Sensors and Actuators B 177 (2013) 172–177

Contents lists available at SciVerse ScienceDirect

Sensors and Actuators B: Chemical

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

Ultrasensitive and label-free detection of annexin A3 based on quartz crystal microbalance

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ARTICLE INFO

Article history:
Received 13 July 2012
Received in revised form 21 October 2012
Accepted 26 October 2012
Available online 3 November 2012

Keywords:
Label-free nanoimmunosensor
Annexin A3 (ANXA3)
Lung cancer
Prostate cancer
Quartz crystal microbalance (QCM)

ABSTRACT

Cadmium sulfide (CdS) quantum dots (QDs) were coupled to an Au nanoimmunosensor on a quartz crystal microbalance (QCM) to form the basis of an ultrasensitive and label-free sensor of annexin A3 (ANXA3), a lung and prostate cancer biomarker protein. Polyclonal anti-ANXA3 antibody was covalently immobilized on the CdS QDs, which had previously been functionalized with carboxyl groups and bound to a cystamine self-assembled monolayer on the Au/QCM. Frequency changes induced by the binding of ANXA3 to the anti-ANXA3 on the probe's surface allowed the very sensitive detection of GST-ANXA3 with a detection limit of 0.075 ± 0.01 ng/mL. The sensor could detect ANXA3 at 0.1 ng/mL in spiked human blood and urine samples in less than 15 min without any interference from other proteins.

1. Introduction

The early diagnosis of cancer is crucial for patient survival [1]. In most cases, early detection (stage I) is associated with over 90% five-year survival rates [2]. Lung cancer is the leading cause of cancer death in many countries [3]. Prostate cancer (PCa) is the second leading cause of male cancer-related death; it affects 1 in 9 males over 65 [4]. Lung adenocarcinomas (AdCs) are one of the eight major groups of lung cancer recognized by the World Health Organization (WHO) in 2004 [5]. AdC and PCa are showing increasing rates of recurrence and poor prognosis [6]. Prognosis is related to the metastasis of lung AdC and PCa patients [7]. Stage I lung AdC and PCa can be detected using appropriate biomarkers for diagnosis, assessing recurrence, and therapeutic treatment to improve long term survival rates [8,9].

Few clinically approved biomarkers are available for the early diagnosis of cancers [10]. Annexin As (ANXAs) and prostate specific antigen (PSA) are considered potential biomarkers for monitoring lung AdC and PCa [8,11,12]. Annexin A1 (ANXA1), annexin A2 (ANXA2), and annexin A3 (ANXA3) are expressed abundantly in most AdC and squamous cell carcinomas in a diffuse manner [13]. The detection of PSA for early diagnosis of PCa can be imprecise in that an elevated PSA level in the range 4.0–10.0 ng/mL is not always due to PCa but can be associated with other prostate conditions including benign prostatic hyperplasia, inflammation, and prostatitis, while low PSA levels of <4.0 ng/mL can also be shown by men with PCa [14]. The ambiguity of the test's results has led to large numbers of apparently unnecessary biopsies being performed [15].

Western blot analyses have identified ANXA3 as a highly specific noninvasive biomarker for PCa [16,17]. ANXA3 is stable in exprimate urine samples for more than 48 h at 25 °C and is stable during the course of reiterate measurement within at least 24 h [17]. Therefore assaying for ANXA3 is potentially useful for the detection of the early stages of lung AdC and PCa in blood serum and urine samples, obviating the drawbacks of PSA detection.

Protein biomarkers are commonly detected by conventional immunoassay techniques such as enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and western blotting. However, these techniques are complicated, time consuming, expensive, and labor intensive and the development of a new class of immunosensor based on optical [18] or electrochemical [19] methods would expedite analysis. While electrochemical and optical methods can achieve good sensitivities, they are not suitable in many cases due to their reliance on fluorescence tags and enzymes as labels for signal generation. Therefore, the development of a label-free immunosensor would represent significant progress in detecting cancer biomarker proteins.

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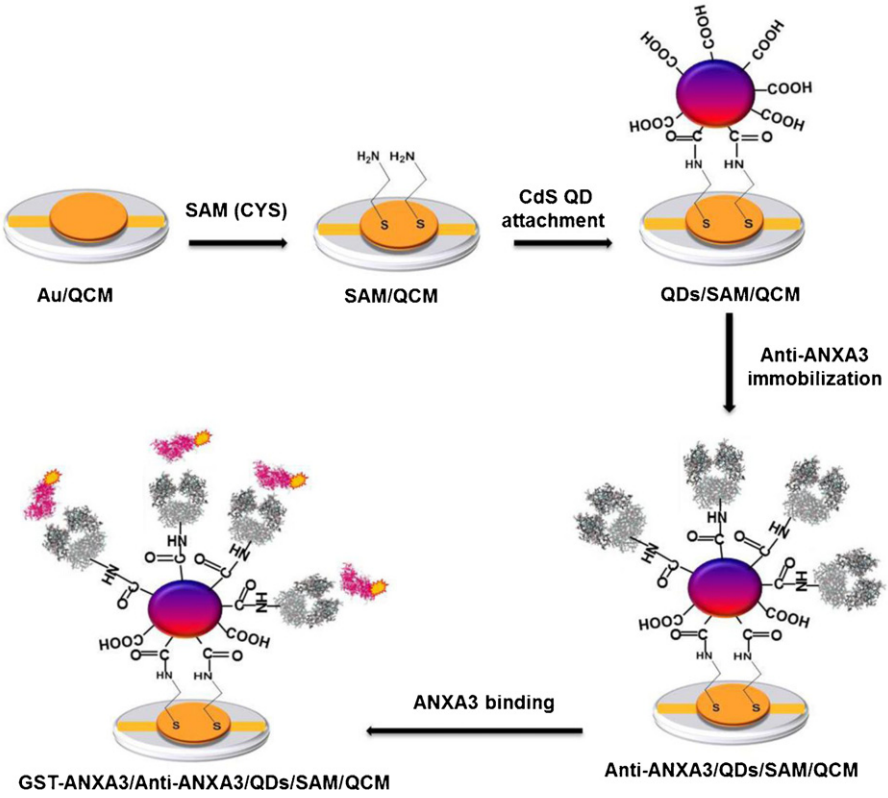
http://dx.doi.org/10.1016/j.snb.2012.10.117



Scheme 1. Schematic representation of the ANXA3 nanoimmunosensor.

To the best of the authors' knowledge, there are no reports of the label-free detection of ANXA3. The use of quartz crystal microbalances (QCMs) is a promising area of label-free detection of cancer biomarker proteins. A QCM can be designed to give a frequency response that characterizes *in situ* an immunointeraction between antigen and antibody [20]. For this, either the antigen or the antibody must be immobilized on to the Au electrode on quartz. Annexin A1 and annexin A2 at over 200 nM have been shown to bind effectively with 5 MHz QCMs at solid supported lipid membranes, usually reaching saturation within ~10 min [21,22]. Considering that ANXA3 is physiologically present in human blood at less than 2 ng/mL (<0.06 nM) [17], stable QCM-based immunosensors with fine biomimetic interfaces are required for the strong immobilization of antigens to allow high sensitivity. In this regard, quantum dot (QD) nanocrystals, QD coated silica (SiO_2) particles, and magnetic particles functionalized with QDs can be used as appropriate biomimetic interfaces to improve the immobilization of antigens or antibodies for higher sensitivity [23,24].

This work reports the fabrication of a QD-based, simple, label-free, and ultrasensitive QCM nanoimmunosensor to detect annexin A3 (ANXA3), a lung and prostate cancer biomarker protein. Conditions affecting the quantitative detection of ANXA3 were optimized and the sensor was applied to human serum and urine samples for the detection of ANXA3.

2. Experimental

2.1. Chemicals and apparatus

Polyclonal anti-ANXA3 (rabbit) and recombinant glutatione S-transferase (GST)-tagged ANXA3 (GST-ANXA3, human) were from Abnova Co. (Walnut, CA, USA). Human serum albumin (HSA), immunoglobin G (IgG, human), bovine serum albumin (BSA), 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC), sodium dihydrogen phosphate (NaH_2PO_4), and disodium hydrogen phosphate (Na_2HPO_4) were from Sigma (St. Louis, MO, USA). Cystamine dihydrochloride (CYS), 11-mercaptoundecanoic acid (MUA), mercaptoethanol, sodium acetate, glacial acetic acid, and sodium chloride (NaCl) were from Aldrich (St. Louis, MO, USA). *N*-Hydroxysuccinimide (NHS) was from Fluka (St. Louis, MO, USA). Cadmium sulfide nanocrystals (CdS QDs 2–7 nm) were from NN-Labs (Fayetteville, AR, USA). Phosphate buffer saline (PBS) solution was prepared by mixing 10 mM NaH_2PO_4 and 10 mM Na_2HPO_4 with 0.9% sodium chloride (NaCl). Solutions of ANXA3 were freshly prepared prior to each use by diluting the stock solution with 10 mM PBS. Acetate buffer solution was prepared by adjusting 0.2 M sodium acetate (Aldrich) to the desired pH with 0.2 M glacial acetic acid. All other chemicals were of analytical grade and used without further purification. All aqueous solutions were prepared with doubly distilled water from a Milli-Q water purifying system (18 MΩ cm).

Square wave voltammetric (SWV) and quartz crystal microbalance (QCM) experiments were performed using a CHI430A electrochemical workstation with an extra module for electrochemical quartz crystal microbalance testing (CH Instruments, USA). A thin gold (Au) layer on an AT-cut quartz crystal (A = 0.21 cm^2; 8 MHz) was used for QCM experiments under steady conditions. SWV experiments used a working electrode of the self-assembled monolayer (SAM) and QDs/SAM modified gold electrodes (area = 0.07 cm^2). Ag/AgCl (*aq*, saturated KCl) and a platinum (Pt) wire were used as reference and counter electrodes, respectively. Potential was scanned from −1.0 to 0.0 V with 5 mV increments, 25 mV amplitude, and frequency 10 Hz.

2.2. Calibration of the QCM electrode

The initial frequency of an Au-coated 8 MHz AT-cut quartz crystal was recorded after the crystal was placed in a QCM cell. 10 μL of 0.5–1.5 μg/mL (5.0–15 ng) aqueous CYS was deposited on the