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Separation of biomacromolecules by electrofiltration through gel layers

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Abstract

A straightforward method for concomitant separation and isolation of biomacromolecules from a mixture in solution was developed. Three gel layers that comprise a middle separation layer of 10% polyacrylamide gel were constructed. This gel system was formed in an electroconcentration apparatus above a collection chamber surrounded at the bottom by a dialysis membrane. The mixture is applied over the gel layers where biomacromolecules are caused to migrate by electrophoresis through the gel system, where they are separated into discrete bands and electroeluted into the collection chamber without dismantling the apparatus. The isolated biomacromolecules are removed from the chamber in a highly pure and concentrated form ready for further investigations. Cooling can be applied throughout the whole process, and the setup and conditions of run can be modified according to the characteristics of the biomacromolecules to be purified. The components of a mixture containing the glycoprotein ovalbumin and bovine serum albumin monomer, dimer, and tetramer were successfully isolated as concentrated and highly pure fractions with good recoveries ranging from 70 to 89%. Other proteins were successfully isolated under denaturing conditions in the presence of sodium dodecyl sulfate (SDS) or 6 M urea.

Keywords: Electrofiltration; Electroelution; Protein purification; Polyacrylamide gel; Agarose gel

Isolation of biomacromolecules from a mixture is a valuable tool and widely used practice in biosciences. In general, proteins and protein complexes can be separated by a wide range of techniques ranging from the nonspecific precipitation by a precipitating agent, such as concentrated ammonium sulfate, to the highly specific separation by immunoaffinity chromatography. These methods usually include more than one step to get the required protein in a reasonably concentrated form. Electroelution of proteins after their electrophoretic separation in polyacrylamide or agarose gels is a known practice [1–8], but most of the methods used require a long running time and suffer from low recoveries and undue dilution of the isolated macromolecule. Electrofiltration has been used to isolate peptides and proteins. Käppler and Posten [9] reported the isolation of lysozyme from a mixture of lysozyme and bovine serum albumin (BSA)[1] by two-sided electrofiltration using pressure and electric field gradients, but the isolated protein was in a diluted form. Other researchers [10–12] reported incomplete isolation of single peptides from protein hydrolysates by electrofiltration with pressure and electric field gradients. Atmeh and coworkers [13] reported an efficient method by which elution and concentration of the isolated biomacromolecules can be achieved simultaneously with high recovery. However, this requires a preseparation of the biomacromolecules on a gel, followed by cutting the required band and its elution from the gel. Here we present an improved method that does not require the presepara-

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[1] *Abbreviations used:* BSA, bovine serum albumin; SDS, sodium dodecyl sulfate; TEMED, *N,N,N′,N′*-tetramethylethylenediamine; apoA-I, apolipoprotein A-I; apoA-II, apolipoprotein A-II; PAGE, polyacrylamide gel electrophoresis.

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tion of biomacromolecules on a gel but rather depends on a single step that involves separation and electrofiltration of a solution of biomacromolecules through a preformed gel filter system using an electroconcentration apparatus [14]. The method is a straightforward separation according to the size of the biomacromolecules under nondenaturing conditions or in the presence of a denaturing agent, namely, sodium dodecyl sulfate (SDS) or 6 M urea. The method is efficient, fast, relatively simple, and economic, and it produces concentrated biomacromolecule solutions of high purity and could be sterile. The properties of the gel filter can be adjusted according to the properties of the molecules to be isolated.

Materials and methods

Materials

Chemicals are of electrophoresis grade. Glycine and acrylamide were obtained from Scharlau (France), bisacrylamide was obtained from Fluka Chemicals (Switzerland), *N,N,N′,N′*-tetramethylethylenediamine (TEMED) was obtained from Acros (USA), and agarose was obtained from BDH Chemicals (UK). Carbonic anhydrase was purchased from Nentech (UK), apolipoprotein A-I (apoA-I) and apolipoprotein A-II (apoA-II) were purchased from Fitzgerald (USA), and BSA, alkaline phosphatase, cytochrome *c*, and ovalbumin all were purchased from Sigma (USA).

Construction of the electrofiltration apparatus

Electrofiltration was performed in a modified form of the electroconcentration apparatus designed previously by Atmeh [14]. In brief, the apparatus consists of two compartments: upper and lower (Fig. 1). The upper compartment contains a cooling coil and the buffer (14 mM Tris and 110 mM glycine, pH 8.3) in which the cathode is immersed. The lower end of this compartment is in the form of a threaded glass cylinder to which a screw cap is attached. The cap has a wide hole in the middle where a collection chamber (300 µl capacity) is formed between the two faces of the hole by fixing a dialysis membrane (molecular mass cutoff = 15,000) at the bottom of the cap held by a rubber band. Buffer layer is added over the dialysis membrane, followed by placing a nitrocellulose membrane (0.2 µm pore size) above the buffer layer. The cap is then screwed to the threaded end of the upper compartment. The lower compartment contains a cooling coil and the buffer in which the anode is immersed. Two designs of the collection chamber were used: (i) a closed chamber in which the collected fraction is carefully withdrawn from the collection chamber by a small syringe and (ii) a modified chamber with two small holes made at opposite sides of the cap (Figs. 1B and C) to act as an inlet and an outlet for the buffer, permitting washing out of the collected fraction from the chamber, with the flow of buffer through the inlet (connected to a buffer reservoir) and outlet (leading to collection tubes) being controlled by valves. The latter design enables both continuous and discontinuous washing of the chamber content, and the flow rate of the washing buffer is regulated by adjustment of the height difference between the reservoir and the collection tube. The electrofiltration membrane system is constructed from three layers of gels as follows: (i) a mixture of 1% agarose and 4% polyacrylamide (1:1) is poured inside the lower part of the upper compartment over the cellulose nitrate membrane to form a support gel layer with a thickness of 5 mm, (ii) a separation and filtration gel layer (10% polyacrylamide, 20 mm thick) is placed over this layer, and (iii) a concentration gel layer (5% polyacrylamide, 5 mm thick) is formed above the other two layers. The two compartments are filled with the buffer, and the electrodes are immersed in the buffer. The sample (dissolved in the buffer containing 10% [w/v] sucrose) is carefully placed under the buffer over the 5% polyacrylamide gel layer. Sucrose is added to increase the density of the sample so as to prevent its diffusion during the underlayering and electrofiltration process.

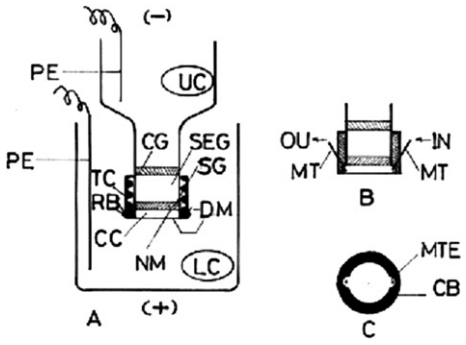

Fig. 1. Electrofiltration apparatus. (A) Scheme of a longitudinal section of the apparatus used for stepwise electrofiltration. (B) Scheme of a longitudinal section of the lower part of the apparatus used for continuous and discontinuous electrofiltration. (C) Scheme of a traverse section of the lower part of the apparatus used for continuous and discontinuous electrofiltration. CB, cap bottom; CC, collection chamber; CG, concentration gel layer (5% polyacrylamide); DM, dialysis membrane; IN, buffer inlet; LC, lower compartment (filled with buffer); MT, capillary metallic tube; MTE, metallic tube end; NM, nitrocellulose membrane; OU, buffer outlet; PE, platinum electrode; RB, rubber band; SEG, separation gel layer (10% polyacrylamide); SG, support gel layer (1% agarose and 5% polyacrylamide, 1:1); TC, threaded cap; UC, upper compartment (filled with buffer). Cooling coils can be inserted in the upper and lower compartments.

The components of the apparatus do not trap appreciable amounts of proteins, as was verified in a previous work [13] where the gel layer trapped less than 0.2%, the nitrocellulose membrane trapped 1.9%, and the dialysis membrane trapped 1.5%.

Electrofiltration conditions

Nondenaturing conditions

Electric field is applied at 1330 $V.m^{-1}$ for 15 min to concentrate the biomacromolecules and then at 2330 $V.m^{-1}$