Journal of Chromatography B, 1004 (2015) 30–36

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Capillary electrophoresis coupled with 1,1′-thiocarbonyldiimidazole derivatization for the rapid detection of total homocysteine and cysteine in human plasma

Alexander Vladimirovich Ivanov*, Edward Danielevich Virus, Boris Petrovich Luzyanin, Aslan Amirkhanovich Kubatiev

Federal State Budgetary Scientific Institution "Institute of General Pathology and Pathophysiology", Baltiyskaya Str., 8, Moscow 125315, Russia

ARTICLE INFO

Article history:
Received 1 July 2015
Received in revised form
22 September 2015
Accepted 23 September 2015
Available online 28 September 2015

Keywords:
Capillary electrophoresis
Homocysteine
Cysteine
1,1′-Thiocarbonyldiimidazole

ABSTRACT

A simple and rapid approach is described for the determination of total plasma cysteine and homocysteine using capillary electrophoresis. Human plasma samples were reduced with dithiothreitol and then processed with 1,1′-thiocarbonyldiimidazole in acetonitrile. After centrifugation, the sample supernatant was injected directly into a capillary by applying negative voltage and analytes were stacked after alkaline post-injection. Using a 50 μm i.d. silica capillary of 35 cm total length, filled with 0.1 M triethanolamine, 0.15 M formic acid, and 50 μM hexadecyltrimethylammonium bromide (pH 3.9), we reached a limit of quantification of 2.5 μM for homocysteine. Accuracy was 94.7–105.1%, intra- and inter-day imprecisions were <2.5 and <3%, respectively. The total analysis time was 6 min. Furthermore, liquid-phase extraction with isopropanol led to a fourfold increase in sensitivity.

1. Introduction

Low molecular weight aminothiols (Cys, Hcy, CysGly, glutathione, and others) play important roles in several biological processes. Particular attention has been given to studying Hcy metabolism. Increased levels of Hcy in blood plasma or serum (hyperhomocysteinemia) is an independent risk factor for many vascular diseases (e.g., infarction, stroke, thrombophilia, coronary artery disease, and others) [1–3]. Many aspects of the action of Hcy have been investigated, and some concepts explaining Hcy pathogenicity and cytotoxic activity are offered [4–7]. Homocysteinylation of different proteins (fibrinogen, fibronectin, annexin A2, proaccelerin, apoB-100, amyloid β-peptide and other) alter their structure and functions [6,8]. Also hyperhomocysteinemia reduce the intensity of methylation reactions in cells because Hcy inhibit SAH utilization, which, in turn, is an inhibitor of transmethylases. But the diagnostic relevance of total Hcy determination remains a topic of discussion [9,10] because it does not account for fractional composition of homocysteine and influence of other metabolites. To overcome this weak points, other markers (for example, SAH [11] or the Cys/Hcy ratio [12]) have been proposed. Cys is metabolite and second (after albumin) carrier of Hcy. Because Cys is major low molecular plasma thiol, it is a major competitor of Hcy in many homocysteinylation reactions. Thus, Cys/Hcy ratio reflects bioavailability homocysteine [12].

For many years, the analysis of total aminothiols has been used in clinical and fundamental research, and new analytical procedures have been developed. Today, ELISA is generally used for total Hcy analysis in clinical laboratories [13]. This method is based on the enzymatic conversion of Hcy to SAH; hence, it does not provide the opportunity to determinate other aminothiols.

Over the few decades, various HPLC and CE methods have been developed using fluorescence or electrochemical detectors with sensitivity $\leq 10^{-9}$ M [14]. New HPLC–MS and CE–MS methods have emerged. MS enables the direct analysis of blood plasma, but the sensitivity is only adequate for determining total or bound forms of aminothiols [15,16]. Derivatization enables us to enhance the sensitivity, up to 10–30 nM [17]. HPLC–UV is used for analysis of total aminothiols using pre-column derivatization [18,19] and for

Abbreviations: BGE, background electrolyte; CE, capillary electrophoresis; CTAB, hexadecyltrimethylammonium bromide; Cys, cysteine; CysGly, cysteinylglycine; CZE, capillary zone electrophoresis; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); DTT, dithiotreitol; EI, electrokinetic injection; ELISA, enzyme-linked immunosorbent assay; EDTA, ethylenediaminetetraacetic acid; Hcy, homocysteine; HPLC, high performance liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; MS, mass spectrometry; PA, D-penicillamine; PBS, phosphate buffered saline; RT, retention time; SAH, *S*-adenosylhomocysteine; SPE, solid-phase extraction; TCDI, 1,1′-thiocarbonyldiimidazole; TEA, triethanolamine; PA, penicillamine; UV, ultraviolet.

* Corresponding author. Fax: +7 495 601 2366.
E-mail address: Ivanov_av82@mail.ru (A.V. Ivanov).

http://dx.doi.org/10.1016/j.jchromb.2015.09.036
1570-0232/

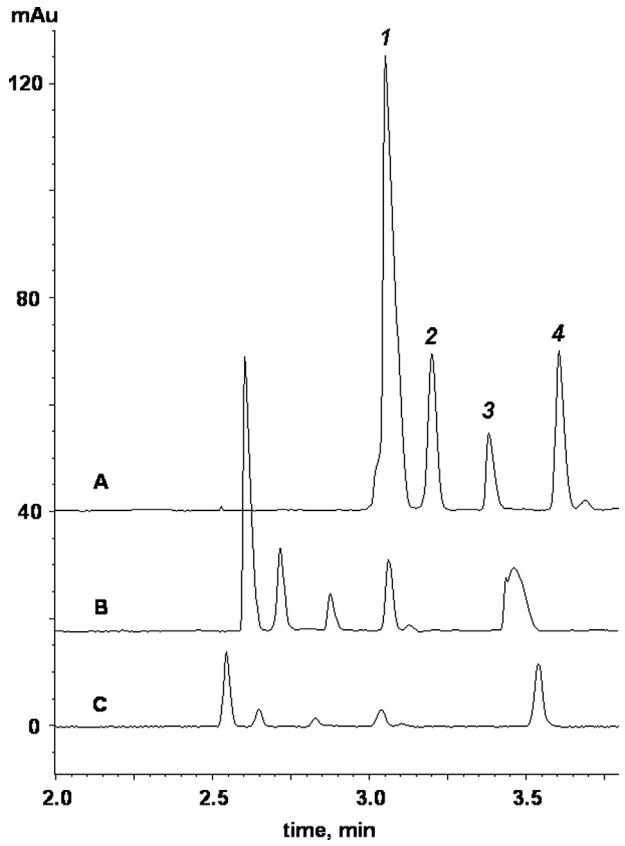

Fig. 1. Electropherograms of water solution (250 μM Cys (peak 1), 50 μM Hcy (peak 2), 50 μM PA (peak 3) and 100 μM CysGly (peak 4)) treated with TCDI. (A) EI (−13 kV 30 s), (B) the same with 10 mM NaCl addition, (C) hydrodynamic injection (1500 mbar × s). A postinjection (–13 kV 60 s) of 100 мM KOH was done in all cases. CE was performed using 35 cm total length capillary.

analysis of their reduced forms using post-column derivatization [20].

CE–UV has advantages over HPLC–UV (separation power, small volumes of samples and eluents/BGE, fast analysis, and regeneration); hence, CE could be an alternative method for clinical diagnosis. To date, only a few examples of CE–UV application for the analysis of plasma aminothiols have been described [21]. Zinellu has reported that aminothiols can be determined in clean solutions with sufficient sensitivity (~1 μM) without derivatization [22]. This approach is, however, unsuitable for such a complex matrix as plasma. Attempts have been made to use CE–UV for the analysis of various aminothiol derivatives with 2,2′-dithiopyridine [23], DTNB [24], and monobromobimane [25]. However, the sensitivity not only considerably concedes to the HPLC–UV, but is also insufficient for the accurate determination of Hcy in blood plasma. This shortcoming is explained by common weak points associated with CE (e.g., small injection volume and optical path length of detectors). The successful use of CE–UV for total plasma aminothiols analysis is reported for the case of transient isotachophoretic (acetonitrile stacking) or pH-mediated concentration of its 2-chloro-1-methyl chinoline tetrafluoroborate derivatives [26,27]; however, opportunities for application are limited. This reagent requires independent synthesis. A highly sensitive method (LOD 0.01–0.065 μM) has been reported by Chang and Tseng [28]. They used direct CZE–UV analysis with extraction of thiols by gold nanoparticles. However, this method is very time consuming (up to 30–40 min for separation) and laborious probe preparation is required.

In 2003, Amarnath et al. [29] used TCDI derivatization for HPLC–UV analysis of aminothiols in plasma and urine. This reagent binds the $–NH_2$ and –SH groups of the analytes via the thiocarbonyl moiety. As a result, cyclic *S,N*-thiocarbonyl derivatives (or cyclic dithiocarbamates) are formed, which have high absorbance in the 270–290 nm region. Probe preparation is, however, fairly labour-intensive and includes a SPE step. Unlike with TCDI, its derivatives of Hcy, Cys and CysGly have an acid character as their carboxyl groups are free. This affords the possibility of using analytes in capillary electrophoresis concentration (for example, pH-dependent alkaline stacking [30,31]) to increase the sensitivity of the analytical method.

Table 1
Recovery of the CE–UV assay ($N = 5$).

Analyte	Init. level, μM	Added, μM	Found, μM	Recovery, %
Cys	194 ± 5	45	236 ± 3	98.8 ± 3.9
		200	373 ± 5	94.7 ± 3.9
Hcy	8.5 ± 0.2	8	16.4 ± 0.2	98.8 ± 3.6
		25	34.9 ± 0.8	105.6 ± 4.6

In this paper, we present an approach for the determination of total plasma Hcy and Cys using TCDI derivatization and CE–UV analysis. It is also possible to determine CysGly.

2. Materials and methods

2.1. Equipment

A capillary electrophoresis system CE 3D (Agilent, Waldbronn, Germany) was used with an unbound silica capillary of 50 μm i.d. and 35 or 30 cm total (26.5 or 21.5 cm effective) length. The absorption signal at 285 (reference 330 nm) with width 10 nm was registered at a frequency of 3.3 s^{-1}. The temperature of the capillary was 25 °C. The following equipment was used for HPLC