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On-line preconcentration of fluorescent derivatives of catecholamines in cerebrospinal fluid using flow-gated capillary electrophoresis

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ABSTRACT

Flow-gated capillary electrophoresis (CE) coupled with microdialysis has become an important tool for in vivo bioanalytical measurements because it is capable of performing rapid and efficient separations of complex biological mixtures thus enabling high temporal resolution in chemical monitoring. However, the limit of detection (LOD) is often limited to a micro- or nano-molar range while many important target analytes have picomolar or sub-nanomolar levels in brain and other tissues. To enhance the capability of flow-gated CE for catecholamine detection, a novel and simple on-line sample preconcentration method was developed exclusively for fluorescent derivatives of catecholamines that were fluorogenically derivatized with naphthalene-2,3-dicarboxaldehyde (NDA) in the presence of cyanide. The effective preconcentration coupled with the sensitive laser-induced fluorescence (LIF) detection lowered the LOD down to 20 pM for norepinephrine (NE) and 50 pM for dopamine (DA) at 3-fold of S/N ratio, and the signal enhancement was estimated to be over 100-fold relative to normal injection when standard analytes were dissolved in artificial cerebrospinal fluid (aCSF). The basic focusing principle is novel since the sample plug contains borate while the background electrolyte (BGE) is void of borate. This strategy took advantage of the complexation between diols and borate, through which one negative charge was added to the complex entity. The sample derivatization mixture was electrokinetically injected into a capillary via the flow-gated injection, and then NE and DA derivatives were selectively focused to a narrow zone by the reversible complexation. Separation of NE and DA derivatives was executed by incoming surfactants of cholate and deoxycholate mixed in the front BGE plug. This on-line preconcentration method was finally applied to the detection of DA in rat cerebrospinal fluid (CSF) via microdialysis and on-line derivatization. It is anticipated that the method would be valuable for in vivo monitoring of DA and NE in various brain regions of live animals on flow-gated CE or microchip platforms.

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1. Introduction

Catecholamines (CAs) including dopamine (DA), norepinephrine (NE), and epinephrine (E) are important neurotransmitters and neurohormones in nervous systems and adrenal glands [1,2]. The levels of CAs in healthy human beings maintain in specific statistical ranges. Levels below the lower limits or above the upper limits often reflect health problems [3]. In addition, the level variation with time or upon stimulation can be employed as a major indicator for pharmaceutical and physiological investigation. However, the concentration levels of CAs are in nano- or pico- molar ranges. To detect this low level accurately and rapidly, powerful tools are highly wanted. Currently, laser-induced fluorescence (LIF) and electrochemical (EC) detection represent the major two formats of the most sensitive detectors for CAs. For example, HPLC coupled with electrochemical or LIF detection is able to obtain pico-molar limits of detection (LODs) for CAs [4,5]. However, HPLC separation usually takes time and requires a large volume of samples, which is infeasible to the hard-to-obtain biological fluids such as cerebrospinal fluids (CSF). Compared with HPLC, capillary electrophoresis (CE) consumes much smaller sample volumes and facilitates high-throughput analysis.

During last two decades, CE and microchip-based CE separation platforms have been applied to in vivo neurotransmitter monitoring [6–9]. The temporal resolution has been shortened to as low as 2 s when the rapid CE separation was coupled with segmented flow transport of dialysate [9,10]. However, the application of these platforms is often limited to analytes with high basal levels such as glutamate and taurine [11]. CAs such as DA and NE

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have sub-nanomolar concentrations in brain regions, and the low concentrations pose a challenge in detection after CE separation. Shou et al. have developed a sensitive CE method for DA monitoring in which a 1.0 nM LOD was achieved [12]. Nonetheless, the DA signal may often be immersed in interferences, which requires optimal and stable conditions to secure DA detection after separation. Therefore, further improved LODs such as in pico-molar are strongly desired to expand the CE applicability in tackling complex biological samples.

To improve LODs, a sensitive detection method such as LIF should be used. Alternatively, sample preconcentration is a promising strategy and has been extensively used to enhance the detectability of analytes using various strategies [13]. Off-line sample preconcentration such as solid phase extraction (SPE) lacks automation and flexibility although it may effectively enrich specific analytes such as CAs [14,15]. The on-line preconcentration mode is more flexible and convenient to couple with rapid CE separations. Strategies for on-line sample preconcentration in CE including sample stacking, sweeping, isotachophoresis (ITP), and dynamic pH junction, are usually based on the variation of electrophoretic velocities of analytes when located in two distinct buffer zones, sample and BGE [16,17]. This variation in electrophoretic velocity can be manipulated by establishing an electric field gradient or by varying the charges on analyte molecules or analyte-involving entities. Analytes may electrophoretically migrate fast in the sample plug but slow down or stop at the boundary of the sample plug and the BGE, and finally all analytes are piled up and concentrated (e.g. sample stacking and ITP). Alternatively, analytes may be electrophoretically stagnant (for neutral molecules) or migrate slowly; when a voltage is applied across the capillary, the components in the BGE invade into the sample plug and interact with analytes thus varying their electrophoretic velocities (e.g. sweeping). These methods can be hyphenated or combined to have improved concentration enhancement. For instance, dynamic pH junction-sweeping hyphenation has been employed to enhance focusing and selectivity for a complex mixture [18,19]. Field-amplified stacking injection followed by sweeping has been widely used [20,21]. However, these on-line preconcentration techniques are developed mainly for conventional CE [13], but such techniques often pose challenges when conducted on flow-gated and microchip CE that equally or more urgently requires on-line sample preconcentration to enhance the detection sensitivity.

This article reports a novel and simple on-line technique for selective focusing of fluorescent CA derivatives on a flow-gated CE platform. Briefly, DA and NE in CSF or aCSF are first fluorogenically derivatized with naphthalene-2,3-dicarboxaldehyde (NDA) in the presence of cyanide in borate buffer, and then a sample plug is electrokinetically introduced into the separation capillary through the flow-gated injection. The fluorogenic derivatization mixture contains borate buffer while the BGE is void of borate. The basic principle for focusing CA derivatives is based on the complexation of CAs with borate, which generates anionic complexes. In an electric field, these complexes electrophoretically migrate to the rear boundary of the borate plug where complexes may dissociate to neutral CA derivatives due to lower borate concentrations. On the other hand, electrophoretic migration of borate plug increases the local borate concentration which allows reformation of complexes in the concentrated zone. This process continues and all the CA derivatives will be focused to a narrow zone.

In practice, NDA-derivatization of primary amines in the presence of cyanide requires a basic buffer such as pH 9.2, while the medium for LIF detection of DA prefers a lower pH such as 7.4 due to the pH-dependence of fluorescence as reported [12]. The multi-section electrolyte systems may have complicated effects including pH junction, transient ITP, conductivity variation, and surfactant sweeping. To verify the proposed focusing mechanism, individual effects were experimentally studied and theoretically discussed, and the capability of the method was demonstrated by applying it to the measurement of DA in CSF via microdialysis sampling. The technique is anticipated to be easily adapted to microchip platforms which provide additional advantages for sensitive detection and rapid separation of CAs.

2. Experimental

2.1. Chemicals and reagents

Dopamine hydrochloride, D/L-norepinephrine hydrochloride, sodium tetraborate, and all amino acids were purchased from Sigma (St. Louis, MO, USA). Potassium cyanide, boric acid (BA), dimethylformamide (DMF), sodium hydroxide, sodium chloride, tetrasodium salt of ethylenediaminetetraacetic acid (EDTA), and chemicals (NaCl, KCl, $MgSO_4$, $CaCl_2$, and Na_2HPO_4) for preparing artificial cerebrospinal fluid (aCSF) were purchased from Fisher Scientific (Chicago, IL, USA). NDA was ordered from Invitrogen (Eugene, OR, USA). Cerebrospinal fluid (CSF) collected from the striatum of Sprague Dawley rats was purchased from BioreclamationIVT (Long Island, NY, USA). Fused silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA).

The NDA stock solution at 20.0 mM was prepared in DMF, and the working NDA was prepared by diluting the stock solution to 10.0 mM with deionized (DI) water on a daily basis. The stock solution of KCN (100.0 mM) was prepared in DI water, and the working KCN was diluted to 5.0 or 10.0 mM. The stock borate buffer (pH 9.2) was prepared by dissolving tetraborate ($Na_2B_4O_7{\cdot}10H_2O$, 100.0 mM) in DI water, and the borate buffer concentrations in the following text indicate the total concentrations of tetrahydroxyborate (THB) anions and neutral boric acids (BAs) by supposing tetraborate dissociates to equal moles of THB and BA in aqueous solutions. BA stock solution at 500.0 mM was prepared in DI water, and its pH was adjusted by using 100.0 mM NaOH. Stock solutions of NaCl at 2.0 M and EDTA at 100.0 mM were prepared in DI water without pH adjustment. DA and NE stock solutions at 10.0 mM were separately prepared in 20 mM HCl solution at the pH 2.5 on a weekly basis, and they were stored in a refrigerator. Amine solutions (glycine, arginine, glutamine, glutamate, and aspartate) at 10 mM were prepared in DI water, and were diluted to appropriate concentrations with aCSF. The aCSF consisted of 145.0 mM NaCl, 2.7 mM KCl, 1.0 mM $MgSO_4$, 1.2 mM $CaCl_2$, and 2.0 mM Na_2HPO_4, and its pH was adjusted to 7.4 with NaOH [10]. The final cyanide solution contained 5.0 or 10.0 mM KCN, 20 mM EDTA, 200.0 mM NaCl, and 240.0 mM borate unless otherwise stated.

2.2. Instrumentation

The custom-built detection system has been described [22]. Briefly, a 442-nm laser (Laserglow Technologies, Toronto, ON, Canada) beam was spectroscopically filtered through an interference bandpass filter at 442 ± 5 nM and then focused on the separation capillary through a 40x oil-immersion objective with the numerical aperture of 1.3 (Carl Zeiss Microscopy, Thornwood, NY, USA). The fluorescence was collected by using the same objective and then transmitted to a photomultiplier tube (PMT) with the part # R11540 (Hamamatsu Photonics, Japan) after emission filtration with two bandpass filters (485 ± 12.5 nm) in the series arrangement. The current signal generated by the PMT was pre-amplified and then converted to voltages by an SR570 current preamplifier (Stanford Research Systems, Sunnyvale, CA, USA). Voltage signals were finally recorded by a LabVIEW program. Sample injection and separation were performed by applying a negative voltage on the

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