Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 843 (2006) 194–201
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Site-specific sampling of taurine from rat brain followed by on-line sample pre-concentration, throughout in-capillary derivatization and capillary electrophoresis

Shigeyuki Oguri *, Michiko Nomura, Youko Fujita

Laboratory of Food Hygiene, Department of Home Economics, Aichi-Gakusen University, 28 Kamikawanari, Hegoshi-cho, Okazaki-city 444-8520, Japan

Received 4 November 2005; accepted 1 June 2006
Available online 18 July 2006

Abstract

A method of pinpoint-sampling followed by on-line pre-concentration of the sample, throughout in-capillary derivatization and capillary electrophoretic separation was evaluated by demonstrating the detection of taurine, 2-aminoethanesulfonic acid at a specific location of a rat brain. The direct sampling of taurine from the rat brain was accomplished by using voltage injection associated with two kinds of driving forces, electrophoretic flow and electroosmotic flow (EOF). The capillary tube (75 μm of inner diameter × 375 μm of outer diameter) of the capillary electrophoresis (CE) apparatus was already filled with a CE run buffer, viz., 40 mM phosphate–borate buffer (pH 10) containing 2 mM *o*-phthalaldehyde (OPA)/*N*-acetylcysteine (NAC) as the derivatization reagent. One end of a platinum wire (0.5 mm o.d.), used as the anode, and the inlet end of capillary tube (from which a 1.0 cm long polyimide coating was removed), were pricked down onto the surface of either the cerebrum or cerebellum of a rat brain at a location of very small dimension. When a low voltage (5 kV, 30 s) was applied, taurine began to move from the rat brain into the capillary tube, and, simultaneously, electric focusing of taurine occurred by the action of "the pH-junction effect" at the inlet end of the capillary tube. After completing the injection, both the platinum wire and capillary tube were detached from the brain and dipped into the run buffer in an anode reservoir filed with the same solution as that in the capillary tube for the CE apparatus. Then, by applying a high voltage (20 kV) between the ends of the capillary tube, taurine was automatically derivatized to yield the fluorescent derivative, separated and detected with fluorescence (E_x = 340 nm, E_m = 455 nm) during migration throughout the capillary tube. The migration profiles obtained from cerebrum and cerebellum appeared to be different, but the peak corresponding to taurine was identified on both electropherograms. The efficacy of the present method including sample on-line pre-concentration prior to throughout in-capillary derivatization CE was first verified with several preliminary experiments by using samples of taurine in water, saline and a piece of 1.5% agar–gel block, as an alternate standard for the rat brain used in this study.

Keywords: Capillary electrophoresis; On-line pre-concentration; Taurine; Throughout in-capillary derivatization; Rat brain

1. Introduction

For the past several decades [1], in vivo microdialysis or its variations have become the method of choice in the study of unbound tissue concentrations of both endogenous and exogenous substances at site-specific locations of the brain or other organs. For example, in 1958, Kalant [2] first described the microdialysis procedure for extraction and isolation of corticosteroids from peripheral blood plasma. More recently, have used methods combined with on-line coupling to micro separation technique such as capillary electrophoresis (CE) [3], because less than 1 μl of dialysate or perfusion solution could be examined. Shippy and co-workers [4] and his group introduced a low flow push–pull perfusion system that provided chemical information from small volumes of the central nervous system (CNS) tissue without sacrificing neurochemical recovery. They succeeded in the in vivo monitoring of glutamate levels with CE examination of a 0.5 μl perfusion solution in the striatum of an anesthetized rat. Kennedy and co-workers [5,6] developed a method for monitoring primary amines in vivo using the microdialysis technique coupled with an on-line CE and micellar electrokinetic chromatograph (MEKC) with

Abbreviations: CE, capillary electrophoresis; EOF, electroosmotic flow; 2ME, 2-mercaptoethanol; NAC, *N*-acetylcysteine; OPA, *o*-phthalaldehyde

* Corresponding author. Tel.: +81 465 34 1212; fax: +81 564 34 1270.
E-mail address: s-oguri@gakusen.ac.jp (S. Oguri).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.06.008

laser-induced fluorescence detection. Dialysates were derivatized on-line with OPA/2-mercaptoethanol (2ME) and automatically transferred to a separation capillary by a flow-gated interface. By using such a system, they succeeded in monitoring taurine (2-aminoethanesulfonic acid) in rat ventral striatum [7]. This systematically designed method seemed to be very attractive and potentially effective for in vivo monitoring of biological components at a small-sized locations. However, the system was much too big and required using special techniques during its operation. Furthermore, the microdialysis method might not be proper for a reliable distribution in vitro study of a compound in a mammalian tissues or organs. For example, the distribution study in each organ (or tissue) should be carried out when the pre-clinical test for a new therapeutic drug enrollment is scheduled. In such a case, each organ is separated form the body and the target compound is separately extracted from each organ after administration of the compound to the body. After chemical modification of the compound (if necessary), an instrumental analysis is carried out by one method or another. By using this off-line method, the time required for analysis for is quite long. Moreover, there is currently an interest in analytical biochemistry regarding just how much of a biological compound is distributed at a restricted or regional site in an individual organ or tissue sample, rather than in the organ or sample as a whole, because the biological compound may not be distributed uniformly throughout. To meet the demands of speed, accuracy and efficiency, and to overcome the problems associated with the previously employed methods, it is important to reduce equipment size and to streamline the system by unifying the individual steps, e.g., sampling, chemical modification, separation and detection. In the work described in this paper, we designed a direct sampling method of the rat brain at small, site-specific location by mean of a voltage injection method that is generally used for the CE method. Then, the method was further modified to add sample on-line pre-concentration and throughout in-capillary derivatization CE method employed to uncouple the special on-line derivatization device from the method. The sample on-line pre-concentration prior to throughout in-capillary derivatization method was also evaluated with reference to our previous work, throughout in-capillary derivatization CE [8,9] and sample on-line pre-concentration prior to on-column derivatization capillary electrochromatography (CEC) [10,11], in order to apply them to the present method. By using the new method, we demonstrated an in vitro detection of taurine at small-sized site-specific locations in rat cerebrum and cerebellum for the first time.

2. Experimental

2.1. Chemicals

Taurine, OPA and *N*-acetyl cysteine (NAC) were of the highest grade. These reagents were purchased from Wako Pure Chemical Co. (Osaka, Japan), and used without further purification. The other reagents used were of the HPLC grade or the highest grade commercially available. All aqueous solutions were prepared by using water purified with a Milli-Q purifier system (Millipore, Milford, MA, USA). Taurine and a 100 μM 17-component amino acid solution were prepared by diluting a 2.5 mM 17-component L-amino acid solution (Type H; Wako Pure Chemical Co.) with water to make an adequate concentration. The run buffer was prepared by mixing equal molar amounts of 20 mM (or 40 mM) sodium dihydrogenphosphate and 20 mM (or 40 mM) sodium tetraborate, and was adjusted to pH 10, with 1 M sodium hydroxide. The phosphate–borate buffers solution were stored at room temperature prior to use. Then, 13.4 mg of OPA and 16.3 mg of NAC were each separately added to 50 ml quantities of the phosphate–borate buffers (pH 10) and dissolved by sonification for 3 min, respectively. A portion of each solution was filtered with a disposable syringe filter unit DISMIC-13cp (ADVANTEC, Tokyo, Japan). The run buffer was prepared just before use. The 1.5% agar–gel block containing taurine was prepared as follows: 3 g of granulated agar (Wako Pure Chemical) was suspended in 100 ml of 0.9% sodium chloride (or saline) in a glass flask. The glass flask was then heated until the suspension became clear. Subsequently, a 10 mM taurine solution in 0.9% sodium chloride solution was added to make the adequate concentration. Approximately 5 ml of the solution was poured into a 10 ml plastic vessel, and placed at room temperature to make an agar–gel block (ca. 2 cm circle in diameter, 3 cm in height).

2.2. Apparatus

The CE system consisted of an 890-CE stabilized high voltage power supply (Jasco, Tokyo, Japan) with a FP-920 fluorescence detector (Jasco) equipped with a capillary flow-cell unit for CE and a model 807-IT data processor (Jasco). A 75 μm (i.d.) × 375 μm (o.d.) capillary tube of fused silica (Polymicro Technologies, Phoenix, AZ, USA) was used throughout the work. The detection window (1.0 cm) was made by removing the polyimide coating at the 20 cm position from the cathodic end of the tube. The electropherograms were recorded by monitoring the fluorescence intensity at 455 nm after excitation with light of 340 nm. For homogenization of the rat brains, an Iuchi model HOM digital homogenizer (AsOne Co., Osaka, Japan) was used. For centrifugation of the samples, a Hitachi model Himac-CR 20 refrigerated centrifuge (Ibaragi, Japan) was employed.

2.3. CE set-up

Two reservoirs were placed in the CE system, one at the anodic site and another at the cathodic site. The anodic reservoir contained 1 ml of run buffer in which the derivatizing reagent (2 mM OPA/NAC) was present, while the cathodic reservoir contained 1 ml of run buffer that lacked the derivatizing reagent. Both the ends, one of a piece of platinum wire (0.5 mm of outer diameter, anode) and one end of the capillary tube (from which a 1.0 cm long polyimide coating was removed) at the anodic site of CE apparatus were fixed in a Teflon-holder as shown in Fig. 1. Before the sample solution was injected into the capillary at the anodic end, any remaining residue in the capillary was swept out with the run buffer and subsequently filled with the same buffer at the cathodic end. When the CE system was not in the