Analytical Biochemistry 449 (2014) 158–163

Contents lists available at ScienceDirect

Analytical Biochemistry

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

Simultaneous determination of phenylethanoid glycosides and aglycones by capillary zone electrophoresis with running buffer modifier

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ARTICLE INFO

Article history:
Received 16 September 2013
Received in revised form 8 November 2013
Accepted 11 November 2013
Available online 19 November 2013

Keywords:
Capillary electrophoresis
Phenylethanoid glycosides and aglycones
β-Cyclodextrin buffer modifier
Lamiophlomis rotata
Cistanche

ABSTRACT

Although the separation efficiency of capillary electrophoresis (CE) is much higher than that of other chromatographic methods, it is sometimes difficult to adequately separate the complex ingredients in biological samples. This article describes how one effective and simple way to develop the separation efficiency in CE is to add some modifiers to the running buffer. The suitable running buffer modifier β-cyclodextrin (β-CD) was explored to fast and completely separate four phenylethanoid glycosides and aglycones (homovanillyl alcohol, hydroxytyrosol, 3,4-dimethoxycinnamic acid, and caffeic acid) in *Lamiophlomis rotata* (*Lr*) and *Cistanche* by capillary zone electrophoresis with ultraviolet (UV) detection. It was found that when β-CD was used as running buffer modifier, a baseline separation of the four analytes could be accomplished in less than 20 min and the detection limits were as low as 10^{-3} mg L^{-1}. Other factors affecting the CE separation, such as working potential, pH value and ionic strength of running buffer, separation voltage, and sample injection time, were investigated extensively. Under the optimal conditions, a successful practical application on the determination of *Lr* and *Cistanche* samples confirmed the validity and practicability of this method.

During recent years, with the development of traditional Chinese medicine research, much attention has been devoted to its chemical composition. Phenylethanoid glycosides as a principal chemical compound are widely distributed in Chinese herbal medicine [1]. It is well known that phenylethanoid glycosides possess variable antioxidant properties; for example, verbascoside is reported to exhibit antimicrobial, anti-inflammatory, immunosuppressive, antitumor, diuretic, and antibacterial activities [2]. With the development of chemical composition research for *Lamiophlomis rotata*[1] (*Lr*) [3], a number of phenylethanoid glycosides have been found in *Lr*. In 1995, the Yi group obtained two phenylethanoid glycoside compounds from *Lr*'s roots [4] and, according to the chemical method and spectral analysis, determined the structure of them (lamiophlomioside A and cistanoside B). Wang and his coworkers obtained three phenylethanoid glycoside compounds (forsythoside B, betonyoside A, and verbascoside) from *n*-butyl alcohol extract of *Lr* [5]. However, there is little related study on phenylethanoid glycosides in *Lr*. Therefore, the goal of the current work was to separate and analyze these kinds of compounds and further evaluate the analgesic effect of phenylethanoid glycosides in this plant. Many separation techniques have been developed to study various kinds of phenylethanoid glycoside compounds in different medicine plants [6], including thin-layer chromatography (TLC) [7], high-performance liquid chromatography (HPLC) [8], gas chromatography (GC) [9], and capillary electrophoresis (CE) [10]. However, owing to the highly polar and basic properties as well as highly structural similarity of compounds, the conventional chromatographic approaches could not always afford satisfactory results. For example, HPLC gives poor separation resolutions and peak shapes for these kinds of compounds. Moreover, it requires long analysis time and needs an unfavorable pH modifier that will potentially lead to column contamination and damage. CE is a very economical technique and has the advantages of remarkable separation efficiency, rapid analysis, and minimal consumption of samples and solvents [11]. In CE, many modes have been developed, including capillary zone electrophoresis (CZE) [12], micellar electrokinetic chromatography (MEKC) [13], capillary gel electrophoresis (CGE) [14], and cation exchange chromatography (CEC) [15]. However, CE is sometimes still very difficult to separate some

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[1] *Abbreviations used: Lr*, *Lamiophlomis rotata*; HPLC, high-performance liquid chromatography; CE, capillary electrophoresis; CZE, capillary zone electrophoresis; β-CD, β-cyclodextrin; UV, ultraviolet; RSD, relative standard deviation.

0003-2697/$ - see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ab.2013.11.014

complicated systems completely. To solve this problem, the addition of modifier is an effective solution. There are many effective means of achieving the separation of complex systems with the addition of modifiers [16–20]. However, up to now, most articles have reported the use of modifiers in the different separation systems, but most of them were only for the separation of chiral compounds or the improvement of resolution efficiency, not for the solution of other problems such as longer separation time and lower sensitivity.

In this study, we attempted to establish the quantification of these phenylethanoid glycoside compounds in *Lr* by CE with β-cyclodextrin (β-CD) as running buffer modifier. But due to a great variety of phenylethanoid glycosides and difficulties in preparing and purchasing standard substances, they could not be quantified accurately. In this regard, we separated and detected homovanillyl alcohol, hydroxytyrosol, 3,4-dimethoxycinnamic acid, and caffeic acid, which were obtained by hydrolysis of the phenylethanoid glycoside compounds. The chemical structures of the phenylethanoid glycosides and four hydrolyzed productions are shown in Fig. 1. It is clear that the hydrolyzed aglycones have simple molecular structures and are easy to obtain, so the hydrolysis method can be used to accomplish the difficult tasks of quantification by hydrolysis reaction molar ratios of aglycones and phenylethanoid glycosides. However, the structures of the aglycones were similar, and the separation efficiency was poor. Therefore, it was necessary to establish rapid and effective methods for the determination of the phenylethanoid glycosides and aglycones in *Lr* by CE. The addition of β-CD as modifier not only can improve the separation efficiency but also can shorten the migration time of the four analytes. According to the detection of the hydrolysis productions, we can estimate the amount of phenylethanoid glycosides in *Lr*. This method greatly simplifies the complex research process. The proposed method is also used to investigate the amount of the phenylethanoid glycosides in *Cistanche*, for which the main active ingredients were confirmed as phenylethanoid glycosides, and the detection results were satisfactory. It is shown that the method has convenient preparation, high sensitivity, and good repeatability and could be used in the rapid determination of practical samples. It is possible to get new insights into the quality control of traditional Chinese medicine.

Materials and methods

Apparatus

A CE system with a diode array detector (Beckman P/ACE MDQ, USA) was used. CE was performed using a 68.5 cm × 75 μm (i.d.) capillary with an effective length of 60.0 cm. Fused silica capillary was purchased from Yongnian Photoconductive Fiber (Hebei Province, China). The CE system was interfaced with a computer and controlled using Beckman 3.2 Karat software (version 7.0).

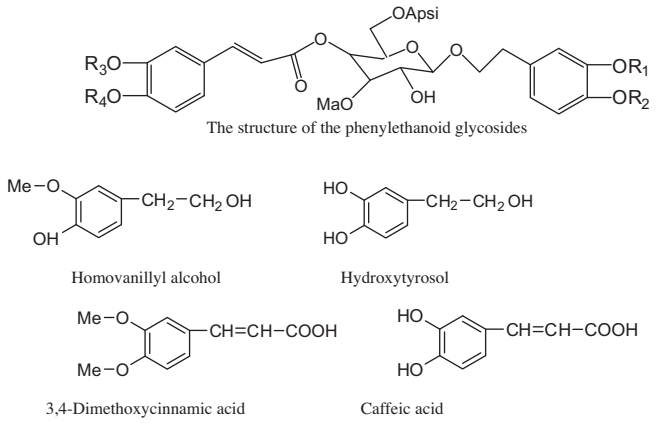

Fig.1. Molecular structures of the phenylethanoid glycosides and four hydrolyzed productions.

Reagents

The standards of homovanillyl alcohol, hydroxytyrosol, and 3,4-dimethoxycinnamic acid were obtained from Aldrich (Milwaukee, WI, USA). Caffeic acid was purchased from Toshima (Tokyo, Japan). Disodium tetraborate decahydrate and β-cyclodextrin were purchased from J&K Scientific (Beijing, China). Methanol, ethanol, and all other chemicals were obtained from Shanghai First Reagent (Shanghai, China), and all of them were of analytical reagent grade. Double distilled water was used throughout the experiment. Herbs were purchased from a local medicine supermarket.

Preparation of standard solution

The four standard analytes were accurately weighed and dissolved in absolute ethanol to a concentration of 5.0 mg ml^{-1} stock solution of each. They were stored in the dark at 4 °C. Before the CE experiment, they were diluted with buffer solution. All solutions were filtered through a 0.45-μm polypropylene Acrodisc syringe filter (Xinya Purification Instrument, Shanghai, China) and degassed by agitation in an ultrasonic bath for 5 min to remove bubbles.

Sample preparation

Lr and *Cistanche* were pulverized with a shredder, and then 20.0 g of the powder was accurately weighed and soaked in a flask with 90% (v/v) ethanol aqueous at room temperature for 2 h. Then the mixed liquid was sonicated at 45 °C for 1 h. Next the extract solution was decanted and the residues in the flask were heated to reflux at 75 °C again with 100 ml of 90% (v/v) ethanol aqueous for 2 h. The combined extract solution was filtered through qualitative filter paper that had been soaked with 90% (v/v) ethanol aqueous solution and then was concentrated by a rotary evaporator. Hydrochloric acid (1.0 ml, 10% HCl) was added to concentrated solution (10 ml), and the mixed solution was diluted to 100 ml by distilled water and then heated in boiling water for 1 h. Next the cooled mixture (500 μl) was diluted to 1.0 ml with buffer solution, filtered through a 0.45-μm filter, and analyzed by CE.

Procedure

Prior to CE experiments, the capillary was sequentially syringed with 0.1 mol L^{-1} hydrochloric acid, doubly distilled water, and 0.1 mol L^{-1} sodium hydroxide for 10 min each and then with corresponding running buffer until the inside current of the capillary remained stable. This was crucial to get a reproducible electroosmotic flow (EOF).

CE was performed at the separation voltage of 15 kV, 0.05 mol L^{-1} borate–0.10 mol L^{-1} phosphate mixture solutions (pH 9.5) were used to make a running buffer, and 5 mmol L^{-1} β-CD was used as buffer modifier. The standard samples were prepared with the buffer solution and pressure injected at 0.5 psi for 5 s. All experiments were carried out at room temperature.