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Simple device for electroelution of proteins from a large number of pieces of polyacrylamide gel

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ABSTRACT

A device for electroelution of macromolecules that handles up to % different pieces of gel was constructed. The basic unit is the electroelution plate containing the % electroelution chambers, which is made from a 96-well tissue culture plate with holes bored in each individual well, both through the base of the plate and through the lid covering the plate. The electroelution plate can be used either with a buffer tank equipped with platinum electrodes or with flat graphite electrodes. Using standard conditions, 60–70% of the proteins with molecular mass less than 70 000 were eluted in 1 h.

INTRODUCTION

Preparative electrophoretic techniques include preparative isoelectric focusing (IEF) in granulated gels and focusing in gels containing immobilized pH gradients. The recovery of proteins using these methods usually involves a second zone electrophoresis into an ion exchanger followed by column chromatography [1]. Although these methods have advantages such as high resolution, even at high protein load, the procedure is cumbersome and time consuming, especially when several samples are to be processed.

One-dimensional [sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)] [2] and two-dimensional (IEF–SDS-PAGE) [3] gel electrophoresis of proteins are powerful and widely used techniques for the separation of proteins, especially for analytical purposes [4,5]. These high resolution techniques are also used for preparative purposes when the amounts of proteins to be isolated are small.

In order to recover the separated proteins from polyacrylamide gels, electroblotting is usually performed when amino acid sequencing or detection by antibodies is later to be carried out. However, if biological activities such as inhibition of cell growth or antigen stimulation of T-cells are to be tested, it would be favourable to elute the proteins into a buffer solution [6]. Such electroelution of macromolecules from polyacrylamide gels is usually time consuming and needs some care in implementation, e.g., when putting small pieces of gel into dialysis tubes for electroelution [7]. Currently available commercial equipment for electroelution handles only a relatively small number of pieces of gel, and other methods are designed to process several pieces of gel containing the same protein [8] or whole polyacrylamide gels [6]. In this paper, we describe a simple device for the electroelution of proteins that handles a variable number of pieces of gel (l-96) and that can easily be made in any laboratory.

EXPERIMENTAL

Chemicals

Standard protein molecular mass markers (SDS-6H) (Sigma, St. Louis, MO, USA) con-

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taining myosin (M_r 205 000), β-galactosidase (116 000), phosphorylase B (98 000), bovine albumin (66 000), ovalbumin (45 000) and carbonic anhydrase (29000) were used for quantitative analysis. The standard proteins were dissolved in Laemmli sample buffer [2] giving a protein concentration of 2.0 mg/ml. Prestained molecular mass markers (SDS-7B) (Sigma), used for the preliminary testing of the device, ranged from M_r 26 000 to 180 000.

The electroelution buffer was 190 m*M* glycine–25 m*M* Tris base as described previously for electroblotting [9].

Preparation of pieces of gel

Pieces of polyacrylamide gel were made by polymerizing acrylamide-bisacrylamide (30:0.8) solution containing 0.4 *M* Tris-HCl (pH 8.8) and 0.1% SDS in a glass tube of 5 mm I.D. In the present experiments, 7% polyacrylamide gels containing 1.0 mg/ml of total protein were used. After polymerization, 2 mm pieces of gel were cut from the gel cylinder and placed in the electroelution chambers. Each piece of gel contained *ca.* 45 μg of total protein.

Description of the device

The main part of the device is the electroelution plate containing the electroelution chambers, which is made from a flat-bottomed 96-well tissue culture plate (Costar, Cambridge, MA, USA). The electroelution plate can be used in a buffer tank made of acrylic plastic (Fig. 1B) equipped with platinum electrodes, where the cathode is in the bottom of the tank and the anode in the top lid. When electroelution is carried out, the chambers are separated from the outside buffer by agarose plugs in the bottom and a semipermeable membrane at the top. In order to press the membrane tightly to the wells, the plate lid with the two glued acrylic rods is pressed on to the electroelution plate using rubber bands (Fig. 1A, top view).

The electroelution plate can alternatively be used with flat graphite electrodes (Novablot) (LKB-Pharmacia, Uppsala, Sweden), with the cathode as the lower and the anode as the upper electrode (Fig. 1C). With the semipermeable membrane covering the top of the wells, the electroelution plate is placed between wet filter-papers. The plate lid is not required as the membrane is pressed tightly to the wells by the mass of the graphite electrode (anode).

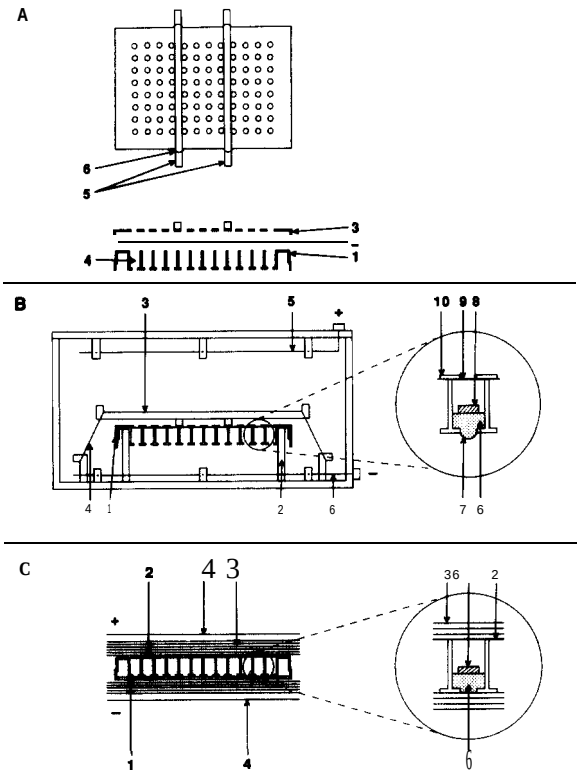

Fig. 1. Description of the electroelution device. (A) Below is a side view of the electroelution plate with the electroelution chambers (wells). The plate (1), membrane (2), lid with holes (3) and electroelution chamber (4) are shown. Above **is a top view of the plate lid showing the holes and the acrylic rods (5) used to keep the lid close to the plate with rubber bands (6). (B) Side view of the set-up using the buffer tank. The plate with the membrane and lid (1) is kept in place on the stand (2) by the acrylic rod (3) and rubber bands (4). The electrodes (5) are in the bottom of the tank and on the tank lid. The enlarged diagram of one electroelution chamber shows the agarose plug (6) with an agarose droplet (7), the piece of gel (8), the membrane (9) and the lid (10). (C) Side view of the set-up using the graphite electrodes. The plate (1) with the membrane (2), the wetted filter-papers (3) and the electrodes (4) are shown. The enlarged diagram of one electroelution chamber shows the agarose plug (5), the gel piece (6), the membrane (2) and the wetted filter-papers (3).**

Set-up for electroelution

In order to prepare the device for use in the buffer tank, the bottom of the electroelution

plate was first sealed with Parafihn (American Can, Greenwich, CT, USA) and molten 1% agarose (in electroelution buffer) was transferred into the wells that were to be used for electroelution. The amount of agarose depends on the desired elution volume, and in the present experiments 100-μl agarose plugs were used. After the agarose had solidified, the plate was turned upside down and the Parafilm removed. Droplets of molten agarose were placed on the plugs to prevent gas bubbles from adhering to the agarose plugs during the electroelution process. After the agarose droplets had solidified, the plate was again turned and the pieces of gel containing the macromolecules to be electroeluted were placed in the wells, which subsequently were filled with electroelution buffer. A semipermeable membrane was placed over the wells, taking care not to introduce any air bubbles, and the lid was pressed on to the membrane and tightened to the plate with rubber bands. The whole plate was immersed in the buffer tank containing buffer just above the plate level, and the plate was anchored to the tank with rubber bands and an acrylic rod as shown in Fig. 1B. Finally, the buffer tank was filled with the electroelution buffer and the top lid with the anode was put in place.

When the electroelution plate was used with flat graphite electrodes, agarose plugs were made as described for the application of the buffer tank. However, to ensure a flat surface of the base of the plate, agarose droplets were not used (Fig. 1C, enlargement). Ten Whatman (Maidstone, UK) 1MM filters were wetted in electroelution buffer and placed on the lower cathode. The electroelution plate, containing the pieces of gel and buffer, was then placed on top of the filters and the semipermeable membrane was carefully fitted on top of the plate, making sure that no air bubbles were trapped inside the chambers. Ten wet filter-papers were laid on the membrane and finally the anode was put on top.

Electroelution and analysis of eluate

To investigate the time course of the process, electroelution was carried out in the buffer tank with a constant current of 200 mA (0.7 mA/cm'). At time intervals, 25- and 100-μl samples were collected using a Hamilton syringe to penetrate the semipermeable membrane. The polarity of the current was reversed for 30 s prior to sample taking in order to reduce the amount of protein bound to the membrane. The collected samples were mixed with an equal volume of twofold concentrated sample buffer (2xSB) and subjected to analysis by 10% SDS-PAGE [2]. *The* gel was stained with Coomassie Brilliant Blue and the proteins were determined by videodensitometric scanning (CREAM) (Kern En Tec , Copenhagen, Denmark).

When graphite electrodes were used, electroelution was performed with a current of 0.8 mA/cm^2 as described for electroblotting by the manufacturer. Electroelution was carried out for 1 h, after which the polarity of the current was reversed for 30 s. The anode, the wet filter-papers and the semipermeable membrane were carefully removed and samples of 50 μl of the eluted proteins were collected, mixed with an equal volume of 2xSB and subjected to analysis by SDS-PAGE as described.

RESULTS

In order to follow the electroelution process visually, preliminary experiments were performed in the buffer tank using pieces of gel containing prestained markers. It was observed, as expected, that the eluted proteins concentrated just below the semipermeable membrane. Initially, some leakage between the electroelution chambers and the membrane was observed, but this was avoided by applying a thin layer of silicone grease around the top of each chamber.

When the time course of the electroelution process was investigated, pieces of gel containing standard protein mixture were placed in the wells and electroeluted using the buffer tank. Fig. 2 shows a plot of the amount of the various proteins eluted versus electroelution time. The results clearly show that electroelution proceeded more rapidly with low- than with high-molecular-mass proteins. After 60 min, 55–61% of the molecules with $M_r <$ 70000 were eluted, whereas only 13% of phosphorylase B (98000) and 11% of β-galactosidase (116 000) were eluted. When electroelution was carried out

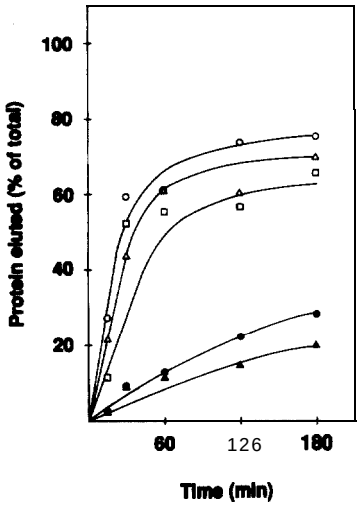

Fig. 2. Time course of the electroelution process using the buffer tank. Standard proteins were electroeluted for the times indicated and determined by SDS-PAGE and densitometric scanning. 0 = carbonic anhydrase (M_r 29000); △ = ovalbumin (45000); □ = bovine serum albumin (66 000); ● = phosphorylase *b* (98 000), A = β-galactosidase (116000).

for an additional 2 h, 65–70% of the proteins with $M_r < 70\,000$ were eluted, hence a relatively small additional yield of these proteins was observed. Regarding the higher molecular mass proteins, 28% of phosphorylase B (98000) and 20% of β-galactosidase (116 000) were recovered in 3 h whereas no myosin (205000) was detected.

When electroelution was performed using the graphite electrodes, 60–70% of the proteins with M_r 29000, 45 000 and 66 000 were eluted in 1 h (Fig. 3). Electroelution of the proteins with M_r 98 000 and 116 000 was not very efficient, releasing only 5–15% of the total protein in 1 h. Myosin (205 000) was not detected. The results obtained with the graphite electrodes were thus similar to those obtained by applying the buffer tank.

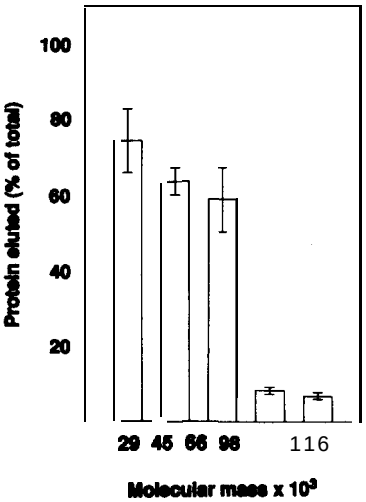

Fig. 3. Electroelution using graphite electrodes. Standard proteins were electroeluted for 1 h and determined by SDS-PAGE and densitometric scanning. The average values (±S.D.) obtained from five different pieces of gel from the same run are shown.

DISCUSSION

We have described a device for the electroelution of macromolecules from a large number of pieces of gel. The device has a simple construction and can easily be made in any laboratory. It is also flexible in use, as any number of pieces of gel between 1 and 96 can be processed. In addition, when electroelution is carried out with the graphite electrodes (Novablot), three electroelution plates can be placed adjacent to each other, allowing up to 288 pieces of gel to be processed simultaneously.

Even though electroelution of molecules with $M_r > 70\,000$ was a slow process, the device has proved to be beneficial for the elution of molecules with $M_r < 70000$, especially when total recovery is not important. In 1 h, more than 60% of these molecules were eluted. An improved recovery of the high-molecular-mass proteins might be obtained by prolonging the electroelution time or by modifying the buffer, e.g., by adding SDS.

The format of the electroelution plate is compatible with enzyme immunoassay plates and cell culture plates, hence samples can easily be transferred to these for further testing or processing. We suggest that the device described here can also be used for electroelution of DNA fragments from pieces of agarose gel as well as for rapid buffer exchange of samples containing macromolecules.

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