Biosensors and Bioelectronics 29 (2011) 210–214

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Short communication

Field-effect amperometric immuno-detection of protein biomarker

Jiapeng Wang[a], Siu-Tung Yau[a,b,*]

[a] Department of Electrical and Computer Engineering, Cleveland State University, Cleveland, OH 44115, USA
[b] The Applied Bioengineering Program, Cleveland State University, Cleveland, OH 44115, USA

ARTICLE INFO

Article history:
Received 10 July 2011
Accepted 28 July 2011
Available online 4 August 2011

Keywords:
Biosensor
Immunosensor
Field-effect enzymatic detection
Biomarker detection

ABSTRACT

The field-effect enzymatic detection technique has been applied to the amperometric immunoassay of the cancer biomarker, carcinoma antigen 125 (CA 125). The detection adopted a reagentless approach, in which the analyte, CA 125, was immobilized on the detecting electrode, which was modified using carbon nanotubes, and the detection signal was obtained by measuring the reduction peak current of the enzyme that was used to label the antibody. A gating voltage was applied to the detecting electrode, inducing increase in the signal current and therefore providing amplification of the detection signal. The voltage-controlled signal amplification of the detection system has increased the sensitivity and lowered the detection limit of the system. A detection limit of 0.9 U/ml was obtained in the work.

1. Introduction

Biomarkers used for clinical testing of certain types of cancer are specific proteins such as prostate-specific antigen (PSA) (for prostate cancer), carcinoma antigen 125 (CA 125) (for ovarian cancer), cancer antigen 15-3 (CA 15-3) (for breast cancer), and carcinoembryonic antigen (CEA) (for colon cancer). Development of sensitive detection techniques for the detection of low-concentration cancer biomarkers present in the early stage of the disease is crucial for the outcome of the treatment of the disease (Rasooly and Jacobson, 2006; Warsinke, 2009). Electrochemical immunosensors appear to be a suitable platform for establishing biomarker detection techniques (Wang, 2006). In particular, since they allow fast real-time measurements and device miniaturization, electrochemical immunosensors are potentially suitable for the point-of-care approach to biomarker assay (Warsinke, 2009).

Recent development in the detection of biomarkers includes several noted works. In a bio-barcode assay for PSA (Thaxton et al., 2009), gold nanoparticles, functionalized with DNA and PSA-specific antibodies, were used to interact through immuno-reaction with magnetic microparticles that had been first functionalized with PSA-specific antibodies and subsequently mixed with the serum sample containing the PSA target. After magnetic separation, the PSA-specific DNA barcodes were released into a solution and detected using scanometric assay. The detection limit of this bio-barcode PSA assay was 330 fg/mL serum. An amperometric immunosensor for the simultaneous detection of two cancer biomarkers has been constructed on two iridium oxide electrodes (1-mm diameter) patterned on a glass substrate (Wilson, 2005). Capture antibodies were immobilized on the porous iridium oxide electrodes by covalent attachment using (3-aminopropyl) triethoxysilane and glutaraldehyde. The spatial separation of the electrodes (2.5 mm) enabled simultaneous electrochemical immunoassays of CEA and α-fetoprotein to be conducted without cross-talk between the electrodes. The biomarkers were measured using electrochemical ELISA, and detection was achieved by electrochemically oxidizing alkaline phosphatase-generated hydroquinone. The sensor had detection limits of 1.2, and 1 ng/mL for CEA and α-fetoprotein, respectively. A novel CA 125 immunosensor was prepared by co-immobilization of CA 125 and thionine, which was used as an electron transfer mediator, on a modified carbon electrode (Wu et al., 2007). Antibodies labeled with horseradish peroxidase (HRP) were used to bind CA 125 via immuno-conjugation. Hydrogen peroxide (H_2O_2), the substrate of HRP, was released to the sensor, and CA 125 was detected by measuring the electrode current produced due to the HRP-catalyzed reduction of H_2O_2. The sensor showed a linear range of 2–75 U/ml and a detection limit of 1.8 U/ml.

Recently, a novel field-effect enzymatic detection (FEED) technique, in which an external gating voltage was used to induce an electric field at the enzyme–electrode interface to amplify the signal current of an enzyme-based biosensor, has been developed (Choi and Yau, 2009). This technique was used to lower the detection limit of molecular analytes from the milli-molar (10^{-3} M) level obtained in the absence of the gating voltage down to the pico-molar (10^{-12} M) level achieved when the gating voltage was

* Corresponding author at: Department of Electrical and Computer Engineering, Cleveland State University, Cleveland, OH 44115, USA. Tel.: +1 216 875 9783; fax: +1 216 687 5405.
E-mail address: s.yau@csuohio.edu (S.-T. Yau).

0956-5663/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2011.07.072

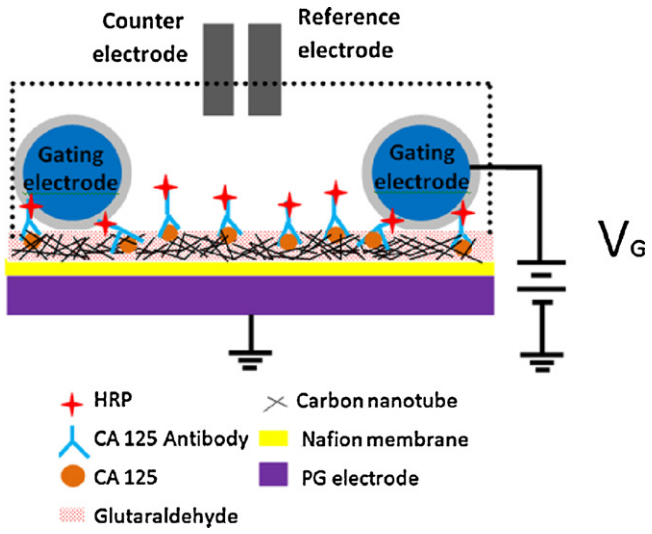

Fig. 1. A cross-sectional view of the detection system. The PG electrode with the multilayers of materials deposited on it depicts the structure of the detecting electrode. The gating electrodes are represented by the circular structures, which consist of a copper wire (the blue circles) and a thin layer of insulator (the shaded shells made of enamel). The gating voltage V_G is used to induce an electric field at the interface between the sample solution and the detecting electrode. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

applied. Compared to the conventional field-effect-transistor (FET) biosensor, which employs the FET to provide a reference current, the FEED technique features a voltage-controlled amplification of the signal current. The signal current is produced by quantum tunneling of electrons, which depends critically on the distance electrons travel by means of tunneling and the potential energy profile of the tunnel structure. The FEED technique allows the energy profile of the tunnel structure to be changed using the gating voltage to increase the electron transfer rate. Therefore, the main advantage of the new technique is the voltage-controlled amplification of the signal current. In the work reported here, the FEED technique was applied to the enzyme-linked amperometric immuno-sensing scheme and the feasibility of using this new detection approach as a biomarker detection technique was demonstrated by the detection of the biomarker CA 125, using electrodes modified with carbon nanotube. The incorporation of the two detection methods has resulted in a reagentless assay approach (Wang, 2005; Weiss et al., 2007), another advantage of the reported technique, which does not use electron transfer mediators and the substrate of the enzyme used to label the secondary antibody. As the result of the signal amplification, the detection limit of the detection system was lowered from 4.9 U/ml to 0.9 U/ml.

2. Materials and methods

Fig. 1 shows a cross-sectional view of the detection system used in the experiment. The detection system consists of a conventional three-electrode (counter electrode, reference electrode and working/detecting electrode) electrochemical cell modified with additional gating electrodes for applying a gating voltage V_G to the detecting (working) electrode. A piece of 0.5 mm-diameter copper wire coated with a thin layer of insulator (enamel) was bent to form a U-shaped structure and was attached on the detecting electrode close to the exposed area using nonconductive epoxy as the gating electrode. A variable dc power supply was connected between the gating electrode and the detecting electrode to provide the gating voltage V_G. The principle of the field-effect enzymatic detector is described previously (Choi and Yau, 2009). Disks of pyrolytic graphite (PG) (1 cm × 1 cm) were used as the basic electrode. The PG electrode was modified first with a thin layer of Nafion (Nafion 117, Fluka). The Nafion membrane was used to enhance the binding of the nanotube layer to the PG electrode. Single-walled carbon nanotubes (Carbon Solutions) were dissolved in dimethylformamide. The nanotube solution was then mixed with 1% glutaraldehyde solution (Sigma–Aldrich) and a drop of the mixture was placed on the Nafion layer to host the biomarker. The preparation of nanotube solution and its handling were carried out in a chemical hood in order to eliminate exposure to aerosol release. A mask was used to expose a 0.5 mm × 0.5 mm area of the modified PG electrode. An 8 μl drop of a solution containing CA-125 (CA-125 ELISA, Signosis), the analyte, was deposited on the exposed area and the electrode was incubated for 22 h, after which the electrode was rinsed with de-ionized water. The detecting electrode was formed by placing a 25 μl drop of solution containing 1 ng/ml of antibody labeled with HRP (CA-125 ELISA, Signosis) on the exposed area and incubating the electrode for 45 min. The same antibody concentration (1 ng/ml) was used for the incubation of all electrodes. The CA 125 antibody was monoclonal and has an affinity constant of $1.0 \times 10^9\ M^{-1}$ (Boerman et al., 1990). This protein immobilization method has been used to immobilized other biomarkers (Bollo et al., 2007; Yu et al., 2005). The antibody was bound to CA-125 due to immuno-reaction. In Fig. 1, the PG electrode with the multilayers of materials deposited on it depicts the structure of the detecting electrode. The advantage of immobilizing the antigen on the electrode without using the primary antibody is that the passage for electron transfer from the enzyme used to label the secondary antibody to the electrode is shortened and therefore signal current is increased. Note that without nanotubes the signal would become weak. This is likely to be due to the better electrical conductivity provided by the nanotubes and the fact that CA 125 was immobilized on the electrode by being entrapped in the nanotubes.

Voltammetric (cyclic and linear) measurements were made to obtain the detection signal. A commercial Ag/AgCl (3 M KCl) electrode was used as the reference electrode, and a platinum wire was used as the counter electrode. The volume of the electrochemical cell was 1 ml. The cell was driven by a commercial electrochemical controller (CHI 660C Work Station). A potential scan rate of 50 mV/s was used in recording voltammograms. Phosphate buffer solution (PBS, 0.1 M at pH 7) was prepared using de-ionized water (18.2 MΩ-cm). Commercially available hydrogen peroxide (Fisher scientific, 30% concentration) was diluted to the concentrations used in the experiment and was stored in plastic containers. All measurements were made with deaerated PBS at room temperature. Reproducible results were obtained by repeating each measurement multiple times.

3. Results and discussion

In carrying out the detection, control experiments have been performed repeatedly using different types of electrode in order to avoid possible misinterpretation of the measurement results. Fig. S1(a) shows several cyclic voltammograms (CVs), which are used to validate the detection signal of CA-125. The CVs were obtained in PBS under different electrode conditions. CV1 was obtained with a modified PG electrode, upon which CA-125 (200 U/ml) was immobilized. The CA-125-immobilized electrode was then incubated with the HRP-labeled antibody and then rinsed with de-ionized water. The electrode now contained the antigen–antibody immuno-structure on its surface and it is referred to as the detecting electrode. CV2 was obtained with the detecting electrode. CV1 is featureless while CV2 shows an increased charging current and a pair of redox peaks with a formal potential of −0.42 V and a peak-to-peak separation of 20 mV. Previous works on the