Chapter 19

The Electrophoretic Elution of Proteins from Polyacrylamide Gels

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Introduction

The analytical power of acrylamide gel electrophoresis is one of the keys of modern protein chemistry. It is not surprising, therefore, that many methods have been described for converting that analytical power into a preparative tool. None of the available methods is entirely satisfactory for general use since loss of resolution or low recovery is often involved. The method described here has given both high resolution and good recovery but suffers from the disadvantage of being relatively laborious (*1,2*). In addition, although the recovered proteins are good for

peptide analysis or amino acid composition determination, we have found very low yields on Edman degradation of proteins eluted from gels (*3*).

The method described below works well for eluting proteins from acid–urea–Triton gels and should work equally well with acid–urea gels (Chapter 8). Wu et al. (*1*) describe an alternative set of buffers that can be used for SDS gels.

The method uses the principle of isotachophoresis, as in the stacking gel portion of a discontinuous gel electrophoresis system (*see*, for example, Chapter 6). The gel pieces containing the protein of interest are embedded in agarose above an agarose gel column. A detergent, CTAB, is used to displace the Triton (or SDS) bound to the protein that is electrophoretically eluted into the agarose gel column. In the column, the protein is concentrated by stacking between the leading ion (Na^+) and the trailing ion (betaine). The protein dye (Coomassie blue or Amido black) stacks ahead of the protein. The concentrated protein band is cut from the agarose gel and recovered.

Materials

1. Lyophilizer
2. Vacuum pump
3. Electrophoresis apparatus capable of running tube gels, preferably in glass tubes with small funnels like the BioRad Econocolumn #737-0243 (which is 5 mm inner diameter and 20 cm long) with the lower end cut off.
4. Staining solution (Coomassie): 0.1% (w/v) Coomassie Brilliant blue R, 5% (v/v) acetic acid, 40% (v/v) ethanol, 0.1%; (w/v) cysteamine in distilled water.
5. Destaining solution (Coomassie): 5% (v/v) acetic acid, 40% (v/v) ethanol, 0.1% (w/v) cysteamine.
6. Staining solution (Amido): 0.1% (w/v) Amido black in destaining solution (Amido) (note: add the cysteamine immediately before use).
7. Destaining solution (Amido): 7% (v/v) acetic acid, 20% (v/v) methanol, 0.1% (w/v) cysteamine.
8. Equilibration buffer: 1*M* acetic acid, 50 m*M* NaOH, 1% (w/v) cysteamine.

9. Siliconizing solution: 1% (v/v) Prosil-28 in distilled water.
10. LMT agarose solution: 0.5% (w/v) low melting temperature agarose in 1*M* acetic acid, 50 m*M* NaOH.
11. HMT agarose solution: 1% (w/v) high melting temperature agarose, 1*M* acetic acid, 50 m*M* NaOH, 0.0005% (w/v) methyl green in distilled water (*see* note 3).
12. Upper reservoir buffer: 1*M* acetic acid, 0.1*M* betaine, 0.15% (w/v) Cetyl Trimethyl Ammonium Bromide (CTAB) in distilled water. Lower reservoir buffer: 1*M* acetic acid, 50 m*M* NaOH.
13. Acidified acetone: add conc. HCl to acetone to give a concentration equivalent to 0.02*N*.
14. Elution buffer: 0.02*N* HCl with optionally 0.1% (w/v) cysteamine, 0.1*M* *N*-methyl-morpholine-acetate, pH 8.0
15. Agarose gels: (can be done 1 d before needed):
    (a) Thoroughly clean and dry the tubes and then siliconize them, for example, by immersing in 1% Prosil-28 followed by thorough rinsing with water and drying.
    (b) Soak small pieces (3 cm square) of dialysis membrane in distilled water and fix one piece over the end of each tube with an elastic band. This is intended to hold the agarose in the tube.
    (c) Melt LMT agarose solution by heating to 65–100°C. Use 7 mL per tube. Fill each tube carefully, avoiding trapping air bubbles, just to the bottom of the funnel. Let the agarose gel at 4°C.

Method

1. Stain the gel just enough to visualize the band(s) of interest. Either Coomassie blue or Amido black staining may be used. If the gel is loaded heavily enough, then 15 min in either staining solution and no destaining is recommended.
2. Cut out the bands of interest and soak them for at least 1 h, or overnight, in equilibration buffer.
3. Chop the gel bands into small pieces with a razor blade and transfer them to the funnels on the tops of the agarose gels in the electrophoresis apparatus.

4. Melt the HMT agarose solution in a 100°C bath and add between 2 and 5 mL to each funnel. Stir to remove air bubbles and achieve a uniform distribution of gel pieces in the agarose.
5. Let the agarose solution gel either at room temperature or at 4°C.
6. Place the bottom reservoir of the electrophoresis apparatus in a tray containing melting ice as coolant. Fill the upper and lower reservoir with upper reservoir buffer and lower reservoir buffer, respectively. Connect the negative terminal of the power supply to the lower electrode and the positive terminal to the upper electrode.
7. Start the electrophoresis, using 2.5–5 mA per tube (about 200 V).
8. During electrophoresis, the staining dye (Coomassie or Amido) migrates fastest, followed by the buffer discontinuity, the protein, and the methyl green marker dye. Continue electrophoresis at least until the staining dye front reaches the middle of the gel. If the buffer discontinuity is clearly separated from the staining dye, then proceed to step 9. Otherwise, continue electrophoresis until the staining dye is clearly separated.
9. Immediately remove the gel from the tube as follows:
   (a) Use a spatula to remove the acrylamide gel pieces and their supporting HMT agarose gel.
   (b) Push the gel column with a plunger from a disposable syringe out of the tube onto a clean glass plate.
10. Immediately cut the gel about 3 mm below the buffer discontinuity (above the staining dye) and about 5 mm above the buffer discontinuity. The resulting 8 mm section of gel contains the protein and the methyl green marker dye; the remainder is discarded. Place the gel section in a 1.5 mL polypropylene centrifuge tube.
11. Use the following procedure (steps 12–15) if the presence of agarose, and protein denaturation during drying, do not interfere with subsequent analysis. Otherwise proceed to step 16.

12. Add 1 mL of acidified acetone and leave overnight in the freezer, at −20°C.
13. Centrifuge (10,000*g*; 5 min) and slowly decant the acetone, which contains the dye, buffer salts, and detergent.
14. Cap the tube, pierce the cap, and dry the gel under vacuum using a vacuum pump until the vacuum falls below 100 mtorr.
15. Rehydrate the agarose with 20–50 μL of distilled water, or appropriate buffer, by incubation at 65°C for a few minutes. This solution will remain liquid at 37°C, allowing enzymatic digestion, or it may be diluted to give less than 0.1% agarose when it will no longer gel. This is the end of this procedure.
16. This step follows step 11 if steps 12–15 were unsuitable. If this procedure is used, then the methyl green marker dye should not be used (*see* note 3).
17. To the gel slice, add 0.5 mL elution buffer. Mix very gently without breaking up the agarose. Leave at room temperature for at least 1 h and preferably overnight.
18. Equilibrate a small (~1 × 10 cm) desalting column of Sephadex G-25 with 0.1*M* *N*-methylmorpholine acetate, pH 8.0.
19. Centrifuge (10,000*g*; 5 min). Carefully decant or pipet off the supernatant and set it aside.
20. Repeat steps 17 and 19, combining the supernatants. The extracted gel may be discarded.
21. Load the combined supernatants onto the Sephadex desalting column equilibrated with 0.1*M* *N*-methylmorpholine acetate, pH 8.0 (step 18). Elute with 0.1*M* *N*-methylmorpholine acetate and collect the material eluting in the excluded volume.
22. Lyophilize the protein to dryness. The resulting protein is salt-free since *N*-methylmorpholine acetate is volatile. This ends the alternative procedure.

Notes

1. Gels that have been normally stained and destained may be used but the time of agarose gel electrophore-

sis may have to be increased or the capacity of the system will be reduced. (Note that cysteamine should not be included for Coomassie staining of histone H1.)

2. For Amido black, but not for Coomassie, the equilibration step (step 2) also acts as a destaining step.
3. The presence of methyl green in the HMT agarose makes it easy to locate the buffer discontinuity during the subsequent electrophoresis and is mostly removed by acetone precipitation of the protein. Small residual amounts do not interfere with subsequent peptide mapping. However, if the protein is to be isolated and desalted by the Sephadex method (step 16 on) the methyl green should be omitted from the HMT agarose solution.(If methyl green *were* included it would elute partly with the protein and partly immediately after the protein). In this case the buffer discontinuity must be located directly from the refractive index change.
4. At step 4, use the minimum volume of agarose required to suspend the gel fragments since the time of electrophoresis depends greatly on the volume of agarose plus gel fragments.
5. Betaine (rather than glycine) is used as the trailing ion because of its higher acetone solubility. It also increases the capacity of the system.
6. If ice cooling is not used during electrophoresis, reduce the current to 2.5 mA/tube.
7. The distance between the lower edge of the staining dye and the buffer discontinuity depends on the amount of stain and how full the funnels are on top of the gel, varying from about 5 to about 40 mm. The distance between the upper edge of the staining dye and the buffer discontinuity is fairly constant at 5–10 mm (reduces to 2–3 mm if the betaine buffer is replaced by glycine).
8. The time of electrophoresis will be 2–4 h at 4 mA/tube for low amounts in the funnels, and up to overnight at 2.5 mA/gel for full funnels. Different tubes may require different running times. In this case, remove the tubes whose electrophoresis is complete as follows: (a) turn off the power, disconnect the power supply, and empty the upper reservoir buffer into a beaker.

(b) Remove the required tube(s), block the resulting hole(s) with rubber bung(s), replace the upper reservoir buffer, and continue the electrophoresis, adjusting the power supply as necessary.

If large volumes of reservoir buffers (e.g., 2 L) are used, then reservoir buffer changes are not needed.

9. It is important to remove the gel immediately after eletrophoresis since the staining dye diffuses rapidly once the current is turned off. It may be possible to remove the gel in other ways (e.g., *see* ref. *1*), but we have found the method described in step 9 to be rapid and reliable.
10. Slice the gel immediately after electrophoresis, to prevent loss of resolution by diffusion. In our experience, this 8 mm section of the gel contains at least 98% of the protein.
11. The simple acetone extraction and drying procedure (steps 12–15) is suitable if the protein is to be digested with enzymes and the products analyzed by gel electrophoresis. Note that the procedure described by Cleveland (*see* Chapter 22) provides another peptide mapping approach for some applications. The alternative procedure (step 16 on) is recommended if the protein is to be analyzed for amino acid composition or characterized by nuclear magnetic resonance or other techniques, for example, thin layer analysis of tryptic digests.
12. The protein will precipitate in the gel at step 12, possibly forming a white band, but precipitation may not be quantitative with extremely small amounts of protein.
13. Drying of the extracted gel (step 14) takes several hours. This procedure may result in irreversible denaturation of the protein, but it will probably still be solubilized by enzymic digestion.
14. At step 17, do not vortex or mix vigorously or you will get agarose in the final protein solution. Allow the protein to diffuse out. Do not include urea or other agents that would solubilize the agarose in the elution buffer.
15. The second extraction (step 20) need only be 1 h. In our tests, less than 5% of the protein remained in the

gel after the two extractions, although the white appearance persisted. The protein concentration in the eluate cannot be determined by the Bradford method (*4*) since CTAB strongly interferes.

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