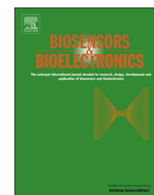
Biosensors and Bioelectronics 83 (2016) 85–90

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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



Multifunctional nanocatalyst-based ultrasensitive detection of human tissue transglutaminase 2

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ARTICLE INFO

Article history:
Received 29 January 2016
Received in revised form
14 April 2016
Accepted 16 April 2016
Available online 16 April 2016

Keywords:
TGM2
Multifunctional nanocatalyst
Electrochemical method
Signal amplification

ABSTRACT

Tissue transglutaminase 2 (TGM2) is a novel tumor marker, which can be used as the complementary marker for alpha-fetoprotein to improve overall diagnostic performance of hepatocellular carcinoma at the clinical level. In this work, we have developed a multifunctional nanocatalyst-based electrochemical method for TGM2 assay with an ultrahigh sensitivity. Here we firstly functionalize carboxyl-modified graphene oxide with poly-lysine and copper ion to form an electrochemical nanocatalyst. On the one hand, the nanocatalyst can function as the substrate of TGM2. On the other, the nanocatalyst can be applied for signal amplification to enable high sensitivity of the detection. With the specific glutamine-donor-peptide of TGM2 modified on the electrode, the exertion of the transamidation activity by TGM2 can lead to the tethering of the nanocatalyst with the peptide on the electrode, inducing obvious changes of the electrochemical signals. Therefore, simple and sensitive detection of TGM2 can be achieved. Moreover, TGM2 is also detected effectively in complex serum samples, suggesting potential diagnostic applications of the new method proposed in this work.

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1. Introduction

Hepatocellular carcinoma (HCC) is a common worldwide disease with extremely poor prognosis due to failure in early diagnosis (Llovet et al., 2002; Sung et al., 2012). Currently, alpha-fetoprotein (AFP) is the only available biomarker for HCC diagnosis (Hann et al., 2013; Maggs et al., 2012); however, its use in the early detection of HCC is limited (Durazo et al., 2008), because many patients suffered from HCC have normal levels of serum AFP (Tomimaru et al., 2012). Thus, additional biomarker that can be used in combination with AFP to improve early detection of HCC is greatly needed. Many studies have suggested that tissue transglutaminase 2 (TGM2) may serve as a novel histological/serologic candidate of HCC, especially for the individuals with normal serum AFP (Sun et al., 2008; Zhu et al., 2014). Moreover, increased expression and transamidation activity of TGM2 are associated with various types of liver injury (Kojima et al., 2012). The increase in TGM2 expression may also be useful as a potential marker for early cancer detection or an independent prognostic factor for increased drug resistance, metastasis and poor patient survival (Antonyak et al., 2011). So, for a more detailed investigation of HCC and its accurate diagnosis, sensitive techniques for quantification of TGM2 protein should be available.

The current methods for quantification of TGM2, such as ELISA, fluorometric transamidation assay, are effective but require labor-intensive and time-consuming labeling of proteins or complicated preparation of signal reporters (Gnaccarini et al., 2009). In addition, these assays mainly use a combination of mono- and polyclonal antibodies against TGM2 that are not of human origin, so shortcomings with the sensitivity and anti-interference ability of these methods may limit their applications (Gnaccarini et al., 2009; van Geel et al., 2012; Wolf et al., 2011). Besides, the antibody-based probes suffer from costly preparation and purification processes, poor storage stability, and they are easily inactivated by improper treatment (Chen et al., 2015; Truta et al., 2014; Yoshimoto et al., 2010). Although great efforts have been devoted to overcome such shortcomings, the development of sensitive, reliable, and cost-effective TGM2 assay method still remains a great challenge.

Herein, we have proposed a novel nanocatalyst-based electrochemical method for TGM2 assay. We firstly functionalize carboxyl-modified graphene oxide (GO–COOH) with poly-lysine (PL) to form the hybrid nanocomposite GO/PL. PL has been proven to be particularly attractive as effective ligands in the synthesis of nano-

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http://dx.doi.org/10.1016/j.bios.2016.04.045
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scaled materials because of their multiple chelating ligands and plentiful active amino groups imparting unique functionalities (Liu et al., 2014; Lv et al., 2014; Ma et al., 2014; Mondragón et al., 2014). Because of the plentiful epoxy groups of GO–COOH (Li et al., 2008, 2015; Liu et al., 2008; Shen et al., 2012), some free amino groups on the PL molecular chains cross-link with those epoxy groups, and other residual amino groups can act as a relative amicable and soft linker between the graphene sheet and bioactive molecules. Therefore, the prepared GO/PL nanocomposite may introduce additional capabilities, including affinity to specific biomolecules TGM2 and chelating copper ions to produce the electrochemical active GO/PL/Cu^{2+} nanolabel. Moreover, since the chelated copper ions can catalyze the oxidation of o-phenylenediamine (OPD) to electroactive 3-diaminophenazine (DAP) (Li et al., 2013), the nanolabel can be applied as a nanocatalyst for signal amplification to improve the sensitivity of the detection. Notably, the nanocatalyst allows the combination of properties of three different materials for increased electronic conductivity, biocompatibility and catalytic efficiency. Benefited from this design, the exertion of the transamidation activity of TGM2 leads to the tethering of the nanocatalyst on the electrode, inducing obvious change of the electrochemical signal due to the catalytic activity of the nanocatalyst. Therefore, detection of TGM2 can be achieved, and the experimental results show that the sensitivity is better than the current available methods. In addition, TGM2 is also detected effectively in complex biological samples. Since TGM2 shows similar diagnostic values for AFP-negative HCC cases, this method may have great potential for improving overall diagnostic performance at the clinical level.

2. Experimental section

2.1. Reagents and chemicals

Recombinant human TGM2 (> 97%) was purchased from Sino Biological Inc. The peptides [11-mercaptoundecanoic acid (MUA)-TVQQEL, lyophilized powder, purity > 98%] was synthesized by ChinaPeptides Co., Ltd. PL, N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-Hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (USA). GO–COOH was purchased from Nanjing XFNano Materials Tech. Co. Ltd. (Nanjing, China), and further fractionated by ultrasonic wash and centrifugation filtering. Other chemicals used in this work were of analytical grade and directly used without additional purification. The stock solution of the peptide was prepared by dissolving the powder with 10 mM phosphate buffer solution (PBS, pH 7.4) to 10 μM. The standard sample of TGM2 was prepared by dissolving the powder with 10 mM Tris–HCl (pH 7.4) and diluted it to desired concentrations. All solutions were prepared with Milli-Q water (18 $M\Omega\,cm^{-1}$) from Millipore system. Human venous blood samples from HCC patients were obtained from the local hospital and were approved by the ethical committees. The blood samples were centrifugated at 2500 rpm for 10 min and then the supernatants were collected and ready for assay.

2.2. Synthesis and characterization of GO/PL/Cu^{2+} nanocatalyst

The obtained GO–COOH was first fractionated to remove large flakes and impurities. Firstly, GO–COOH flakes were dispersed in deionized water (2 $mg\,ml^{-1}$) and sonicated in a bath-type sonicator (Scientz-IID) for 180 min Then, the solution was centrifuged at 1000 rpm for 10 min to remove large precipitates. After that, GO sheets were washed several times by centrifugation and filtration. To synthesize the GO/PL composite, GO–COOH (1 $mg\,ml^{-1}$), EDC (12.5 $mg\,ml^{-1}$), and NHS (7.5 $mg\,ml^{-1}$) were mixed together for 15 min under ultrasonication. PL (2 $mg\,ml^{-1}$) was then quickly added into the solution and the mixture was stirred gently at room temperature for 3 h. After that, the GO/PL was collected and purified by centrifugation for subsequent coordination with 1 mM Cu^{2+} at ambient temperature for 1 h. Finally, the GO/PL/Cu^{2+} nanocatalyst was treated by consecutive washing/centrifugation, and re-suspended to a concentration of 0.30 $mg\,ml^{-1}$. For the construction of the control label PL/Cu^{2+}, PL (1.5 $mg\,ml^{-1}$) was mixed with 1 mM Cu^{2+} and incubated at ambient temperature for 1 h.

All the samples were dispersed in deionized water for characterization. Transmission electron microscopy (TEM) was conducted using a JEOL 2000 transmission electron microscope operating at 200 kV. The TEM specimens were prepared by dispersing the nanocatalyst on 400 mesh size copper grids. The X-ray photoelectron spectroscopy (XPS) analysis was performed on a UIVAC-PHI 5000 Versa Probe scanning microprobe device. The specimens for XPS were deposited onto silicon wafers before characterization. Ultraviolet-visible (UV–vis) spectra were recorded using a Shimadzu UV-2450 spectrophotometer. Zeta potentials were measured by dynamic light scattering (Malvern Nano-ZS, U.K.).

2.3. Gold electrode treatment and modification

These steps were essentially the same as previously reported (Huang et al., 2014). Briefly, the gold electrodes (3 mm diameter) were cleaned by immersing them in bath of freshly prepared piranha solution for 5 min (*Caution: piranha reacts violently with organic compounds*). Then the electrodes were rinsed with double-distilled water and polished with 1 μm and 0.3 μm alumina slurry in sequence. After that, the electrodes were ultrasonicated in both ethanol and water to remove residual alumina powder, and finally electrochemically cleaned with 0.5 M H_2SO_4.

After being dried with nitrogen, the prepared gold electrodes were immerged in 50 μL assembly solution (5 μM substrate peptide and 5 mM TCEP in 10 mM PBS, pH 7.4) for 16 h at 4 °C, followed by being dipped in 1 μM 9-mercaptononanol for 1 h at room temperature to block the nonspecific binding site on the electrode surface. Finally, the modified electrodes were rinsed with double-distilled water and dried under mild nitrogen stream.

2.4. Detection of TGM2

The above modified electrodes were incubated with the reaction solution (10 mM Tris–HCl, 2 mM $CaCl_2$, pH 7.4) containing TGM2 and the nanocatalyst mixture for 1 h at 37 °C. After that, the electrodes were gently rinsed with double-distilled water and dipped in 5% Tween-20 for 30 min to exclude non-specific adsorption. Signal response of the coordinated copper ion was then recorded. For signal amplification, the electrodes were dipped in a 1 mM HCl solution (1 ml) containing 1.0 $mg\,ml^{-1}$ OPD. The solution was then placed in 50 °C water bath for 30 min After being cooled to room temperature, this solution was buffered with 3 ml 10 mM PBS (pH 7.4). Signal response of the generated DAP was then recorded in the buffered reaction solution.

2.5. Electrochemical measurements

All electrochemical measurements were performed on a CHI660D Potentiostat (CH Instruments) work station at room temperature. Cyclic voltammograms (CVs), electrochemical impedance spectra (EIS) and square wave voltammograms (SWVs) were recorded using a conventional three-electrode system, which included a gold electrode as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum

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