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Sensors and Actuators B 106 (2005) 455–460
SENSORS AND ACTUATORS B CHEMICAL
www.elsevier.com/locate/snb

Development of a piezoelectric immunosensor for the detection of alpha-fetoprotein

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Received 15 January 2004; received in revised form 26 August 2004; accepted 14 September 2004

Abstract

A piezoelectric immunosensor was developed for the detection of α-fetoprotein (AFP) in human serum. A number of immobilization schemes via amine-functionalized coating have been studied and evaluated for their potential use in fabricating a piezoelectric immunosensor. These included polyethyleneimine (PEI) and two different types of self-assembled monolayers (SAM), cystamine and cysteamine. Antibody was immobilized onto the amine-coated surface via glutaraldehyde (GA) cross-linking. Antibodies cross-linked at 1.25% GA for 40 min showed the best activity. The amount and the reaction activity of bound antibody on PEI film were better than those on SAM. The calibration graph for AFP obtained by the immunosensor was linear up to 800 ng/ml. Application of the immunosensor to clinical samples demonstrated that results were in good agreement with ELISA. No cross-reactivates were observed with the common endogenous substances in serum, such as glucose (1 mg/ml), ascorbic acid (1 mg/ml) and uric acid (0.25 mg/ml). The antibody-modified crystals showed no significant loss in activity over 3 days under storage at 4 °C.

Keywords: α-Fetoprotein; Piezoelectric quartz crystal; Immobilization; Immunosensor

1. Introduction

α-Fetoprotein (AFP) is an oncofetal glycoprotein and is widely used in diagnosis, in monitoring therapy and in follow-up of patients with germ cell tumors and hepatocellular carcinoma [1]. Elevated AFP concentration in adult plasma may be an early indication of hepatocellular carcinoma and teratoblastoma. Numerous immunological methods for determining the concentration of AFP have been described [2–5]. An amperometric immunosensor using glucose oxidase as a label has been reported as an alternative to the conventional methods [6]. These methods can be precise but usually require labels and several incubation and washing steps. Therefore, they are time-consuming and labor-intensive.

The development of a rapid, simple, label-free method for the detection of proteins has been a long-standing goal since the beginning of immunoassay technology. Piezoelectric immunosensing methods, being a label-free assay, can obviate complications of conventional immunoassay methods and offer an alternative to existing methods. Therefore, we aim to develop a piezoelectric immunosensor for the determination of AFP. The use of piezoelectric devices as potential sensors is an application of the Sauerbray equation, which describes the relationship between the resonant frequency of a crystal and mass deposited on its electrodes:

$$\Delta F = \frac{-2.3 \times 10^{-6} F^2 \, \Delta M}{A}$$

where ΔF is the measured frequency shift, F the resonant frequency of the piezoelectric crystal (PZ), A the area coated and ΔM the mass change due to surface deposition.

Antibody immobilization is a vital step in successful development of a piezoelectric immunosensor. The immobilization process must preserve the biological activity of the antibody and a more efficient binding. Although many immobilization methods have been studied and applied to

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doi:10.1016/j.snb.2004.09.007

piezoelectric immunosensor development, it is usually necessary to determine a suitable immobilization method for each biological material for a particular application [7]. Polymer membrane, such as polyethyleneimine (PEI) was reported to provide a uniform thin coating with complete coverage [8] and has shown many successful applications in piezoelectric immunosensor [9,10]. The self-assembled monolayer (SAM) technique offers one of the simplest ways to provide a reproducible, ultra thin and well-ordered layer suitable for further modification with antibodies, which has potentials in improving detection sensitivity, speed and reproducibility [11–13]. Therefore, immobilization methods based on PEI and SAM were examined in this study. PEI and aminoalkanethiols (cystamine and cysteamine) were utilized for the modification of a gold surface to introduce amino groups on it. The amino groups can provide reaction sites for covalently bonding to glutaraldehyde. Antibodies were then immobilized through Schiff base via glutaraldehyde cross-linking. The immobilization procedure is schematically shown in Fig. 1. Finally, in detection of the target antigen, a specific binding event occurs between the immobilized antibodies and antigens.

Hepatocellular carcinoma is prevalent in Taiwan [14], so the development of a convenient and simple method for the determination of AFP should be of importance for public health. The objectives of this study were: to optimize the immobilization methods; and to apply the proposed piezoelectric immunosensor to AFP determination.

2. Experimental

2.1. Reagents

α-Fetoprotein (AFP) from human core serum was purchased from Cortex Biochem Inc. (USA), anti-human AFP antibody from Fitzgerald Industries International (USA) and glutaraldehyde, polyethyleneimine, glycine, cysteamine hydrochloride and cystamine dihydrochloride from Sigma Chemical Co. (St. Louis, USA). All other chemicals used were of analytical grade.

2.2. Apparatus

An amount of 10 MHz AT-cut quartz crystals (8 mm in diameter) with gold electrodes (4 mm in diameter) on both sides were obtained from Mercury Company (Taipei, Taiwan). The electrode surface was pretreated with 1.2 M NaOH for 30 min and 1.2 M HCl for 30 min, in sequence, to obtain a clean gold surface. After the pretreatment, the crystal was rinsed with ethanol and water successively, and air-dried [15]. The electrode was then ready for use.

The frequency measurements were carried out in a static system using a quartz crystal microbalance system (QCM) (SB01B, Smell, Taiwan) and controlled by a computer under Windows environment. The resonant frequency of the same crystal was measured in gas phase before and after each step. The Smell program was used for data collection, storage and processing.

2.3. Methods of surface modification

2.3.1. Adsorption [16,17]

An aliquot of 10 μl of antibody solution (1 mg/ml) was applied onto the electrode surface for 1 h. The crystal was washed three times with PBS then three times with distilled water. After air-drying, the frequency values were taken.

2.3.2. PEI method [9,15]

A 5 μl aliquot of a methanol solution containing 1% PEI was dispersed onto the PZ crystal surface for 15 s at room

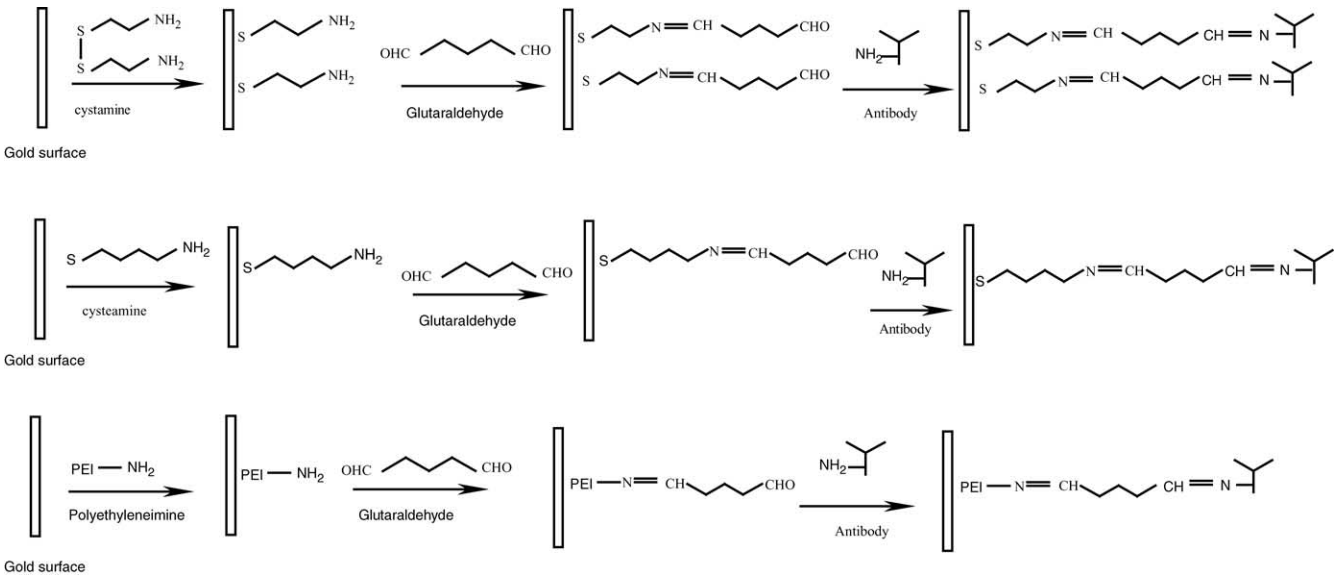

Fig. 1. Schematic diagram for piezoelectric immunosensor fabrication.