Electrochemical impedance behavior of DNA biosensor based on colloidal Ag and bilayer two-dimensional sol–gel as matrices

Yingzi Fu, Ruo Yuan*, Lan Xu, Yaqin Chai, Yan Liu, Dianping Tang, Ying Zhang

Chong qing Key Laboratory of Analytical Chemistry, College of Chemistry and Chemical Engineering, Southwest China Normal University, Chongqing, 400700, PR China

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Abstract

A novel method for fabrication of DNA biosensors has been developed by means of self-assembling colloidal Ag (Ag) to a thiol-containing sol–gel network. The thiol groups of 3-mercaptopropyltrimethoxysilane (MPTS) serve as binding sites for the covalent attachment to gold electrode surface. Then the one-dimensional network of silane unites (1dMPTS) was combined together into a two-dimensional sol–gel network (2dMPTS) by dipping into aqueous NaOH. The second silane layer (B2dMPTS) was formed by immersing electrodes back into the MPTS solution overnight, and then the Ag nanoparticles were chemisorbed onto the thiol groups of the second silane layer. Finally, the mercapto oligonucleotide was self-assembled onto the surface via the Ag nanoparticles. The modified process was characterized by electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). In addition, we utilized the impedance spectroscopy as a platform for DNA sensing assay. The factors influencing the performance of the resulting biosensor were studied in detail. The linear range of the biosensor was from 8.0×10^{-9} to 1.0×10^{-6} M with a detection limit of 4.0×10^{-9} M at 3σ. In addition, the experiment results indicate that oligonucleotide immobilized on this way exhibits a good sensitivity and selectivity, high stability and a long-term maintenance of bioactivity.

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* Corresponding author. Tel.: +86 23 68252277; fax: +86 23 68254000.
E-mail address: Yuanruo@swnu.edu.cn (R. Yuan).
1. Introduction

At present, many new electrochemical procedures are under intense investigation in order to get a fast, sensitive and selective quantification of nucleic acids and to meet the new challenges connected to the application of DNA biosensors in different fields [1,2]. Solid electrodes have been demonstrated to be very useful for this task [3]. Immobilization of DNA probes on a solid surface is an important initial step for the preparation of a DNA biosensor. Various methods are available for immobilizing DNA on a gold electrode, include biotin–streptavidin interaction [4], direct adsorption [5–10] and self-assembly of thiol-capped DNA [11–16]. To a large extent, the selectivity, sensitivity and reproducibility of a DNA recognition interface will depend on the immobilization of the DNA probe strands. When hybridization occurs, the probe and target strands must be free to coil around each other. Immobilization of the probe strand onto a transducer surface will inevitably cause a decrease in configurational freedom. Therefore, minimization of the decrease in this freedom is required to achieve efficient hybridization. Direct adsorption is the simplest way to produce a DNA-modified gold surface. However, neutron reflectivity studies [16] indicated that the ss-DNA was lying flat on the surface of electrode with multiple adsorption points as the DNA bases complexed with the surface. The resultant hybridization efficiency was low. In order to improve the hybridization efficiency, ss-DNA immobilization on transducer surfaces using single-point attachment is recommended by preventing the nonspecific adsorption of the DNA bases. Self-assembled monolayer (SAMs) is one kind of useful technique, since it can provide simple organizing procedure and regular orientation to immobilize a stable DNA on various metal and oxide surfaces [17], and it provides an elegant method to preparation of well-define assemblies on solid surfaces. Sol–gel technique [18–20] has been recognized as a valuable way to prepare stable inorganic host materials via the sequential hydrolysis and polycondensation of an appropriate metal or semimetal alkoxide at room temperature. The sol–gel derived electrodes possess a number of advantages, including simplicity of fabrication, tunability of physical characteristics, mechanical rigidity (surface renewal), porosity, chemical and thermal stability, etc. These attractive features would be further enhanced by means of various modification procedures or by the use of bi-functional sol–gel precursors, such as 3-mercaptopropyltrimethoxysilane (MPTS), which is a heterobifunctional molecule that contain both thiol and silane functional group, therefore, it has been immobilized on glass [21], glass carbon [22], Pt [23], Cu [24], Ag [25] and Au [26–30] substrates via Sol–gel procedure. Sol–gel technology provides a unique means to prepare a two-dimensional network suited for the encapsulation of a variety of biomolecules ascribed to the gel possesses a large internal surface area. Recently, nanomaterials have been used in bioanalytical chemistry for their unique properties. Colloidal Ag is a kind of nanomaterial which possesses tremendous specific surface area, good biocompatibility and electrically activity to shuttle electrons from the surface into the conduction band [25,31,32]. Ag particles functionalized with DNA would route to new diagnostic systems of electrochemical analysis.

This paper describes the use of bilayer two-dimensional-network of MPTS (B2dMPTS) and Ag nanoparticles to self-assemble 5’-thiol-capped ssDNA for fabricating a DNA biosensor. The high activity of Ag to thiols [25,32] was led it successfully and hard anchored on B2dMPTS and coupled thiol-capped DNA. This assembly procedure offers
several major advantages such as simple, tunable and improved mechanical properties, chemical and thermal stability and single-point attachment onto the miniaturized surface. We used the impedance spectroscopy as a platform for DNA sensing assay, the experiment results indicate that oligonucleotide immobilized on this way exhibits a good sensitivity, selectivity, stability and a long-term maintenance of bioactivity.

2. Experimental

2.1. Materials

The oligonucleotides used in this study were purchased from Shenggong Bioengineering (Shanghai, China). Oligonucleotide probe with a mercaptohexyl group at its 5'-phosphate end, abbreviated HS-ssDNA, is the sequence as follows: 21-mer 5'-HS-(CH₂)₆-ACT GCT AGA GAT TTT CCA CAT-3'; the complementary target oligonucleotide have the sequences: 5'-ATG TGG AAA ATC TCT AGC AGT-3'; three-mismatch containing oligonucleotide sequences: 5'-ATG AGG AAA ACC TCT AGG AGT-3', and the non-complementary control is the same sequence as the 21-mer HS-ssDNA only without the HS-attachment at the 5' end. 3-Mercaptopropyltrimethoxysilane (MPTS) was purchased from Sigma (St. Louis, MO, USA) and used as received. Tris(2,2'-bipyridyl)cobalt (III) perchlorate (Co(bpy)₃⁺) was prepared according to a published procedure [33]. K₃Fe(CN)₆ and other chemicals were all of analytical reagent grade. The supporting electrolyte was 0.1 M phosphate buffer solution which was prepared with KH₂PO₄ and Na₂HPO₄ and containing 0.1 M KCl (PBS, pH 7.0). The pH (4.5–8.5) was adjusted with additional Na₂HPO₄ solution. Water used was twice distilled and sterilized. The colloidal Ag was prepared according to the literature [34].

Electrochemical impedance spectroscopy (EIS) measurements were performed with a Model IM6e (ZAHNER Elektrick, Germany). Cyclic voltammetry (CV) was performed with a CHI660A electrochemical workstation (Shanghai Chenhua Instrument, China). The Electrochemical cell consisted of a three-electrode system where bare or modified gold electrodes were used as a working electrode, platinum wire as an auxiliary electrode and a saturated calomel as a reference electrode (SCE). All measurements were carried out at a temperature of 25 °C. The size of Ag colloids was estimated from transmission electron microscopy (H600, Hitachi Instrument, Japan).

2.2. Preparation of working electrodes

A bulk gold disk electrode (Φ=4 mm) was carefully cleaned as follows: first working electrode was polished to a mirror-like surface with 3.0, 0.3-μm alumina slurry on microcloth pads. After sonicating in ethanol and double distilled water, the layer was cleaned with a hot mixture of piranha solution (7: 3 mixture of concentrated sulfuric acid and 30% hydrogen peroxide) and then washed in double distilled water. The polished gold substrate was immersed in a 40 mM solution of MPTS in methanol for 3 h to produce a self-assembled monolayer (1dMPTS). After thorough rinsing, the silane units were polymerized into a 2d-network by dipping into aqueous 0.01 M NaOH for 2 h (2dMPTS). A second silane layer
(B2dMPTS) was then formed by immersion back into the MPTS solution overnight. The surface, now containing exposed thiol moieties (Scheme 1), was dipped into a 15-nm Ag nanoparticles solution for 4 h at room temperature (Ag-B2dMPTS). At last, the modified gold electrode was obtained by transferring a droplet of 10 µl 6.38×10⁻⁵ M HS-ssDNA solution onto the Ag nanoparticles-modified electrode surface, followed by drying at 4 °C overnight. It was denoted ssDNA-Ag-B2dMPTS and stored at 4 °C when not used.

2.3. Hybridization

Hybridization reaction was performed by dipping the probe DNA into the stirred solution (0.1 M PBS, pH 7.0) containing different concentration of the target DNA for 5 min while holding the potential at +0.5 V. After that, the electrode was washed with the same PBS buffer solution. The same procedure as above was repeated by using the mismatch containing oligonucleotide or noncomplementary oligonucleotide instead of the target oligonucleotide.

2.4. Electrochemical detection

The electrochemical characteristics of the modified electrode were characterized by using electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) during
the self-assembled process. CV were measured in 0.5 mM Co(bpy)$_3^{3+}$ solution (0.1 M PBS, pH 7.0). EIS were measured in the presence of a 2.5 mM [Fe(CN)$_6^{4-/3-}$] (1:1) mixture as a redox probe in 0.1 M PBS (pH 7.0) at the frequency range from $10^{-2}$ to $10^6$ Hz in a bias potential of 0.18 V vs. SCE, amplitude was 10 mV. The change of electron-transfer resistance is calculated as the following equation:

$$\Delta R_{et} = R_{dsDNA} - R_{ssDNA}$$

where $R_{ssDNA}$ is the impedance value of electron-transfer resistance of the immobilized HS-ssDNA, $R_{dsDNA}$ is the value after hybridization, and the $\Delta R_{et}$ is the difference of the impedance value before and after hybridization.

3. Results and discussion

3.1. Electrochemical characteristics of the modified electrode

Cyclic voltammograms (CV) is a valuable and convenient tool to monitor the barrier of the modified electrode, because the electron transfer between the solution species and the electrode must occur by tunneling either through the barrier or through the defects in the barrier. Therefore, it was chosen as a marker to investigate the changes of electrode behavior after each assembly step. When electrode surface has been modified by some materials, the electron-transfer kinetics of Co(bpy)$_3^{3+}$ is perturbed. Fig. 1 illustrates the CV of different modified electrodes in 0.5 mM Co(bpy)$_3^{3+}$. As expected, Co(bpy)$_3^{3+}$ exhibits reversible behavior with peak-to-peak separation ($\Delta E_p$) of 70 mV at bare gold electrode (curve a). When 1dMPTS formed on electrode, the cathodic and anodic peak current

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**Fig. 1.** Cyclic voltammograms (CV) of the different electrodes in a 0.5 mM Co(bpy)$_3^{3+}$ solution (0.1 M PBS, pH 7.0) of (a) bare gold electrode, (b) 1MPTS-, (c) 2dMPTS-, (d) Ag-B2dMPTS-, and (e) ssDNA-Ag-B2dMPTS modified gold electrode. Scan rate, 100 mV s$^{-1}$. Scan range, $-0.2$ to $0.4$ V. Starting potential, $-0.2$ V (vs. SCE).
decreased obviously (curve b). The reason is that the -SH of the MPTS combining to gold electrode surface blocked electron transfer. After dipping the 1dMPTS-modified electrode back into the MPTS solution, one can notice a near disappearance of the peak current (curve c). It is shown that 2d-network strongly blocked the electrode reaction. When B2dMPTS modified electrode is immersed in colloidal Ag, since the high activity of silver to oxygen and to thiols established the assembly procedures [32], the modified gold substrate would be quickly sensitized. Therefore, Ag colloids could deposit both inside the network and on the surface of the B2dMPTS which would act as tiny conduction center and could facilitate the transfer of electron. Curve d exhibits a reversible redox with an increasing the cathodic and anodic peaks current. At last step of assembly, the HS-ssDNA were adsorbed onto the Ag nanoparticles. Curve e shows that the HS-ssDNA molecules adsorbed onto the nanoparticles can enrich the response of Co(bpy)$_3^{3+}$ by its strongly electrostatic interaction with Co(bpy)$_3^{3+}$ [7].

It is well known that electrochemical impedance spectroscopy (EIS) is an effective tool for studying the interface properties of surface-modified electrodes. In EIS, the total impedance was determined by several parameters: (1) electrolyte resistance, $R_s$; (2) the lipid bilayer capacitance, $C_{dl}$; (3) charge transfer resistance, $R_{ct}$; (4) Warburg element, $Z_w$. The complex impedance can be presented as the sum of the real, $Z_{re}$, and imaginary, $Z_{im}$, components that originate mainly from the resistance and capacitance of the cell. For the sake of giving more detailed information about the impedance of the modified electrode, a modified Randles equivalent circuit (insert of Fig. 2) was chosen to fit the measured results. The two components of the scheme, $R_s$ and $Z_w$, represent bulk properties of the

![Fig. 2. Electrochemical impedance spectroscopy (EIS) of (a) bare gold electrode, (b) 1dMPTS-, (c) 2dMPTS-, (d) Ag-B2dMPTS-, and (e) ssDNA-Ag-B2dMPTS modified gold electrode in 2.5 mM [Fe(CN)$_6$]$_{4-3}^-$ solution (0.1 M PBS, pH 7.0) at a bias potential of 0.18 V vs. SCE. The alternative voltage was 10 mV and the frequency range was $10^{-2}$–$10^6$ Hz. Inset: equivalent circuit used to model impedance data, (1) $R_s$, electrolyte resistance; (2) $Z_w$, Warburg impedance; (3) $R_{ct}$, electron-transfer resistance; (4) $C_{dl}$, double-layer capacitance.](image-url)
electrolyte solution and diffusion of the applied redox probe, respectively. Thus, they are not affected by chemical transformations occurring at the electrode interface. The other two components of the circuit, $C_{dl}$ and $R_{et}$, depend on the dielectric and insulating features at the electrode/electrolyte interface. In EIS, the semicircle diameter equals the electron-transfer resistance, $R_{et}$. This resistance controls the electron-transfer kinetics of the redox probe at electrode interface. Its value varies when different substances are adsorbed onto the electrode surface.

The electrochemical impedance measurements were carried out in a background solution of 2.5 mM $[\text{Fe(CN)}_6]^{4-/3-}$ PBS (0.1 M, pH 7.0) at a bias potential of 0.18 V. The alternative voltage was 10 mV and the frequency range was $10^{-2}$–$10^6$ Hz. In order to view the procedure of DNA immobilization and amplified biosensor clearly, we only considered the relation between $R_{et}$ and the concentration of DNA. Fig. 2 shows the results of Faradic impedance spectroscopy on a bare gold electrode, 1dMPTS-, B2dMPTS-, Au-B2dMPTS-, and ssDNA-Au-B2dMPTS modified gold electrode. Significant differences in the electron-transfer resistances ($R_{et}$) were observed upon the stepwise formation of the modified electrode. It can be seen (Fig. 2a) that the bare gold electrode exhibits no semicircle but an almost straight line that is characteristic of a diffusional limiting step of the electrochemical process. The $R_{et}$ increased from 3036.6 $\Omega$ (Fig. 2b) to 5298.9 $\Omega$ (Fig. 2c) upon the formation of the 1dMPTS and B2dMPTS. As expected, the silanization with B2dMPTS has the effect of increasing the network impedance when compared to 1dMPTS [21]. They both obstructed electron transfer of the electrochemical probe. However, when the B2dMPTS-modified gold electrodes were dipped into Ag nanoparticles, we can be surprised to find that the $R_{et}$ was decreased ($R_{et}=476.3$ $\Omega$, Fig. 2d), implying that Ag colloids were entered inside the network of B2dMPTS and adsorbed on SAMs substrate through chemical bonds. Since the polar terminal groups (-SH) were exposed at the surface, the surface hydrophobicity was lowered and Ag colloids were allowed to attack and react with the terminal groups. That is to say the Ag nanoparticles were attached to B2dMPTS via a S–Ag bonds [25,32,35]. Ag nanoparticles are facilitated to transfer electron. When the HS-ssDNA was absorbed on the surface via Ag colloids, the $R_{et}$ increased again ($R_{et}=673.7$ $\Omega$, Fig. 2e), indicated that the HS-ssDNA has strongly bound to Ag nanoparticles to generate a negatively charged interface that electrostatically repels multi-charged negative redox probe, $[\text{Fe(CN)}_6]^{4-/3-}$. The repulsion of the redox label is anticipated to inhibit the interfacial electron transfer and thus leads to enhanced $R_{et}$ [36]. On the basis of the CV and EIS results, we can conclude that HS-ssDNA is successfully immobilized on the gold electrode surface via the self-assembly of bilayer two-dimensional-network of MPTS and Ag nanoparticles.

3.2. Optimization of experimental conditions

The effect of experimental parameters was investigated and optimized in order to establish optimal conditions for the probe preparation and hybridization detection. The immersing time of B2dMPTS-modified electrode in Ag nanoparticles was investigated by EIS in 2.5 mM $[\text{Fe(CN)}_6]^{4-/3-}$ solution. It was found (Fig. 3a) that the resistance ($R_{et}$) of the modified electrodes decreased with the increment of immersing time, and then leveled off after 4 h, implying that the B2dMPTS-modified electrodes were
saturated with Ag nanoparticles. Therefore, 4 h was selected as the optimum immersing time.

The effect of pH on modified electrode hybridization with complementary DNA (cDNA) was investigated over the range of pH 4.5–8.5. The impedance response at the electrode is influenced by the pH value (Fig. 3 b). The modified electrode can be measured over the pH range 5.5–8.0 and achieve a maximum difference of impedance value ($\Delta R_{et}$) before and after hybridization (with $4.0 \times 10^{-7}$ M cDNA in 0.1 M PBS) at pH 7.0. Highly acidic or alkaline surroundings would damage the electrode, especially in alkalinity. The possible explanation may be that the formed dsDNA would be denatured in acidic or alkaline media causing the dsDNA layer damaged. So pH 7.0 was selected as the optimum pH value.

The efficiency of hybridization of DNA probe with cDNA is dependent on the hybridization time. As hybridization proceeded (with $4.0 \times 10^{-7}$ M cDNA in 0.1 M PBS, pH 7.0), $\Delta R_{et}$ increased because the increased local concentration of dsDNA on the electrode surface would generate a negatively charged interface that electrostatically repels an anionic redox probe $[\text{Fe(CN)}_6]^{4-/3-}$ and form electron-transfer barrier. The hybridization reaction appears complete after about 5 min, since a plateau or maximum value of the $\Delta R_{et}$ was obtained (Fig. 3c).
3.3. Analytical performance

DNA hybridization was monitored by EIS, before and after the probe electrode dipped into the stirred solution (0.1 M PBS, pH 7.0) containing different target DNA for 5 min, while holding the potential at +0.5 V. Impedance spectra of the electrodes were measured in 2.5 mM [Fe(CN)₆]₄⁻/₃⁻ solution. The results were shown in Fig. 4. When the hybridization reaction occurs, an increase of \( R_{\text{et}} \) in the impedance spectrum, that is, in the presence of an anionic redox probe, the DNA hybridization with a nucleic acid-functionalized electrode will be accompanied by an increase in the interfacial electron-transfer resistance, \( R_{\text{et}} \), that will be reflected in the \( Z_{\text{re}} \) component of the impedance spectrum [36]. As shown in inset of Fig. 4, the calibration curve indicates that the values of \( \Delta R_{\text{et}} \) were linear with the amount of cDNA ranging from \( 8.0 \times 10^{-9} \) to \( 1.0 \times 10^{-6} \) M with a detection limit of \( 4.0 \times 10^{-9} \) M at 3\( \sigma \) (where \( \sigma \) is the standard deviation of a blank solution, \( n=11 \)). The \( \Delta R_{\text{et}} \) were recorded with each measurement repeated three times. The regression equation was \( y=1643.7x+305.61 \) (\( x \) is the concentration of target DNA, \( y \) is the \( \Delta R_{\text{et}} \)) and the regression coefficient (\( r^2 \)) of the linear curve was 0.9921.

The selectivity of this assay was explored by adding a three-base mismatch sequences oligonucleotide (\( 1 \times 10^{-8} \) M) together with the stirred cDNA (\( 8 \times 10^{-9} \) M) solution (0.1 M PBS, pH 7.0), while holding the potential at +0.5 V for 5 min. If this mismatch sequences oligonucleotide were to interfere with the analysis, obvious increase of impedance values would be received, since it would have been hybridized with probe. On the contrary, we have obtained approximate result with the probe (Fig. 5). The \( \Delta R_{\text{et}} \) was only 2.4 Ω.

![Fig. 4. Impedance curves after hybridizing with different concentrations of cDNA: (a) 0; (b) 8.0 \times 10^{-9} \) M; (c) 2.0 \times 10^{-7} \) M; (d) 4.0 \times 10^{-7} \) M; (e) 6.0 \times 10^{-7} \) M; (f) 8.0 \times 10^{-7} \) M; (g) 1.0 \times 10^{-6} \) M in a 2.5 mM [Fe(CN)₆]₄⁻/₃⁻ solution at \( 10^{-2} - 10^6 \) Hz vs. SCE. Hybridization condition: with cDNA for 5 min while holding the potential at +0.5 V. Inset: the relationship between the impedance value (\( \Delta R_{\text{et}} \)) and the concentration of cDNA.](image-url)
Another sample ssDNA (1.0 × 10⁻⁸ M) having a non-complementary sequence to the probe did not have the difference of ΔRₑᵣ. These results suggest that in this system, a good selectivity of target DNA capturing can be reached.

3.4. Stability and reproducibility of the modified electrodes

The stability of the modified electrodes was studied. The operational stability of the modified electrodes was measured by monitoring the CV in 0.5 mM Co(bpy)₃³⁺ solution response for a 3-h period of continuous use and little decrease in sensitivity was observed. To investigate the reproducibility of the current response of the modified electrode, repetitive measurements were carried out. They can be used continuously if stored in the dry state at 0–4 °C after use. Only 3.8% deterioration of peak currents was found during a 2-month storage of the modified electrode, since the B2dMPTS as a sol–gel derived matrix is cast on the electrode surface, which can actually stabilize the biomolecule and protect it from denaturation under extreme conditions [37]. By immersing the hybridized electrode in 0.01 M NaOH for 120 s, the dsDNA was denatured and an decrease of impedance values was immediately observed, which shows the regenerative characters of the DNA probes. Experiments show that this DNA biosensor can be reproduced for seven to eight times without losing its sensitivity. Thus, the modified electrodes exhibit good stable characteristics and can be used for repetitive measurements.

4. Conclusions

In this paper, we have introduced a versatile approach for the fabrication of DNA biosensor based on self-assembly B2dMPTS, and colloidal Ag can be easily assembled into B2dMPTS network and the HS-ssDNA strongly bound to the Ag nanoparticles. The
nanoparticles both inside the network and on the surface increased the surface area of the modified electrode which increases the DNA anchor. The DNA biosensor obtained has high sensitivity, good selectivity and reproducibility. In addition, the biosensors are stable with prolonged use.

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