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Research Article

Self -Assembled Monolayers with Peptide Nucleic Acid (PNA) Probe to Develop Electrochemical Biosensors

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ABSTRACT

Alkanethiol self-assembled monolayers (SAMs) with Peptide Nucleic Acid (PNA) were proposed as a novel probe design in electrochemical biosensors. This paper investigates the optimization of PNA probe by using two different concentrations of SAMs, immobilization temperature, and two-stage immobilization process. Alkanethiol self-assembled monolayers with PNA probe immobilization and cDNA (complementary DNA) hybridization were measured via AC voltammetry method. The small negative shift in formal potential (-17 mV) was occurred after cDNA hybridization at high ionic strength concentration.

Keywords: Alkanethiol self-assembled monolayers, PNA, Electrochemical biosensors, AC voltammetry

Elektrokimyasal Biyosensörlerin Geliştirilmesinde Alkanetiol Kendiliğinden Oluşan Tek Katmanlar ile Peptid Nükleik Asit (PNA) Probu

ÖZET

Peptid Nükleik Asit (PNA) içeren alkanetiol kendiliğinden oluşan tek katmanlar (SAM), elektrokimyasal biyosensörlerde prob olarak önerilmiştir. Bu makale, PNA probunun optimizasyonunu iki farklı SAM konsantrasyonu, immobilizasyon sıcaklığı ve iki aşamalı immobilizasyon prosesi kullanarak araştırmıştır. Alkanetiol kendiliğinden oluşan tek katmanlar ile PNA prob immobilizasyonu ve cDNA (tamamlayıcı DNA) hibridizasyonu AC voltametre metodu ile ölçülmüştür. Yüksek iyonik kuvvet konsantrasyonunda, cDNA hibridizasyonundan sonra formal potansiyelde küçük negatif kayma (-17 mV) meydana gelmiştir.

Anahtar Kelimeler: *Alkanetiol kendiliğinden oluşan tek katmanlar, PNA, Elektrokimyasal biyosensörler, AC voltametre*

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I. INTRODUCTION

Electrochemical devices as a transducer have received significant interest due to they are high sensitive, compatible with modern microfabrication technologies, portable, and low cost. Electrochemical transducers measure DNA interactions directly so they are simple, rapid and appropriate for DNA sensing [1-3]. This label-free detection not only simplifies the electrochemical sensing protocols but also offers instantaneous detection of the cDNA hybridization. Researchers have studied on electrochemical DNA biosensors to explore novel protocols. Therefore, Aoki et al. used PNA as a probe to detect DNA hybridization by using redox markers in the solution with ion-channel protocol [1, 4].

Peptide nucleic acid (PNA) has attracted attention as a probe in biosensors. Because PNA is a DNA mimics which has neutral N-(2-aminoethyl) glycine (AEG) unit instead of negatively charged sugar-phosphate backbone of DNA. This makes PNA as a probe more selective, stable and strong hybridization with cDNA than conventional DNA-DNA duplexes in biosensors [1, 5-8].

Several useful nucleic acid attachment protocol as a probe was developed. One of the attractive for fabricating reproducible probe surfaces with high hybridization activity is alkanethiol self -assembled monolayers (SAMs) [1]. Alkanethiol self -assembled monolayers (SAMs) have been used widely in DNA electrochemical biosensors to obtain sensing interface because of their many advantages. First, SAMs are easy preparation with well-designed composition, structure and thickness. Second, they are compatible with metal substrates to measure electrochemically. Last, they are chemically stable and suitable interface to immobilize biomolecules as a probe in biosensors. The performance of biosensors is affected from the design of the interface and its fabrication process. Thus, well-organized SAMs with biomolecules can be obtained by changing some parameters such as immersion time, concentration of adsorbate, temperature, chain length which effects the SAMs formation and properties [9-14].

In this study, we aim to develop electrochemical biosensors based on self-assembled monolayers with PNA as a probe to detect nucleic acids. 11-Ferrocenyl-1-Undecane thiol as a longer chain length redox species and high ionic strength (19 mM) as an electrolyte was used different from the previous paper [15]. Two different concentrations of 11-Ferrocenyl-1-Undecane thiol: 6-Mercapto-1-hexanol (FcUT:MCH) were examined as 1:1 and 1:20. 11-Ferrocenyl-1-Undecane thiol: 6-Mercapto-1-hexanol (FcUT:MCH) with probe PNA (thiol modified single strand PNA) was immobilized on bulk gold electrodes by using two-stage immobilization procedure. The first step of the immobilization procedure, FcUT:MCH was preheated at 43.5°C. Measurements with AC voltammetry method were used to describe the shift in formal potential after cDNA hybridization process. Experimental results were evaluated by using the model developed by Ho et al. [16].

II. MATERIALS AND METHODS

A. CLEANING PROCEDURE

A radius of 1mm bulk polycrystalline Au electrodes (CH Instruments, USA) was used as a substrate. They were cleaned both physically and chemically before immobilization procedure. As a physically,

they were sonicated with 3% Decon for 10 minutes and then polished with 0.3 μm alumina powders for 10 minutes followed by sonication in water. They were polished without powder again for 3 minutes to remove the residual powders from the surface followed by sonication in water. As an electrochemically, electrodes were cleaned in 0.5 M H_2SO_4 by cyclic voltammetry method. The working electrode (WE), the reference electrode (RE) and the counter electrode (CE) were chosen Au electrode, Hg/Hg_2SO_4 (K_2SO_4 saturated) and Pt wire, respectively. The potential was changed between (-0.05V) and (+1.1V) vs Hg/Hg_2SO_4 for 60 cycles by using Autolab PGSTAT302/FRA2 potentiostat (Eco Chemie, The Netherlands). At the end of the cleaning procedure, electrodes were rinsed with water followed by dried with nitrogen. The cleaned electrodes were placed into the immobilization solution directly [15].

B. TWO-STAGE IMMOBILIZATION PROCEDURE

Two-stage immobilization procedure was applied as mentioned in the previous paper [15]. 11-Ferrocenyl-1-Undecane thiol (FcUT) was used as a redox species. 6-Mercapto-1-hexanol (MCH) was mixed with FcUT to prevent nonspecific adsorption of cDNA hybridization and help to produce well-organized SAM. As a first-stage of immobilization, two different molar ratios of 1:1 and 1:20 FcUT:MCH were immobilized on bulk gold electrodes. FcUT:MCH was dissolved in ethanol to produce SAM. The cleaned gold electrodes were placed into this solution for immobilization 19 h in a humidity chamber. The temperature of 43.5°C was applied at the first 10 minutes of immobilization procedure. Then it was cooled at 22°C (room temperature). Electrodes were rinsed with ethanol and (1:1) (v/v) DMSO:H_2O followed by dried with nitrogen. As a second-stage of immobilization, 5μM probe PNA (20 base sequence) was immobilized on bulk gold electrodes with 1:1 M ratio of FcUT:MCH at 22°C for 19 h in a humidity chamber. Before PNA immobilization, 5μM probe PNA (20 base sequence) in 1:1 DMSO (Dimethyl Sulfoxide):H_2O was preheated to 50°C for 10 min. Then MCH was backfilled on bulk gold electrodes with mixed SAMs (FcUT:MCH+ probe PNA) for 1 hour. After backfilled, electrodes rinsed with (1:1) (v/v) DMSO:H_2O and 10 mM phosphate buffer (PB, pH = 7) followed by dried with nitrogen.

C. CDNA HYBRIDIZATION PROCEDURE

cDNA (20 base sequence) in TE buffer was diluted with 10mM PB to obtain the concentration of 5μM. Then the electrode with mixed SAMs was hybridized with cDNA at 22 °C for 2 hours in a humidity chamber. The electrode was rinsed with 10 mM PB followed by dried with nitrogen.

D. MEASUREMENT WITH AC VOLTAMMETRY METHOD

The electrode cell was set for immobilization and hybridization measurements. The electrolyte was chosen 10mM Phosphate Buffer (PB) pH=7 with the ionic strength of 19mM. The three electrodes (WE, RE, CE) placed into the cell were connected to an Autolab PGSTAT302/FRA2 potentiostat (Eco Chemie, The Netherlands). AC voltammetry method was made use of the measurement of the electrodes. The ac signal amplitude (E_{ac}) was 10 mV. The potential scan range and scanning frequency range were from -0.6 to 0.25 V vs Hg/Hg_2SO_4 and from 1 to 1000 Hz, respectively.

III. Results And Discussion

The formal potential (E°') vs time after baseline correction of the graphs was determined at 30Hz in Figure 1. The formal potentials of 1:1M FcUT:MCH were the same in Electrode 1 and 2. Although different formal potential values were observed between each electrode in 1:20M FcUT:MCH, it was seen the stabilization in formal potential values about 2 hours later.

The surface coverage of SAMs (N_{total}) on the bulk gold electrodes was calculated from the ferrocene (Fc) species by using in Eq. 1 [17].

$$I_{pk} = 2nfFN_{total}\frac{sinh(nFE_{ac}/RT)}{\cosh(nFE_{ac}/RT)+1} \quad (1)$$

In the equation, I_{pk} is the peak current of the graphs. f, n, F, E_{ac}, R, and T are the frequency, the number of electrons, Faraday's constant, the ac signal amplitude, the molar gas constant, and the absolute temperature, respectively. N_{total} is the total moles of redox species immobilized on a substrate. The surface coverages of 1:1M FcUT:MCH and 1:20M FcUT:MCH were calculated around (4.50-2.20).10^{-12} molcm^{-2} and (3.10-1.70).10^{-12} molcm^{-2}, respectively.

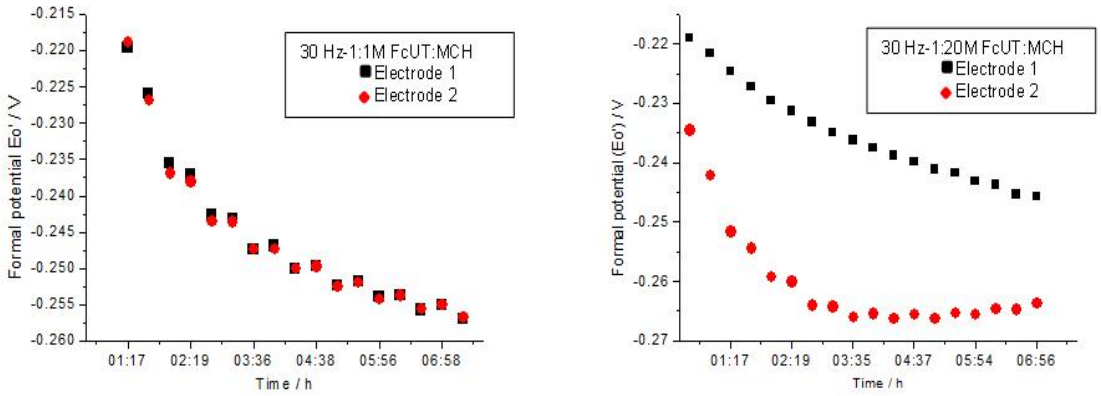

Figure1. *Graphs of formal potential (Eo') vs time of 1:1M FcUT:MCH and 1:20M FcUT:MCH SAMs. Black and red colors were at the same time measurements of Electrode 1 and 2, respectively*

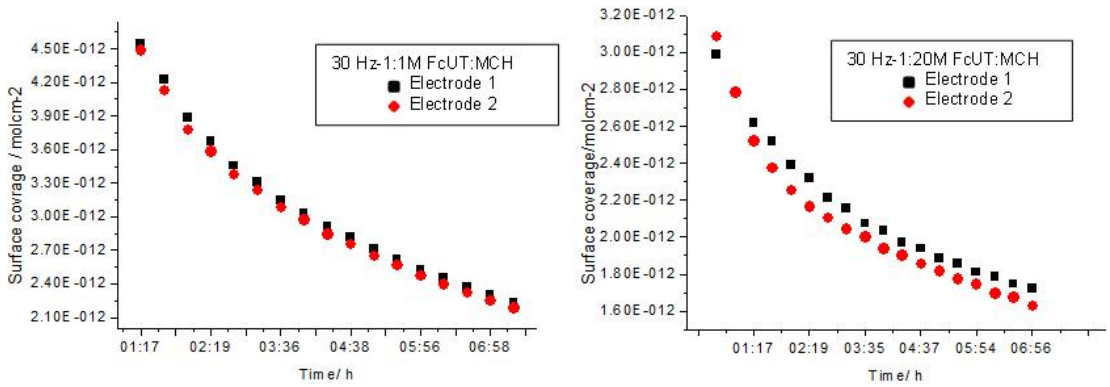

Figure 2. *Graphs of surface coverage vs time of 1:1M FcUT:MCH SAM and 1:20M FcUT:MCH SAM at 30Hz. Black and red colors were at the same time measurements of Electrode 1 and 2, respectively.*

In Figure 2 shows the graphs of surface coverages versus time of two electrodes in 1:1M FcUT:MCH and 1:20M FcUT:MCH. The surface coverage decreased with time relating to decrease of the peak current according to Eq. 1. Nevertheless, the same values of surface coverage between two electrodes especially in 1:1 M FcUT:MCH was observed by imposed the electrodes in 43.5˚C at first 10 minutes of immobilization. The applied temperature above 25 °C improves the SAMs formation at the first few minutes of the immobilization process [11, 18]. 1:1 M FcUT:MCH was used in probe design to detect cDNA hybridization.

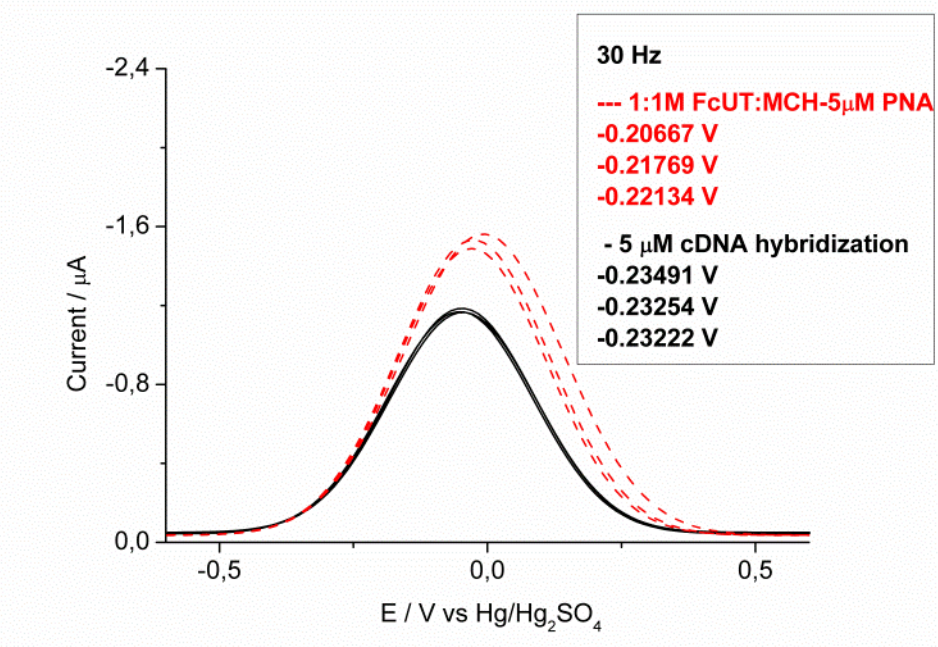

Figure 3. *Graph of immobilization (red dash) and hybridization (black line) measurements with AC voltammetry at 30 Hz after baseline correction.*

Figure 3 shows the current vs potential (E) graphs of mixed SAMs (1:1M FcUT.MCH+ probe PNA) immobilization and cDNA hybridization after baseline correction. The three consecutive immobilization and hybridization measurements were taken into account for the average formal potential shift. After cDNA hybridization, the potential shifts became smaller especially the two of cDNA hybridization peaks were overlapped and the current decreased. This can be explained by neutralization of charged head groups with cDNA hybridization and the potential became stable at 19 mM of ionic strength concentration. The potential lowered after cDNA hybridization. The distance between charged head groups came to be much greater than the Debye length. The small negative change of formal potential (-17 mV) was detected after 5 μM cDNA hybridization (black line) process. This results support the measurements of formal potential vs ionic strength concentrations in the previous paper [15]. The stable but more lowered potential value after cDNA hybridization was obtained at 19mM of ionic strength concentration. Ionic strength and the surface coverage in probe design effect the detection of cDNA hybridization. High ionic strength and/or low surface coverage lead to small shift in formal potential [19].

IV. CONCLUSION

This paper shows that the change of immobilization steps, temperature above 25℃ in SAMs formation and also high ionic strength concentrations effect the final results in respect with cDNA hybridization as a biosensors platform. The importance of the immobilization temperature and two-step immobilization procedure on mixed SAMs was supported with this study. The stable and reproducible measurements after cDNA hybridization were obtained by applying immobilization temperature (43.5˚C) and two-step immobilization procedure. It was obtained small but stable negative change of formal potential (-17 mV) after cDNA hybridization with the high ionic strength concentration.

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