Journal of Chromatography B, 879 (2011) 3818–3822

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

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

Capillary electrophoretic determination of DNA damage markers: Content of 8-hydroxy-2′-deoxyguanosine and 8-nitroguanine in urine

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ARTICLE INFO

Article history:
Received 4 July 2011
Accepted 23 October 2011
Available online 29 October 2011

Keywords:
Capillary electrophoresis
Amperometric detection
8-Hydroxy-2′-deoxyguanosine
8-Nitroguanine
DNA damage marker
Urine

ABSTRACT

A sensitive and low-cost analytical method has been developed to determine 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 8-nitroguanine (8-NO_2Gua) based on capillary electrophoresis with amperometric detection (CE–AD) after solid phase extraction (SPE). Under optimized condition, these two markers were well separated from other components coexisting in urine, exhibiting a linear calibration over the concentration range of 0.1–50.0 μg/mL with the detection limits ranging from 0.02 to 0.06 μg/mL. The relative standard deviations (RSDs) were in the range of 0.1–2.1% for peak area, 0.1–1.5% for migration time, respectively. The average recovery and RSD were within the range of 100.0–108.0% and 0.1–1.7%, respectively. It was found that the urinary contents of 8-OHdG and 8-NO_2Gua in cancer patients were significantly higher than those in healthy ones.

1. Introduction

In recent years, the mechanism of oxidative DNA damage has attracted a great interest because of its physiological effects. Extensive reports have demonstrated that various reactive oxygen species (ROS) and reactive nitrogen species (RNS) play significant roles in DNA oxidative damage [1]. Superoxide dismutase catalyzes ROS to hydrogen peroxide, producing hydroxyl radicals by the Fenton reaction [2]. Then the reactive free radicals attack the guanine at its C-8 position, generating 8-hydroxy-2′-deoxyguanosine (8-OHdG) [3]. In addition, among various RNS, peroxynitrite ($ONOO^-$) [4] often reacts with guanine to form 8-NO_2Gua, also at the C-8 position [5]. Considerable evidence suggests that both 8-OHdG and 8-NO_2Gua may have pathophysiological consequences owing to their mutagenic properties, namely, introducing ganciclovir (G) to thymine (T) transitions [6,7]. Nevertheless, DNA lesions can be repaired by nucleotide excision repair, the damaged guanines (including 8-OHdG and 8-NO_2Gua) are transported mostly out of the cells and finally excreted into urine without further metabolism [8].

Increasing reports have shown that, compared with healthy individuals, patients with various cancers have significantly higher levels of urinary 8-OHdG and 8-NO_2Gua. Mei and co-workers [9] found that the excretion levels of urinary 8-OHdG in cancer patients were much higher than those in healthy persons, indicating that a follow-up of urinary 8-OHdG might be a useful tool to evaluate the response to therapy. Sawa and co-workers [10] reported increased levels of 8-NO_2Gua in urine of smokers compared to nonsmokers. Kuo and his fellow workers [11] divided the breast cancer patients into three groups based on the stages of their cancer, revealing that urinary 8-OHdG levels apparently correlated with the development of breast cancer. Hence, 8-OHdG and 8-NO_2Gua have been increasingly considered as sensitive biochemical markers of oxidative DNA damage, as well as potential indicators for a wide variety of cancers.

As a noninvasive assay of oxidative DNA lesions in vivo, the detection of urinary 8-OHdG and 8-NO_2Gua has been used in health screening. However, due to the low level of the analytes and the complexity of the coexisting interfering substances in urine, the determination of these two compounds is a real challenge [12]. A variety of methods have been introduced to determine 8-OHdG and 8-NO_2Gua, including gas chromatography–mass spectrometry (GC–MS) [13], enzyme-linked immunosorbent assay (ELISA) [14–16], high performance liquid chromatography–mass spectrometry (HPLC–MS) [17], capillary electrophoresis (CE) and HPLC coupling with electron capture detector (ECD) [18,19,10,20–23]. However, most reported methods have their specific drawbacks. For example, the ELISA method is less quantitative; LC–MS system is expensive and requiring complicate operational procedures; as for the GC–MS method, 8-OHdG has to be derivatized to increase its volatility.

Among the analytical methods mentioned above, CE has been a rapidly growing separation technique due to its speed, separation efficiency, ultra-small sample size requirement, little solvent

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doi:10.1016/j.jchromb.2011.10.026

consumption, and ease of clearing up contaminants [24]. Combined with amperometric detection (AD), CE–AD offers high sensitivity and good selectivity for electroactive species. Weiss and Lunte reported that 8-OHdG could be quantified in urine by CE–AD with a detection limit of 17 μM [25]. Several methods for 8-OHdG determination have been developed by CE following solid phase extraction (SPE), which could concentrate the purified urine samples [26,27].

However, to our best knowledge, although some similar work was reported in the literature, most of them either involved only one of the two markers, or required tedious and time-consuming analytical procedures [3,11]. In this work, the two DNA damage markers in urine samples were analyzed successfully through an ingenious method for the first time. In order to avoid the matrix effect, the urine samples were purified with the SPE method before analysis [28]. Determination of urinary 8-OHdG and 8-NO_2Gua was achieved by comparing the migration times with those of the standards, spiking approach and the external standard method. Data were expressed as mean ± standard deviation (SD).

2. Experimental

2.1. Chemicals

8-OHdG ($C_{10}H_{13}N_5O_5$; MW = 283.24; CAS 88847-89-6), 8-NO_2Gua ($C_5H_4N_6O_3$; MW = 196.12; CAS 168701-80-2) and guanine ($C_5H_5N_5O$; MW = 283.2; CAS 73-40-5) were obtained from Sigma (St. Louis, MO, USA). Methanol, disodium tetraborate decahydrate, boric acid were purchased from China National Pharmaceutical Group Corporation (Shanghai, China). SPE cartridges (60 mg, 3.0 mL, ProElut PXC) were products of Dikma Technologies Inc.

The stock solution of 8-OHdG (40 mg/mL) was prepared with twice distilled water, while 8-NO_2Gua was dissolved in a 0.1 M NaOH aqueous solution to reach the final concentration of 200 mg/mL. All stock solutions were kept in the dark at 4 °C for storage. Before use, all sample solutions were diluted to the desired concentrations with the running buffer (H_3BO_3–$Na_2B_4O_7$, pH 9.23, 40 mM). Other aqueous solutions were prepared with twice distilled water. All other reagents were of analytical grade or higher and used without any further purification.

2.2. Equipment

In this work, the laboratory-built CE–AD system was adopted [29]. Briefly, a ±30 kV high-voltage power supply (Shanghai Institute of Applied Physics, Shanghai, China) provided a voltage between two ends of the capillary. The inlet of the capillary was held at a positive potential while the outlet was connected with ground. A fused-silica capillary (90 cm × 25 μm i.d. × 360 μm o.d., Polymicro Technologies, Phoenix, AZ, USA) was used for separation. Before its first use, the capillary was conditioned by washing with 1 M NaOH for 15 min, deionised water for 15 min, and finally with the running buffer for 15 min. In order to protect the operator from accidental exposure to high voltages, the entire capillary, the running buffer reservoirs for CE, and all electrodes were enclosed in a Plexiglas box.

A 300 μm diameter carbon disc working electrode was positioned at the outlet of the capillary. A saturated calomel reference electrode (SCE), a platinum auxiliary electrode, and a grounding electrode were also placed into the cell besides the working electrode. The three-electrode electrochemical cell was used in combination with a BAS LC-3D amperometric detector (Bioanalytical Systems Inc., West Lafayette, IN, USA). Before using, the carbon disc electrode was polished with emery sand paper, sonicated in twice distilled water, and finally positioned carefully opposite capillary outlet with an Oriel Corp. (Stratford, CT, USA) Model 14901 micropositioner. Data acquisition and analysis were preformed through HW2000 software package, Version 2.16 (Shanghai Qianpu Company, China).

2.3. Sample preparation

Urine samples were collected from various cancer patients in Changhai Hospital (Shanghai, China) and stored frozen at −20 °C [30]. Before analysis, the urine was thawed at room temperature and was centrifuged for 10 min (10,000 rpm/min) in order to remove precipitates; then the supernatant was filtered through a 0.22 μm micropore filter membrane. The cartridge was preconditioned with 4× 3 mL of methanol and 4× 3 mL of water in turn, and then 4 mL of urine was applied. The pH of urine sample was adjusted to 5.0 by adding 0.1 M HCl. Then the column was washed with 4× 3 mL of water, 4 mL methanol/water (10:90), and 4 mL methanol/water (50:50). 8-OHdG stock solution was eluted with 10% methanol, while 8-NO_2Gua stock solution with 50% methanol. The solutions were evaporated to dryness by a rotary evaporator [31]. The concentrated sample solutions were dissolved into 400 μL with running buffer, thus a 10-fold concentrated sample solution was obtained for analysis. After preconcentration, the solutions were injected to the CE–AD system for analysis.

3. Results and discussion

3.1. Optimization of the CE–AD conditions

3.1.1. Effect of the potentials applied to the working electrode

In this study, two kinds of working electrodes (copper-disc and carbon-disc electrodes) were investigated in different buffer systems to obtain the best electrochemical response, including $Na_2B_4O_7$–KH_2PO_4, $Na_2B_4O_7$–H_3BO_3 and $Na_2B_4O_7$–NaOH, respectively. Experimental results showed that 8-OHdG and 8-NO_2Gua generated no electrochemical response at copper electrode in any of the above running buffers, and had low response at carbon electrode in $Na_2B_4O_7$–KH_2PO_4 or $Na_2B_4O_7$–NaOH buffer solution, while had higher electrochemical response at carbon electrode in $Na_2B_4O_7$–H_3BO_3 buffer solution. Therefore, the $Na_2B_4O_7$–H_3BO_3 buffer solution and carbon electrode was selected as the optimum running buffer and working electrode, respectively.

Since the 8-OHdG and 8-NO_2Gua can be readily oxidized electrochemically, AD was based on this feature. In amperometric detection, the potential applied to working electrode directly affects the sensitivity, detection limit and stability of the method. Therefore, hydrodynamic voltammograms (HDVs) experiment was investigated to obtain optimum detection. As shown in Fig. 1, when the applied potential exceeded +800 mV (vs. SCE), these two analytes could generate oxidation current at the working electrode, and the peak area of both analytes increased rapidly. However, when the applied potential was over +950 mV (vs. SCE), both the baseline noise and the background current increased significantly, which were drawbacks for sensitive and stable detection. As a result, the applied potential of +950 mV (vs. SCE) was adopted where the background current was not too high and the S/N ratio reached highest. Moreover, the working electrode exhibited good stability and high reproducibility at this optimum potential.

3.1.2. Effect of the pH and concentration of the running buffer

As for capillary zone electrophoresis (CZE), the acidity of running buffer plays a key role because of its effect on the zeta-potential (ζ), the electro-osmotic flow (EOF) as well as the overall charge of the analytes, and finally affects the migration time and the separation of the analytes. Therefore, it is important to study the influence of