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Direct electrochemistry of myoglobin at reduced graphene oxide-multiwalled carbon nanotubes-platinum nanoparticles nanocomposite and biosensing towards hydrogen peroxide and nitrite

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ABSTRACT

We described the preparation of a novel nanobiocomposite, reduced graphene oxide- multiwalled carbon nanotubes-platinum nanoparticles/myoglobin (RGO-MWCNT-Pt/Mb) for the direct electrochemistry of myoglobin and its application towards determination of hydrogen peroxide (H_2O_2) and nitrite (NO_2^-). RGO-MWCNT-Pt nanocomposite has been prepared by simple solution based approach and its structure was characterized. RGO-MWCNT-Pt/Mb nanobiocomposite was prepared and attained the direct electrochemistry of Mb with pair of well-defined redox peaks with the formal potential of −0.33 V and peak to peak separation of 22 mV. Amount of electroactive protein (Γ) and heterogeneous electron transfer rate constant (k_s) were calculated to be 1.04×10^{-9} mol cm^{-2} and 9.47 s^{-1}. The sensor displayed lowest detection limit (LOD) of 6 pM which is the lowest LOD ever achieved for the detection of H_2O_2. Two linear ranges were observed for the detection of H_2O_2: (1) 10 pM–0.19 nM with sensitivity of 1.99 ($\pm$0.058) μA pM^{-1} cm^{-2} and (2) 0.25 nM–2.24 μM with sensitivity of 0.037 ($\pm$0.081) μA nM^{-1} cm^{-2}. In addition, the biosensor offered good analytical parameters towards determination of NO_2^- with wide linear range of 1 μM to 12 mM and high sensitivity of 0.1651 ($\pm$0.026) μA μM^{-1} cm^{-2}. The sensor acquires good selectivity, repeatability, reproducibility and stability. The practical feasibility of the sensor has been addressed.

1. Introduction

Graphene, an allotrope of carbon is a two dimensional nanomaterial which is densely arranged in a honeycomb lattice structure with sp^2 hybridized carbon (Novoselov et al., 2004). Graphene based nanocomposite materials find widespread applications, owing to their peculiar physicochemical properties (Soldano et al., 2010). Though graphene can be prepared by number of strategies, chemical method is very attractive method to prepare bulk quantity of graphene via cost effective oxidation-reduction approach (Stankovich et al., 2007). Graphene oxide (GO), oxygenated derivative of graphene is important intermediate and precursor compound in the chemical preparation of graphene and graphene based composite materials, respectively (Dreyer et al., 2010). Inexpensive production from graphite, easy processing in aqueous solutions and available sites for functionalization make GO as a feasible material for the preparation of any kind of new graphene based composite materials (Huang et al., 2012). Interestingly, GO is one of the best dispersant for high level dispersion of CNTs and thereby producing a unique hybrid GO-CNT (Mani et al., 2013). Assembling graphene and CNTs as a hybrid via non covalent $\pi-\pi$ stacking interaction offer great opportunity to collectively harvest the exceptional properties of these two important nanomaterials (Yen et al., 2011). Graphene (or reduced graphene oxide, RGO)–CNT hybrid delivered superior performance over graphene and CNTs and therefore find extensive applications in various research fields (Yen et al., 2011; Deng et al., 2012; Devadas et al., 2012). Recently, our research group reported highly enhanced electrocatalytic performance of RGO-CNT hybrid towards sensor (Unnikrishnan et al., 2012), biosensor (Mani et al., 2013) and biofuel cell applications (Devadas et al., 2012). Owing to the 3D hierarchical arrangement, graphene-CNT hybrid delivered highest edge density per unit normal area compared with all other carbon nanostructures (Stoner and Glass, 2012). On the one hand, CNTs inhibits the restacking of graphene sheets, on the other hand, graphene inhibits the aggregation of individual tubes of CNTs and eventually rendering high stability to the hybrid.

All over the past years, significant efforts were made for the exploitation of carbon nanomaterials as Supporting material to anchor metal nanoparticles (Gopalan et al., 2009; Dey and Raj, 2010). Owing to the large surface area and high stability, graphene-CNT hybrid could be a better Supporting material to anchor the metal

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nanoparticles (Rajesh et al., 2013). Therefore, herein we utilized RGO-MWCNT hybrid as a platform for the decoration of Pt nanoparticles via chemical reduction. A previous work pioneered the application of GO as molecular templates of Pt nanoparticle growth and significantly improved biosensor performance was brought by the hybrid nanocomposite owing to the excellent electrocatalytic ability of Pt nanoparticles and unique properties of RGO-CNT hybrid (Shi et al., 2012). In addition with providing good electrocatalytic ability, Pt nanoparticles help to prohibit the aggregation and restacking of graphene sheets and CNTs.

Myoglobin (Mb) a heme protein found in skeletal muscles plays vital role in biological process and therefore it was studied as model system for the electron transfer reactions of heme proteins (Liu and Ju, 2003). Mb exhibited peroxidase-like activity and hence catalytic reaction between Mb and hydrogen peroxide (H_2O_2) was well established in the literature (Carlsen et al., 2003). During the past decades, numerous efforts were made for the exploration of direct electrochemistry of Mb by immobilizing it into various modified electrodes (Yue et al., 2011). Though several CNTs and graphene based modified electrodes were reported for the immobilization of Mb towards determination of H_2O_2, very few offer low detection. In the present work, we report the preparation of a novel nanobiocomposite RGO-MWCNT-Pt/Mb for the direct electron transfer of Mb and determination of H_2O_2. The developed Mb based biosensor achieved low detection limit of 6 pM, which is the lowest LOD achieved for the detection of H_2O_2. Determination of nitrite has profound impact since it is extensively used in food preservation and fertilizing agents. But its excess level has severe health effects and therefore several methods were proposed for the detection of nitrite (Huang et al., 1996). Among all, electrochemical methods are simple and sensitive (Yue et al., 2011). Herein we fabricated Mb immobilized electrochemical biosensor for the sensitive determination of nitrite.

2. Experimental

2.1. Reagents

Myoglobin, graphite, MWCNT were purchased from sigma-Aldrich and used as received. Commercially available contact lens cleaning solution containing 3% H_2O_2 has been acquired from a local drug store in Taipei, Taiwan to demonstrate the practicality of the sensor. All the other reagents used were of analytical grade and used without any further purification. Supporting electrolytes used for the electrochemical studies were 0.1 M Phosphate buffer solutions (PBS), prepared using Na_2HPO_4 and NaH_2PO_4 and the required pH were adjusted either using H_2SO_4 or NaOH. Prior to each experiment, electrolyte solutions were deoxygenated with pre-purified nitrogen for 15 min unless otherwise specified.

Electrochemical measurements were carried out using CHI 611 A work station in a conventional three electrode cell using modified GCE as a working electrode (area 0.071 cm^2), saturated Ag/AgCl as a reference electrode and Pt wire as a counter electrode. Amperometric measurements were performed with analytical rotator AFMSRX (PINE instruments, USA) with a rotating disc glassy carbon electrode (RDGCE) of area 0.21 cm^2. Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) studies were carried out with Hitachi S-3000H scanning electron microscope and Hitachi H-7000, respectively. Energy-dispersive X-ray (EDX) spectra were recorded using HORIBA EMAX X-ACT (Sensor+24 V=16 W, resolution at 5.9 keV). Powder X-ray diffraction (XRD) studies were performed in a XPERT-PRO (PANalytical B.V., The Netherlands) diffractometer using Cu $K\alpha$ radiation (k=1.54 Å).

2.2. Preparation of RGO-MWCNT-Pt nanocomposite

Schematic representation for the preparation of RGO-MWCNT-Pt/Mb nanobiocomposite is given in Fig. 1. Graphite oxide was prepared from graphite by Hummer's method (Hummers and Offeman, 1958) and exfoliated to GO (1 mg mL^{-1}) via ultrasonication for 1 h. GO was subjected to centrifugation for 30 min (4000 rpm) to remove the unexfoliated graphite oxide. Subsequently CNTs were added to the GO dispersion (1:1 weight ratio) and ultrasonicated for 2 h. Then, the formed GO-CNT hybrid was separated, washed with water, dried overnight and redispersed in water. Afterwards, GO-CNT hybrid was mixed with H_2PtCl_6 and stirred for 30 min. Then HCl was slowly added until pH becomes <2 and then $NaBH_4$ was slowly added with vigorous stirring and

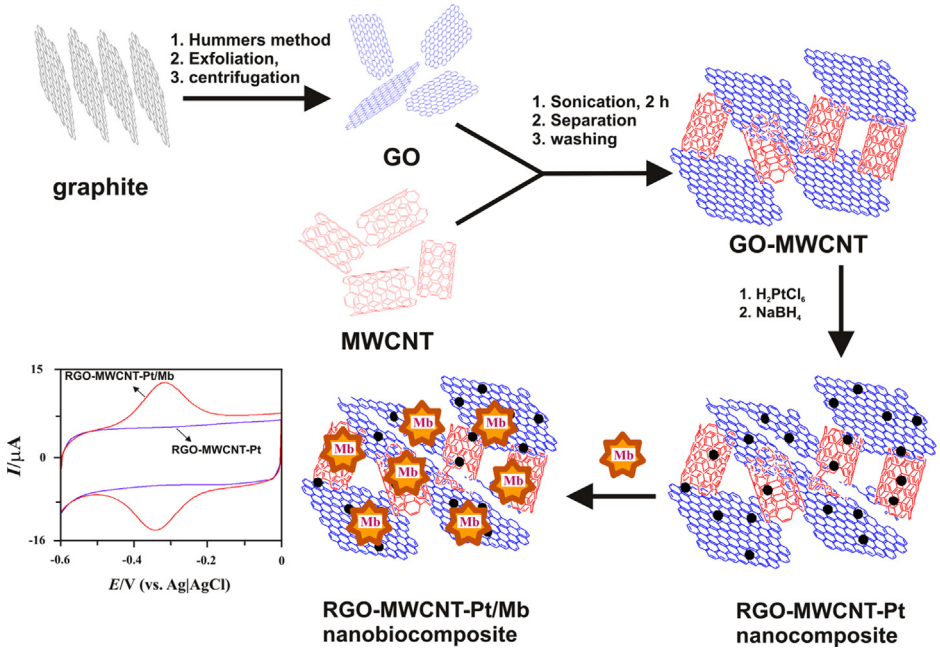

Fig. 1. Schematic representation for the preparation of RGO-MWCNT-Pt/Mb nanobiocomposite. Conditions for CV analysis: CVs were obtained at the respective film modified GCEs in PBS (pH 7) at the scan rate of 50 mV s^{-1}.