Chapter 18

Recovery of Proteins from Dried Polyacrylamide Gels after Fluorography

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Introduction

Several methods are available for recovering proteins electrophoretically from polyacrylamide gels directly after electrophoresis or after staining for protein (*see*, for example, Chapter 19). Fluorography (*see* Chapter 17) is used to detect small amounts of proteins or specifically modified forms of proteins by labeling these with specific precursors such as ^{3}H-lysine or ^{3}H-acetate.

However, fluorography requires dried gels and thus prevents the use of existing methods for protein recovery from gels. We have found that dried gels containing PPO

(2,5-diphenyloxazole) can rapidly be reswollen in acetic acid. The gels may then be eluted electrophoretically so that the labeled (and also the unlabeled) proteins can be analyzed for amino acid composition, tryptic peptide analysis, and even Edman degradation to determine amino acid sequence (*1*,*2*)

Materials

1. Swelling solution: glacial acetic acid with 0.001 % (w/v) Coomassie Brilliant Blue.
2. Equilibration buffer (*see* Chapter 19): 1*M* acetic acid, 50 m*M* NaOH, 1% cysteamine.

Method

1. Determine the position of the protein band(s) of interest in the polyacrylamide gel, dried on Whatman 3MM paper, after fluorography (Chapter 17).
2. Use scissors or a razor blade to cut out the required piece of dried gel together with its paper backing.
3. Place up to 10 mL of gel pieces (*see* Note 4) in a 50 mL polypropylene tube with screw cap. Add 10 volumes of swelling solution to the tube, and gently rock, roll, or invert for 15 min.
4. Decant the solution from the swelling gel pieces and discard.
5. Repeat steps 3 and 4 once.
6. Equilibrate the swollen gel pieces for 30 min in 10 volumes of equilibration buffer, decant the equilibration buffer from the gel pieces, and discard.
7. Repeat step 6 once and use the reswollen and equilibrated gel pieces in the electrophoretic elution procedure described in Chapter 19.

Notes

1. This protocol for reswelling dried and fluorographed polyacrylamide gels is ineffective on dried gels that do not contain PPO.

2. Labeled protein bands can be precisely located in the dried gel by placing the fluorograph film on top of the dried gel. Generally, a marker pen injected with some ^{14}C-labeled compound is used to mark the filter paper at several positions next to the gel with sufficient cpm to give a small dark spot on the film after the fluorography. In gels previously stained with Coomassie Brilliant Blue, the residual stain can assist in precisely superimposing the film on the gel. Although gel staining prior to fluorography is not required when only labeled proteins are to be recovered, unlabeled proteins can only be isolated when they can be localized by their residual stain.
3. Cut the pieces of gel-on-paper small enough to assure homogeneous swelling, e.g., diameter less than 0.5 cm.
4. The "volume" of the pieces is measured as a loose layer. During the first wash the gel pieces will be seen to rapidly swell to up to twice the original gel thickness.
5. The gel pieces, in equilibration buffer, will generally display a white center of reprecipitated PPO. Gel pieces from thick gels may even be completely white. This PPO will not interfere with the subsequent electrophoretic elution, and reproducible and complete protein recovery is obtained when the gels are completely reswollen. For very thick gels (more than 2 mm thick) steps 4 and 5 may need to be repeated twice, and care should be taken to reduce the size of the gel pieces as much as possible. Loss of protein may occur wen the number of washes in swelling solution is increased, especially when the trace of Coomassie is omitted from the swelling solution.

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