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Extraction of proteins from gels-A brief review

Biji T. Kurien[§,¶,&], **R. Hal Scofield**[§,¶,&]

[§]Department of Medicine, Diabetes and Endocrinology Section, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, OK 73104.

[¶]Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, OK 73104.

[&]Department of Veterans Affairs Medical Center, Oklahoma City, Oklahoma, OK 73104.

Summary

Gel electrophoresis is an important methodology employed for protein analysis. It is often necessary to elute and recover proteins separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The procedure involves localizing the protein of interest on the gel following SDS-PAGE, eluting the protein from the gel, removing SDS from the eluted sample and finally renaturing the protein (enzymes, for example) for subsequent analysis. Proteins are extracted from gels by several methods. These include dissolution of the gel matrix, passive diffusion and electrophoretic elution. Proteins eluted from gels have been used successfully in a variety of downstream applications, including protein chemistry, proteolytic cleavage, determination of amino acid composition, polypeptide identification by trypsin digestion and matrix assisted laser desorption ionization-time of flight mass spectroscopy, as antigens for antibody production, identifying a polypeptide corresponding to an enzyme activity and other purposes. Protein yields ranging from nanogram levels to 100 μg have been obtained.

Keywords

Elution; SDS PAGE; Passive diffusion; Electroelution; Renaturation

1. Introduction

To recover a specific protein of interest from a gel, it has to be liberated out of the gel matrix that imprisons them. In principle, one has to just simply reverse the way these molecules entered the gel. But for all practical purposes this process is easily beset with problems, especially when working with large protein complexes (1). Omitting the gel matrix and performing preparative electrophoresis in solution is an alternative to the arduous process of recovering proteins from gels (2). However, free-flow electrophoresis is even more challenging with respect to the technical requirements compared to the traditional method of gel electrophoresis followed by protein elution.

Correspondence to: Biji T. Kurien, Oklahoma Medical Research Foundation, 825 NE 13th Street, MS 24, Oklahoma City, OK 73104, Phone: (405) 271-7394, Fax : (405) 271-7063, biji-kurien@omrf.org.

Proteins recovered from gels have been used in protein chemistry, proteolytic cleavage, determination of amino acid composition, polypeptide identification by trypsin digestion and matrix assisted laser desorption ionization-time of flight mass spectroscopy, as antigens for antibody production, identifying a polypeptide corresponding to an enzyme activity and other purposes. Protein yields ranging from nanogram levels to 100 μg have been obtained. Denatured enzymes obtained in this manner are often amenable to renaturation and regain enzymic activity (3).

The procedure basically involves (a) gel electrophoresis (b) localizing the protein of interest on the gel (c) protein elution from the gel (d) SDS removal from the eluted sample and (e) protein renaturation for subsequent analysis (3).

In this chapter we report the various methods that have been employed to elute proteins from gels.

2. Elution procedures

2.1 Diffusion mediated elution of proteins from gels

This method, known as elution by diffusion (4) or passive elution, is the simplest method to release protein molecules from gel matrix and can be accomplished by adding water (or buffer) to a piece of gel containing the protein of interest. Trapped protein molecules, generally proteins below 60 kDa (5), diffuse out of the gel matrix into the surrounding medium if one waits sufficiently long and can be separated from the gel by centrifugation or filtration. Contaminants such as acrylamide monomers or remainders of the polymerization initiators which also diffuse out of the matrix may necessitate additional purification procedures (such as ion exchange chromatography). In addition, this method does not work well with protein complexes in acrylamide gels (6), unless harsh detergents like sodium dodecyl sulfate (SDS) is added to the elution process (7). Complete elution by passive diffusion has been achieved by crushing a gel slice with a small Teflon pestle in an elution buffer containing 0.1% SDS and incubating the crushed gel fragments for 4 h (for a 36 kDa protein) and 16-24 h (for a 150 kDa protein) on a rotator (3).

2.1.1. SDS removal and concentration—SDS removal following elution has been effectively achieved by acetone precipitation. This procedure also concentrates the protein (3).

2.2 Protein recovery by dissolving gels

The easiest way to elute proteins from a gel matrix would be to dissolve this matrix. However, the ability of acrylamide to form stable and chemically inert matrix becomes a disadvantage in this context. Very harsh conditions are required to recover proteins from acrylamide gels containing the standard cross-linker *N,N'*-methylene bisacrylamide. Typically a gel slice is incubated in 30% hydrogen peroxide at 50°C for this purpose. This results in irreversible damage to all proteins. Other cross-linkers, such as *N,N'*-diallyltartardiamide (gel slice that has this cross-linker needs to be dissolved with 2% periodic acid), *N,N'*-(1,2-dihydroxyethylene)bisacrylamide (protein recovery requires harsh conditions) and ethylene diacrylate (gel slice needs to be dissolved with 1 M sodium

hydroxide) have been used. However, these methods have serious problems as well (as noted above). Cleavable disulfide cross-linkers have been used that require milder dissolving conditions. However, the cleavable bond in unstable under alkaline conditions (1).

2.3 Electroelution devices

Several methods have been described to elute proteins out of gels by the use of an electric field *(reviewed in 6)*. The simplest method requires no special device, except for a dialysis tubing and a flat-bed electrophoresis chamber. The proteins are trapped by the large surface of the tubing as they migrate electrophoretically out of the gel. The non-specific adsorption by the dialysis tube may be problematic.

Other electroelution methods include devices like the vertical-type eluter, horizontal-type electroeluter, bridge-type electroeluters and methods such as reversed electrophoresis using a discontinuous conductivity gradient, elution by employing a steady-state stacking buffer system and continuous elution devices. These methods have been reviewed nicely by Seelert and Krause (1).

As described, several interesting methods have been employed to elute proteins out of gels. An interesting method to elute proteins out of gels has been described in detail in this book (*see* **Chapter 35**). The method is simple, convenient, and easy to perform.

References

1. Seelert H, and Krause F (2008) Preparative isolation of protein complexes and other bioparticles by elution from polyacrylamide gels. Electrophoresis 29, 2617–2636. Review. [PubMed: 18494038]
2. Ressler N (1958) Application of direct photographic photometry to preparative electrophoresis. Nature 182, 463–464.
3. Burgess RR (2009) Elution of proteins from gels. Methods Enzymol 463, 565–572. Review. [PubMed: 19892192]
4. Lewis UJ, and Clark MO (1963) Preparative methods for disk electrophoresis with special reference to the isolation of pituitary hormones. Anal Biochem 6, 303–315. [PubMed: 14082693]
5. Jacobs E, and Clad A (1986) Electroelution of fixed and stained membrane proteins from preparative sodium dodecyl sulfate-polyacrylamide gels into a membrane trap. Anal Biochem 154, 583–589. [PubMed: 2425657]
6. Shoji M, Kato M, and Hashizume S (1995) Electrophoretic recovery of proteins from polyacrylamide gel J Chromatogr A 698, 145–162.
7. May LT, Santhanam U, and Sehgal PB (1991) On the multimeric nature of natural human interleukin-6. J Biol Chem 266, 9950–9955. [PubMed: 2033081]