Electrochemistry Communications 31 (2013) 129–132

Contents lists available at SciVerse ScienceDirect

Electrochemistry Communications

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

A magnetic bead-based bienzymatic electrochemical immunosensor for determination of H9N2 avian influenza virus

Chuan-Hua Zhou, Yan-Min Long, Bao-Ping Qi, Dai-Wen Pang, Zhi-Ling Zhang *

Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), College of Chemistry and Molecular Sciences, State Key Laboratory of Virology, Wuhan University, Wuhan 430072, PR China
Wuhan Institute of Biotechnology, Wuhan 430075, PR China

ARTICLE INFO

Article history:
Received 28 January 2013
Received in revised form 6 March 2013
Accepted 15 March 2013
Available online 26 March 2013

Keywords:
Virus detection
Immunosensor
Magnetic bead
Electrochemical detection
Bienzymatic strategy
Layer-by-layer assembly

ABSTRACT

A novel electrochemical immunosensor based on the integration of immunomagnetic separation and bienzymatic amplification for sensitive detection of virus particles was fabricated in this work. The bienzymatic strategy was realized by using the first enzyme as tracer tagged on immunomagnetic beads which could be accumulated on the magneto controlled home-made Au electrode (m-AuE) and the second enzyme immobilized on the m-AuE by layer-by-layer (LBL) assembly technique. The proposed immunosensor not only provides a rapid, simple, cost-effective and on-site platform with high sensitivity, selectivity, and reproducibility for early diagnosis but also presents a new approach for sensitive magneto immunoassay.

1. Introduction

Viral pathogen detection techniques are always of great importance. To control the infection and spread of viral epidemics, rapid and sensitive detection techniques which are suitable for early diagnosis are urgently needed. Conventional virus diagnosis methods usually include virus isolation, serological test, polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA) and so on [1]. These methods are widely accepted and used for the clinical diagnosis, but their application is limited by the long analysis time as well as the low sensitivity or the requirement for sophisticated instruments, expensive reagents and facilities operators.

As one of the predominant analytical techniques with high sensitivity, low cost and low power requirement, electrochemical immunoassay is widely used in clinical diagnosis [2]. However, the electrochemical signals are always affected by the activity of the electrodes, thus the application in matrix samples is limited. Great efforts have been contributed to searching for simple and sensitive methods for on-site diagnoses.

Magnetic bead-based electrochemical immunoassay which combines the advantages of immunomagnetic separation and electrochemical detection, displays its unique advantages. It possesses the high sensitivity of electrochemical method. More importantly, the use of magnetic beads (MBs) as reaction carrier can not only avoid the electrode fouling but also greatly improve the performance of immunological reaction due to the unique advantages of MBs [3].

In order to improve the sensitivity, various signal amplification materials have been used, including quantum dots [2], metal nanoparticles [4] and enzymes [5]. Among them, enzyme labeling is the most commonly employed process not only for the intrinsic catalytic activity of the enzyme but also for the convenience to get catalytic signals directly on the magneto controlled electrode in magnetic-bead based electrochemical immunosensing. Moreover, the co-immobilization of two enzymes could obtain a high amplification factor of more than 3000 times [6]. Therefore, the application of multienzymatic system to improve the sensitivity of biosensor has attracted extensive attention.

Herein, the strategy of combining immunomagnetic bead, bienzymatic amplification and electrochemical detection was first proposed for high sensitive virion detection. This strategy avoided the accumulation of H_2O_2, which could cause enzyme inactivation, and allowed a low detection potential with the use of hydroquinone (HQ) as mediator. The regeneration procedure of the m-AuE was also simplified. This novel immunosensor displayed rapid detection of H9N2 virus with high sensitivity, good selectivity and could be used in complex samples. Moreover, this approach could be expanded to other multienzymatic amplification systems to construct more sensitive immunosensor for on-site medical diagnostic application.

* Corresponding author. Tel.: +86 27 68756759; fax: +86 27 68754067.
E-mail address: zlzhang@whu.edu.cn (Z.-L. Zhang).

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http://dx.doi.org/10.1016/j.elecom.2013.03.024

2. Experimental section

2.1. Chemicals and apparatus

H9N2 avian influenza virus (AIV), inactivated H5N1 AIV, newcastle disease virus (NDV), porcine pseudorabies (PRV), and baculovirus (BAV) were obtained from Wuhan Institute of Virology, Chinese Academy of Sciences. Anti-influenza A H9N2 hemagglutinin (HA) mouse monoclonal antibody (mAb) and rabbit polyclonal antibody (pAb) were purchased from Sino Biological Inc. (Beijing, China). *N*-(3-dimethylamino-propyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), horseradish peroxidase (HRP), and concanavalin A (Con A) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Superparamagnetic MBs (300 nm in diameter) was purchased from Ademtech SA (Pessac, France). Glucose oxidase-conjugated avidin D (GOD-A) was purchased from Vector (Burlingame, CA). Bovine serum albumin (BSA) was obtained from Biosharp. All other chemical reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All electrochemical measurements were performed on a CHI 660a electrochemical workstation (Shanghai, China). Platinum wire and Ag/AgCl electrode worked as counter and reference electrode, respectively. A Digital Instruments Nanoscope IV atomic force microscope (AFM) (Veeco Instruments Inc.) was used in tapping mode to measure the surface structure and roughness of the electrode.

2.2. Fabrication and modification of the m-AuE

The working electrode m-AuE was designed in our laboratory previously for electrochemical immunosensing. The electrode had an Au pattern which used as working electrode and a poly(dimethylsiloxane) (PDMS) well as shown in scheme 1B. Au electrode was modified with HRP through biospecific interaction between Con A and HRP by layer-by-layer (LBL) self-assembly technique. The first layer of Con A was covalently conjugated to the β-mercaptoethylamine modified Au electrode using glutaraldehyde as coupling agent. LBL modification procedure was according to the literature method [7] until the desired number of HRP layers (n) was obtained. Finally, the electrode was blocked with 0.1 M pH 7.4 Tris–HCl buffer containing 1% BSA and 1% glucose solution. The HRP covalently modified electrode Au/HRP was prepared as control.

2.3. The immunoreaction and sensing procedure

The detection protocol based on a sandwich immunoassay on MBs was illustrated in Fig. 1A. First of all, MBs were conjugated with mAb according to our previous report [8]. The immunoreaction was performed in 1.5 mL centrifuge tube for three steps which was similar to our previous report [9] except GOD-A was used as the tracer. Then immunocomplex-coated magnetic beads (IMBs) were accumulated on the m-AuE with an external magnet underneath for electrochemical measurement as shown in Fig. 1B. A 0.1 M pH 6.1 phosphate buffer solution (PBS) containing 16 mM glucose as substrate and 40 mM HQ as mediator was used as detection solution.

3. Results and discussion

3.1. Characterization of the as prepared LBL electrodes

With LBL self-assembly technique, a uniform and stable multilayer HRP modified electrode with high enzyme activity and loading capacity was fabricated. It could be clearly seen from Fig. 2A that the electrodes constructed based on Con A-HRP biospecific interaction displayed more excellent catalytic properties compared with the HRP covalently modified electrode and the catalytic signals of 100 μM H_2O_2 increased with increasing number of the bilayer (Con A/HRP). The catalytic

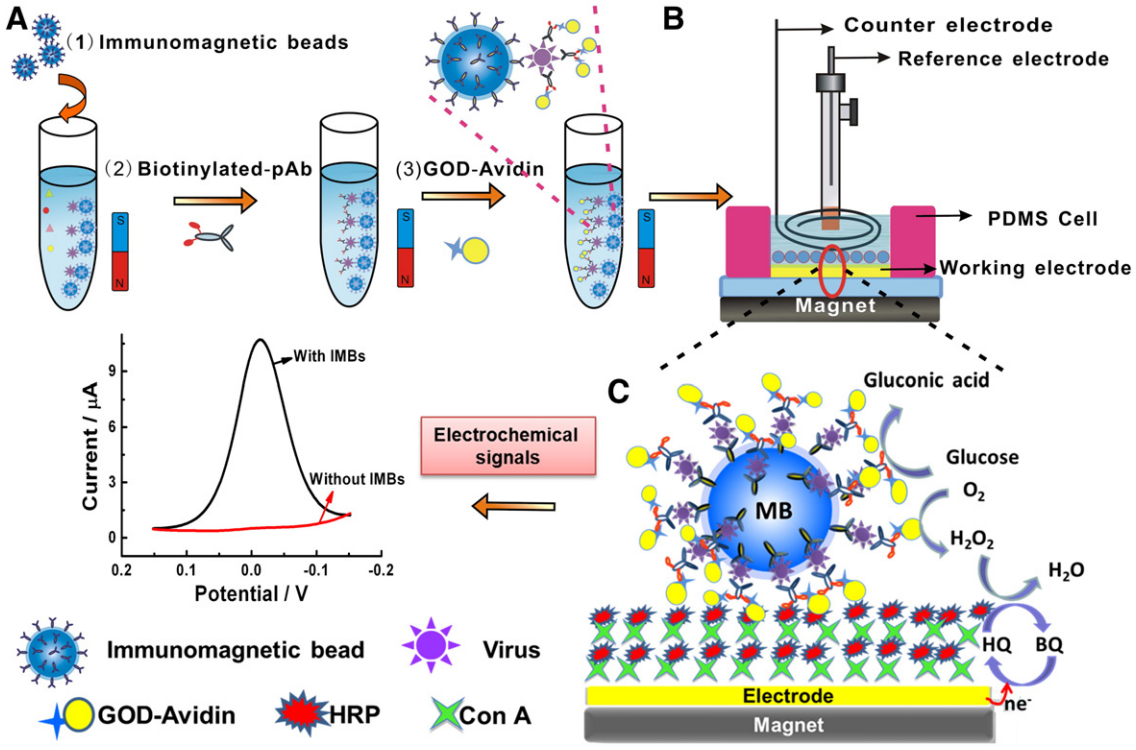

Fig. 1. Schematic illustration of the magnetic bead-based bienzymatic electrochemical immunosensor: (A) immunoreaction strategy in a centrifuge tube; (B) electrochemical detection on a m-AuE; (C) chemical reactions occurring at the multilayer HRP modified m-AuE surface in the presence of detection solution.