New Technique for Rapid Purification, Entrapment, and Recovery of Enterotoxin A from a Liquid Chamber by Polyacrylamide Gel Electrophoresis


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Electrophoresis of enterotoxin into a middle liquid section of a polyacrylamide gel column enhances recovery for serological assay.

Disc electrophoresis in polyacrylamide gel has been useful for separating and purifying proteins including enterotoxin A (2). Enterotoxin A is cathodic-moving, whereas about 93% of the accompanying proteins move toward the anode. One of the problems encountered in using the technique for isolation of protein fractions is that, in many instances, the gel has an affinity for the enterotoxin A and it is difficult to elute the protein from the gel. Serology can be accomplished by using the total gel column with the Ouchterlony plate technique (3, 4); however, considerable antisera must be used in the test. A simple technique has been developed for isolating and recovering staphylococcus enterotoxin A in a single step during electrophoresis on polyacrylamide gel without the usual need for elution from the gel column. This technique consists of entrapping the fractionated enterotoxin band in a sucrose-filled liquid chamber in the middle of the gel column (Fig. 1). A dense sucrose solution (20%) is used because it does not readily mix with the polymerizing layer. Essentially the procedure of forming the column consists of polymerizing the bottom gel, adding the sucrose, delicately adding the top layer of acrylamide and repolymerizing the column. The operating procedure is much the same as in a standard disc electrophoresis experiment (6); however, the electrical current is turned off when the particular protein fraction reaches the liquid chamber. The protein-containing sucrose layer then can be easily drawn out with a syringe fitted with polyethylene tubing. Serological assays such as the Oudin tube technique (5) or the microslide technique (1) can easily be performed on this liquid material.

The following procedure has been used giving approximately 40% recovery of enterotoxin A. The monomeric solution from which the polyacrylamide gel was derived was made up to contain 7.5% acrylamide, 0.2% N,N',N'-methylenebisacrylamide, 0.06% N,N',N',N'-tetramethyl-ethylenediamine, 0.001% riboflavin, 0.03% potassium persulfate, and 0.34% potassium hydroxide, and titrated to pH 4.4 with glacial acetic acid. One milliliter of this monomeric solution was introduced into a glass column (77 mm in length and 6.8-mm inside diameter) sealed at one end with a piece of moistened dialysis membrane which was tautly stretched across the end and held with several turns of a small rubber band. Then 0.1 ml of distilled water was carefully layered on top of the monomeric solution by using a 1-ml tuberculin syringe which was fitted with a short piece of polyethylene tubing. This water layering procedure is used to form a flat meniscus on the gel surface. The glass column was aligned vertically, positioned within 1 inch (2.54 cm) of a daylight fluorescent lamp containing two G.E. F6T5 bulbs, and left for 1 hr. A photopolymerized bottom gel resulted from this reaction. After photopolymerization, the small volume of excess liquid above the surface of the polyacrylamide gel was discarded.

To create a liquid sucrose chamber, 0.3 ml of a 20% sucrose solution (riboonuclease-free) made up with the lower electrode buffer was layered on top of the polyacrylamide gel. The lower electrode buffer was prepared by titrating 120 ml of 1.0 N KOH with glacial acetic acid to pH 4.3 and then diluting this solution to 1.0 liter with distilled water. The upper wall of the liquid sucrose chamber consisted of a column of polyacrylamide gel polymerized on top of the sucrose layer. This was achieved by slowly layering 0.2 ml of the monomeric solution with an ultramicro-pipet, followed by the more rapid addition of another 0.8 ml of the monomeric solution on top of the sucrose layer. Extreme caution should be exercised at this stage to prevent mixing of the sucrose layer with the monomeric solution.
A 0.1-ml amount of distilled water was next layered on the surface of the monomeric solution by the procedure used for the bottom polyacrylamide gel. The column was then subjected to a second photopolymerization for 1 hr in the usual manner. At the end of this period, the excess liquid on the surface of the upper polymerized gel was discarded. A 1.0-mg sample of lyophilized enterotoxin A-containing bacterial supernatant fluid [prepared from Staphylococcus aureus strain 100 in N-Z amine NAK in the manner previously described for strain 196-E (2)] was dissolved in 0.4 ml of 5% sucrose (ribonuclease-free) made up with lower electrode buffer. A slight trace of methyl green dye was mixed with this sample. The sample was then layered on top of the polyacrylamide gel column, and the column was placed inside an ordinary disc electrophoresis apparatus (such as Canaco model 6).

The upper electrode buffer (pH 5.0) was a 1:10 dilution of a stock solution containing 31.2 g of \(\beta\)-alanine and 8.0 ml of glacial acetic acid made up to 1 liter with distilled water. The lower electrode buffer was the previously characterized potassium acetate buffer (pH 4.3). The positive electrode was immersed in the upper buffer, and the negative electrode was immersed in the lower buffer. Several polyacrylamide gel electrophoresis columns may be run simultaneously in this apparatus, by using a constant current of 6 ma per column. For the entrapment of the enterotoxin A band, the migration of the methyl green leading band was followed visually until it had penetrated and reemerged from the sucrose chamber. When the dye band had migrated about 4 mm from the lower edge of the sucrose chamber (as measured by vernier caliper), the power supply was turned off. At this point, the enterotoxin band was trapped inside the liquid sucrose chamber. The glass column containing the gels and sucrose was removed from the apparatus. The top gel was then punctured with a 0.5-mm (inside diameter) piece of polyethylene tubing attached to a 1-ml tuberculin syringe, and the liquid contents of the chamber were removed from the column. The recovered enterotoxin A was assayed immediately by the Oudin gel diffusion test or stored in a frozen or lyophilized form and later assayed.

Several trials were necessary to determine the exact amount of time and band movement necessary for the optimal recovery of enterotoxin A. A specific protein isolated by this procedure will be concentrated but not necessarily pure. Purity depends on the degree of separation of the bands during polyacrylamide gel electrophoresis.

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LITERATURE CITED


