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Sensitive analysis of amino acids with carrier-mediated single drop microextraction in-line coupled with capillary electrophoresis

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ABSTRACT

In order to analyze amino acids sensitively without derivatization, we have developed carrier-mediated single drop microextraction (SDME). Nonane-1-sulfonic acid was added to an acidic sample donor solution as a carrier to form neutral ion pair complexes with amino acids. The ion pair complexes were extracted to the organic phase, covering a drop of an aqueous basic acceptor phase hanging at the tip of a capillary, and then back-extracted to the basic acceptor phase, where both the amino acids and the carrier have negative charges and the ion pair complexes are broken. The resulting extract of enriched amino acids was injected into the capillary and analyzed by capillary electrophoresis. With 20-min SDME with agitation of the donor phase, enrichment factors of four aromatic amino acids were up to 120-fold, yielding the LOD of 70–500 nM. The linear dynamic ranges for corrected peak areas were 1–100 μM with linear correlation coefficients larger than 0.9959. With internal standardization, the intraday RSDs of migration times and corrected peak areas were 0.01–0.04% and 2.0–3.7%, respectively. The capabilities of sample cleanup including desalting and preconcentration of carrier-mediated SDME were demonstrated with the analysis of human urine after minimal pretreatment of acidification and centrifugation.

1. Introduction

Capillary electrophoresis (CE) is very favorable to analysis of amino acids in biological samples with complex matrices because of its high separation efficiency [1,2]. However, the limits of detection (LODs) of amino acids in CE using a narrow-bore capillary are relatively low [3]. Besides, since biological samples are usually highly conductive, peak broadening often occurs [4]. Recently a method for sample enrichment and pretreatment through in-line coupling of single drop microextraction (SDME) and CE was reported [5–8]. A drop of an aqueous acceptor phase covered with a thin organic layer is formed at the tip of the capillary inlet. Then, through liquid–liquid–liquid extraction, a neutral analyte in the sample donor phase is partitioned to the organic layer and transferred to the acceptor phase where the analyte becomes charged. SDME allows high enrichment in a short time since the volumes of the acceptor and organic phases are much smaller than those of other liquid phase microextraction techniques. Desalting also takes place, since inorganic ions cannot easily pass through the organic layer.

Although the SDME method is applicable to most acidic and basic compounds, it is difficult to apply to an amino acid that has both the carboxyl and amino groups. This is because the amino acid is charged at all pH values, even at the isoelectric point (p*I*) with no net charge but existing as a zwitterion. One solution to this problem is to block one of the two functional groups through derivatization to make the amino acid either acidic or basic [9–11]. Furthermore, if a fluorescent agent is used for the derivatization, the detection sensitivity may be enhanced by employing laser induced fluorescence detection. However, this method may require long overnight reaction or heating, or the reaction may not proceed cleanly and thus some unknown peaks can occur. Also, amino acids undergo permanent changes, hampering subsequent analysis such as mass spectrometry.

Another solution that does not involve permanent derivatization is to use carriers as a way to enhance liquid phase extraction of analytes. In reverse micellar microextraction, an amino acid is transferred into hydrophilic reverse micelles and partitioned in an organic phase [12]. Alternatively, a carrier forms an ion pair with the amino acid in either the donor phase or the organic phase, thereby promoting carrier-mediated transport into the acceptor phase [13–15]. These methods are frequently used in pretreatment for GC and HPLC of various metal ions [16–20], drugs [21–26], chemicals [27,28], organic acids [29], and biological molecules

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including amino acids [13,30], peptides [14,31], and metabolites [15], with proper carriers.

In this report, carrier-mediated transport was applied to SDME for sensitive CE analysis of amino acids. The selected carrier was nonane-1-sulfonic acid. When it was mixed with the sample and the pH was adjusted properly, the amino acids and the carrier were charged positively and negatively, respectively, to form ion pairs. Thus, SDME of amino acids could be performed without derivatization, and the amino acids in a biological fluid such as urine could be analyzed quite conveniently with 100-fold enhancements in the UV detection sensitivity.

2. Materials and methods

2.1. Chemicals

Homophenylalanine (HPA), tryptophan (Trp), phenylalanine (Phe), tyrosine (Tyr), lysine (Lys), histidine (His), alanine (Ala), valine (Val), glutamic acid (Glu), aspartic acid (Asp), 1-octanol, octadecyltrimethoxysilane (ODTS), ethanol, sodium dihydrogen phosphate, *n*-octane, hexyl ether, and sodium tetraborate decahydrate were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetic acid was from Merck (Darmstadt, Germany) and phosphoric acid from Fluka (Buchs, Switzerland). Hexane-1-sulfonic acid (C6), heptane-1-sulfonic acid (C7), octane-1-sulfonic acid (C8), nonane-1-sulfonic acid (C9), decane-1-sulfonic acid (C10), 1-heptanol, and 1-nonanol were from TCI (Tokyo, Japan). Deionized water was prepared with a NANO Pure II System (Barnstead, Dubuque, IA). 200, 400, and 600 mM sodium borate buffers were prepared by adjusting the pH of sodium borate solutions with 0.1 M NaOH to pH 10.0 and used as acceptor phases. 10 mM stock solutions of amino acids were prepared in 0.1 M HCl and used as standard samples by diluting with the 600 mM borate buffer. A phosphate run buffer of pH 2.0 for CE was prepared by adding 100 mM phosphoric acid to 100 mM sodium dihydrogen phosphate. The pH of the aqueous donor phases containing amino acids and a carrier was adjusted using 0.1 M HCl. 20 mL of urine collected from a healthy volunteer was mixed with 630 μL of 0.1 M HCl and centrifuged. A urine sample for CE without SDME was prepared by spiking the supernatant with 50 μM amino acids. A urine donor phase for SDME was prepared by spiking the supernatant to contain 5 μM amino acids and a carrier. The pK_a values of amino acids and alkanesulfonic acids were calculated by using ACD/Lab software (Advanced Chemistry Development, Toronto, Canada).

2.2. Capillary electrophoresis

CE was performed with an MDQ CE system (Beckman, Fullerton, CA, USA) equipped with a UV detector. The detection wavelength was set at 200 nm. A bare fused silica capillary of 60 cm (50 cm to the detector) × 40 μm id × 360 μm od from Polymicro Technologies (Phoenix, AZ, USA) was conditioned by rinsing with 0.1 M NaOH, water, and run buffer for at least 1 min at 80 psi prior to each run. The voltage applied across the capillary was held at 20 kV during separation. The temperature was maintained at 25 °C.

2.3. Carrier mediated SDME procedures

The end surface of a capillary inlet tip was made hydrophobic so as to form a drop stably attached to the tip, daily or as needed. After washing with ethanol for 3 min, the capillary inlet tip was immersed in a coating solution for about 5 s and then dried for 10 min to complete the condensation reaction. The coating solution was a mixture of 5 vol% ODTS and 0.1 vol% acetic acid in ethanol. The schematic of carrier mediated SDME procedures is shown in Fig. 1. (1) The capillary was filled with a run buffer, the acceptor phase was injected into the capillary for 8 s at 2 psi, and (2) octanol was injected for 5 s at 6 psi. (3) Then, the capillary inlet was transferred to a sample solution (donor phase) and a backpressure of 1 psi applied for 0.59 min to form a drop of the acceptor phase covered with octanol. (4) During extraction, a backpressure of 0.3 psi was applied for 0.3 min every 2.06 min of extraction to prevent changes in the drop size. (5) After extraction, the concentrated acceptor phase was injected into the capillary at 0.5 psi for 5 s. (6) The capillary inlet was then placed in a run buffer vial and electrophoresis was carried out. A laboratory-made microstirrer (7 mm in height and 14 mm in diameter) was installed on the CE instrument in order to agitate the donor phase to speed up the extraction process [6].

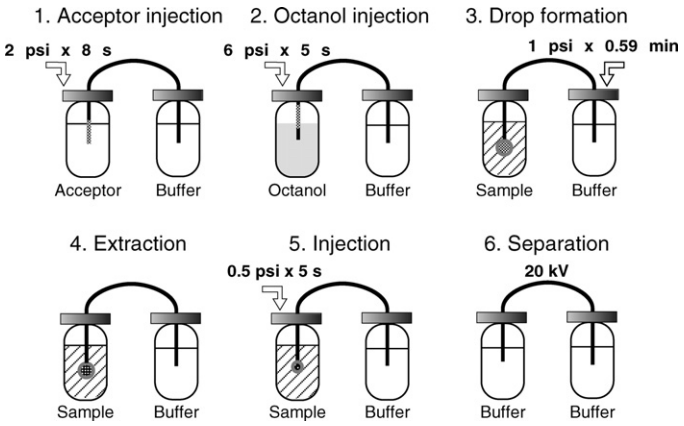

Fig. 1. Schematic of carried-mediated SDME.

3. Results and discussion

3.1. Carrier-mediated extraction

By adjusting the pH of the donor and acceptor phases, liquid–liquid–liquid extraction, can be carried out in two steps whereby analytes are extracted from the aqueous donor phase to an organic phase and then back-extracted from the organic phase to the aqueous acceptor phase. However, in the case of an amino acid having both carboxyl and amino groups, the first step of transferring the amino acid from the donor phase to the organic phase does not occur well with the pH control only. When a positively charged amino acid at low pH forms an ion pair with a negatively charged carrier, the neutral ion pair can be extracted into the organic phase. If the pH of the acceptor phase is sufficiently high, both the amino acids and the carrier are negatively charged and the ion pair dissociates. The overall effect is enrichment of the amino acid from the acidic donor phase to the basic acceptor phase, mediated by a carrier.

Let us consider an amino acid A^+H_2 having acid dissociation constants K^A_{a1} and K^A_{a2} which is extracted from an acidic aqueous donor phase (1) to an organic phase (o) as an ion pair A^+H_2-R^- with a carrier RH having an acid dissociation constant K^R_a, and then back-extracted into a basic aqueous phase (2) as shown in Fig. 2. The enrichment factor (EF) at equilibrium, defined as the ratio of the analytical concentration of the amino acid in the acceptor phase at equilibrium $C^A_{2,eq}$ to the one in the donor phase initially $C^A_{1,init}$, is given by [32]:

$$EF_{eq} \equiv \frac{C^A_{2,eq}}{C^A_{1,init}} = \frac{1}{D_2/D_1 + D_2(V_o/V_1) + V_2/V_1} \quad (1)$$