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Electroosmotic pump-assisted capillary electrophoresis of proteins

Liang Xu, Xiao-Yan Dong, Yan Sun*

Department of Biochemical Engineering, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China

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ABSTRACT

A new method for protein analysis, that is, electroosmotic pump-assisted capillary electrophoresis (EOPACE), is developed and demonstrated to possess several advantages over other CE-based techniques. The column employed in EOPACE consists of two linked sections, poly(vinyl alcohol) (PVA)-coated and uncoated capillaries. The PVA-coated capillary column is the section for protein electrophoresis in EOPACE. Electroosmotic flow (EOF) is almost completely suppressed in this hydrophilic polymer coated section, so protein electrophoresis in the PVA-modified capillary is free of irreversible protein adsorption to the capillary inner wall. The uncoated capillary section serves as an electroosmotic pump, since EOF towards cathode occurs at neutral pH in the naked silica capillary. By the separation of a protein mixture containing cytochrome *c* (Cyt-*c*), myoglobin and trypsin inhibitor, we have demonstrated the advantages of EOPACE method over other relevant ones such as pressure assisted CE, capillary zone electrophoresis (CZE) with naked capillary and CZE with PVA-coated capillary. A significant feature of EOPACE is that simultaneous separation of cationic, anionic and uncharged proteins at neutral pH can be readily accomplished by a single run, which is impossible or difficult to realize by the other CE-based methods. The high column efficiency and good reproducibility in protein analysis by EOPACE are verified and discussed. In addition, separation of tryptic digests of Cyt-*c* with the EOPACE system is demonstrated.

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1. Introduction

The need for high-resolution and efficient protein separation has been of increasing importance due to the rapidly developing field of proteomics [1,2]. Today, various separation techniques are available for the qualitative and quantitative analysis of proteins. Frequently used methods are liquid chromatography, slab-gel electrophoresis as well as capillary electrophoresis (CE) [3–6]. Compared with other two techniques, protein separation with CE offers advantages of faster separation, higher efficiencies, lower consumption of both samples and reagents as well as ease of automation. Therefore, CE is projected to play a vital role in proteomic research [6–9]. However, CE in protein analysis is still limited due to the irreversible adsorption of proteins to naked silica capillary inner wall. The limitations usually lead to many problems, such as loss of efficiency, tailing of peak, instability of baseline as well as lack of reproducibility of the migration time [10,11]. Thus, coated capillaries have been wildly applied to prevent or to minimize the protein–wall interactions [12–18]. For example, poly(vinyl alcohol) (PVA) coated capillary columns have been fabricated and widely used for the high-efficiency separation of proteins by CE [15,16,18].

Another problem associated with protein CE is that not all proteins (either cationic or anionic proteins) can be analyzed simultaneously [19]. It is attributed to the fact that coated capillary columns with very low electroosmotic flows (EOFs) are usually employed for high efficient protein separation by CE. Moreover, under certain experimental conditions, some proteins are positively charged while the others are negatively charged. Therefore, only a portion of proteins can be electrically driven to the detector and then analyzed. To solve this problem, some capillary columns were designed to offer good resolution for protein separations and maintain significant EOF. Unfortunately, most of these columns are either unstable in basic solutions or incapable of high efficient separation of basic proteins (e.g., peak tailing of analytes) [20–22]. Recently, Liu et al. developed a "one-sample-two-separation" approach for comprehensive protein profiling; one separation handles the cationic proteins and the other separation takes care of the anionic proteins. Clearly, simultaneous separation of all proteins cannot yet be accomplished with the method [19].

Electroosmotic pump (EOP), utilizing the electric-induced EOF for fluid delivery, is a new delivery system appeared in the late 1990s. The advantages of EOP are: no moving parts and motors, no pistons and check valves, no dynamic sealing, no material failure or wear out and so on [23,24]. So far, EOP has been used for microflow-injection analysis [23,25,26] and microfluidic liquid chromatography [27,28]. However, it has not been used as an assisted pump for protein CE. In this work, we developed an

* Corresponding author. Tel.: +86 22 27404981; fax: +86 22 27406590.
E-mail address: ysun@tju.edu.cn (Y. Sun).

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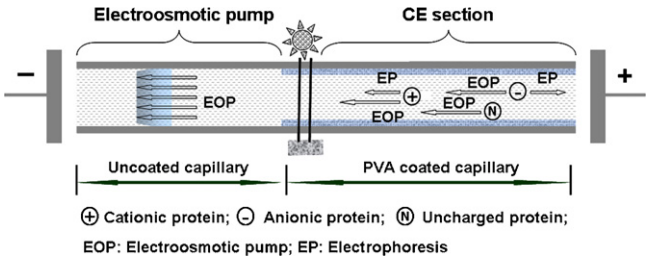

Fig. 1. Conceptual illustration of electroosmotic pump-assisted capillary electrophoresis (EOPACE).

electroosmotic pump-assisted capillary electrophoresis (EOPACE) approach for protein separation. The principle of the approach is conceptually illustrated in Fig. 1. In the EOPACE, an uncoated silica capillary is employed as the EOP. The 'pump' is linked to a PVA-coated capillary in which adsorption-free protein electrophoresis occurs. By this new CE approach, simultaneous separation of positively, negatively and uncharged proteins at neutral pH can be accomplished. Herein, we report the method for protein analysis and compare it with other relevant ones such as pressure assisted CE, capillary zone electrophoresis (CZE) with naked capillary and CZE with PVA-coated capillary column to reveal the advantages of the EOPACE.

2. Experimental

2.1. Materials

Myoglobin [MB, isoelectric point (p*I*): 7.1], trypsin inhibitor (TI, p*I*: 4.6), trypsin and PVA (average molecular weight: 70 000–100 000) were purchased from Sigma (St. Louis, MO, USA). Cytochrome *c* (Cyt-*c*, p*I*: 10.3) was from Lianxing Chemical Co. (Tianjin, China). (3-Aminopropyl)trimethoxysilane (APTS, 97%) was obtained from Jingchun Chemical Co. (Shanghai, China). Glutaraldehyde was received from Guangfu Institute of Fine Chemical Industry (Tianjin, China). Other chemicals were all analytical grade from local sources. Deionized water was used in all of the experiments. Untreated fused-silica capillaries of 50 μm I.D. and 375 μm O.D. were provided by Yongnian Optic Fiber (Hebei, China).

2.2. Instrumentation

All CE experiments were performed on a P/ACE system MDQ capillary electrophoresis instrument (Beckman Coulter, Fullerton, CA, USA) equipped with a UV detector connected to an IBM (NY, USA) personal computer. The Beckman P/ACE MDQ system CE software named 32 Karat was installed to control the instrument functions and to process the CE data.

2.3. Preparation of PVA-coated capillary

There were four steps in the PVA coating to the silica capillaries. The first step was pretreatment of the capillary inner wall. It was the same as that reported by Qu et al. [29]. The second step was to introduce amino groups. It was performed as described by Kitagawa et al. with some modifications [30]. Briefly, 20% APTS solution dissolved in dry acetone was introduced into the pretreated capillary at room temperature for 60 min. Thereafter, the capillary was rinsed with acetone and then blown dry with nitrogen. The third step was aldehyde-group functionalization of the amino groups. 10% glutaraldehyde dissolved in 50 mM borate buffer (pH 9.0) was used to wash the capillary at room temperature for 60 min. The obtained capillary was flushed with water for 15 min and then transferred into the last step for PVA immobilization. In this step, the aldehyde-modified capillary column was rinsed with an acidified PVA solution (a mixture of 900 μL of 5% aqueous PVA solution and 100 μL of 5.0 M HCl) at room temperature for 60 min. Subsequently, the capillary was washed with water for 15 min and then flushed with methanol for another 15 min. The prepared capillary was finally blown dry with nitrogen and used for the subsequent CE experiments.

2.4. Protein separations

Protein sample containing 0.1 mg/mL Cyt-*c*, 0.1 mg/mL MB and 0.5 mg/mL TI was prepared with 20 mM phosphate buffer (pH 7.0). The protein mixture was separated by EOPACE, pressure assisted CE (PACE), as well as CZE, respectively. In all CE experiments, 60 mM phosphate buffer of pH 7.0 was used as the running buffer. To perform EOPACE, an uncoated capillary of 45 cm in length was linked with a PVA-coated capillary of 15.2 cm in length with a Teflon tube sleeve. The linking method of two capillaries was the same as that reported by Ding et al. [31]. In EOPACE, protein CE was carried out in the PVA-coated capillary and the effective CE length (from column inlet to the detector) was 10.2 cm. The applied voltage was +20 kV. Between runs, the column was flushed with the running buffer containing 0.5 M NaCl for 2 min to elute the proteins adsorbed to the naked capillary, and then rinsed with the running buffer for 5 min. PACE of proteins was performed with a PVA-coated capillary of 60.2 cm in total length and 10.2 cm effective CE length. In PACE, a voltage of +20 kV was added between the PVA-coated capillary associated with a vacuum of 0.8 psi added to the outlet of the capillary column. In CZE separations, both uncoated and PVA-coated capillaries were used for comparisons. The applied voltage was +20 kV or −20 kV. The detection wavelength was 200 nm in all CE experiments. After experiments, capillary columns were stored in deionized water at room temperature for short term storage (within 2 days) or at 4 °C in the refrigerator for long term storage (more than 2 days).

2.5. Separation of tryptic digests of Cyt-c

Preparation of the tryptic digests of Cyt-*c* was similar with that reported by Gao and Liu [14]. Briefly, 10 mg/mL trypsin was prepared as a stock solution by dissolving 2 mg lyophilized trypsin in 200 μL of 50 mM acetic acid. Then, a 10-mg sample of Cyt-*c* was dissolved in 1.0 mL of 50 mM Tris–HCl buffer (pH 8.5). For digestion, 100 μL of the Cyt-*c* solution and 2 μL of the trypsin stock solution were mixed and incubated at 37 °C for 20 h. This solution was then diluted to 1.0 mL with the running buffer (60 mM phosphate buffer, pH 7.0), and the diluted solution was used as the sample for EOPACE separation. The applied voltage was 17 kV. Other conditions were the same as those described for the EOFACE operation in Section 2.4.

3. Results and discussion

3.1. Principle of EOPACE for protein separations

The EOPACE method was developed and applied for simultaneous separation of cationic, anionic, and uncharged proteins with a running buffer of neutral pH. As shown in Fig. 1, the column used in EOPACE consists of two sections, PVA-coated and uncoated capillaries. PVA-coated capillary column is highly hydrophilic and uncharged, so no EOF and protein adsorption takes place in the capillary. Therefore, pure protein electrophoresis occurs in the PVA-coated section. These properties of the PVA-coated capillary for protein CE have been demonstrated by preliminary experiments, and it was shown that the capillary kept stable after over 100 times of CE analyses of proteins (data to be published elsewhere). The

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