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Electrochemical Sensing of DNA with Porous Silicon Layers

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Abstract: *The nanostructure known as porous silicon is an excellent material for sensing applications. Due to its large internal surface, it is capable of adsorbing an enormous amount of different compounds. The average porous size can be easily adjusted to allow the penetration of molecular compounds with different sizes. In this work we show a sensing application for porous silicon. We have fabricated a biosensor from DNA. The biosensor is an electrochemical device that transduces the hybridization of DNA into a chemical oxidation of guanine by* $Ru(bpy)_3^{2+}$*, the reduced form of which is then detected electrochemically. The anodic peak current of* $Ru(bpy)_3^{2+}$ *was linearly related to the target DNA sequence in the range* 0.5×10^{-10} *-* 500×10^{-10} *M with a detection limit of* 0.5×10^{-10} *M. In addition the ruthenium bipyridine indicator was able to selectively discriminate against different DNA sequences; a necessary property for sensing applications.*

Keywords: biosensor, DNA hybridization, ruthenium bipyridine, porous silicon

1. INTRODUCTION

Porous silicon (PSi) possesses a large internal surface area and it has been proposed as the substrate material for sensing applications because it can readily be integrated with silicon technology [1]. The sensitivity of the surface to chemical species has been demonstrated by exposing porous silicon layers to different compounds, such as vapors, molecules with different dipole moments, DNA, small viruses and bacteria [2-7]. In the development of a sensor, selectivity is a very important issue because the sensor must be able to distinguish the element being sensed. For biosensors the bioselective element is usually immobilized on some sort of support [4], PSi in this case. The sensor is then exposed to the species being sensed and the output signal is detected. To obtain a high quality sensor the PSi layers must be made by choosing the appropriate anodization conditions. DNA biosensors based on nucleic acid recognition processes are rapidly being developed with the goal of rapid and inexpensive testing of genetic and infectious diseases. The use of DNA recognition layers represents an exciting development in analytical chemistry [8]. Electrochemical biosensors have received a great deal of attention due to their high sensitivity and rapid speed of detection. In addition, electrochemical techniques are ideally suited to miniaturization and have the potential to simplify nucleic acid analysis using low-cost electronics [9]. Nowadays, they are being used in many reports for detecting the DNA hybridization event, due to their high sensitivity, small dimensions, low cost, and compatibility with microfabrication technology [9-15]. This paper describes the use of porous silicon layers to transduce hybridization of DNA into a chemical oxidation of guanine by $Ru(bpy)_3^{2+}$, the reduced form of which is then detected electrochemically. Nucleic acid modified PSi electrodes were used in combination of voltammetry for the detection of DNA. We have used $Ru(bpy)_3^{2+}$ as electrochemical hybridization indicator for DNA detection. It has been shown [16-19] that Ruthenium $Ru(bpy)_3^{2+}$ is a better indicator for quantitative determination of short gene sequence such as the ones used here. The DNA probe sequence, immobilized at the electrodes, was engineered in a similar way as in [19] to decrease the oxidation of the probe DNA with $Ru(bpy)_3^{2+}$. Such a sequence does not contain the guanine but inosine. Inosine is three orders of magnitude less reactive than guanosine and still recognizes cytidine which is very important for sensing all four bases in the DNA target sequence.

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2. EXPERIMENTAL

2.1. Porous Silicon

Porous silicon samples were prepared from p+-type, boron doped silicon wafers with a resistivity of 0.008-0.012 Ohm cm by standard anodization (electrolyte: 15% of HF) at a current density of 30 mA.cm^{-2}. The porosity was measured by the gravimetrical method given a porosity of approximately 62%. The pore size was estimated by TEM and ranged from 50 nm-75 nm in diameter. These diameters are large enough to allow the sensing molecules to penetrate and attach. For DNA, the diameter of the nucleotides is approximately 5Å , which is small enough to fit into the porous matrix. Stabilization of PSi is necessary to passivate its surface and this was done by thermal oxidation. Thermal oxidation of PSi requires several precautions and high temperatures (>700ºC). Covering the whole internal surface with a thin SiO_2 layer stabilizes the structure, permits water penetration into the pores [22], and facilitates probe and target penetration [5]. All PSi samples were thermally oxidized in oxygen ambient at 900ºC for 10 minutes.

2.2. Measurement set up

The electrochemical instrumentation used for these experiments includes a BAS 100B/W Electrochemical Analyzer. A BAS VC-2 voltammetry cell (model MF-1065) was used for the electrochemical experiments. It is well suited to small sizes and has a special micro-cell for volumes as small as 50µL. The micro-cell, which includes the working electrode, separates a small volume containing the sample from a bulk solution containing the reference and auxiliary electrode with a salt bridge. A platinum wire serves as auxiliary electrode and the modified PSi samples function as working electrodes. It is important to mention that PSi, especially oxidized PSi, is not conducting and it is in fact the p+ doped silicon that is conducting the electrical current. The top area of the exposed PSi samples was 0.8 cm^2 and all lateral areas were insulated with a commercial epoxy resin (see figure1). The epoxy resin was deposited very carefully and dried for one hour. The samples were attached to the electrochemical system as shown in figure 1. Potentials were measured relative to an aqueous, saturated Ag/AgCl double junction (reference electrode). The voltammetry experiments were carried out at different scan rates in an electrochemical buffer solution composed by 50 mM sodium phosphate (pH 7) with 0.7 M NaCl. A schematic representation of the electrochemical measurement set up and the electrode arrangement is shown in fig. 1.

2.3. Reagents

Three different synthetic oligonucleotides were obtained from MWG Biotech, INC, and have the following sequences: (probe): 5'-TAI-CTA-TII-AAT-TCC-TCI-TAI-ICA-3',(target):5'-GCC-TAC-GAG-GAA-TTC-CAT-AGC-T-3' and (two-base mismatch target):5'-GCC-TAC-GAG-GAA-TTG-GAT-AGC-T-3. Tris(2,2'-bypyridyl) ruthenium (II) chloride hexahydrate was purchase from Strem Chemicals. All other chemicals were of analytical grade and purchased from Aldrich and Fluka. Deionized distilled water was obtained from Millipore.

2.4. Procedure

The detection of DNA consists of the following steps: PSi silanization, probe immobilization, hybridization, and voltammetric detection.

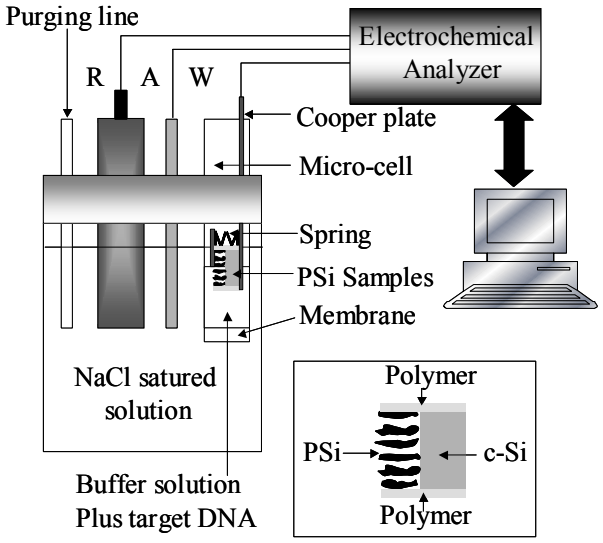

Figure 1. Measurement system: the PSi electrode is used as working electrode. A platinum wire is the auxiliary electrode and Ag/AgCl the reference electrode. Inset: cross section showing the different parts of the working electrode.

2.4.1. PSi Silanization

Several methods maybe employed to bind DNA to different supports [20]. One method commonly used for binding DNA involves silanization of an oxidized surface. The function of silane coupling agents is to provide stable bond between two non-bonding surfaces: for example, an inorganic surface to an organic molecule. 3-glycidoxypropyltrimethoxysilane was used to silanize the oxidized PSi. A 5% aqueous solution of silane was prepared (pH 4.0). This converts silane into a reactive silanol through hydrolysis. The PSi samples were then immersed into the continuosly stirred solution and left overnight. 3-glycidoxypropyltrimethoxysilane is hydrolyzed to a reactive silanol by using double distilled water (pH 4). PSi samples were then submerged into silanol solution for approximately 17 hours. Constant stirring of the solution was necessary to continuously mix the solution.

2.4.2. Probe immobilization

After successful silanization, DNA was immobilized onto the surface of PSi through diffusion. Aqueous solutions of DNA containing 150 µl of DNA (50 µM) were carefully placed directly above de PSi layer. The DNA molecules covalently bond to the silanized surface, where they become immobilized. The samples were then placed in a steam container where they were heated in an oven at 37ºC for approximately 20 hours. The DNA attached samples were then rinsed in double distilled water and dried with nitrogen.

2.4.3. Hybridization

The DNA attached to PSi was exposed to its complementary strand DNA (target), the mismatch sequence (mismatch probe) and itself (probe). Binding was allowed to proceed for 1 hour at room temperature into hybridization buffer containing 1 M NaCl,

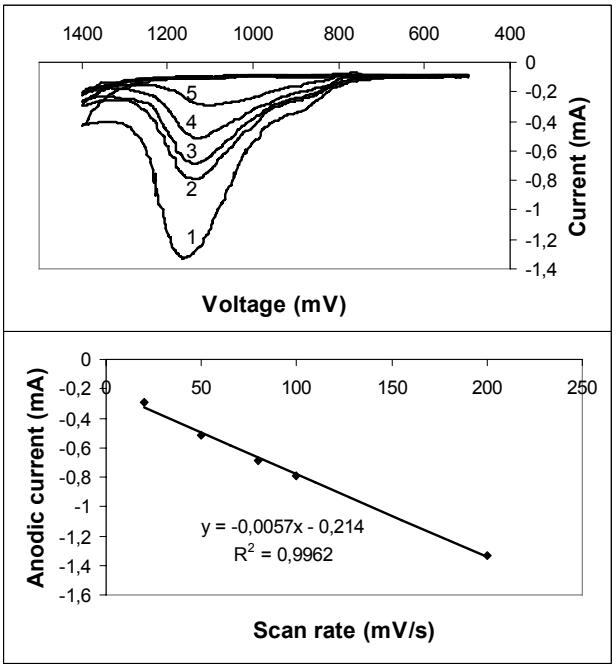

Figure 2. (top) Cyclic voltammograms of the probe-target DNA sequence in 0.1 μM $Ru(bpy)_3^{2+}$ solution at different scan rates ($mV.s^{-1}$): (1) 20; (2) 50; (3) 80; (4) 100; (5) 200. (bottom) The anodic current change with the scan rate. The target concentration was 0.5×10^{-10}M.

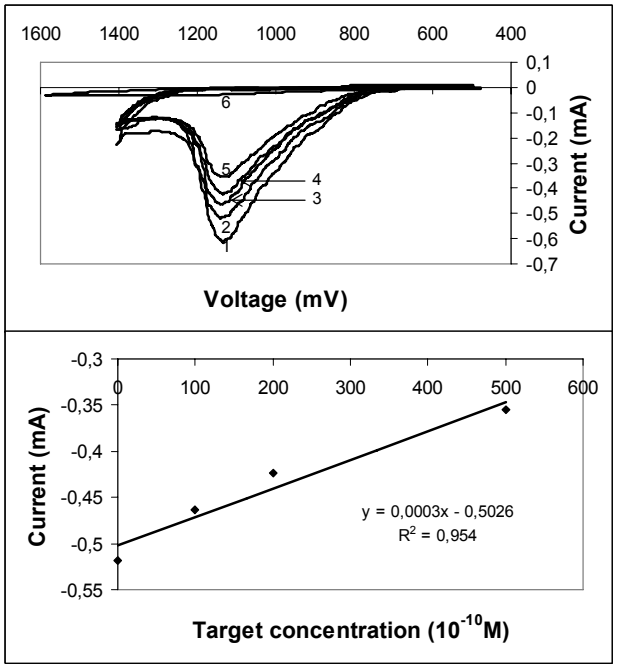

Figure 3. (top) Cycled voltammograms of different concentrations of target DNA: (2) 0.5×10^{-10}M, (3) 100×10^{-10}M, (4) 200×10^{-10}M, (5) 500×10^{-10}M. Curve 1 shows the CV for the probe-mismatch target DNA sequence (0.5×10^{-10}M) and curve 6 for the probe-probe DNA sequence (0.5×10^{-10}M). (bottom) The anodic current change with the concentration of the target DNA. In all cases we have used 0.1 μM of $Ru(bpy)_3^{2+}$.

10-20 mM sodium cacodylate, 0.5 mM EDTA, 150 mM KCl and 5 mM $MgCl_2$. Throughout the steps, binding was confirmed using Fourier Transform Infrared Spectroscopy (results not shown in here).

2.4.4. Voltammetric detection

Cyclic voltammetry (CV) was carried out having the DNA-modified, PSi electrode as working electrode, an Ag/AgCl as the reference electrode, and platinum wire as the counter electrode. 6 μl of $Ru(bpy)_3^{2+}$ (0.1μM) was poured into 150 μl of electrochemical buffer solution. After allowing the solution to diffuse into the samples for 15 minutes, CV was performed. Solutions were deoxygenated via purging with nitrogen for 10 minutes prior to measurements.

3. RESULTS AND DISCUSSION

PSi DNA-electrodes and $Ru(bpy)_3^{2+}$ were used for specific gene detection. $Ru(bpy)_3^{2+}$ exhibits a reversible redox couple at 1.05 V and oxidizes guanine in DNA at high salt concentration [19] according to:

$$Ru(bpy)_3^{2+} \longrightarrow Ru(bpy)_3^{3+} + e^- \quad (1)$$

$$Ru(bpy)_3^{3+} + DNA \longrightarrow DNA_{ox} + Ru(bpy)_3^{2+} \quad (2)$$

where DNA_{ox} is a DNA molecule in which guanine has been oxidized by $Ru(bpy)_3^{3+}$. If the DNA probe contains guanine then $Ru(bpy)_3^{3+}$ will oxidize guanine in DNA, even without the presence of the target DNA. In order to prevent that the DNA probe reacts with $Ru(bpy)_3^{2+}$ the guanine has been replaced for another less reactive nucleotide. Some previous results show that the addition of an oligonucleotide that does not contain guanine produces a small enhancement in the oxidation current [19]. Those results have shown that the inosine 5'-monophosphate is 3 orders of magnitude less electrochemically reactive than guanosine 5'-monophosphate and still recognizes cytidine (19). This fact is very important if we want to recognize all four bases in the target sequence. Nevertheless there is a drawback that can have consequences on the hybridization efficiency. Since the deaminated hypoxanthine in the ionosine can only form two of the three hydrogen bonds in a Watson-Crick base pair, it may be desirable to use a guanine derivative that is redox-inert but capable of forming all three hydrogen bonds. However some studies have shown (19) that the specificity afforded by inosine substitution was sufficient but they propose 7-deazaguanine as alternative. For this reason the DNA probe sequence does not contain the guanine base but the target does. Figure 2 (top) shows the CV obtained in solution for the hybrid DNA (probe-target) at different scan rates (target DNA concentration of 0.5×10^{-10}M). Figure 2 (bottom) shows that the anodic current of $Ru(bpy)_3^{2+}$ is linearly proportional to the scan rate. This result is congruent with a process that is controlled by adsorption. Figure 3 (top) shows the CV (scan rate of 50 $mV.s^{-1}$) of varied concentrations of target DNA (probe-target sequence, curves 2 to 5) and

different targets (probe-mismatch target sequence, curve 1 and probe-probe sequence, curve 6). In curve 1, the mismatch target sequence contains two more pairs of base G than the target sequence and that is why the current in this case is bigger than the current obtained in the probe-target sequence cases (curves 2 to 5) or the probe-probe sequence (curve 6). Moreover in curve 6 the current intensity decreases as a consequence of the absent of the base G in the probe. Nevertheless a significant increase in current was observed for curves 2 to 5 where the target DNA undergoes hybridization to the complementary DNA. This current increase suggests that the hybridization was successful and that the electron transfer from the guanines of the hybridized strand to $Ru(bpy)_3^{2+}$ is responsible for the increase in the current. If we compare curves 1, 2 and 6 (same DNA concentration but different target sequence) we observe that the sensor responds differently to each target and therefore a good selectivity is achieved. Figure 3 (bottom) shows the anodic peak currents of $Ru(bpy)_3^{2+}$ at four different concentrations (curves 2 to 5). The peaks are linearly related to the concentration of the target DNA sequence between 0.5×10^{-10} and 500×10^{-10}M. The detection limit of this approach was 5×10^{-11}M. The sensitivity achieved in this work is similar to the one obtained in reference (9), where a sensitivity of $9.0\text{x}10^{-11}$M was reported for a sensor that uses gold substrates instead.

In summary our results clearly show that the PSi sensor shown here has a good selectivity and sensitivity to the target compound, two very important characteristics that a sensor ought to have.

4. CONCLUSIONS

Porous silicon has been demonstrated as a suitable host for different sensing applications. PSi has been proved to be an ideal sensor material for various compounds and the variety of sensing applications ranges from alcohols to viruses. The specificity of DNA and the sensitivity of electrochemical detection have been used to develop a novel, silicon compatible detection sensor for the identification of DNA. DNA hybridization has been detected voltammetrically by using ruthenium bipyridine in PSi samples. In order to prevent the DNA probe from reacting with $Ru(bpy)_3^{2+}$ the guanosine 5'-monophosphate has been replaced from the DNA probe sequence for inosine 5'-monophosphate, which is much less reactive to oxidization. Ruthenium bipyridine showed a catalytic effect on the anodic peak current which is related to the concentration of target DNA in the hybridization reaction. The results confirmed that the DNA sensing method using here is a quick and convenient way for the specific and quantitative detection of DNA. Finally, further investigation on the properties of this biosensor, including selectivity to a different DNA sequences, detection of small viruses and large bacteria will be done in the future.

5. ACKNOWLEDGMENTS

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