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Short communication

A simple peptide mapping method by partial filling micellar electrokinetic capillary chromatography with a zwitterionic–nonionic mixed micelle

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Abstract

A partial filling micellar electrokinetic capillary chromatography (PF-MEKC) method with a mixed micelle system composed of a zwitterionic surfactant named 3-(*N,N*-dimethylhexadecylammonium)propanesulfonate (PAPS) and a nonionic surfactant polyethylene glycol dodecyl ether (Brij 35) for peptide mapping is described. The method was demonstrated by the separation of tryptic digestion of bovine serum albumin (BSA). The optimal mixed micelle solution was 50 mM NH_4OH–HCOOH buffer (pH 2.0) containing 32 mM PAPS and 0.6% (m/v) Brij 35. It was found that the mixed micelle system permitted a highly selective separation of the tryptic digestion. The high separation selectivity was probably due to the ion-pairing interaction between the zwitterionic surfactant molecules and the peptides.
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Keywords: Mixed micelle; Micellar electrokinetic capillary chromatography; Peptide mapping

1. Introduction

Peptide mapping represents one of the most important works for protein identification [1–6]. Currently, high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) is the commonly used tool for peptide mapping [1,2]. During the past 2 decades, capillary electrophoresis (CE) has been widely used for separation of various biological substances including peptides, proteins, nucleic acid, and oligosaccharides. Nowadays, CE has become a complementary separation tool to reversed-phase HPLC in peptide mapping [3–11].

Since introduced by Terabe, micellar electrokinetic chromatography (MEKC) has become an important tool for the separation of neutral and charged analytes due to its high separation efficiency, simplicity, minute sample requirement and high selectivity [12–14].

So far, sodium dodecyl sulfate (SDS) is the most widely used surfactant in MEKC as the pseudostationary phase. In order to improve the selectivity, other types of surfactant have also been used in MEKC [15–17]. Although mixed micelle systems composed of two or more surfactants are not commonly used in MEKC, they can display unique selectivity in some cases [17–22]. In our previous work, a mixed micelle system consisting of a zwitterionic surfactant 3-(*N,N*-dimethylhexadecylammonium)-propanesulfonate (PAPS) and a nonionic surfactant polyethylene glycol dodecyl ether (Brij 35) permitted a highly selective separation of polypeptide antibiotics from its impurities [22].

The aim of the present work is to explore the possibility of using this mixed micelle system for peptide mapping. The feasibility was demonstrated by the separation of the tryptic digestion of bovine serum albumin (BSA). Experimental conditions such as surfactants concentration and electroosmotic flow (EOF) were optimized. Furthermore, the partial filling MEKC was also demonstrated for the separation of the tryptic digestion of BSA.

2. Experimental

2.1. Materials

Bovine serum albumin (BSA), trypsin and Sudan III were from Sigma (St. Louis, MO, USA). 3-(*N,N*-dimethylhexadecylammonium)-propanesulfonate (PAPS), polyethylene glycol dodecyl ether (Brij 35) were purchased from Fluka (Buchs, Switzerland). Sodium dodecyl sulphate (SDS), ammo-

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nium hydroxide (25–28%), formic acid, dimethyl sulfoxide (DMSO), hydrochloric acid and sodium hydroxide were purchased from the Shanghai Chemical Co. (Shanghai, China). All reagents are used as received.

2.2. Running buffer and sample preparation

A saturated Sudan III solution in DMSO was prepared as the marker of the micelles. Trypsin solution was prepared at a concentration of 1 mg/mL. BSA was dissolved in 0.1 M Tris–HCl (pH 8.5) buffer to give a concentration of 5 mg/mL. For denaturing BSA, 1 mL BSA solution was heated at 100 °C for 8 min. After cooling down to the room temperature, 0.1 mL trypsin solution was added to the denatured BSA solution (the weight ratio between trypsin and BSA was 0.02:1). The mixed solution was incubated at 37 °C for 6 h for digestion. The background electrolyte (BGE) was composed of 50 mM NH_4OH, its pH was adjusted by adding 0.1 M formic acid to the solution until pH 2.0. The micelle solutions were prepared with this BGE solution. All buffers were prepared with the deionized water and were filtered through a 0.45 μm nylon filter prior to use.

2.3. Instrumentation and electrophoresis procedure

All experiments were carried out on a P/ACE^{TM} MDQ CE system (Beckman Coulter, CA, USA). Fused silica capillary with a dimension of 50 μm i.d. (370 μm o.d.) × 60 cm (50 cm to the detection window) were purchased from Yongnian Optical Fiber Inc. (Hebei, China). A new capillary column was treated with 0.1 M NaOH solution for 30 min, followed by flushing with water and running buffer for 5 min, respectively. Between two runs, the capillary was rinsed with 0.1 M HCl, water and running buffer for 2 min, respectively. In the partial-filling MEKC (PF-MEKC) experiment the capillary was first rinsed with the buffer without the micelle for 3 min, then Sudan III solution in DMSO was injected at 2.07 kPa for 2 s to trace the front of the micelle plug. The mixed micelle solution was then introduced by pressure at 34.47 kPa for various period of time from 30 s to 107 s (the linear velocity of the micelle solution was determined as 0.328 cm/s). For all experiments, the capillary was thermostatted at 25 °C; voltage of 15 kV was applied, and detection was carried out at UV wavelength of 200 nm. Unless indicated elsewhere, sample was injected by pressure at 3.45 kPa for 5 s.

3. Results and discussion

3.1. Method development and optimization

In our previous work [22], the zwitterionic micelle system showed a highly selective separation of polypeptide antibiotic bacitracin and its relative impurities. Logically, this zwitterionic micelle system might be also a good separation medium for the separation of tryptic digestion peptides of proteins. Initially, separations of tryptic digestion peptides of BSA by three CE models were compared: CZE method with a buffer composed of 50 mM NH_4OH–HCOOH (pH 2.0); MEKC method with SDS micelle system and MEKC method with PAPS–Brij 35 mixed micelle system. Both micelle solutions consisted of the same BGE as that used in CZE except the type of surfactant. Because of the complexity of the tryptic digestion of BSA, it is easy to use the sum of separated peaks as the criteria for experimental optimization. The integrator was set to reject peaks with an area of less than 120 units, and thus only peaks above this threshold were counted. As shown in Fig. 1, the SDS method provided a very poor separation selectivity to the tryptic digestion of BSA under acidic condition (only 29 peaks was obtained), and the separation was even worse when using it under basic condition (data not shown); CZE approach gave about 67 peaks; whereas, the PAPS–Brij 35 mixed micelle system gave the best separation giving about 77 peaks. The separation of the tryptic digestion blank showed that only one peak appeared at 11.63 min was obtained. This means that the peaks obtained with PAPS–Brij 35 mixed micelle system were not from autoproteolysis of trypsin or the buffer components, but only from the digestion of BSA. The use of the acidic buffer at pH 2.0 allows all peptides to possess positive charge and to effectively depress the adsorption of peptides on the capillary wall, therefore most of the peptides can be detected.

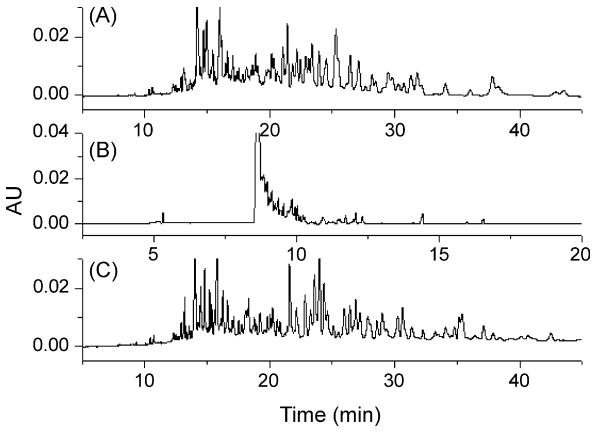

Fig. 1. Comparison of separations of tryptic digestion of BSA with CZE (A), MEKC with SDS (B), MEKC with PAPS and Brij 35 mixed micelle (C). Conditions: fused silica capillary, 50 μm i.d. × 60 cm total length (50 cm effective length); running buffer, 50 mM NH_4OH–HCOOH (pH 2.0) (A); 50 mM NH_4OH–HCOOH (pH 2.0) containing 32 mM SDS (B); 50 mM NH_4OH–HCOOH (pH 2.0) containing 32 mM PAPS and 0.6% (m/v) Brij 35 (C); applied voltage, 15 kV (A and C), −15 kV (B); column temperature, 25 °C; detection wavelength, UV 200 nm; sample injection, 3.45 kPa for 5 s.

The presence of the zwitterionic surfactant PAPS in the running buffer provided the separation selectivity, however, it was also enhanced the EOF by 1.5 times than that obtained with the buffer without micelles. This could be due to the modification of the capillary wall by the zwitterionic surfactant. High EOF is undesirable when partial filling MEKC is performed. Therefore, it is necessary to depress the EOF as low as possible. It was found that the EOF was obviously minimized by addition of the nonionic surfactant Brij 35 in the PAPS micelle solution. Effect of the concentration of Brij 35 on the magnitude of EOF was investigated in a range from 0.1% to 1.0% (m/v). The EOF was reduced to 2.82×10^{-9} m^2 V^{-1} s^{-1} corresponding 120 min when 0.6% (m/v) Brij 35 was added to the running buffer containing 32 mM PAPS. The reduced EOF should be

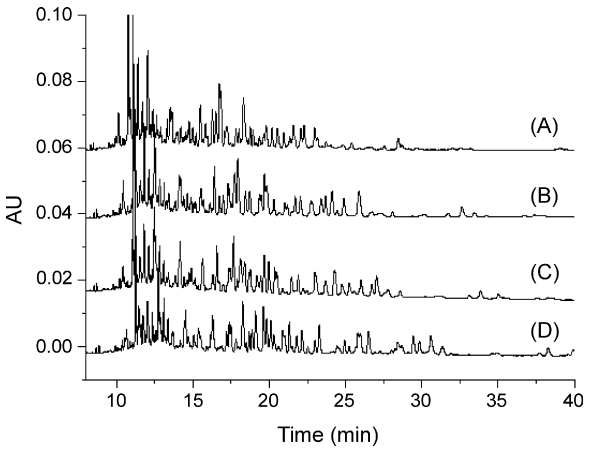

Fig. 2. Effect of PAPS concentration on the separation of the tryptic digestion of BSA. Conditions: running buffer, 50 mM NH_4OH–HCOOH (pH 2.0) containing 0.6% (m/v) Brij 35. PAPS concentration: 20 mM (A), 32 mM (B), 40 mM (C), 60 mM (D). Other conditions as in Fig. 1.

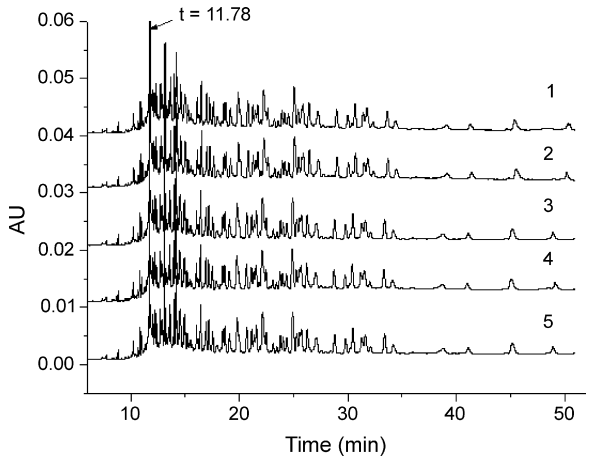

Fig. 3. The repeatability of the separation of tryptic digestion of BSA. Conditions: running buffer, 50 mM NH_4OH–HCOOH (pH 2.0) containing 32 mM PAPS and 0.6% (m/v) Brij 35; applied voltage, 15 kV; current, 18.0 μA. The plug length of the mixed micelle: 25 cm. Other conditions as in Fig. 1.

due to the dynamical modification of the capillary wall by Brij 35 [23]. Furthermore, effect of the concentration of PAPS on the separation was investigated in a concentration range from 10 to 60 mM. As shown in Fig. 2, the separation was improved with the increase of the PAPS concentration. However, the separation time also increased obviously. By trade-off between the resolution and separation time, 32 mM PAPS was selected for the following experiments. Finally, the optimal buffer composition was established as: 50 mM NH_4OH–HCOOH (pH 2.0) containing 32 mM PAPS and 0.6% (m/v) Brij 35.

3.2. Partial filling technique for peptide mapping and repeatability

The partial filling experiment with the mixed micelle system was investigated based on the consideration that the method can be potentially coupled with MS for peptide mapping. The linear velocity of the micelle solution driven by pressure of 34.47 kPa was measured as 0.328 cm/s. Thus, changing the filling time can precisely control the plug length of the micelle solution. Theoretically, because the net charge of the mixed micelle is zero, the micelles should migrate with the EOF. For proving this supposition, the saturated Sudan III solution in DMSO was injected to trace the migration of the micelles. Herein, Sudan III and DMSO represent the marker of the micelles and the EOF, respectively. In the experiment, no separation between Sudan III and DMSO was achieved during the separation time of 140 min. Therefore, it can be concluded that the mixed micelles migrated with the same velocity of the EOF. Effect of the plug length on the separation was investigated in a range from 10 cm to 35 cm. It was found that a 25 cm long plug of the micelle gave a satisfactory separation and long enough time gap (about 10 min) between the last peak of the peptides and the front of the micelle plug. Under this condition, the front of the micelle plug, which was marked by DMSO, spent about 60 min to reach the UV detection window, whereas the separation of peptides only needed about 50 min.

The repeatability of PF-MEKC for the separation of tryptic digestion of BSA was also evaluated. Electropherograms for five successive injections were shown in Fig. 3. The migration time of the highest peak (marked with an arrow in Fig. 3) and the number of peaks were used for evaluation of the repeatability. The intra-day ($n = 6$) and inter-day ($n = 4$) relative standard deviations (% RSD) of the migration time were measured as 0.39% and 1.09%, respectively. The number of peaks is completely repeatable for intra-day and inter-day experiments. Therefore, the method displayed a satisfactory repeatability.

4. Conclusion

A mixed micelle system composed of a zwitterionic surfactant PAPS and a nonionic surfactant Brij 35 in a volatile acidic buffer for peptide mapping has been developed. The separation selectivity of the tryptic digestion of BSA by the present method was proved to be superior to the MEKC with SDS and the CZE methods. The presence of Brij 35 in the buffer not only improved the separation selectivity, but also significantly depressed the EOF. Furthermore, the partial filling technique with the mixed micelle system was established to be a satisfactory and repeatable separation method, which can be potentially coupled with MS for peptide mapping.

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