Measurement 94 (2016) 1–4

Contents lists available at ScienceDirect

Measurement

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

# Application of mixed self-assembled monolayers (Mixed SAMs) for nucleic acid detection

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ARTICLE INFO

*Article history:*
Received 20 October 2014
Received in revised form 16 July 2016
Accepted 19 July 2016
Available online 20 July 2016

*Keywords:*
Alkanethiol
Peptide Nucleic Acid (PNA)
AC voltammetry
Amperometric biosensors

ABSTRACT

*Mixed self-assembled monolayers* (Mixed SAMs) consisting of 8-Ferrocenyl-1-octanethiol and 6-Mercapto-1-hexanol (FcOT:MCH) with probe PNA on gold electrodes were fabricated by using two-step after the optimization of immobilization temperature of FcOT:MCH SAMs. Using AC voltammetry, a novel nucleic acid detection platform, with mixed SAMs, was proposed. A negative formal potential shift was observed after complementary ssDNA hybridization while there was no significant difference after non-complementary ssDNA hybridization. Compatible results were obtained with the measurement of formal potential differences between mixed SAMs and target DNA (complementary and non-complementary DNA) in different target DNA concentrations. The formal potential difference between mixed SAMs immobilization and complementary ssDNA hybridization was measured in different ionic strength concentrations.



## 1. Introduction

DNA-based biosensors apply oligonucleotides as probes for target recognition. While DNA-based biosensors have presented an encouraging substitute for fast, simple and cheap detection of target molecules, there still exist key challenges such as poor stability and reproducibility that delay their competition with the current gold standard for DNA assays [1]. PNA can be used as a novel biosensor recognition element to detect toxins to fabricate stable and reproducible DNA biosensors. Specific PNA probe with indirect approach has been developed to detect toxic chemicals, some specific gene producing environmental toxin, and toxin producing organisms [2].

Alkanethiol self-assembled monolayers (SAMs) have been investigated for many years. Recently, for the detection of streptavidin as a target, mixed SAMs were fabricated by co-immobilization on Au electrodes of thiolated alkane chains with three different head groups: hydroxy terminating head group, ferrocene head group, and a functional head group such as biotin. It has been observed that generic label-free biodetection platform can be obtained by using different head group alkanethiols with biomolecules on gold electrodes by detecting a change of charge [3].

Theoretical description of the interfacial potential distribution of electrodes coated with monolayers was proposed by Smith and White [4]. Then an improvement model including the discreteness of charge and ion association effects was projected by Fawcett and co-workers [5,6].

Creager and Rowe [7,8] have also used these models to interpret their data in their experiments. They have depicted these models in which double layer effects can offer a viable explanation for apparent shifts in formal potential which is affected by the electrostatic potential distribution across the electrode/monolayer/solution interface. The potential distribution is a strong function of the electrolyte concentration, the surface coverage of electroactive species, the charge and fractional degree of oxidation of the electroactive species, and the position of those species in the monolayer. Therefore, experimental factors as a type of solvent, temperature, concentration of adsorbate, immersion time, cleanliness of the substrate, and chain length, can effect of the resulting SAM and the rate of formation [9–11].

In this study, we aim to optimize the thiol modified ssPNA (probe PNA) with FcOT:MCH SAMs on Au electrodes to detect nucleic acids by using two-step immobilization process after the optimization of immobilization temperature of FcOT:MCH SAMs. AC voltammetry measurements were used to characterize the formal potential shift after immobilization and hybridization process. We used a previously developed model by Ho et al. [3], based on Smith and White's [4] theory, to explain our experimental results.

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http://dx.doi.org/10.1016/j.measurement.2016.07.060
0263-2241/

## 2. Methods

### 2.1. Chemicals

Complementary and non-complementary single stranded (ss) DNA oligonucleotides (target DNA) were purchased from the Protein and Nucleic Acid Chemistry facility of Cambridge University Biochemistry Department. Thiol modified ssPNA (probe PNA) oligonucleotides were purchased from Panagene, Korea. 8-Ferrocenyl-1-octanethiol (FcOT) as a redox species was purchased from Dojindo Molecular Technologies, Inc. 6-Mercapto-1-hexanol (MCH), Ethanol, Dimethylsulfoxide (DMSO, $(CH_3)_2$ SO), Potassium sulfate ($K_2SO_4$), Potassium monobasic phosphate ($KH_2PO_4$), Potassium dibasic phosphate ($K_2HPO_4$), and TE buffer were purchased from Sigma–Aldrich, UK. 18.2 MΩ cm ultra-pure water (Millipore, Billerica, MA, USA) with a Pyrogard filter (Millipore) was used for preparation of aqueous solutions. Decon, the cleaning agent, was purchased from Veltek Associates.

### 2.2. Electrode cleaning

First, bulk polycrystalline Au electrodes with a radius of 1 mm (CH Instruments, USA) were sonicated with 3% Decon for 10 min. Second, electrodes were polished with 0.3 μm alumina powders for 10 min followed by sonication in water. Then electrodes were polished with polishing pad without powder for 3 min to remove the residual powders from the surface. After sonication with water, electrodes were cleaned electrochemically in 0.5 M $H_2SO_4$ by cyclic voltammetry. Gold electrode, $Hg/Hg_2SO_4$ ($K_2SO_4$ saturated) and Pt wire were used as the working electrode (WE), the reference electrode (RE) and the counter electrode (CE), respectively. The potential was varied between (−0.05V) and (+1.1V) vs $Hg/Hg_2SO_4$ for 60 cycles by using either Autolab PGSTAT302/FRA2 potentiostat (Eco Chemie, The Netherlands) or a Gamry Instruments Femtostat (Gamry Instruments, USA). At the end of the cleaning procedure, electrodes were rinsed with water and ethanol, respectively then dried with nitrogen. The cleaned electrodes were placed into the immobilization solution immediately.

### 2.3. Immobilization procedure & temperature

A two-step immobilization process was applied. As the first step, the cleaned electrodes were immersed into the 1:20 M ratio of FcOT:MCH in ethanol for 2 h at four different temperatures (25 °C, 50 °C, 60 °C and 70 °C) in a humidity chamber. Different immobilization temperatures were used to obtain well-organized SAMs. After rinsing with ethanol and (1:1) (v/v) DMSO:$H_2O$, electrodes were dried with nitrogen. In the second step, 5 μM probe PNA (20 base sequence) in (1:1) (v/v) DMSO:$H_2O$ which was preheated to 50 °C in 10 min, was immobilized on electrodes with FcOT:MCH SAMs for 19 h at 25 °C in a humidity chamber. After rinsing with (1:1) (v/v) DMSO:$H_2O$ and 10 mM phosphate buffer (PB, pH = 7), mixed SAMs (FcOT:MCH + probe PNA) on electrodes were dried with nitrogen.

### 2.4. Hybridization procedure

The target DNA in TE buffer (20 base sequence) was diluted with 10 mM PB (pH = 7) to obtain the concentrations of 0.05, 0.5, 2, and 5 μM target DNA. The mixed SAMs on electrodes were hybridized with the target DNA for 2 h at 25 °C in a humidity chamber. After rinsing with 10 mM PB, electrodes were dried with nitrogen.

### 2.5. Ionic strength measurements

0.5 M $K_2SO_4$ was used to measure the effect of different ionic strength concentrations on the immobilization and hybridization process. 5 mM PB (pH = 7) with the ionic strength concentration of 9 mM was used as the electrolyte. 0.5 M $K_2SO_4$ was added to the electrolyte yielding ionic strength concentrations between 9 and 80 mM in each measurement.

### 2.6. Sample characterization

After immobilization and hybridization process, the electrode cell was prepared for measurements. PB (pH = 7) with concentrations of 10 mM and 5 mM were used as the electrolyte for the measurement of immobilization and hybridization process and the measurement of the effect of the ionic strength concentrations. The three electrodes (WE, RE, CE) were placed into the cell and were connected to an Autolab PGSTAT302/FRA2 potentiostat (Eco Chemie, The Netherlands). AC voltammetry was used to measure the electrodes. The ac signal amplitude was 10 mV. The potential scan range was from −0.6 to 0.25 V vs $Hg/Hg_2SO_4$. The scanning frequency range was from 1 to 1000 Hz.

## 3. Results and discussion

Fig. 1 shows the AC voltammograms after the two-step immobilization process. The aim of the process was to accomplish well-organized mixed SAMs with two-step immobilization and the effect of immobilization temperature on FcOT:MCH SAMs. Four different temperatures (25 °C, 50 °C, 60 °C and 70 °C) on separate electrodes were used as the immobilization temperatures for FcOT:MCH SAMs (Fig. 1(a–d)). In each graph, three repetitive measurements were taken at 00:46 h, 01:27 h, and 02:09 h. When the temperature increased above 25 °C, the peaks became stable at three repetitive measurements especially at 50 °C (Fig. 1(b–d)). The effect of temperature on the SAMs formation was studied by many researchers [9,11–15]. It was pointed out that the temperatures above 25 °C improve the formation of SAMs and reduce the number of defects [9,12–15]. Lee et al. [15] have found that the closely packed, well-ordered OT (octanethiol) SAMs with long-range ordered structure formed in ethanol at 50 °C while studying the solvent effects on SAMs. Delamarche et al. [16] have studied the annealing temperature of 50 °C and revealed that thermal annealing of preassembled SAMs improves the molecular packing, enabling them to be studied with better accuracy. It was also pointed out that the currents fell gradually through increasing the temperature from 50 °C to 70 °C. Therefore, in our study, we used 50 °C as the immobilization temperature for FcOT:MCH SAMs.

Fig. 2 shows the AC voltammograms after baseline correction of the immobilization and hybridization process. After 2 μM complementary ssDNA hybridization, a negative shift in formal potential by −25 mV was observed while there was no significant shift in the formal potential of 2 μM non-complementary ssDNA hybridization (−0.15 mV). Beside the negative shift in potential, the current fell with the complementary ssDNA hybridization. This is since negative charge of ssDNA backbones in DNA/PNA duplex can change the surface charge on the electrode and passivate the redox reaction of Fc. Aoki and Tao [17] have reported that the hybridization of a complementary target DNA decreased the flexibility of the Fc-PNA probe and inhibited the access of the terminal ferrocene moiety to the electrode surface, resulting in a decrease in the observed current intensity. Aoki et al. [18] have demonstrated the use of ion channel sensors for DNA detection and indicated that PNA as a probe has a large effect of DNA binding on the surface charge and DNA binding in PNA/DNA duplex changed the surface