Biosensors and Bioelectronics 26 (2011) 4779–4784

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Gold nanoparticles-coated magnetic microspheres as affinity matrix for detection of hemoglobin A1c in blood by microfluidic immunoassay

Shao-Peng Chen, Xiao-Dong Yu, Jing-Juan Xu*, Hong-Yuan Chen

Key Laboratory of Analytical Chemistry for Life Science, Ministry of Education of China, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China

ARTICLE INFO

Article history:
Received 31 March 2011
Received in revised form 17 May 2011
Accepted 7 June 2011
Available online 12 June 2011

Keywords:
Hemoglobin A1c (HbA1c)
Immunoassay
Magnetic particles
Microchip

ABSTRACT

A novel microfluidic immunoassay system for specific detection of hemoglobin A1c (HbA1c) was developed based on a three-component shell/shell/core structured magnetic nanocomposite Au/chitosan/Fe_3O_4, which was synthesized with easy handling feature of Fe_3O_4 by magnet, high affinity for gold nanoparticles of chitosan and good immobilization ability for anti-human hemoglobin-A1c antibody (HbA1c mAb) of assembled colloidal gold nanoparticles. The resulting HbA1c mAb/Au/chitosan/Fe_3O_4 magnetic nanoparticles were then introduced into microfluidic devices coupled with a gold nanoband microelectrode as electrochemical detector. After that, three-step rapid immunoreactions were carried out in the sequence of HbA1c, anti-human hemoglobin antibodies (Hb mAb) and the secondary alkaline phosphatase (AP)-conjugated antibody within 20 min. The current response of 1-naphtol obtained from the reaction between the secondary AP-conjugated antibody and 1-naphthyl phosphate (1-NP) increased proportionally to the HbA1c concentration. Under optimized electrophoresis and detection conditions, HbA1c responded linearly in the concentration of 0.05–1.5 $\mu g\,mL^{-1}$, with the detection limit of 0.025 $\mu g\,mL^{-1}$. This system was successfully employed for detection of HbA1c in blood with good accuracy and renewable ability. The proposed method proved its potential use in clinical immunoassay of HbA1c.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Hemoglobin-A1c (HbA1c), which is a stable minor hemoglobin derivative formed by a non-enzymatic reaction of glucose with the amino-terminal valine of hemoglobin (Hb) β-chains (Bunn et al., 1976) and presents the long-term average blood glucose level (Nathan et al., 2007), has become an established procedure in the management of diabetes mellitus in both diagnosis and monitoring (Goodall, 2005; Koenig et al., 1976; Sacks et al., 2002). Recently, many techniques based on HbA1c affinity matrices such as ion-exchange chromatography (IEC), boronate affinity chromatography (AC), electrophoresis (EP) or immunochemical methods (IM) (Goldstein et al., 1986; John, 1997) have been used to determine HbA1c, most of which require a separation step to get rid of Hb variants or glucose prior to a specific HbA1c detection step. Besides, most of their HbA1c affinity matrices cannot be renewed because they are fixed onto the device. Therefore, the construction of immunsensor with a matrix which can not only sensitively recognize, selectively immobilize HbA1c and efficiently maintain their functionality but also be renewed without destroying the interface between the matrix and the device remains a challenging task.

Recently, magnetic beads have attracted considerable attention due to their easy handling by using magnets or magnetic coils (G.D. Liu et al., 2004; Miao and Bard, 2004; Fan et al., 2005; Zacco et al., 2006). Hence, considerable efforts have been made to prepare magnetic beads and the magnetic beads-based materials have been exploited in a number of applications such as biomedical diagnosis (Josephson et al., 1999), concentration and separation of trace amounts of specific targets, information storage (Chakraborty, 1999), color imaging, microwave absorption (Pinho et al., 2001), cell separation (Sieben et al., 2001), and signal amplification. However, naked magnetic beads such as Fe_3O_4 nanoparticles are very sensitive to oxidation for their high chemical reactivities and prone to aggregate for their large ratio of surface area to volume, which result in poor magnetism and dispersibility (Lu et al., 2006, 2007; Zhao et al., 2006; Deng et al., 2003) and limit their further applications. Protecting naked magnetic beads with shells has been proved to be one of the approaches to overcome these limitation (Zhao et al., 2005, 2006; Deng et al., 2003; Lu et al., 2006; Lin et al., 2003; Qiu et al., 2006; Jeong et al., 2007; Yang et al., 2007; Park et al., 2007; Wang and Zhong, 2005; Lyon et al., 2004; Xu et al., 2007).

Chitosan, which is a kind of attractive biocompatible matrix for the immobilization of various biomolecules due to its excellent membrane-forming ability, good biocompatibility, low cost, nontoxicity, high mechanical strength and hydrophilicity (Krajewska, 2005; Betigeri and Neau, 2002), can be used to protect naked mag-

* Corresponding author.
E-mail address: xujj@nju.edu.cn (J.-J. Xu).

0956-5663/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2011.06.001

netic beads. The protective chitosan layer can efficiently prevent the magnetic beads from aggregation. Particularly, its positively charged nature has a high affinity for adsorption of nanoparticles (Huang and Yang, 2003) such as colloidal gold, which can provide an efficient scaffold of nanoscale with a large variety of nanoparticles and provide a useful platform for further functionalization. Gold nanoparticles (GNPs) have shown widespread use in fundamental research, particularly in biological and sensing applications (Du et al., 2005; Danniel and Astruc, 2004; Hone et al., 2002; Cao et al., 2002). Their good interface-dominated properties offer a friendly environment to immobilize protein molecules. Therefore, GNPs have been exploited in protein immobilization such as Hb (Gu et al., 2001, 2002), HRP (Jia et al., 2002), MP-11 (Patolsky et al., 1999), tyrosinase (Z.M. Liu et al., 2004), and oxidase (Kang et al., 2007).

Here, combining easy handling feature of Fe_3O_4 as magnetic beads, high affinity for colloidal gold nanoparticles of chitosan and protein immobilization ability of gold nanoparticles, we prepared a new kind of three-component shell/shell/core structure magnetic nanocomposite Au/chitosan/Fe_3O_4, which was then used as a nanoscale anchorage substrate to immobilize HbA1c mAb. The assembled colloidal gold nanoparticles on chitosan provide an excellent interface with good biocompatibility and show good immobilization ability for HbA1c mAb. The presence of chitosan improves the stability and biocompatibility of GNPs. The resulting HbA1c mAb/Au/chitosan/Fe_3O_4 magnetic nanoparticles were introduced into microfluidic devices for process controlled and low-volume immunoassay system for HbA1c. By means of an external magnetic field, the magnetic nanoparticles can be easily immobilized on the devices and renewed. Furthermore, the HbA1c mAb/Au/chitosan/Fe_3O_4 magnetic nanoparticles can sensitively recognize and selectively immobilize HbA1c and present excellent analytical performance for HbA1c detection. The proposed immunoassay system based on Au/chitosan/Fe_3O_4 magnetic nanoparticles possess potential applications in clinical diagnosis and therapy of diabetes mellitus.

2. Experimental

2.1. Reagents

Hemoglobin-A1c (HbA1c), anti-human hemoglobin antibodies (Hb mAb, H4890), the secondary alkaline phosphatase (AP)-conjugated goat anti-rabbit antibody (0.1 mg) and chitosan (Chit, from crab shells, minimum 85% deacetylated) and poly(ethylene glycol)-*block*-poly(propylene glycol)-*block*-poly(ethylene glycol) (P123) were purchased from Sigma–Aldrich. Anti-human hemoglobin-A1c antibodies (HbA1c mAb, AB31152) were purchased from Abcam. 1-Naphthyl phosphate (1-NP) was the product of the Acros. Iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), sodium acetate (NaAc), ethylene glycol (EG), sodium dodecylbenzenesulfonate-6 (NaDBS), and hydrogen tetrachloroaurate hydrate ($HAuCl_4{\cdot}4H_2O$), Span-80 were purchased from Shanghai Chemical Reagent Co. Ltd. (China). Reagents were used as received without further purification. A phosphate buffer solution (PBS) (20 mM, pH 7.4) was prepared by mixing solutions of Na_2HPO_4 and NaH_2PO_4. Other chemicals were of analytical grade. All solutions were prepared with twice distilled water.

2.2. Preparation of Au/chitosan/Fe_3O_4 nanocomposites

The entire preparation procedure involves three major steps. The first step involved magnetic microspheres being synthesized using a microwave-assisted solvothermal reaction (Zhang et al., 2008). In the second step, the chitosan particles were employed for coating the magnetic microspheres to form a shell–core structure via a suspension cross-linking method (Li et al., 2008). After that, chitosan/Fe_3O_4 solution was added to the Au colloid under stirring leading to formation of Au/chitosan/Fe_3O_4 nanocomposites (third step). A detailed description of these steps is summarized in Supporting information section (SI1).

2.2.1. Characterization of Au/chitosan/Fe_3O_4 composite nanoparticles

Particle size and morphology of Fe_3O_4, chitosan/Fe_3O_4 and Au/chitosan/Fe_3O_4 nanoparticles were studied on FEI Tecnai-12 transmission electron microscope using an accelerating voltage of 120 kV. The magnetic property of Au/chitosan/Fe_3O_4 was measured by a vibration sample magnetometer (VSM, Lakeshore, Model 7300). X-ray diffraction (XRD) analyses of Fe_3O_4, chitosan/Fe_3O_4 and Au/chitosan/Fe_3O_4 were carried out on a Philips X' pert Pro X-ray diffractometer (Cu Kα radiation, $\lambda = 0.15418$ nm).

2.3. Immunoassay arrangement and electrophoresis procedures

2.3.1. Temperature control device

To keep a constant temperature at the reaction areas on the microchip, we developed a temperature control device (Chen et al., 2010). The device was designed and fabricated to keep in contact with the bottom plate of the microchip and control the temperature of this area individually. Both of two Ni/Cd heater bands (I: 5 mm × 3 cm, II: 5 mm × 4.5 cm) were arranged above the heat sink ca. 1 cm. The thermistor glued to the middle of the heater bands with thermally conductive adhesive was connected to temperature controllers. Heater band II was used in this paper.

2.3.2. Fabrication and integration of the microchip device

Standard photolithographic and wet chemical etching techniques were used for fabricating channels onto a 1.7-mm-thick 6 × 9 mm glass plate with chromium and photoresist coating (Shaoguang Microelectronics Corp., Changsha, China). Fig. 1A shows the layout of the microfluidic chip fabricated in this work. It consists of seven inlet channels (with 120 μm wide and 10 μm high). The chip with this design was fabricated using a procedure detailed elsewhere (Jia et al., 2004), which was also provided in Supporting information section (SI2). This microchip was then integrated with the gold nanoband microelectrode as the end-column mode for the following experiments (Fig. 1A). The fabrication of gold nanoband microelectrode was provided in the Supporting information section (SI2). The alignment between the outlet of electrophoresis microchannel and the electrode was set to be 40 μm under an optical microscope. In order to promise the good alignment between the outlet of microchannels and the nanoband working electrode, the thickness of PDMS plate containing the gold film applied for working electrode was set to be 1.7 mm, as the same as the glass plate.

2.3.3. Electrophoresis procedure

Conjugation of HbA1c mAb to Au/chitosan/Fe_3O_4 nanoparticles was performed using the following protocol. 20 μL of HbA1c mAb was added into 1.5 mg of Au/chitosan/Fe_3O_4 nanoparticles for linking during 30 min on a rotator. After washing three times with 20 mM PBS (pH = 7.4), 1 mL of 1 mg mL^{-1} BSA was added to block nonspecific adsorption sites on the surface of magnetic beads for 30 min. The final product was stored in 20 mM PBS (pH = 7.4) with 0.02% sodium azide at 4 °C.

Before electrophoresis, the channels of the microchip were sequentially rinsed with water for 10 min and finally running buffer (20 mM PBS, pH = 7.4) for 30 min. 5 μg magnetic nanoparticles coated with HbA1c mAb suspended in 1 mL of running buffer were sonicated for 10 min, then were injected into the channel in the

Download English Version:

https://daneshyari.com/en/article/867996

Download Persian Version:

https://daneshyari.com/article/867996

Daneshyari.com