Biosensors and Bioelectronics 24 (2009) 1213–1218

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Detection of cadmium by a fiber-optic biosensor based on localized surface plasmon resonance

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ARTICLE INFO

Article history:
Received 13 April 2008
Received in revised form 7 July 2008
Accepted 8 July 2008
Available online 18 July 2008

Keywords:
Localized surface plasmon resonance
Biosensor
Gold nanoparticle
Cadmium
Phytochelatin

ABSTRACT

A novel transmission-based localized surface plasmon resonance (LSPR) fiber-optic probe has been developed to determine the heavy metal cadmium ion (Cd(II)) concentration. The LSPR sensor was constructed by immobilizing phytochelatins (PCs), (γGlc-Cys)$_8$-Gly, onto gold nanoparticle-modified optical fiber (NM_{Au}OF). The optimal immobilizing conditions of PCs on to the NM_{Au}OF are 71.6 μg/ml PCs in pH 7.4 PBS for 2 h. The absorbability (change of light absorption) of the PC-functionalized NM_{Au}OF sensor increases to 9% upon changing the Cd(II) level from 1 to 8 ppb with a sensitivity of 1.24 ppb^{-1} and a detection limit of 0.16 ppb. The sensor retained 85% of its original activity after nine cycles of deactivation and reactivations. In addition, the sensor retains its activity and gives reproducible results after storage in 5% D-(+)-trehalose dehydrate solution at 4 °C for 35 days. The dissociation constant (K_d) of the immobilized PCs with Cd(II) was about 6.77×10^{-8} M. In conclusion, the PCs-functionalized NM_{Au}OF sensor can be used to determine the concentration of Cd(II) with high sensitivity.

1. Introduction

Detection and quantitation of toxic heavy metals is an important issue with the increasing environmental concern about drinking water safety. Traditionally, atomic absorption (AA) spectroscopy, inductively coupled plasma (ICP) optical emission spectrometry, and inductively coupled plasma mass spectrometry (ICPMS) are widely used methods for the determination of heavy metals (Anderson et al., 1996; Burlingame et al., 1996; Jackson and Chen, 1996). These methods are sensitive and allow for the discrimination of different metal ions but require tedious sample pretreatments (e.g., desalting, filtration and concentration). Therefore, it is essential to develop a rapid and sensitive screening method to ascertain the presence of metal ions for in situ environmental monitoring.

A lot of efforts have been devoted to develop inexpensive enzymatic or peptide methods for the detection of heavy metals for industrial processes and environmental monitoring. The enzyme or peptide may be immobilized on a suitable surface or supporter, and its binding or inhibition can be measured by different detection techniques such as ion-sensitive field effect transistors, potentiometric and piezoelectric devices, amperometric electrodes, and optical-based biosensors (Bontidean et al., 1998, 2003; Saber and Piskin, 2003; Lee and Russell, 2003; Blake et al., 1998). In this study, cadmium ion (Cd(II)) has been chosen as the target analyte, since it is representative of a ubiquitous environmental pollutant. Phytochelatins (PCs) as the heavy metal ion binding peptides consist of L-glutamic acid, L-cysteine and a carboxy-terminal glycine. These compounds, occurring in plants and some fungi with the general structure (γ-Glu-Cys)$_n$-Gly (n = 2–11), are metallothionein-like substances (Grill et al., 1985, 1986; Rauser, 1995). They are capable of chelating Cd(II) by thiolate coordination because of induction by the addition of Cd(II), high capacities of Cd-binding, and a high content of Cys-residues (Kondo et al., 1984). Since it is tedious and time consuming to isolate and purify PCs from plants or organisms, PCs can be massively produced up to 26 mg/l by using recombinant DNA and fermentation technologies.

Recently, a novel fiber-based biosensor based on the particular optic properties of gold nanoparticles has been developed (Lin et al., 2006; Chau et al., 2006). When an optical field is incident upon noble metal nanoparticles, absorption occurs if the optical frequency is resonant with the collective oscillation of the conduction electrons, which is known as the localized surface plasmon resonance (LSPR), which is absent in the bulk metal. The local environment of the metal nanoparticles has a significant influence on the resonance frequency and absorption of LSPR (Jensen et al., 1999; Nath and Chilkoti, 2002). The light absorption level by gold nanoparticles is sensitive to the refractive index (RI) of the surrounding solvent and, in addition, to the binding events of those functionalized nanoparticles. With a suitable receptor

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doi:10.1016/j.bios.2008.07.013

immobilized at the surface of the gold nanoparticles, the resulting LSPR fiber-optic sensor can detect the corresponding analyte even if there is no emission and no absorption in the UV–vis region. Therefore, the presence of analytes can be determined directly without the use of labeled molecules (Lin et al., 2006; Nath and Chilkoti, 2002).

For this study, a PCs-functionalized fiber-based biosensor utilizing the LSPR effect was developed to evaluate the concentration of Cd(II). First, a layer of the gold nanoparticle was immobilized on the outside of the fiber to couple with the evanescent wave. By using a self-assembling technique, a bioactive layer consisting of genetically synthesized PCs was immobilized by covalent coupling onto the gold nanoparticle layer and the optimal conditions of immobilization were examined. When the PCs binding with Cd(II) takes places, the local refractive index was altered and hence the transmission. Based on the correlation between binding rate and light attenuation, the concentration of Cd(II) can be determined. Several factors, including reactive rate, stability, and binding constant, were investigated as well.

2. Materials and methods

2.1. Reagents and materials

Multimode plastic-clad silica optical fiber (0.37 NA, model F-MBC) was purchased from Newport (Irvine, CA) with core and cladding diameters of 400 and 430 μm, respectively. Phytochelatins ($(\gamma$Glc-Cys$)_8$-Gly) were provided by the Development Center for Biotechnology, Taipei, Taiwan. The following chemicals, *n*-hexadecyltrimethylammonium bromide (CTAB, Fluka), sodium borohydride (Lancaster), 3-(mercaptopropyl)-trimethoxysilane (MPTMS, Acros), cystamine dihydrochloride (Sigma), phosphate buffered saline (PBS, Sigma), *N*-(3-dimethylaminopropy)-*N*-ethylcarbodiimide hydrochloride (EDC, Sigma), dithiothreitol (DTT, Sigma), *N*-hydroxysuccinimide (NHS, Sigma), D-(+)-trehalose dehydrate (Sigma), were used as received. All aqueous solutions were prepared with water purified using YMDI-100 water purification unit (Yeameei Membrane) with a specific resistance of 18 MΩ cm.

2.2. Production of PCs

The cells of *Escherichia coli* (*E. coli*) Top10 and BL21x transfected by vectors pCR2.1-TOPO and pTrcHis containing the PCs were cultured at 37 °C in 1.5 l of 2YT medium (10 g/l yeast extract, 16 g/l tryptone and 5 g/l NaCl) with ampicillin (100 μg/ml). When the absorbance (OD_{600}) reached 0.5, 2.5 ml IPTG (isopropyl-β-D-thiogalactopyranoside) (100 μg/ml) was added into the culture medium. After 16 h of protein induction, 20 μg/ml Cd(II) was added after an overnight growth period in 2YT medium. Then, the cells were lysed to break the cell walls using lysozyme (1 mg/ml) and ultrasonic probe. The lysed cells were centrifuged at 4000 × *g* at 4 °C for 30 min to obtain a clarified supernatant for immobilized metal-chelated affinity chromatography. The desired peptide (i.e., PCs) was isolated by Ni-NTA column metal-chelated affinity chromatography techniques. The purity of the peptide was monitored by sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using the Mini-Protean II apparatus and a Tris-glycine buffer system was used to monitor purification during chromatography. The gels were run under reducing conditions with heat treatment of the samples (95 °C, 5 min) and electrophoresis was performed for 45 min at 200 V using 12% polyacrylamide gels. Detection was performed with Coomassie brilliant blue R250 staining.

2.3. Preparation of gold nanoparticle-modified optical fiber

First, colloidal Au solution was prepared by mixing hydrogen tetrachloraurate (1.78 ml, 25.4 nM), 8.22 ml of chloroform, and 0.4 ml of a 0.02 M ethanol solution of CTAB for 10 min to form a 4.52×10^{-4} M hydrogen tetrachloroaurate solution. Freshly prepared $NaBH_4$ ethanol solution (0.8 ml, 0.15 M) was added to the hydrogen tetrachloroaurate solution with vigorous stirring for 30 min. Histograms derived from TEM image analysis showed that the mean diameter of Au nanoparticles was 9.6 ± 2.3 nm. Then, the unclad portion (5 cm) of the optical fibers was cleaned for 30 min in a bath consisting of 3 vol of 30% H_2O_2 and 7 vol of concentrated H_2SO_4. The clean unclad portions of the optical fibers were then submerged into vials of 1% solution of MPTMS in toluene. After 8 h, the optical fibers were rinsed with methanol to remove unbound monomers from the surface. After thorough rinsing, the unclad portions of the optical fibers were immersed in Au solution for 5 h to form a self-assembled gold nanoparticle monolayer (NM_{Au}) on the core surface. Subsequently, the modified optical fibers were rinsed sequentially with water, methanol, and chloroform. The AFM surface topography of the prepared gold nanoparticle-modified optical fiber is shown in Fig. 1(a).

2.4. Immobilization of PCs onto NM_{Au}

First, NM_{Au} was modified to form a self-assembled monolayer (SAM) of cystamine by immersing NM_{Au} in 0.02 M cystamine dihydrochloride (pH 7.4 in PBS) for 2 h to form an amine functional group. The cystamine-modified NM_{Au} was further immersed in a PBS solution containing 450 mM EDC, 90 mM NHS, and various concentrations of PCs for 2 h at room temperature, rinsed with PBS, and air-dried at room temperature. Then, the amine group of cystamine modified on the NM_{Au} can couple with the activated succinimide esters reacting from PC and NHS, and finally form an amino bond between cystamine and PC, as shown in Fig. 1(b). The concentration of PCs, pH of the solution, and incubation time were varied to find the optimal immobilization conditions. Prior to the following tests, the PCs-immobilized LSPR sensors were activated by 2% DTT for 20 min, and rinsed with DI water. Fig. 1(a) shows an AFM surface topography of PCs-modified NM_{Au} probe. The 4-μm^2 image contained a random distribution of PCs on the surface with 13 nm roughness. The morphology clearly indicates that the PCs immobilizing treatments were successfully performed on the surface of NM_{Au}.

2.5. Cd(II) detection by PCs-modified sensors

In the LSPR sensors, the light attenuation will be affected upon capturing or reaction of analyte molecules by a molecular recognition element immobilized on the sensor surface. For this case, the reaction was carried out in a standard solution. When Cd(II) binds with PCs, the decrease in light intensity is correlated to the concentration of Cd(II). The degree of absorbability can be calculated according to the following formula:

$$\text{absorbability}\,(\%) = \frac{I_0 - I_1}{I_0} \times 100 \quad (1)$$

where I_0 and I_1 are the average light intensity measured at the initial PCs activity without and with Cd(II), respectively. According to World Health Organization (WHO), the Cd(II) concentration in drinking water should be less than 5 ppb. In this study, the detection range of Cd(II) is chosen from 0 to 8 ppb in 0.02 M Tris buffer solution (pH 6.5).