Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography B, 827 (2005) 51–57
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

On-column preconcentration of glutathione and glutathione disulfide using pH-mediated base stacking for the analysis of microdialysis samples by capillary electrophoresis

Mohammed E. Hoque, Stacy D. Arnett, Craig E. Lunte *

Department of Chemistry, University of Kansas, 3071 Malott Hall, 1251 Wescoe Hall Drive, Lawrence, KS 66045-0046, USA

Received 14 February 2005; accepted 13 May 2005
Available online 5 July 2005

Abstract

Capillary electrophoresis (CE) has become a useful analytical tool for the analysis of microdialysis samples. However, CE with UV detection (CE-UV) does not provide detection limits sufficient to quantify glutathione (GSH) and glutathione disulfide (GSSG) in biological samples such as liver microdialysates, because of the small optical path length in the capillary. To overcome this limitation, an on-column preconcentration technique, pH-mediated base stacking, was used in this study to improve the sensitivity of CE-UV. This stacking technique allowed large volumes of high ionic strength sample injection without deterioration of the separation efficiency and resolution. A 26-fold increase in sensitivity was achieved for both GSH and GSSG using the pH-mediated base stacking, relative to normal injection without stacking. The limit of detection for GSH and GSSG was found to be 0.75 μM ($S/N = 6$) and 0.25 μM ($S/N = 6$), respectively. The developed method was used to analyze GSH and GSSG in liver microdialysates of anesthetized Sprague Dawley male rats. The basal concentrations of GSH and GSSG in the liver microdialysates of male rats were found to be 4.73 ± 2.08 μM ($n = 7$) and 5.52 ± 3.66 μM ($n = 7$), respectively.

Keywords: Capillary electrophoresis; Glutathione; Glutathione disulfide; pH-mediated stacking; On-column preconcentration; Microdialysis samples

1. Introduction

Glutathione is a thiol found intracellularly at high concentrations (1–10 mM) [1] and is also present in small amounts in the extracellular fluid. Glutathione exists as reduced glutathione (GSH) and in an oxidized form as glutathione disulfide (GSSG). GSH is a tripeptide of glycine, glutamate, and cysteine and GSSG is a dimer of GSH, where two GSH molecules are linked through a disulfide bond. A deficiency of glutathione is thought to be associated with a variety of diseases, such as cancer, neurodegenerative disorders, cystic fibrosis, lung diseases, HIV, and liver diseases [1,2]. One of the important functions of GSH in biological systems is antioxidant activity where GSH is oxidized to GSSG as it scavenges reactive oxygen species [2–4]. Therefore, the simultaneous determination of GSH and GSSG in biological fluids, such as microdialysates, is important since the ratio of GSH to GSSG concentration may be used as a biomarker of oxidative stress [2,5].

High performance liquid chromatography (HPLC) has been used widely for the analysis of thiols and disulfides in biological samples [6–10], but this separation technique requires a sample volume in the microliter range. Capillary electrophoresis (CE), however, uses only a few nanoliters of sample and is therefore a good choice to couple to microdialysis sampling which produces samples of only a few microliters total volume. A few papers have reported the analysis of biologically important thiols in microdialysis samples using CE with fluorescence and electrochemical (EC) detection [11,12].

* Corresponding author. Tel.: +1 785 864 4670; fax: +1 785 864 5396.
E-mail address: clunte@ku.edu (C.E. Lunte).

1570-0232/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2005.05.038

UV detection is widely used in CE since it is easy to operate and assemble to the CE system. However, it has poor concentration detection limits because of the small optical path length (25–75 μm) limited by the inner diameter of the capillary and small sample injection volumes (∼nL). To improve the detection limits of UV detection, the analytes can be chemically derivatized by UV labeling agents with strong UV absorption [13]. EC detection offers lower detection limits, but the integration of an EC detection cell with an electrophoretic separation system can be problematic because the separation current has to be separated effectively from the detection circuit [14,15]. Laser-induced fluorescence (LIF) detection offers excellent detection limits, but requires chemical derivatization with fluorescent labels when the analytes do not have native fluorescence.

A simple way to improve the sensitivity of CE with UV detection (CE-UV) without using any chemical derivatization is to use an on-column preconcentration technique so that a larger sample volume may be injected onto the capillary without losing separation efficiency and resolution. The commonly used on-column preconcentration techniques [16–19] are field-amplified stacking [20,21], large-volume sample stacking [22,23], pH-mediated stacking [24–27], transient isotachophoresis (t-ITP) [28–30], transient pseudo-isotachophoresis (tp-ITP) [31,32], and dynamic pH junction [33,34].

pH-mediated stacking has proven to be a simple and useful on-column preconcentration technique for the analysis of high ionic strength samples, such as microdialysates [24–27]. Using this technique, a large volume of high ionic strength sample can be injected directly into the CE capillary without prior dilution or any sample pretreatment. Acid stacking is used for cationic analytes and base stacking for anionic analytes. Both stacking techniques have been used successfully to increase the sensitivity of CE-UV for a variety of analytes in high ionic strength matrices [24–27]. A 66-fold enhancement in sensitivity has been reported using the pH-mediated base stacking method for the analysis of anions, such as *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, and syringic acid [25].

Both GSH and GSSG are anions at physiological pH, hence, base stacking was used in this study. In base stacking, the EOF is reversed using a cationic surfactant in the BGE and the separation is performed with reverse polarity in order to obtain electromigration of anions and EOF in the same direction. The background electrolyte (BGE) consists of salt of a weak base (e.g. NH_4^+). A large volume sample injection is followed by the injection of a strong base. This results in the titration of BGE cations (e.g. ammonium ions in NH_4^+/NH_3 buffer) by hydroxyl ions and creates a low conductivity sample zone where anions move faster and stack at the interface of the sample and highly conductive background electrolyte [25,27].

This paper describes the optimization of a CE-UV method with pH-mediated base stacking for the simultaneous analysis of GSH and GSSG in high ionic strength sample matrix and the application of the developed method to quantify GSH and GSSG in the liver microdialysates of anesthetized male Sprague Dawley rats.

2. Materials and methods

2.1. Materials

Reduced glutathione (∼98%), glutathione disulfide (∼98%), and tetradecytrimethylammonium bromide (99%) were purchased from Sigma-Aldrich Company (St. Louis, MO). All other compounds were reagent grade or better. Distilled-deionized-water (Water Pro Ps, Labconco, Kansas City, MO) was used in the preparation of all solutions. The anesthetics (Isoflurene, Xylazine, and Ketamine) used for the animal studies were supplied by the Animal Care Unit at the University of Kansas.

2.2. Sample preparation

The CE background electrolyte was ammonium buffer and consisted of 100 mM ammonium chloride with 0.5 mM tetradecyltrimethylammonium bromide (TTAB) adjusted to pH 8.4 with 0.1 M sodium hydroxide solution. The Ringer's solution was composed of 155 mM NaCl, 5.5 mM KCl, and 2.3 mM $CaCl_2$ at pH 7.4. Both GSH (10 mM) and GSSG (5 mM) stock solutions were prepared in BGE. GSH stock solution was prepared daily and GSSG stock solution was prepared weekly and stored in the refrigerator. Standard solutions of GSH and GSSG were prepared daily from their stock solutions by multiple dilutions with Ringer's solution. Buffer and Ringer's solutions were bubbled with Argon gas for 20 min to remove dissolved oxygen. All solutions were filtered through a 0.22 μm pore size syringe filter (Millipore Millex™ GP, Fisher Scientific) prior to use. The microdialysis samples were analyzed without any pretreatment.

2.3. CE-UV apparatus

A lab-built CE system with a SpectraPhysics UV1000 UV detector (Thermoseparation, San Jose, CA) was used for this study. Polyimide coated fused silica capillary (Polymicro Technologies, Phoenix, AZ) with 50 μm i.d. and 360 μm o.d. was cut to a total length of 60 cm long with a 45 cm effective length. The analysis was performed in reversed EOF mode using a cationic surfactant, TTAB, in the background electrolyte. Both GSH and GSSG were detected on-column by UV absorbance at a wavelength of 214 nm. A voltage of −10 kV was applied across the capillary using a high voltage power supply unit (CZE1000R, Spellman High Voltage Electronics, Hauppauge, NY, USA) to drive the electrophoresis. All sample injections into the capillary were made electrokinetically at −10 kV, and analyses