Biosensors and Bioelectronics 51 (2014) 310–316

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Electrochemical immunoassay for procalcitonin antigen detection based on signal amplification strategy of multiple nanocomposites

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ARTICLE INFO

Article history:
Received 27 April 2013
Received in revised form
17 June 2013
Accepted 20 July 2013
Available online 26 July 2013

Keywords:
Electrochemical immunosensor
Graphene
Multiwalled carbon nanotubes
Chitosan
Procalcitonin

ABSTRACT

Procalcitonin, as a medium of inflammation, has become a new marker of the identification of severe bacterial infections in recent years and has received high attention due to its most ideal diagnostic indicators of specificity with major types of organism systemic inflammation of bacterial infection in the early stages. Thus, a novel method for the determination of procalcitonin (PCT) was developed based on a sandwich-type electrochemical immunosensor, which combined a simple immunosensor array as well as an effectively designed trace tag. The immunosensor was fabricated by layer-by-layer coating graphene (GC), carbon nanotubes (MWCNTs), chitosan (CS), glutaraldehyde (GA) composite on the working electrode, which can increase the electronic transfer rate and improve the surface area to capture a large number of primary antibodies (Ab_1). The trace tag was prepared by loading high-content signal horseradish peroxidase labeled secondary PCT antibody (HRP-Ab_2) with AuNPs, which were coated with mesoporous silica nanoparticles (MCM-41) through thionine linking. In comparison with conventional methods, the proposed immunosensor for PCT provided a better linear response range from 0.01 to 350 ng/mL and a lower limit of detection (LOD) of 0.5 pg/mL under optimal experimental conditions. In addition, the immunosensor exhibited convenience, low cost, rapidity, good specificity, acceptable stability and reproducibility. Moreover, satisfactory results were obtained for the determination of PCT in real human serum samples, indicating that the developed immunoassay has the potential to find application in clinical detection of PCT and other tumor markers as an alternative approach.

1. Introduction

Procalcitonin(PCT) is a normal precursor of the active hormone calcitonin with a molecular weight of 13 kDa. Various studies have shown that rapidly increasing levels of procalcitonin were closely correlated with severe systemic bacterial infections (Assicot et al., 1993; Dandona et al., 1994). Procalcitonin is considered to be an early inflammatory marker, which reflects the severity of systemic inflammatory and now has been used as a parameter for the diagnosis and survey of sepsis, severe sepsis (Balci et al., 2003; Schroder et al., 1999; Gendrel et al., 1996; Meisner, 1996). Monitoring the PCT concentration not only can be used to provide useful information for the differential diagnosis of infectious and noninfectious diseases as to avoid the abuse of broad-spectrum antibiotics, to reduce the occurrence of drug resistance (Eberhard et al., 1998; Brunkhorst et al., 1998; Hatherill et al., 1997; Hammer et al., 1999), but also for the follow-up of patients with infections and a systemic inflammatory response to improve the rescue success rate of critically patients, to assess the prognosis of the disease (Meisner et al., 1999; Oberhoffer et al., 1999), and to improve the efficacy of therapeutic measures (Reith et al., 1998; Gramm et al., 1995).Therefore, the detection of PCT in serum is crucial for effective early diagnosis and very helpful for further treatments. Extensive efforts and a variety of analytical techniques have been widely employed in this field. However, despite considerable efforts launched in this area, to improve the selectivity and sensitivity of detecting PCT is still a challenge. To explore new strategies and techniques for simple, sensitive, rapid, and reliable detection of PCT is strongly desirable.

At present, the usual techniques used for determination of PCT are chemiluminescence immunoassay (CLIA) (Köszegi, 2002), fluorescence immunoassay (IFA) (Baldini et al., 2009; Manuel et al., 2009; Ursula et al., 2011), and enzyme-linked immunosorbent assay (ELISA) (Kremmer et al., 2012). However, there are few reports about the application of electrochemical immunoassays for procalcitonin biomarker. Electrochemical immunoassays, as a promising approach for selective and sensitive analyses, with simple instrumentation, easy signal quantification, low cost, low power requirements, rapidness, high compatibility and high repeatability, have become important

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http://dx.doi.org/10.1016/j.bios.2013.07.035

analytical tools in different fields such as environmental protection, food safety, and clinical diagnosis (Tsagkogeorgas et al., 2006; Wu et al., 2007; Wei et al., 2010; Zhang et al., 2007; Jin et al., 2011; Jiang et al., 2008; Cai et al., 2011; Zhuo et al., 2011; Tang et al., 2008). As for the construction of an electrochemical immunosensor, it is based on the antigen–antibody interactions with electrochemical transducers. The key step is the efficient immobilization of bimolecular onto the electrode surface (Dempsey et al., 2004). In recent years, nanomaterials and nanotechnologies have enabled significant improvement in the analytical performance of electrochemical immunoassay methods, which possess great advantages of unique physical and chemical properties (Zhuo et al., 2006; Giannetto et al., 2011; Yin et al., 2010; Li et al., 2009; Chen et al., 2009), such as large surface area, excellent conductivity, low cost, and electrocatalytic activity (Liu et al., 2008; Wang et al., 2009; Yu et al., 2012).

Carbon nanotubes (CNTs), both single- and multi-walled, have been widely used in both scientific and industrial electrochemistry due to their great chemical stability, large aspect ratio, wide potential window, excellent electrical conductivity, and electrocatalytic activity (Andrews et al., 2002; Yanez-Sedeno et al., 2010; Tasis et al., 2006). These unique physiochemical properties make carbon nanotubes have substantial potential use in a biosensing field (Du et al., 2007; Antiochia and Gorton, 2007; Yang et al., 2010; Jiang et al., 2010; Zhao et al., 2006; Kauffman et al., 2010). Graphene nanosheets (GS), a single layer of carbon atoms with a closely two-dimensional packed honeycomb lattice, have gained great interest and enormous attention in constructing electrochemical biosensors due to their unique structural features such as large specific surface area, low manufacturing cost, high thermal conductivity and good biocompatibility (Wang et al., 2010; Du et al., 2011; Meyer et al., 2007). Recently, many investigations have demonstrated that graphene-based nanocomposite offer better electrocatalytic activity than sole graphene, which have been used for constructing enhanced electrochemical sensors and biosensors, due to the synergistic effects of two or more functional components, catalytic activity and the other potential applications (Wang et al., 2010; Huang et al., 2011; Sheng et al., 2011; Kim et al., 2011), such as GS/Pt (Wu et al., 2009), GS/AuNP (Shan, et al., 2010; Dong et al., 2011; K.F. Zhou et al., 2010; Y. Zhou et al., 2010), GS/PB (Zhang et al., 2011), GS/CS (K.F. Zhou et al., 2010; Y. Zhou et al., 2010), GS/GO (Sharma et al., 2013), GS/PANI (Wang et al., 2011),GS/MWCNTs (Lu et al., 2012) etc. Chitosan (CS), as an immobilizing matrix, has become one of the most interesting sensing materials because of its excellent film-forming ability (K.F. Zhou et al., 2010; Y. Zhou et al., 2010), biocompatibility, non-toxicity, susceptibility to chemical modification, and retaining activity of immune biomolecules on the electrode surface, etc. (Kaushik, et al., 2009). In this contribution, we tried to use the MWCNTs-GS composite modified electrode, then the CS were dotted on the surface of MWCNTs-graphene composite modified electrode, which can improve the electronic transmission rate and increase the surface area to capture more primary antibodies (Ab_1) through the glutaraldehyde (GA) cross-linker (Lai, et al., 2011; Liang et al., 2012). In addition, we used HRP-Ab_2/Au/TH/MCM-41 as traces and H_2O_2 as enzyme substrates. The large specific surface area of mesoporous silica nanoparticles (MCM-41) coated with a large amount of AuNPs through thionine linking. The interlayer thionine not only had the function to connect MCM-41 and AuNPs but also was an excellent electron mediator, and the AuNPs were used to immobilize the HPR-PCT antibody, which increased the anti-PCT loading and promoted electron transfer among probe and the electrode (Ambrosi, et al., 2010; Ambrosi et al., 2007).

Herein, we designed a novel sandwich-type electrochemical immunosensor for PCT based on the advantages of GS, MWCNTs, CS, GA, MCM-41 and AuNPs in this work. The sensitivity of the sandwich-type electrochemical immunoassay was greatly amplified in this study: one is CS dotted MWCNTs-GS composite immobilized on the working electrode through the GA cross-linker to capture a large amount of primary antibodies (Ab_1) as well as improve the electronic transmission rate; the other is that a large amount of thionine and HRP-Ab_2 was introduced on the electrode surface through the unique properties of AuNPs codified mesoporous nanoparticles TH/MCM-41, providing a promising platform for the detection of high-performance electrochemical immunosensors. In addition, horseradish peroxidase(HRP) as enhancer could catalyze the oxidation reaction of thionine by H_2O_2, which results in great enhancement of the reduction peak current. The proposed immunosensor provided a useful tool for monitoring PCT, which exhibited acceptable specificity, precision, reproducibility and stability. This strategy shows potential applications in clinical immunoassay for other biomolecules (such as IL-6, TNFa, IL-1b, CRP and so on).

2. Experimental

2.1. Chemicals

Procalcitonin (PCT), procalcitonin antibody (anti-PCT), HRP-labeled procalcitonin antibody (HRP-anti-PCT) were made by our own, the synthesis methods and related articles will be published in other journals. Multi-walled carbon nanotubes (MWCNTs) were purchased from Nanoport Co. Ltd. (Shenzhen, China), The natural graphite powder (GS) was obtained from Nanjing XFNANO Materials Tech. Co. Ltd. (Nanjing, China). Chitosan (CS, 99% deacetylation), Bovine serum albumin (BSA), and Glutaraldehyde (GA, 25% aqueous solution) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, US). Chloroauric acid ($HAuCl_4$ $3H_2O$), Sodium borohydride ($NaBH_4$) were obtained from Shanghai Reagent Company (Shanghai, China). The clinical serum samples were from Military Region General Hospital of Guangzhou, China. All other reagents were of analytical grade and used without further purification.

0.1 M PBS of various pHs were prepared by mixing the stock solutions of KH_2PO_4 and K_2HPO_4, and then adjusting the pH with 0.1 M NaOH and H_3PO_4. Double distilled water was used throughout the experiment. The washing buffer was 0.1 M Tris–HCl containing 0.05% (w/v) Tween-20.

2.2. Apparatus

Electrochemical measurements of cyclic voltammetry (CV) were carried out on a Gamry 600 electrochemical workstation (US), electrochemical impedance spectroscopy (EIS) and chronoamperometry (CA) were performed on a CHI 660C electrochemical workstation (Chenhua, Shanghai, China). A three-electrode system was employed with a bare or modified GCE as the working electrode, a platinum wire (Pt) and a saturated calomel electrode (SCE) as the counter electrode and reference electrode, respectively. All of the potentials in this article were with respect to SCE. The scanning electron micrographs were taken with field emission scanning electron microscopy (FE-SEM; Zeiss Ultra55, Germany).

2.3. Acid treatment of MWCNTs

The Multi-walled carbon nanotubes(MWCNTs) were treated and followed a typical procedure: MWCNTs were first carboxyl functionalized and shortened by sonicating in a mixture of concentrated H_2SO_4 and HNO_3 (v/v, 3:1) for 6 h, and filtered, rinsed with double distilled water until the filtrate was at neutral pH, then dried under vacuum (Qiu et al., 2010; Liu and Lin, 2006; Tsang et al., 1994; Jeykumari and Narayman, 2008). The acid-treated MWCNTs were dispersed into double distilled water.