Biosensors and Bioelectronics 50 (2013) 251–255

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Short communication

Graphene-based nanoprobes and a prototype optical biosensing platform

Hui Xu [a,1], Dongfang Wang [b,1], Shijiang He [b], Jiang Li [b], Bianying Feng [c], Peijun Ma [c], Pingping Xu [a], Shanmin Gao [a], Shengxiao Zhang [a], Quanwen Liu [a], Jianxin Lu [c,*], Shiping Song [b,**], Chunhai Fan [b]

[a] School of Chemistry and Materials Science, Ludong University, Yantai 264025, China
[b] Laboratory of Physical Biology, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China
[c] Key Laboratory of Laboratory Medicine, Ministry of Education, Wenzhou Medical University, Wenzhou 325035, Zhejiang, China

ARTICLE INFO

Article history:
Received 11 April 2013
Received in revised form
17 June 2013
Accepted 20 June 2013
Available online 29 June 2013

Keywords:
Graphene oxide (GO)
Nanoprobe
Signal amplification
Immunoassay
Cancer marker

ABSTRACT

Biochemical and biomedical applications of graphene are critically dependent on the interaction between biomolecules and the nanomaterial. In this work, we developed a graphene-based signal-amplification nanoprobe by combining anti-immunoglobulin G (anti-IgG) and horseradish peroxidase (HRP) with graphene oxide (GO). The structure and function of HRP in the nano-interface of GO were firstly investigated, which demonstrated that the enzyme retained 78% of its native activity and 77% of its native α-helix content. HRP and anti-IgG were then co-adsorbed onto GO to form bifunctional nanoprobes. The nanoprobes provide both improved binding ability and signal-amplification ability. Comparing with conventional bioconjugates such as enzyme-linked antibody, co-adsorption could avoid chemical conjugation between biomolecules, keeping their bioactivity well. As an example for their application, the nanoprobes were used to obtain amplified signals in a sandwich-type immunoassay for cancer marker, instead of conventional colorimetric conjugates. This approach provided a detection limit of 10 pg/mL alpha-fetoprotein (AFP), which was much more sensitive than conventional enzyme-linked immunosorbent assay (ELISA) methods. The easily fabricated GO-based nanoprobes have the potential to become universal probes for molecular diagnostics.

1. Introduction

Graphene, an atomic single-layer of graphite, has been intensively studied in the past years because of its unique electronic, optical, thermal and mechanical properties (Novoselov et al., 2004; Zhang et al., 2005; Avouris et al., 2007; Balandin et al., 2008; Lee et al., 2008; Castro Neto et al., 2009; Bonaccorso et al., 2010; Schwierz, 2010). Graphene oxide (GO), the water-soluble derivative of graphene, has been used in the fields of sensing, electric devices (Yun et al., 2009a, 2009b), and biology (Geim, 2009; Wang et al., 2011). However, few studies were focused on the changes of biofunctionalized GO, although the interaction of biological molecules (e.g., proteins and nucleic acids) with this planar nanomaterial is critical for its use in biosensor, drug delivery, and related biological applications (Sun et al., 2008; He et al., 2010; Wang et al., 2010, 2011). So it is very important for the design of optimal biomolecule–GO hybrid structures to assess and understand the potential changes of biomolecular structure and function upon interfacing the biomolecules with the two-dimensional nanomaterials (Niemeyer, 2001).

Early diagnosis of cancer is very important for cancer therapy but still remains challenges facing scientists all over the world (Kitano, 2002). Accurate detection of protein biomarkers is critical but difficult in early diagnosis of cancers because there is only trace protein biomarker in serum of early cancer patients (Welsh et al., 2001; Wilson and Nock, 2003; Ludwig and Weinstein, 2005). Usually, traditional methods such as enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), fluorescence immunoassay (FIA), are used to detect these cancer markers. However, these techniques cannot meet the needs for higher detection sensitivity as earlier diagnosis becomes more and more important (Yu et al., 2006; Zhang et al., 2007). There have been great advances in nano-amplification technologies, yet most of them suffered from obstacles such as complicated assembly processes, insufficient surfaces of some nanomaterials, and lack of stability of biofunctionalized nanomaterials (Giljohann and Mirkin, 2009; Song et al., 2010; Zhang et al., 2011). For example, in order to efficiently immobilize enzymes on nanomaterial's surfaces, complicated and labored work was required to firstly modify the substrate surfaces (Lin et al., 2004; Lee et al., 2006). Zero-dimensional nanomaterials

* Corresponding author at: Key Laboratory of Laboratory Medicine, Ministry of Education, Wenzhou Medical University, Wenzhou 325035, Zhejiang, China
** Corresponding author. Tel.: +86 21 3919 4065; fax: +86 21 3919 4702.
E-mail addresses: jxlu313@163.com (J. Lu)spsong@sinap.ac.cn (S. Song)
[1] These authors contributed equally in this work.

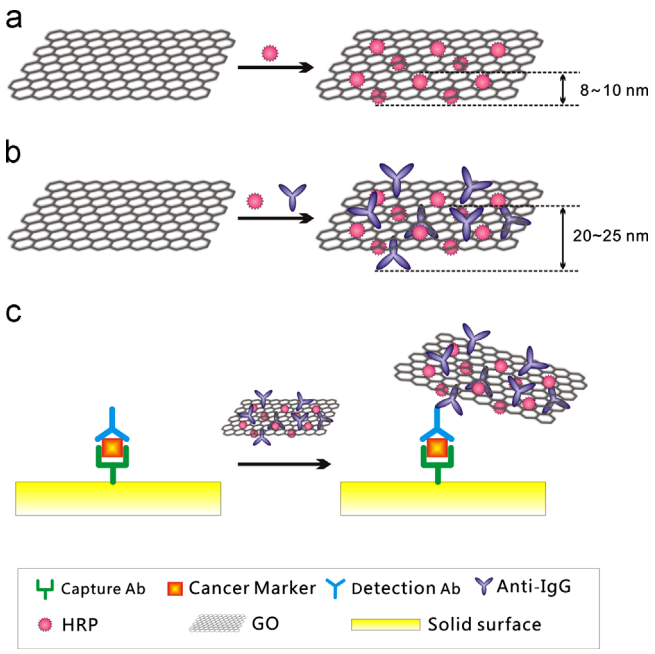
0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.06.039

such as gold nanoparticles often could not meet the requirement for nano-based signal-amplification because of their limited surfaces, especially when enzymes and large biomolecules such as immunoglobulins need to be co-assembled on one nanoparticle (D. Li et al., 2008; J. Li et al., 2008; Sperling et al., 2008; Giljohann et al., 2010). One-dimensional nanomaterials such as carbon nanotubes often could not offer uniformly sized nanostructures and become instable after surface biological functionalization (Wang et al., 2004; Yu et al., 2006; Wan et al., 2011; Zhang et al., 2011). Therefore, fabricating simple and cost-effective functional nanoprobes for high-sensitive biosensors still remains challenging. Herein, we have tried to use two-dimensional GO to fabricate nanoprobes for signal amplification and develop a prototype optical biosensing platform for high-sensitive detection of cancer markers. We firstly evaluated both the structure and function of GO assembled with horseradish peroxidase (HRP). The GO-HRP was characterized by atomic force microscopy (AFM), kinetic analysis and circular dichroism (CD) spectroscopy. Then, we fabricate multifunctional nanoprobes by coassembling HRP and goat anti-rabbit immunoglobulin G (anti-IgG) onto the GO surface. The nanoprobes were used to detect alpha-fetoprotein (AFP), the confirmed biomarker of liver cancer. The protocol of the study was designed as Scheme 1. The prototype biosensing platform has a detection limit of 10 pg/mL for AFP. Importantly, since the anti-IgG can react with rabbit polyclonal antibodies for any antigen, the platform has a potential to become powerful tools for the detection of most of target proteins.

2. Experimental

2.1. Materials

HRP (lyophilized, 99%), bovine serum albumin (BSA), Tween 20 and anti-IgG labeled with HRP (anti-IgG-HRP) were purchased from Sigma-Aldrich. TMB substrate was purchased from Neogen. AFP polyclonal antibody and monoclonal antibody were purchased from R&D Systems (Minneapolis, MN, USA), anti-IgG, AFP were purchased from Fitzgerald (Acton, MA, USA). Antigens and antibodies were dissolved in phosphate buffered saline (PBS, 0.01 M PB, 0.14 M NaCl, 2.7 mM KCl, pH 7.0) unless otherwise noted. The washing buffer was PBST (0.5% Tween in 0.1 M PBS buffer). Bichinchoninic Acid (μBCA) assay reagents used for protein concentrations determination were purchased from Pierce Biotechnology, Inc. (Rockford, IL). GO was synthesized from the natural graphite powder by using the modified Hummer's method (Hummers and Offeman, 1958; He et al., 2010).



Scheme 1. Schematic of GO-based probes and the biosensing platform for cancer markers. (a) The assembly of GO-HRP conjugates; (b) the assembly of anti-IgG-GO-HRP conjugates; and (c) the immunosensing configuration for the detection of AFP, using GO-based probes.

2.2. Instruments

Centrifugation was operated using Centrifuge himac-CF 16RX (Hitachi, Japan). The circular dichroism (CD) spectroscopy was measured by using Chirascan spectropolarimeter (Applied Photophysics). HRP activity was measured by using a microplate reader (Tecan GENios). The morphology of GO was characterized using AFM (Nanoscope IIIa Digital Instrument, USA).

2.3. Preparation of GO-protein conjugates

200 μL GO (0.5 mg/mL) were mixed with different amounts of HRP in phosphate buffer (50 mM, pH 7.0), and the mixture was shaken on an Eppendorf platform shaker for 4 h at 300 rpm at room temperature. After incubation, the mixtures were centrifuged at about 10,000 rpm and the supernatant was removed and then fresh buffer was added. Typically, five times of centrifugation and washing were repeated to remove unbound enzyme. All supernatants were collected and used for protein content determination using the μBCA assay. The amount of enzyme loaded onto the GO was obtained by measuring the difference in the concentration of enzyme in solution before and after exposing it to the dispersion of GO in buffer. To prepare the GO-based probe (anti-IgG-GO-HRP), 200 μL GO (0.5 mg/mL) were mixed with different amounts of HRP and anti-IgG in 0.1 M PBS, and the mixture was shaken on an Eppendorf platform shaker for 10 h at 300 rpm at room temperature. Next, 50 μL of 5% BSA solution was added to passivate the surface of GO for 1 h. After incubation, the mixtures were centrifuged at about 10,000 rpm and the supernatant was removed. The final deposition was suspended in 500 μL of 0.1 M PBS and stored at 4 °C for further use.

2.4. Determination of HRP activity

The activity of HRP was measured using TMB as the substrate. HRP catalyzes the TMB substrate to form a soluble product whose concentration can be quantified by measuring its absorbance at 595 nm.

2.5. Circular dichroism spectroscopy

The secondary structure of HRP and the GO-HRP conjugates was monitored by CD spectroscopy. The far-UV CD spectra (200–250 nm) of the native and immobilized enzymes were recorded at 20 °C using cylindrical quartz cuvettes with a 1 mm path length. In all measurements, the protein concentration was 50 μg/mL. CD spectra of the GO (50 μg/mL) were recorded under the same condition as a control. At least three CD spectra were acquired for each sample. The spectra were then averaged and the α-helix content was calculated on the basis of the mean residue ellipticity at 222 nm (⊖222).

2.6. Detection of AFP with GO-based probe

First, a 96-well was coated with capture antibody (200 μL, 10 μg/mL in PBS). The plate was incubated at room temperature overnight, followed by washing with PBST wash buffer. Then the