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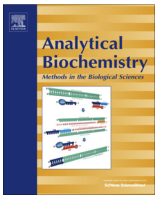

Voltammetric discrimination of mandelic acid enantiomers

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ABSTRACT

We report a novel electrochemical biosensor for direct discrimination of D- and L-mandelic acid (D- and L-MA) in aqueous medium. The glassy carbon electrode (GCE) surface was modified with reduced graphene oxide (rGO) and γ-globulin (GLOB). Electrochemical characterization of the modified electrodes was investigated by cyclic voltammetry and electrochemical impedance spectroscopy. The modified electrode surfaces were also characterized by scanning electron microscopy. Electrochemical response of the prepared electrode (GCE/rGO/GLOB) for discrimination of D- and L-MA enantiomers was investigated by cyclic voltammetry and was compared with bare GCE in the concentration range of 2 to 10 mM. Whereas the bare GCE showed no electrochemical response for the MA enantiomers, the GCE/rGO/GLOB electrode exhibited direct and selective discrimination with different oxidation potential values of 1.47 and 1.71 V and weak reduction peaks at potential values of −1.37 and −1.48 V, respectively. In addition, electrochemical performance of the modified electrode was investigated in mixed solution of D- and L-MA. The results show that the produced electrode can be used as electrochemical chiral biosensor for MA.

Chiral discrimination has become of increasing importance during the past decade due to the racemic switch strategy adapted by nearly all fields of biological, chemical, and pharmaceutical sciences [1]. This process generally includes the selective receptor that selectively forms complex with one of the enantiomers of a chiral molecule by noncovalent interaction such as hydrogen bonding, electrostatic interaction, or hydrophobic interaction. Such receptors not only provide a controlled means for studying the fundamentals of the interactions in nature but also open new ways to develop novel valuable enantioselective sensors, catalysts, and other molecular devices [2–4]. In the enantioselective sensors, electrochemical techniques have been attracted significant attention owing to some advantages over chromatographic and spectrophotometric techniques. In particular, the electrochemical technique offers the advantages of easy preparation and relatively low cost, and it requires only small sample volumes (microliter range) that couple with the very low detection limits. Due to the fact that relatively fewer electroactive interferents often come up than spectroscopic interferents, electrochemical detection in complex samples shows excellent results with high selectivity [5]. For these reasons, many efforts have been devoted to the goal of finding new modification materials and various organic redox mediators, nanoparticles, polymers, self-assembled monolayers, and carbon materials [6].

During recent years, as a new kind of carbon material, graphene has attracted enormous attention in constructing electrochemical sensors due to large specific surface area for the substances [7–11]. Because every atom in graphene is a surface atom, molecular interaction can be highly sensitive for absorbed molecules [12]. The electronic and mechanical properties of graphene can be changed by chemical modification to obtain graphene oxide (GO)[1] for use in graphene-based biosensors [13,14]. The presence of copious oxygen-containing groups on GO, such as alcohols, epoxides, and carboxyl groups, not only can significantly affect the van der Waals interactions between the graphene layers but also can render it strongly hydrophilic [15–17]. In addition, GO-based materials with tunable oxygen functionalities on their basal planes facilitate the surface modification with other materials [8,18]. However, GO is electrically insulating material; thus, it leads to significant losses of the superior properties of graphene [19]. In this respect, GO is generally reduced by reducing agents for the restoration of a graphitic network of sp^2-hybridized carbon. Reduced graphene oxide (rGO) is conductive and has chemically active functional sites, making it a frequently considered active material in molecular sensors [8,20].

The current study deals with a novel graphene oxide-based electrochemical biosensor based on γ-globulin (GLOB) for the

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[1] *Abbreviations used:* GO, graphene oxide; rGO, reduced graphene oxide; GLOB, γ-globulin; MA, mandelic acid; GCE, glassy carbon electrode; CV, cyclic voltammetry; SEM, scanning electron microscopy; EIS, electrochemical impedance spectroscopy; A–PBS, sodium acetate and phosphate-buffered saline; RSD, relative standard deviation.

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direct discrimination of mandelic acid (MA) enantiomers. Glassy carbon electrode (GCE) surface was modified with rGO and GLOB. The electrochemical behaviors of D- and L-MA at the GCE/rGO/GLOB surface were investigated by cyclic voltammetry (CV). The results show that the modified electrode not only can be used for determination of MA but also readily allows the direct enantiomeric discrimination of D- and L-MA in an aqueous medium. It also provides an opportunity to make successful electrochemical enantiomeric percentage analysis for MA.

Materials and methods

Chemical and apparatus

All chemicals were of analytical grade and used without further purification. Graphite powder (99.99 %), concentrated H_3PO_4 and H_2SO_4, H_2O_2 (30%), hydrazine hydrate solution, $KMnO_4$ (99%), D-MA, L-MA, and GLOB were purchased from Sigma–Aldrich. All aqueous solutions were freshly prepared using ultrapure water with a resistivity of 18.2 MΩ cm.

Electrode morphologies were investigated by scanning electron microscopy (SEM) performed on a Zeiss EVO LS 10 SEM at accelerating voltage of 20 kV and 5000× magnification. Before the scanning process, all samples were coated with gold to enhance the electron conductivity. Electrochemical measurements were performed with an Ivium CompactStat potentiostat (Ivium Technologies, The Netherlands) combined with a BAS/C3 electrochemical cell stand using a three-electrode configuration cell. GCE, Ag/AgCl electrode (with Luggin tip) in 3 M KCl, and platinum electrode were used as working electrode, reference electrode, and counter electrode, respectively. Electrochemical impedance spectroscopy (EIS) measurements were recorded in a mixed solution of 50 mM sodium acetate and 50 mM phosphate-buffered saline (A–PBS) containing 1.0 mM $[Fe(CN)_6]^{-3/-4}$ redox couples at pH 7.01. The impedance measurements were performed in a frequency range from 10 Hz to 100 kHz with a 5-mV signal amplitude. CV measurements were carried out in a mixed solution of A–PBS (pH 7.2 as an optimum condition) with 100 mM KCl at ambient temperature (25 °C). To avoid undesired effects, the electrochemical cell setup was freshly prepared and the measurements were performed by purging nitrogen gas for 20 min before each experiment. During the electrochemical experiments, nitrogen atmosphere was maintained over the solution.

Preparation of rGO

GO was synthesized from graphite powder by the improved method [21], which has significant advantages over Hummers and Offeman's method [22], with an additional preoxidation process. Briefly, 3.0 g of graphite was preoxidized in a mixture containing 1.5 g of $K_2S_2O_8$, 1.5 g of P_2O_5, and 15 ml of concentrated H_2SO_4. The mixture was then diluted with ultrapure water, filtered, and dried in a vacuum oven at 50 °C. After this process, the resultant graphite was reoxidized by the improved method containing $KMnO_4$ (18.0 g) in a 9:1 mixture of concentrated H_2SO_4/H_3PO_4 (360:40 ml) for production of GO. To prepare the rGO, 5 ml of GO aqueous suspension (0.5 mg/ml) was added into hydrazine hydrate (0.50 ml, 32.1 mM) in a round-bottom flask and was mixed in an oil bath at 100 °C for 24 h. The product was filtered and washed with ultrapure water (5 × 20 ml) and methanol (5 × 20 ml) and was dried in a vacuum oven at 50 °C [23].

Preparation of modified electrodes

The schematic diagram of a producing electrochemical biosensor for discrimination of MA enantiomers presented in Scheme 1 can be briefly explained as follows. Prior to each experiment, GCE was pre-cleaned with acetone, ethanol, and ultrapure water. Then the GCE surface was polished with 1.0, 0.3, and 0.05 μm alumina powder (PACE Technologies, USA) on a felt pad and was washed with a copious amount of ultrapure water. The GCE was then immersed in water and methanol for 15 min, respectively, in an ultrasonic bath (Sonorex Super RK 106, Germany) in order to remove residual alumina particles by sonication. The electrode was dried under N_2 atmosphere at room temperature before modification steps. After drying under N_2 atmosphere, a 5.0-μl suspension of rGO (0.5 mg/ml) sonicated for 1 h was dropped three times on the GCE surfaces (as an optimum condition) in order to obtain rGO-modified electrode (GCE/rGO). After rinsing with ultrapure water and drying under N_2 atmosphere, the electrode was immersed in a buffer solution containing GLOB (10 mg/ml, pH 7.2) for 40 h (as an optimum condition) at approximately +4 °C in order to prepare the GCE/rGO/GLOB.

The GCE/rGO/GLOB electrode was then washed with ultrapure water and dried under N_2 before use. The electrode was stored in buffer solution at approximately +4 °C in a closed vessel when not in use.

Results and discussion

Surface and electrochemical characterization of modified electrodes

SEM was performed to characterize the surface morphology of the bare and modified GCEs at each step. Whereas the bare GCE has a smooth surface, as can be seen in Fig. 1A, the GCE/rGO surface shows that rGO nanosheets surround the electrode surface, as shown in Fig. 1B. The modification of GLOB on GCE/rGO electrode results in a well coverage of the GLOB molecules wrapped loosely by the sheets of graphene on the surface, as illustrated in Fig. 1C.

The characterization of the modified electrodes was also investigated electrochemically by CV and EIS in A–PBS solution (pH 7.01) containing 1.0 mM $[Fe(CN)_6]^{-3/-4}$ redox couples. The changes at each step of the surface modification of GCE can be easily seen by the peak-to-peak potential difference (ΔE_p) and the peak currents ratio ($I_{p,a}/I_{p,c}$) of the $[Fe(CN)_6]^{-3/-4}$ redox couple in Fig. 2A. The bare GCE shows a typical reversible electrochemical response for the $[Fe(CN)_6]^{-3/-4}$ redox couple at 224 mV with $I_{p,a}/I_{p,c}$ of 1.14 and ΔE_p of 112 mV [24]. The modification of GCE surface with rGO presents a decrease in $I_{p,a}/I_{p,c}$ of 0.81 and a substantial increase for ΔE_p of 281 mV. Considering the related studies, there are two main explanations for these changes. First, when the electrode is coated with GO derivatives, the peak currents decrease greatly, which can be attributed to the negatively charged carboxyl group on rGO edges blocking the diffusion of $[Fe(CN)_6]^{-3/-4}$ from solution to electrode surface [25,26]. Second, ΔE_P is inversely proportional to the electron transfer rate, so the results suggest that the film of GO derivatives acts as an insulating layer, resulting in difficult interfacial electron transfer due to their disrupted sp^2 bonding networks [26–28]. In a further step, GLOB modification on the GCE/rGO caused a significant change on the electrochemical behavior of the $[Fe(CN)_6]^{-3/-4}$ couple, leading to a significant decrease in $I_{p,a}/I_{p,c}$ of 0.43 and an increase in ΔE_p of 405 mV. This result was attributed to the hydrophobic or electrostatic/hydrogen bonding interactions between rGO and GLOB and gives rise to the blockage of the electrode surface for oxidation/reduction of the redox probe ($[Fe(CN)_6]^{-3/-4}$) [7]. EIS was used for confirming and deeply probing the interface properties of surface-modified electrodes. Fig. 2B shows the impedance responses (Nyquist plots) for the bare GCE (curve a), GCE/rGO (curve b), and GCE/rGO/GLOB (curve c) in the presence of 1 mM $[Fe(CN)_6]^{-3/-4}$ in A–PBS solution (pH 7.01). The diameter of the semicircle presents the charge transfer resistance