Analytical Biochemistry 523 (2017) 39–43

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

An innovative ring-shaped electroeluter for high concentration preparative isolation of protein from polyacrylamide gel

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ARTICLE INFO

Article history:
Received 5 December 2016
Received in revised form
22 January 2017
Accepted 25 January 2017
Available online 27 January 2017

Keywords:
Protein
Gel electrophoresis
Preparative isolation
Electroelution
Sample recovery

ABSTRACT

A ring-shaped electroeluter (RSE) was designed for protein recovery from polyacrylamide gel matrix. The RSE was designed in such a way that a ring-shaped well was used to place gel slices and an enrichment well was used to collect eluted protein samples. With HSA as model protein, the electroelution time was less than 30 min with 80% recovery rate, and the concentration of recovered protein was 50 times higher than that of conventional method. The RSE could be reused at least ten times. The developed device makes great advance towards economic electroelution of biomolecules (such as proteins) from gel matrix.

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Due to its high resolution, polyacrylamide gel electrophoresis (PAGE) is not only used for determination of proteins but also applied for preparative isolation of various biomolecules, such as protein complex, nucleic acids, virus particle, nanoparticles and peptides as well [1–4]. Furthermore, the recovery of proteins from PAGE have been successfully used in various downstream purposes, such as protein chemistry, amino acid composition determination and antigen identification etc. [5,6].

The recovery of isolated proteins from polyacrylamide gel is always an issue of interest [7–10]. So far, three main methods, including (i) passive elution [1–4] (ii) gel dissolving [5,11,12] and (iii) electroelution [7,8,13], were used for protein recovery from polyacrylamide gel. For passive elution, active chemicals such as harsh detergent SDS [14], Triton X-100 [15], were applied to accelerate trapped proteins diffusion out of gel slice [16]. This method was time-consuming, requiring 18–36 h and the recovery rate was very poor about 30%–50% [16]. Whereas, the second method, gel dissolving method also needed very harsh conditions, such as 30% hydrogen peroxide [v/v] at 50 °C and 2% β-mercaptoethanol [w/v]to dissolve the cross-linker [17,18], leaving irreversible damage to protein sample and also adding extra contaminants to recovered protein samples [19].

Electroelution is predominant among these methods, because it is fast and not involved in any harsh conditions [20]. For decades, many researchers dedicated to improve devices of electroelution for collecting minute fractions of proteins from gel bands with the minimum sample loss [21–23]. For example, a 3 MM whatman device involving filter paper and collection tube was designed for microscale protein preparation [24]. Witkowski et al. introduced a molecular weight-based fractionation system for rapid recovery (2 h) of protein from gel [25]. Recently, a novel micro-scale preparative gel electrophoresis system was developed for recovery of proteins from gels with up to 92% recovery [26]. In 2012, our research group successfully developed a simple monolithic column electroelution device for protein recovery with 82% recovery [27]. However, the recovered protein in those methods was diluted and concentration step was needed for downstream applications, which unavoidably prolongs experimental time and increases the loss of precious sample.

Herein, a novel ring-shaped electroeluter (RSE) was designed to enrich preparative protein for downstream uses. The RSE has several vantages, such as high concentration of protein recovered,

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http://dx.doi.org/10.1016/j.ab.2017.01.023
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reusability, easy to wash and user-friendly.

The RSE device mainly consists of two parts, the upper cathode and lower anode (Fig. 1). The cathode is a cylinder with a ring-shaped well used for placing the protein band of interest and a small enrichment well in the center (Fig. 1A and B). The i.d. and o.d. of the ring-shaped well (3.0 mm in width and 7.0 mm in depth) are 25.0 mm and 31.0 mm, respectively. The enrichment well is 4.8 mm in diameter and 17.5 mm in depth. The enrichment well with a pore at the bottom is 10.5 mm longer than the ring-shaped well and separated with dialysis membrane (the pore size can be varied with the nature of sample to be separated) to collect the eluted proteins. The enrichment well is immersed into the tank of the cylindrical anode (31.0 mm i.d., 15.0 mm in depth). The details of RSE was given in Fig. S1. The platinum cathode and anode are fixed in the ring-shaped well and the middle of the lower anode.

To carry out the electroelution experiment, proteins were firstly separated by a SDS-PAGE (10% of acrylamide [w/v]). In order to identify the exact position of protein of interest, the first and last lane of SDS-PAGE slab were cut and delivered into staining process as discussed by Driska [28]. With the reference of two standard guide strips, protein band of interest in non-stained gel slab was excised with a scalpel (Fig. 2A) and then rinsed with 50 mL ultra-pure water at shaker for 1 min to remove SDS and buffer salt out of the surface of gel slice. The gel band with protein of interest was then placed directly into the ring-shaped well (Fig. 2B). Ring-shaped well with platinum electrode, enrichment well and the lower anode with platinum electrode well were all filled with elution buffer (2.0 mL in the upper cathode and 7.5 mL in the lower anode). Then, constant voltage was applied. The proteins trapped in the gel were eluted and moved towards the anodic electrode through the enrichment well under electric field due to negative charge (Fig. 2C). After electroelution process, the supernatant in the enrichment well was removed away gently and 80 μL of elution buffer with eluted protein was remained in the enrichment well. The remaining elution buffer with recovered protein in the enrichment well was transferred into 1.5 mL tube.

A series of experiments were carried out to assess the working efficiency of newly fabricated electroeluter. Firstly, the effect of time on the recovery rate was evaluated. Gel slices having 100 μg HSA were electroeluted with elution time ranging from 10 min to 60 min by RSE and conventional method. Conventional electroelution was carried out according to the protocol of Mcdonald et al. [30]. After that 80 μL of elution buffer beyond the bottom of enrichment cell was collected at different time intervals and quantified by commercial protein concentration determining kit (Thermo Fisher Scientific, USA, Massachusetts) using BCA protocol [29]. As shown in Fig. S2, protein recovery rate gradually increased as the first 30 min and then kept almost stable after 30 min. We compared the concentration of recovered protein by RSE with the conventional electroelution (Fig. 3 and Table 1). The results showed

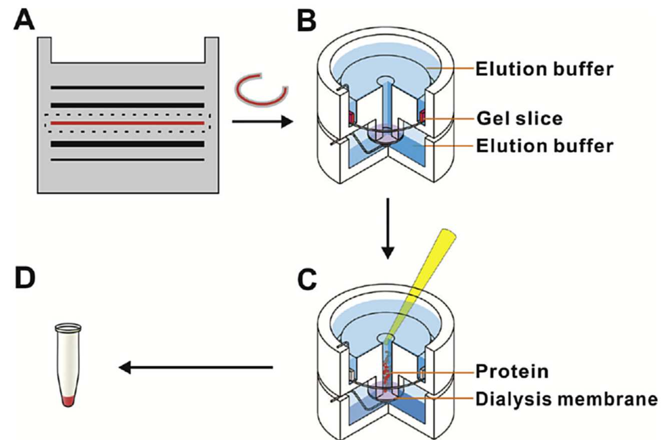

Fig. 2. Schematic illustration of the experiment for protein electroelution by RSE. (A) PAG containing the protein band to be electroeluted; (B) Assembly diagram of RSE having catholyte, anolyte and gel slice; (C) Collection of eluted target protein from RSE after electroelution; (D) Collected eluted protein.

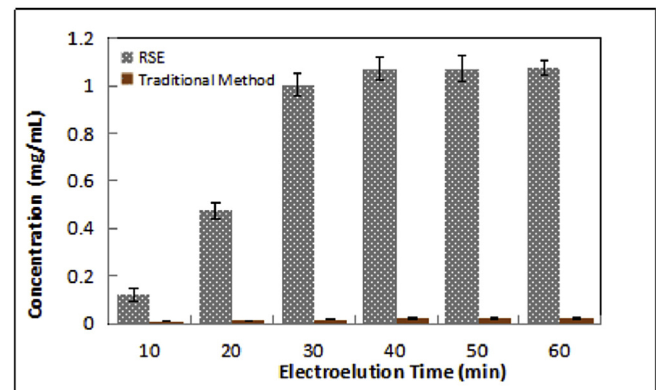

Fig. 3. Effect of time on concentration of HSA electroeluted from polyacrylamide gel matrix. Experiment condition: HSA (100 μg) was electroeluted via RSE and conventional method at 200 V at different time intervals.

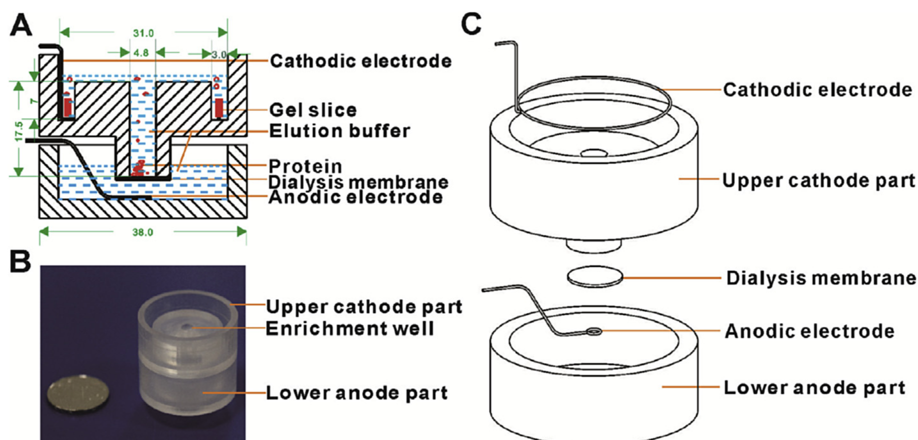

Fig. 1. (A) Schematic diagram of RSE device. (B) Picture of RSE device.(C) Assembly drawing of RSE device.

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