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A comparative study of capacitive immunosensors based on self-assembled monolayers formed from thiourea, thioctic acid, and 3-mercaptopropionic acid

Warakorn Limbut[a,b], Proespichaya Kanatharana[a,b], Bo Mattiasson[c], Punnee Asawatreratanakul[a,d], Panote Thavarungkul[a,e,*]

[a] *Biophysics Research Unit, Biosensors and Biocurrents, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand*
[b] *Department of Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand*
[c] *Department of Biotechnology, Center for Chemistry and Chemical Engineering, Lund University, Box 124, 221 00 Lund, Sweden*
[d] *Department of Biochemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand*
[e] *Department of Physics, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand*

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Abstract

A procedure was developed for the covalent coupling of anti-alpha-fetoprotein antibody (anti-AFP) to a gold surface modified with a self-assembled monolayer (SAM) of thiourea (TU). The performance of the SAM-antibody layer was compared to those of similar layers based on thioctic acid (TA) and 3-mercaptopropionic acid (MPA) by using flow injection capacitive immunosensor system. Covalent coupling of anti-AFP on self-assembled thiourea monolayer (SATUM) modified gold electrode can be used to detect alpha-fetoprotein with high efficiency, similar sensitivity, the same linear range (0.01–10 $\mu g\,l^{-1}$) and detection limit (10 $ng\,l^{-1}$) as those obtained from sensors based on self-assembled thioctic acid monolayer (SATAM) and self-assembled 3-mercaptopropionic acid monolayer (SAMPAM). The system is specific for alpha-fetoprotein and can be regenerated and reused up to 48 times. Therefore, self-assembled monolayer using thiourea which is cheaper than thioctic acid and 3-mercaptopropionic acid is a good alternative for biosensor applications when SAMs are used.

Keywords: Thiourea; Capacitive immunosensor; Thioctic acid; 3-Mercaptopropionic acid; Self-assembled monolayer; Alpha-fetoprotein

1. Introduction

Immunosensors are based on binding interactions between immobilized biomolecules and the analyte of interest and their subsequent detection by appropriate detector (Mattiasson, 1984; Taylor, 1991). Several electrochemical detection principles have been used, such as potentiometric (Tang et al., 2004a; Taylor et al., 1991), amperometric (Ramanaviciene and Ramanavocous, 2004), conductimetric (Yagiuda et al., 1996), and impedemetric (Tang et al., 2004b). Capacitive measurement has also been investigated as a highly sensitive approach (Berggren et al., 1998, 2001; Berggren and Johansson, 1997; Bontidean et al., 1998; Hedström et al., 2005; Hu et al., 2002, 2005).

Capacitive immunosensor is based on the principle that for an electrolytic capacitor the capacitance depends on the thickness and dielectric behavior of a dielectric layer on the surface of a metal (Gebbert et al., 1992). It can be constructed by immobilizing biorecognition elements in a thin layer on an electrode and measuring changes in the dielectric properties when an analyte binds to the biorecognition elements on the electrode, causing capacitance to decrease.

Immobilization is an important part in capacitive immunosensor since the electrode surface has to be electrically insulated. Different immobilization techniques have been developed and biorecognition elements can be immobilized on capacitive sensors via modified semiconductor surfaces (Barraud et al., 1993; Bataillard et al., 1988), metal oxides surfaces (Gebbert et al., 1992, 1994), and self-assembled monolayers (SAMs) of sulfur compounds on gold (Berggren et al., 1998; Berggren and Johansson, 1997; Hedström et al., 2005).

* Corresponding author. Tel.: +66 74 288753; fax: +66 74 212817.
E-mail address: panote.t@psu.ac.th (P. Thavarungkul).

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SAMs is a particularly suitable immobilization technique for capacitive biosensor (Riepl et al., 1999), since it allows electrochemical insulation of the surface of a working gold electrode and it is an excellent immobilization technique for protein, it shields proteins from direct contact with solid surface, thus, reduces the risk of the sensing element denaturation (Wadu-Mesthrige et al., 2000). Furthermore, the proteins, use as the sensing element, are immobilized through covalent binding and they can be exposed to a high or low pH, often uses in regeneration, leading to a reusable system (Frey and Corn, 1996). SAMs can be formed at room temperature by spontaneous adsorption of alkanethiol on gold surfaces (Nuzzo and Allara, 1983; Porter et al., 1987) by the reaction of sulfide

$$RSH + Au \rightarrow RS^{-}Au^{+} \cdot Au + \tfrac{1}{2}H_2$$

or disulfide

$$RS\text{–}SR + Au \rightarrow RS^{-}Au^{+}\cdot Au$$

The affinity between sulfur and gold atoms is extremely high, resulting in the formation of SAMs that are highly stable in air, water, and organic solvents at room temperature (Bain et al., 1989; Chaki and Vijayamohanan, 2002). They are also stable for a wide range of potential, from −400 to +1400 mV versus standard calomel electrode in diluted sulphuric acid solution, which is especially significant for electrochemical sensing (Finklea et al., 1987).

Capacitive biosensors have often been based on SAMs of thioctic acid (TA; $S_2C_7H_{13}$-CO_2H) (Berggren et al., 1998; Berggren and Johansson, 1997; Disley et al., 1998; Hedström et al., 2005; Liu et al., 1999) and 3-mercaptopropionic acid (MPA; $HSC_2H_4CO_2H$) (Disley et al., 1998; Sawaguchi et al., 2001; Vaughan et al., 1999). The carboxylic groups of the SAMs were activated with 1-ethyl-3-(3-diamino)propyl-carbodiimide (EDC) (Akram et al., 2004; Berggren et al., 1998; Berggren and Johansson, 1997; Hedström et al., 2005), and sometimes together with a succinimide, i.e. *N*-hydroxysulfosuccinimide (NHS) (Gooding and Hibbert, 1999; Staros et al., 1986; Vaughan et al., 1999). Then the activated groups were exposed to the protein solution where the activated electrophilic group attached to primary amino group of amino acid residues, forming a new peptide bond between SAM and protein. SAM containing amine-modified entities, such as 2-mercaptoethylamine (MEA; HSC_2H_4-NH_2), was also an effective surface to which protein could be immobilized (Jiang et al., 2003). Glutaraldehyde can also be used as it was introduced to react with the self-assembled MEA monolayer on the gold electrode to covalently immobilize the protein. However, only a few studies on capacitive immunosensors were reported based on the amine-modified SAM (Mirsky et al., 1997).

Since TA, MPA, and MEA are rather expensive, an alternative cheaper thiol reagent, thiourea was investigated. Thiourea (TU; NH_2CSNH_2) was chosen because of its low environmental impact, easier handling of reagent and the fact that it is strongly adsorbed on gold (Holze and Schomaker, 1990; Ubaldini et al., 1998). It has amino groups (R-NH_2) that can be modified to covalently couple to the antibody. To our knowledge no one has applied it to immunosensors.

This paper reports the development of a procedure for the immobilization of antibody to a gold surfaces modified with a SAM of thiourea. The performance was compared with that of the commonly used thioctic acid and 3-mercaptopropionic acid. Alpha-fetoprotein (AFP) and anti-alpha-fetoprotein antibody (anti-AFP) were used as a model system. The evaluation of each method for immobilization was done using a flow injection capacitive immunosensor system. The comparison was done by observing several analytical parameters, such as sensitivity, linear range, limit of detection, specificity, and reproducibility.

2. Materials and methods

2.1. Materials

Anti-AFP and AFP from human fluids were obtained from Dako (Denmark). 3-Mercaptopropionic acid, *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC), and *N*-hydroxysuccinimide (*N*-hydroxy-2,5-pyrrolidinedione, NHS) were obtained from Sigma–Aldrich (Steinheim, Germany), thioctic acid 98% and 1-dodecanethiol were obtained from Aldrich (Milwaukee, USA), thiourea was obtained from BDH laboratory reagents (Poole, England). All other chemicals used were of analytical grade. All buffers were prepared with distilled water treated with a reverse osmosis-deionized system. Before use, the buffers were filtered through an Albet® nylon membrane filter (Albet, Spain), pore size 0.20 μm, with subsequent degassing.

2.2. Methods

2.2.1. Preparation of the gold surface

Gold electrodes (Ø 3 mm, 99.99% purity) were polished (Gripo® 2V polishing machine, Metkon Instruments Ltd., Turkey) with alumina slurries (particle diameters 5, 1, and 0.30 μm) and then cleaned through sonication subsequentially, 15 min each, in distilled water and absolute ethanol to remove any physisorbed multilayer (Yang et al., 1995). They were then washed in distilled water and dried with pure nitrogen gas. Each electrode was pretreated by electrochemical etching in 0.5 M H_2SO_4 solution by cycling potential from 0 to +1500 mV versus Ag/AgCl reference electrode with a scan rate of 0.1 V s^{-1} for 25 scans. Finally they were dried with pure nitrogen gas.

2.2.2. Modification of SAMs formation

A cleaned gold electrode was immediately immersed in a thiol solution (thioctic acid, 3-mercaptopropionic acid, or thiourea) at room temperature for a period of time (see later) before being thoroughly rinsed with distilled water and dried with pure nitrogen gas. In this step self-assembled thioctic acid monolayer (SATAM), self-assembled 3-mercaptopropionic acid monolayer (SAMPAM), or self-assembled thiourea monolayer (SATUM) was formed on the gold surface.

A good formation of SAMs on gold surface depends on both the time (Dubois and Nuzzo, 1992; Kim et al., 1993; Wink et al., 1997) and concentration of thiol solutions (Kim et al., 1993; Liu et al., 1999; Wink et al., 1997). The effects of these factors were