Talanta 106 (2013) 206–211

Contents lists available at SciVerse ScienceDirect

Talanta

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

Electrochemical evaluation of total antioxidant capacities in fruit juice based on the guanine/graphene nanoribbon/glassy carbon electrode

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ARTICLE INFO

Article history:
Received 3 September 2012
Received in revised form
17 December 2012
Accepted 21 December 2012
Available online 29 December 2012

Keywords:
graphene nanoribbon
Guanine
Total antioxidant capacities
Hydroxyl radical
Ascorbic acid

ABSTRACT

Based on electro-immobilization of guanine on graphene nanoribbon (GNR) modified glassy carbon (GC) electrode, a new electrochemical DNA biosensor was developed for the evaluation of total antioxidant capacities (TAC) in fruit juices. The biosensor relies on the guanine damage that is induced by hydroxyl radical (· OH) generated by Fenton-type reaction. Ascorbic acid (AA), which has the ability to scavenge the · OH and to protect the guanine immobilized on the electrode surface, was used as the standard antioxidant to evaluate the TAC in fruit juice. Under optimized conditions, the proposed biosensor has excellent analytical performance for antioxidant capacity assessment: wide linear range (0.1 to 4 mg L^{-1}), high sensitivity (4.16 μA/mg L^{-1}) and low detection limit (0.05 mg L^{-1}). Compared with the other electrochemical sensors developed previously, the proposed electrode demonstrates the improved detection limit of about 5 times to one order of magnitude for antioxidant capacity assessment. Additionally, the biosensor was successfully applied to the determination of the TAC in fruit juices.

1. Introduction

Reactive oxygen species (ROS), such as hydroxyl radicals (· OH), superoxide anions ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2), are highly unstable molecules. In living organisms, ROS are produced by normal metabolism or exogenous stressors (pollution, sunlight exposure, cigarette smoking, excessive alcohol consumption, etc.) [1–3]. At high ROS concentration or an overproduction state, ROS can cause oxidative stress which can induce damage of lipids, proteins or DNA, impeding normal cell functioning [4,5] and leading to numerous human diseases, as well as to the aging process [6,7]. To protect the cells and organ systems of the body against ROS, most living organisms have evolved a highly sophisticated and complex endogenous and exogenous antioxidant protection system [8]. In the endogenous system, superoxide dismutase, glutathione peroxidase, and catalase are the main enzymes involved in the removal of ROS. In the exogenous system, small molecules such as ascorbic acid (AA), α-tocopherol, glutathione, carotenoids, flavonoids, and other antioxidants act as radical scavengers. Since the exogenous antioxidants are naturally present in fresh fruits and vegetables, increasing intake of dietary antioxidant may help to maintain an adequate antioxidant status [9]. Therefore, the determination of total antioxidant capacities (TAC) in natural products is necessary and receives much attention [10,11].

Among the ROS, hydroxyl radical (· OH) is one of the most reactive species. In biological systems, · OH is produced by the reaction between Iron (Fe^{2+}) and H_2O_2, the so-called Fenton reaction. Based on the · OH-induced DNA damage theory, several electrochemical DNA-based biosensors have been developed for the evaluation of TAC in biological and food samples. These biosensors were based on the immobilized double-stranded DNA [12–15], single-stranded DNA [16,17] and purine bases [18–21] as oxidation targets and a Fenton-type reaction was used for · OH generation. However, the performance of these electrochemical DNA sensors for TAC evaluation, such as the linear response range and the detection limit, still needs to be improved further.

On the other hand, it was reported that the immobilized amount of DNA strands directly influences the analytical performance of the electrochemical DNA sensors and high surface area of the sensing materials will result in high-immobilized amount of DNA strands [22,23]. Moreover, the sensing materials with good electrocatalytic properties are beneficial to the sensitive analysis of the electroactive species. Therefore, to improve the performance of the electrochemical biosensor for TAC analysis, we need explore appropriate sensing material with high surface area and good electrocatalytic properties.

Recently, retaining the feature of a high length–diameter ratio and representing a particular kind of graphene-related material,

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0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.12.030

graphene nanoribbon (GNR) prepared from multi-walled carbon nanotubes (MWCNTs) has received wide attention, from experimental to theoretical studies [24–26]. In addition, their large surface area, superior electronic conductivity and edge structure can offer a plenty of space for chemical modification, which would result in good electrocatalytic properties [27–29] and application in biosensors [30–35]. In this work, GNR was produced by longitudinally unzipped MWCNTs and used to construct the guanine electrochemical biosensor for the first time to determine the TAC in commercial fruit juices. Based on the guanine damage which is induced by ·OH generated by Fenton-type reaction and AA as the standard antioxidant, the analytical performance of the proposed biosensor for antioxidant capacity assessment has been investigated.

2. Experimental

2.1. Chemicals

Guanine was purchased from Bio. Basic Inc., Canada. MWCNTs (diameter 20–60 nm, length 5–15 μm) were purchased from Shenzhen Nanotech Port Ltd, China and used without any purification. AA was acquired from Sinopharm Chemical Reagent Co., Ltd, China. All other chemicals were of analytical grade and used as received.

Guanine stock solution (1 g L^{-1}) was prepared by dissolving a definite amount of guanine in NaOH (0.1 mol L^{-1}) and diluting in phosphate buffered solution (PBS, 50 mM, pH 7.4), and then stored at 4 °C. The working standard solutions were prepared daily by diluting the previous stock solution with PBS (50 mM, pH 4.92) just before use. AA standard solution was of 0.5 g L^{-1}, and prepared daily and immediately before measurements by dissolving an amount of the solid AA in water until the desired concentration. Fe^{2+}–EDTA solution was prepared daily by adding the appropriate amount of $FeSO_4 \cdot 7\,H_2O$ to EDTA solution (Fe^{2+}/EDTA 1:3 mol mol^{-1}) in ultrapure water, giving a slightly green and clear solution. Fenton reaction for hydroxyl radical generation was carried out in a mixture of H_2O_2 and Fe^{2+}/EDTA (Fe^{2+}–EDTA/H_2O_2 1:6 mol mol^{-1}). In the Fenton reaction, the concentration of Fe^{2+} is equal to that of ·OH [36]. All solutions used throughout were prepared with ultra pure water obtained from a Millipore system (resistivity >18 MΩ cm).

2.2. Apparatus

The morphology of GNR was characterized by scanning electron microscopy (SEM, JSM 6700F, JEOL, Japan). All electrochemical measurements were performed on a CHI 660B Electrochemical Workstation (Chenhua Instrument Company of Shanghai, China). A conventional three-electrode cell was used with a glassy carbon (GC, with a diameter of 3 mm) electrode as the working electrode, a platinum wire as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. All the potentials in this paper were referred to SCE. Except the specific statement, the electrochemical measurements were carried out in PBS (50 mM, pH 4.92) at room temperature (25 ± 2 °C).

2.3. Preparation of GNR

The GNR was prepared by longitudinal unzipping of MWCNTs according to literatures [37,38] with some modification. First, MWCNTs (150 mg) were treated with concentrated H_2SO_4 (98%, 36 mL) for 1 h. H_3PO_4 (85%, 4 mL) was then added, and the mixture was allowed to stir 15 min before the addition of $KMnO_4$ (750 mg). The mixture was heated at 65 °C for 2 h, cooled down to room temperature, and then poured into ice water (100 mL) containing H_2O_2 (5 mL, 30 wt.%). The resulting light-brown colored precipitate was collected on a 200 nm pore size PTFE membrane, washed 2 times with HCl (20 vol%, 6 mL each) and resuspended in H_2O (60 mL) by stirring for 2 h. Then HCl (30 vol%, 60 mL) was added to coagulate the product, which was then collected on the PTFE membrane, washed 2 times with HCl (20 vol%, 6 mL each), and dispersed in ethanol (40 mL) for 2 h with stirring. Then the product was again coagulated by the addition of anhydrous diethyl ether (60 mL), filtered over the PTFE membrane, washed 2 times with anhydrous diethyl ether (10 mL each), and dried in vacuum.

2.4. Fabrication of the guanine/GNR/GC electrode

The obtained GNR (1.5 mg) was dispersed in PBS (3.0 mL, 50 mM, pH 7.4) and ultrasonicated for 0.5 h to obtain the homogeneous GNR suspension (0.5 mg mL^{-1}). Prior to use, the GC electrode was polished carefully to a mirror-like plane with 0.5 and 0.05 μm alumina powder, rinsed with ultra-pure water, and subsequently sonicated in acetone and ultra-pure water, respectively. Then, the GNR suspension (6 μL, 0.5 mg mL^{-1}) was dropped on the surface of GC electrode. After drying at room temperature, the GNR/GC electrode was obtained.

For preparation of guanine/GNR/GC electrode, the GNR/GC electrode was immersed in PBS (50 mM, pH 4.92) containing guanine (20 mg L^{-1}) and applied an accumulation potential of 0.4 V for an accumulation time of 270 s. The electrode was then rinsed with ultra pure water and denoted as guanine/GNR/GC electrode. For comparison, guanine/GC electrode was also prepared according to the above procedure.

2.5. Assays for guanine damage by ·OH and its protection by AA

Guanine damage was carried out by immersing the guanine/GNR/GC electrode in a freshly prepared Fenton solution (Fe^{2+}–EDTA/H_2O_2 1:6 mol mol^{-1}). After a definite incubation time, the guanine/GNR/GC electrode was rinsed with water and immediately immersed in PBS (50 mM, pH 4.92) to carry out the square wave voltammetry (SWV, frequency 15 Hz, step potential 4 mV and amplitude 0.025 V) experiment. The electro-oxidation peak current of the remaining unoxidized guanine was obtained and used as the detection signal.

For the study of the protection effect of antioxidant AA, the guanine/GNR/GC electrode was immersed in a freshly prepared Fenton solution in the presence of AA. And then the electro-oxidation peak current of the remaining unoxidized guanine was measured according to the above procedure.

2.6. TAC measurement in commercial fruit juices

Three kinds of fruit juices produced by Uni-president company were obtained from local supermarket. The pure fruit juice content of these juices is 10%, according to the package label. Another group of three kinds of fruit juices was homemade and obtained by squeezing the corresponding fruits obtained from local supermarket. Prior to measurement, all fruit juices were centrifuged at 10,000 rpm for 5 min, filtered and the filtrate was stored in the dark at 4 °C. For TAC assay, the fruit juices were diluted in the freshly prepared Fe^{2+} solution (2 mM) to a final volume of 5 mL. The guanine/GNR/GC electrode was immersed in the solution and H_2O_2 (60 μL, 1 M) was added for 180 s. And then the electro-oxidation peak current of the remaining unoxidized guanine was measured according to the above procedure. Measurements were made at least three times and the new prepared guanine/GNR/GC electrode was used for every TAC assay.