamide [5, 10].

a) The sample (50–100 μg) is applied in agarose in the center of the first dimensional gel (as described by Kaltschmidt and Wittmann [1], but substituting aga-

rose for acrylamide); in this way proteins migrating both toward the anode and cathode are resolved in the same run. For this method the longer disc gel tubes (0.45 cm × 12.5 cm) are used. The lower half of the separating gel is poured in the tube and overlayered with water according to standard methods. Agarose (1% in pH 8.2 running buffer) is liquified in a boiling water bath, mixed in a 1 : 1 ratio with the protein sample and kept at 40° in a heating block until it is layered onto the flat surface of the polymerized lower gel. Water is again layered over the agarose–sample mixture until the agarose has hardened; it is then removed, and the tube is filled with more separating gel solution over the sample zone. Electrophoresis is carried out with the cathode above, with pyronine G (0.5%) as the tracking dye.

b) Identical amounts of sample are applied at the top of each of two separate gels in the shorter tubes (0.45 cm × 9.0 cm). Electrophoresis of one of the gels is from the anode to the cathode with pyronine G (0.5%) as the tracking dye; electrophoresis of the other gel is from the cathode to the anode using bromphenol blue (0.1%) as the tracking dye.

By method (a) small losses of proteins occur as material immobilized in the agarose layer. These are however much less than those observed in other systems [5, 10]. By method (b) essentially all the protein is recovered in the bands migrating from the origin. Thus although ostensibly method (b) requires twice as much protein sample as (a), in fact because nothing is lost, the increased sample required is less than double that required when the sample is polymerized in the center of a single disc gel.

In both methods (a) and (b), electrophoresis is performed at 3 mA/gel tube for 30 min to facilitate the stacking of the protein bands; then the current is increased to 6 mA/gel and electrophoresis continued for 5–6 hr. The gels can be removed at once from the glass tubes in preparation for the second dimension, or stored in the tubes at 4° for 24–48 hr before use without significant diffusion of the protein bands or loss of resolution.

The first dimensional gels are sliced in half longitudinally in order to fit between the glass plates in the second dimension. This can be done by placing the gel in a halved piece of tygon tubing matching the diameter of the gel, and then slicing the gel with uniform downward pressure of a thin knife blade.

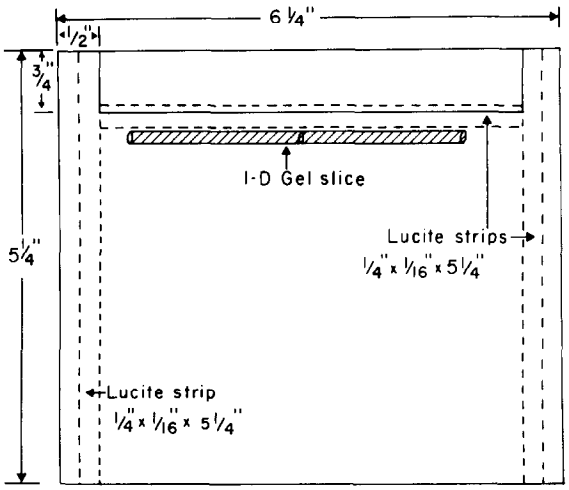

Fig. 1. Placement of the plexiglass spacers and the gel slice from the first dimension between the glass plates used for the second dimension. The two glass plates (one with a notch in the top as indicated) are made from ordinary double strength window glass. The resulting "sandwich" is clamped in place in an apparatus like that referred to in the text.

The first dimensional gel halves are then dialyzed against the "starting buffer" of Kaltschmidt and Wittmann [1] (urea, 480 g/ℓ; glacial acetic acid, 0.74 ml/ℓ; KOH, 0.67 g/ℓ), pH 5.2, for a total of 60 min with at least two changes of buffer.

2.2. Second dimension by slab gel electrophoresis

The apparatus in the second dimension (fig. 1) is an adaption of the thin sheet gel apparatus described by Reid and Bieleski [11], as modified by Studier [12, 13]. Two glass plates are sandwiched together with plexiglass spacers to allow the gel sheet of approx. 2 mm thickness to be formed. A thin coating of petroleum jelly on the spacers is used to prevent leakage of the gel solution prior to polymerization.

The first dimensional slice is placed in position against the top spacer as shown (fig. 1); any excess gel at the ends is trimmed off to allow the gel to fit in the defined width. When the first dimension has been run as two separate short gels in opposite directions, the two origins are placed adjacent to each other in the center as shown in fig. 1. The two glass plates are clamped together at the sides and the top (where the first-dimensional slice is positioned) with foldback binder clips. The plates are inverted 180° and the cavi-

ty thus formed is filled with second dimensional separating gel solution, pH 4.5: (modification of a solution previously reported [1]): urea, 360 g/ℓ; acrylamide, 180 g/ℓ; bis-acrylamide, 2.5 g/ℓ; glacial acetic acid, 53 ml/ℓ; KOH, 2.7 g/ℓ; TEMED, 5.8 ml/ℓ. This solution is filtered and may be stored at 4° for several weeks. It is deaerated immediately before polymerization is initiated by addition of ammonium persulfate: 30 μl of a freshly prepared 10% (wt/vol) solution/1.0 ml of gel solution.

After the gel has polymerized the plates are again turned 180° so that the first dimensional gel slice is on top. The spacer is removed from the top, all excess petroleum jelly is wiped away; the binder clips are carefully removed and the plates with the enclosed gel sheet are clamped to the apparatus referred to above.

The tracking dye (0.1% pyronine G in 20% glycerol) is layered across the top of the first-dimensional slice, under the running buffer, pH 4.0 (modification of the procedure previously reported [1]): glycine, 14 g/ℓ; glacial acetic acid, 1.5 ml/ℓ. Electrophoresis is carried out with the anode on top for 30–60 min at 40 V to allow stacking of the proteins in the first dimensional gel slice; then the voltage is increased to 80–150 V and electrophoresis is continued for 6–12 hr. The exact voltage within the limits tested makes no difference in the resulting separation of the proteins; thus convenience of running time is the major criteria for choosing a particular voltage. At 80 V the dye front moves 1 cm/hr.

Electrophoresis is stopped when the tracking dye is within 1 cm of the bottom of the gel. The top glass plate is loosened from the gel slab by *carefully* prying it upward with a wide spatula. The gel slab is lifted off the lower plate and placed in a tray for staining with Coomassie Brilliant Blue (R-250, 0.1% in 7.5% acetic acid: 50% methanol: H_2O) for 1–4 hr. Occasional agitation may be necessary since the gel slab may adhere to the tray. The gels are destained by slow shaking in a tray of destaining solution (50% methanol: 7.5% acetic acid: H_2O) on a mechanical shaking bath with several changes of the solution for 2–4 hr.

After destaining, the gel slab can be photographed wet, or dried onto filter paper either for storage or for radioautography. Drying is accomplished by using a vacuum or aspirator pump and low heat as described by Maizel [14] (a modification of the procedure of Fairbanks, et al. [15]). The thinness of the gels allows them to be totally dried in about 2 hr without distortion, shrinkage or cracking.

3. Results and discussion

As seen in fig. 2 the method gives separation of the ribosomal proteins of *E. coli* 30 S subunits comparable to that reported by Kaltschmidt and Wittmann [1, 10]. In this case (fig. 2) two separate, short first-dimensional gels were run in opposite directions. No immobilized material appears at the origin. Since this method requires only microgram amounts of ribosomal protein, the use of this "double" first dimension uses only a fraction of the material required by the larger gel systems.

The method described here has been used in this laboratory to study various types of eukaryotic ribosomes. It has been especially useful in that it requires only small amounts of material. We have been able to

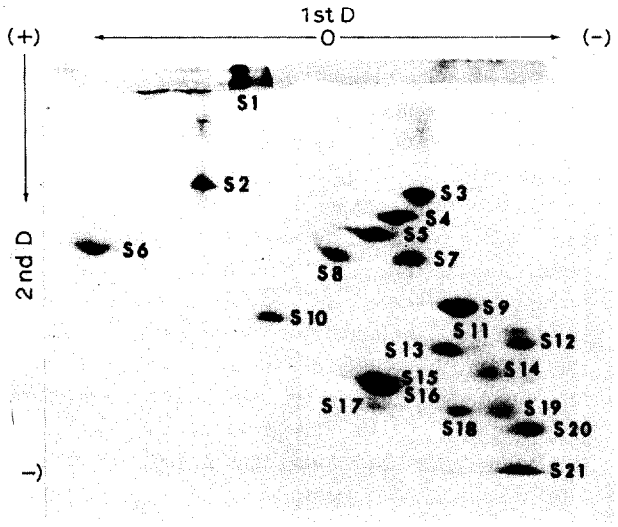

Fig. 2. Two dimensional electrophoresis pattern of *E. coli* MRE 600 30 S ribosomal subunit proteins extracted with 3 M LiCl–4 M urea [19,20]. The separated subunits were obtained by sucrose density gradient sedimentation of ribosomes washed once in 0.5 M NH_4Cl, 0.0 M $MgCl_2$ [21]. Migration directions in each dimension are indicated with arrows. The O (top-center) indicates the origin of the first dimensional gel. In this case two short first-dimensional gels with identical top-loaded samples were run in opposite directions as described in the text. Gel and buffer conditions were as described in the text.

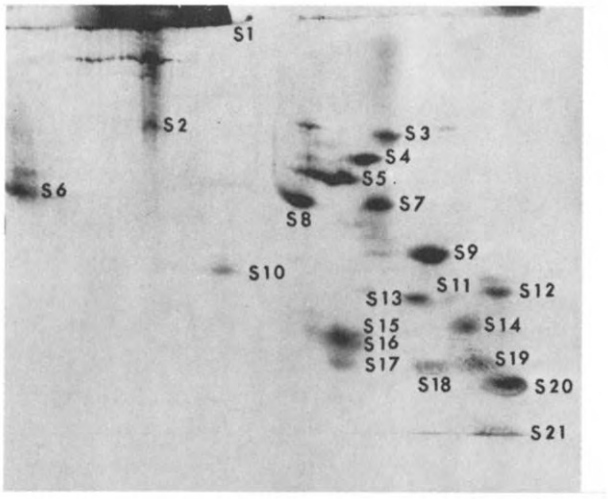

Fig. 3. Autoradiogram of the gel shown in fig. 2, in which the 30 S ribosomal proteins were labeled *in vivo* with ^{35}S [17]. The two-dimensional gel slab was dried as described in the text and the autoradiogram made by placing Kodak No-Screen Medical X-ray film directly against the dried gel.

separate 65–70 proteins from several different eukaryotic 80 S ribosomes [16].

Fig. 3 shows an example of the accurate radioautography facilitated by the use of the thin gel slabs described here. In this experiment the slab gel shown in fig. 2, which contained radioactive 30 S ribosomal proteins obtained from *E. coli* MRE 600 grown in minimal media containing $^{35}SO_4$ [17], was totally dried and placed against Kodak No-Screen X-ray film. Although radioautography of ^{32}P can be made with wet gels, end even the thicker gel slabs described previously [12], that of ^{14}C or ^{35}S requires drying of the gel; this is one of the major advantages of the method described here.

Acknowledgments

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