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Coadsorption optimization of DNA in binary self-assembled monolayer on gold electrode for electrochemical detection of oligonucleotide sequences

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ABSTRACT

Optimization of the probe adsorption has a major key in the preparation of electrochemical sensors for the detection of oligonucleotide sequences hybridization. The role of a mixed monolayer of ssDNA sequences and MCH coadsorbed on a gold electrode surface was studied in this work. The working electrode was modified by chemisorption using a solution of thiol-tethered 33-mer DNA probe and mercaptohexanol (MCH), in a concentration range from 2 nM to 20 μM. The probe surface density was monitored by means of electrochemical impedance spectroscopy (EIS), differential pulse voltammetry (DPV) and chronocoulometry. From EIS measurements, the charge transfer resistance was obtained as a function of the MCH concentration in the immobilization solution. The time dependence of mixed SAM adsorption was also investigated. The SAM adsorption was characterized regarding the electrode surface coverage with DPV and EIS measurements. Moreover, the probe surface density was investigated with chronocoulometry in $Ru(NH_3)_6^{3+}$ solution. Sensor behavior and sensitivity showed significant differences as a function of ssDNA/MCH concentration ratio as hybridization detection efficiency decreases while increasing the MCH concentration. The effect of different probe density in the hybridization detection efficiency was determined. Results demonstrated the effective of the coadsorption of ssDNA and thiols to control the SAM property and the probe density. It was therefore shown the importance to identify the correct density of probes on the electrode, below the saturation value, to ensure both a proper hybridization process and having a high hybridization signal.

1. Introduction

The rapid identification of DNA sequences or the analysis of single nucleotide polymorphisms is of growing scientific and technological importance, as manifested in the growing interest in chip-based characterization of gene expression patterns and the detection of pathogens in clinical, forensic and pharmaceutical application. Even though optical methods, and in particular microarray, retain the main role in DNA investigation for practical use [18], a variety of different electrochemical techniques have been developed, promising significant advantages over previous technologies [11,17,31]. In particular, voltammetric and amperometric techniques, like cyclic voltammetry or differential pulse voltammetry, have been used measuring the current modulated by the presence of complementary DNA sequences [32,12,29]. Moreover, the electrochemical impedance spectroscopy (EIS) has been deeply investigated as it is a powerful tool for hybridization sensing [42,33].

Besides the improvement of measurement techniques, the preparation of the biological substrate, in this case single stranded DNA probes, is a key element for the efficiency of a DNA sensor [21,35,4]. In fact, the organization of the probe self-assembled monolayer, which, in turn, depends on the electrode surface characteristics, strongly affects the hybridization efficiency [35,16,23]. Hence, it is important to study the adsorption process as well as the characteristics of the electrode surface, like the real surface area or the surface roughness factor [6,22]. The optimization of the ssDNA adsorption and the optimization of the active sensing layer is still an open issue, both to improve the reproducibility and sensitivity of the sensors and to allow the use of new materials or electrode layouts [43,36].

Gold electrodes are widely used for ssDNA adsorption. Herne and Tarlov [20] proposed a two step modification of gold electrode with thiol-modified ssDNA with a subsequent exposure to a MCH (6-mercapto-1-hexanol) solution. The self-assembled monolayer of HS-ssDNA and MCH allows to create a more uniform interface and to remove the nonspecifically adsorbed sequences from the surface: to maximize the efficiency of hybridization it is essential to minimize the number of nonspecifically adsorbed probes. Moreover, the spatial position of probe sequences enhances the hybridization, as negatively charged –OH head group of the MCH orients the flat lying HS-ssDNA strands in perpendicular configuration and

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reduces the probe surface density to give high freedom for hybridization [9,3], while the short chains of MCH do not interfere with the hybridization process [34]. Gebala and Schuhmann [13] investigated the post-assembly of a binary MCH/MPA (mercaptopropionic acid) solution on a ssDNA-modified electrode: this work shows that the ratio of concentration of the mixed solution affects the ssDNA probe density and orientation and thus strongly influences the EIS response. Recently, Keighley et al. [24] proposed the direct use of a mixed DNA/thiolated chains solution to modify a gold electrode. Furthermore, Vikholm-Lundin et al. [40] showed that the coadsorption of chains and blocking agents allows a very fast immobilization procedure, with assembly time of about 15 min.

A variety of techniques has been used to characterized the surface of modified gold electrodes, like X-ray photoelectronic spectroscopy (XPS), ellipsometry, ^{32}P-radiolabeling [34], microcantilever [30] chronopotentiometry [1], neutron reflectivity [26], AFM [28] and electrochemical methods, in particular cyclic voltammetry or impedance spectroscopy [41].

In this work we investigate through electrochemical methods the optimization of the self-assembled monolayer adsorption of mixed solution of ssDNA and MCH. Thus, the preparation of the modified gold electrode is a one step process. We studied in details the effect of ssDNA/MCH ratio in the hybridization detection. The adsorption and hybridization quantification are carried out with differential pulses voltammetry and EIS measurements. The sensor hybridization sensitivity is reported as a function of ssDNA/MCH concentration ratio.

2. Materials and methods

2.1. Materials

Ethanol 95% and isopropyl alcohol were from Zetalab (Padova, Italy). Potassium chloride, sulfuric acid, $K_3Fe(CN)_6$, $K_4Fe(CN)_6$, Na_2HPO_4, NaH_2PO_4 and Hexaammineruthenium (III) chloride (RuHex (II)/(III)) and 6-mercapto-1-hexanol (MCH) were all obtained from Sigma–Aldrich (Milan, Italy). Ethanol 95% and isopropyl alcohol were from Zetalab (Padova, Italy). All solutions were prepared with deionized water (Titolchimica, Rovigo, Italy).

Synthetic thiol-modified oligonucleotides were purchased from Diatech (Ancona, Italy). The 33-base sequences are the following:

5′-GTA ACA TCA CAG GCT ATT AGT TGC CAA CGT CCT C-3′

for the *probe* sequence, and

5′-GAG GAC GTT GGC AAC TAA TAG CCT GTG ATG TTA C-3′

for the *target* sequence. The thiolated probe DNA, abbreviated HS-ssDNA, is modified on the 5′ to obtained HS-$(CH_2)_6$-ssDNA. The target sequence is without the thiol modification. All DNA/MCH solutions were made in 0.1 M sodium phosphate buffer (PB), 0.2 M Na_2HPO_4 and 0.2 M NaH_2PO_4, pH 7.0.

2.2. Sample preparation

The working electrode of 1 mm^2 area is made by plasma sputter deposition of ∼80 nm of gold on a polycarbonate substrate (∼1.2 mm thick). Electrodes were cleaned by exposure to 10% sulfuric acid for 5 min. Then, an insulating layer is printed (Dimatix DMP-2800, US) over the device in order to define the electrochemical cell and to insulate the conductive tracks. The electrode is connected to the instruments by means of a micromanipulator.

Just prior to electrochemical experiments, the gold electrode was washed with isopropyl alcohol in a sonicator (Ultrasonic 06, Falc Instruments s.r.l., Treviglio, Italy), and then rinsed with distilled water. Then a drop of 60 μl solution was cast on the active area. Before chronocoulometry measurements, the electrode was allowed to equilibrate with $Ru(NH_3)_6^{3+}$ for 30 s before starting the potential step. All operations were carried out at room temperature (25 °C). The gold working electrode is modified by chemical adsorption by casting 0.7 μl of HS-ssDNA/MCH mixed solution for 2 h (except for kinetics experiments). A longer immobilization time does not have significant effect on the binary mixed monolayer [35,9,10]. The ssDNA solution was in a concentration of 2 μM while the concentration of MCH solution was in a range between 2 nM and 20 μM. The DNA buffer solution was phosphate buffer (PB) 100 mM, pH 7.0. The adsorption process was carried out inside a wet sealed Petri, in order to avoid sample evaporation, at room temperature. Before measurements and hybridization, the electrode surface was washed three times with 20 μl of KCl 100 mM and then was rinsed thoroughly with deionized water.

Before hybridization, the target solution was heated at 90 °C for 1 min. Hybridization was performed by placing 1 μl of target solution directly onto the probe-modified electrode for 3 h, in a wet sealed Petri at 65 °C. The hybridization buffer solution was phosphate buffer (PB) 100 mM, pH 7.0. After hybridization, the electrode surface was washed three times with 20 μl of KCl 100 mM, to remove nonspecifically adsorbed sequences, and then was rinsed thoroughly with deionized water.

2.3. Electrochemical measurements

Electrochemical impedance spectroscopy was carried out with Solartron 1260 impedance analyzer (Solartron, US). A custom-built LabVIEW 2010 (National Instruments) program was developed to drive the Solartron 1260 and to acquire data. Voltammetric measurements were carried out using CH440A potentiostat and CH 8.3 software (CH Instruments Inc., Austin, USA). A commercial Ag|AgCl|KCl 1 M reference electrode (CHI111, CH Instruments Inc., Austin, USA) is used as reference electrode and a platinum wire (CHI129, CH Instruments Inc., Austin, USA) is used as counter electrode.

Two solutions of 1 mM $Fe(CN)_6^{4-/3-}$ in KCl 100 mM and 10 mM $Ru(NH_3)_6^{3+}$ in KCl 100 mM were prepared. EIS measurements were performed with 1 mM $Fe(CN)_6^{4-/3-}$ in KCl 100 mM between the gold electrode and reference electrode in a frequency range between 1 Hz and 100 kHz, using alternating voltage of 5 mV over a +230 mV bias (vs Ag|AgCl) which corresponds to the formal potential of the ferricyanide/ferrocyanide redox couple.

Ciclovoltammetry measurements (CV) were carried out in a potential range from +0.4 to −0.4 V, for hexacyanoferrate (II)/(III) solution with a scan rate of 100 mV s^{-1} (unless otherwise specified). Differential pulse voltammetry (DPV) was performed in the same potential range with a pulse amplitude of 5 mV and a pulse width of 0.2 s. Chronocoulometry were performed in the presence of RuHex (II)/(III) and KCl solutions with a pulse period of 500 ms and a pulse width of 500 mV.

2.4. Sample characterization

The microscopic surface area was investigated with a method described by Trasatti and Petrii [39], that is based on the oxygen chemisorption measurements. The standard reference charge of chemisorbed oxygen layer is assumed to be 390 ± 10 μC cm^2 for polycrystalline Au. The determination of the roughness factor was done by extrapolating the charge from chronocoulometry (CC) measurements. Then, roughness factor is the ratio between the measured charge and the reference charge. In this case, a value of 1.47 ± 0.11 was found for the Au electrode used in our experiments.