Amplified DNA Detection Sensitivity Using Streptavidin-Biotinylated Protein Complex: Characterization by Electrochemical Impedance Spectroscopy

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Thiol-terminated oligonucleotide was immobilized to gold surface by self-assembly method. A novel amplification strategy was introduced for improving the sensitivity of DNA hybridization using biotin labeled protein-streptavidin network complex. This complex can be formed in a cross-linking network of molecules so that the amplification of the response signal will be realized due to the big molecular size of the complex. It could be proved from the impedance technique that this amplification strategy caused dramatic improvement of the detection sensitivity. These results give significant advances in the generality and sensitivity as it is applied to biosensing.

Keywords DNA hybridization, amplification, biotin-streptavidin, electrochemical impedance spectroscopy

Introduction

DNA hybridization is the noncovalent association of two single-stranded (ss) DNA molecules having complementary nucleotide sequences to form a double-stranded (ds) complex. Recently there has been increasing interest in the development of specific DNA sensors in gene analysis, detection of genetic disorders, tissue matching, forensic applications and DNA computing.[1-4] However, the binding reaction of oligonucleotide at the DNA immobilized surface is often insufficient to produce a large signal change and can not be detected by conventional methods. To overcome this drawback, several strategies have been proposed to enhance the response signal by using latex particle, colloidal Au and liposome for biomolecular interaction.[5-7]

Here we report a novel approach for the signal amplification of DNA hybridization based on streptavidin-biotinylated protein complex. In this approach, a molecule with high molecular weight was introduced to the electrode surface. It is well known that electrochemical impedance spectroscopy is an effective method to probe the interfacial properties of modified electrode and often used for understanding chemical transformations and processes associated with the conductive supports.[8-12] The adsorption or desorption of insulating species on conductive supports is anticipated to alter the interfacial electron-transfer features (capacitance and resistance) at the electrode surface. Therefore, impedance technique was used as an electrochemical sensing method in the present work. Surface plasmon resonance (SPR) technique has been used to characterize the mass change on the sensor surface.[13,14] In this paper, SPR was used to test the amplification effect. It should be pointed out that this amplification method could offer a general approach to design immunosensors or DNA sensors.

Experimental

Materials

Streptavidin, biotin and biotinylated bovine serum albumin (biotin-LC-BSA, biotin/BSA = 6) were purchased from PIERCE company (USA). Sensor chip SA, ethanolamine hydrochloride, HBS-EP buffer (BIA certified) (pH = 7.4, consisting of 10 mmol/L 4-[2-hydroxyethyl] piperazine-1-ethane-sulfonic acid, 150 mmol/L sodium chloride, 3.4 mmol/L EDTA, 0.005% (V/V) surfactant P-20), *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N'*-(3-diethylaminopropyl) carbodiimide (EDC) were obtained from Pharmacia Biosensor AB. Four types of oligonucleotides were synthesized on the DNA synthesizer from Sino-Am Biotech. Corporation. (Beijing, China): S1: HS-$(CH_2)_6$-5'-TGC AGT TCC GGT GGC-3', S2: 5'-AGA GTC TCA TGT ATC GTT CGC CGG ACC TGC CTG CAT GCC ACC GGA ACT-3', S3: 5'-ATG CAG GCA GGT CCG GCG AAC GAT ACA TGA GAC TCT AAG CGT-Biotin-3', S5: Biotin-$(CH_2)_8$-5'-TGC AGT TCC GGT GGC-3'.

All other chemicals were of reagent grade and used as

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received. Phosphate buffered saline (PBS) was used. Solutions were prepared from water that had been purified through an Ultrapure water system Milli-Q Plus (Millipore Inc.).

Preparation of the amplify complex

The amplify complex was prepared by mixing the streptavidin and biotin-BSA solution and the concentration of streptavidin in the mixture was 0.1 mg/mL and that of the biotin-BSA was 0.5 mg/mL.[15] The two chemical solutions were mixed thoroughly and placed at 4 ℃ overnight.

Electrode preparation

Gold disk electrode (1 mm diameter) was used for electrochemical measurements. The gold electrode was polished carefully with alumina slurries (1.0, 0.3, 0.05 μm), then cleaned through sonication in distilled water and absolute ethanol. Then it was subjected to cyclic potential sweeps between −0.2 and 1.5 V in 1 mol/L H_2SO_4 until a stable cyclic voltammogram was obtained.

After above treatment, the clean gold electrode was immediately immersed in a solution of 1 mmol/L thiol-terminated DNA (S1) for 4 h. Then, the immobilized S1 electrode was reacted in 0.1 μg/mL S2 solution for 30 min. After rinsed, the electrode was immersed in 1 μg/mL S3 for 30 min. Finally, the electrode was placed in the solution of amplify complex (S4) for 30 min. All the experiments were carried out at room temperature.

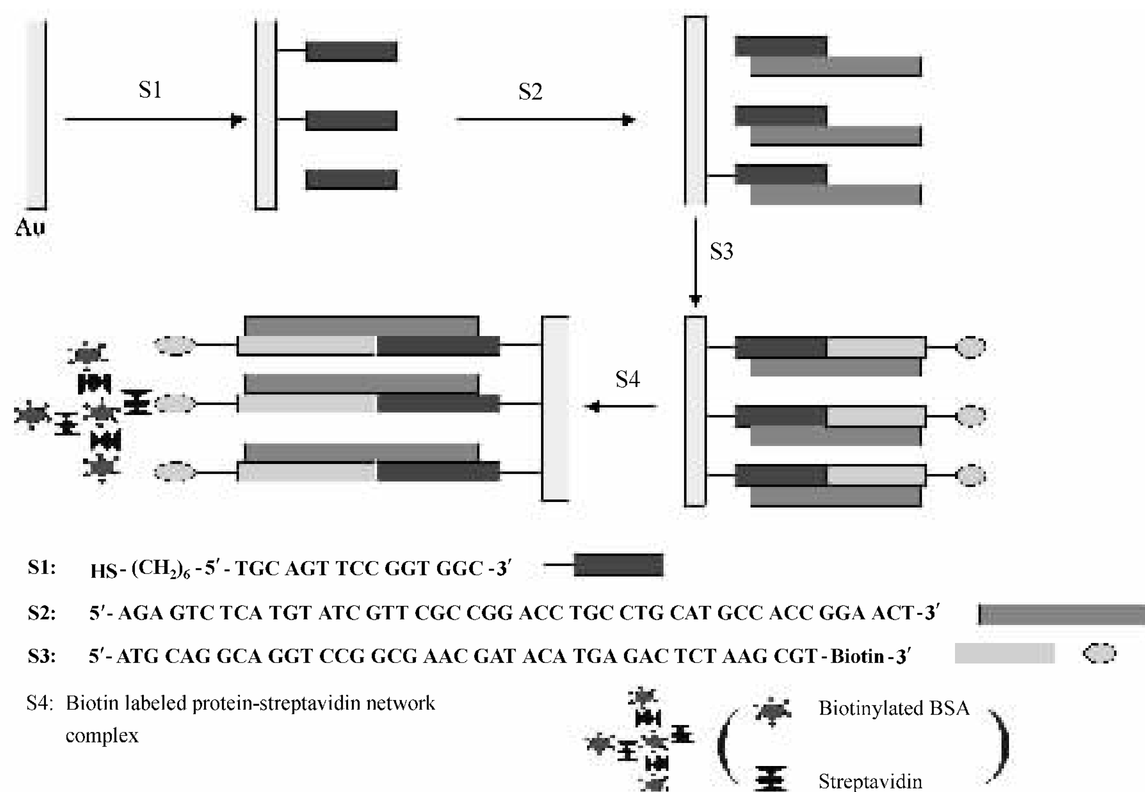
This process is shown in Scheme 1.

Electrochemical measurement

Cyclic voltammetry curves were measured with a CHI 600 voltammetric analyzer (CH instruments, USA) in a conventional three-electrode cell. The working electrodes were homemade polycrystalline gold disk electrodes. A twisted platinum wire was used as the counter electrode. An Ag/AgCl in saturated KCl solution was used as the reference electrode, against which all potentials were measured and reported.

Electrochemical impedance spectroscopy was measured by a Solartron 1250 frequency response analyzer combined with a Solartron 1286 electrochemical interface (Solartron Farnborough, UK). An IEEE-interface (National Instruments, USA) was employed to couple the two Solartron instruments with a PC 586. The impedance spectra were recorded within the frequency range of 0.1—65535 Hz. The amplitude of the applied sine wave potential in each case was 10 mV, while the d.c. potential was 0.24 V in the presence of 5 mmol/L $Fe(CN)_6^{4-/3-}$ and 0.1 mol/L KCl. The same three-electrode configuration as in the above-mentioned cyclic voltammetry experiments was used in the electrochemical impedance spectroscopy measurements.

Scheme 1 Schematic illustration of the sensing and amplified system based on the formation of an amplified complex



Surface plasmon resonance measurements

We used a BIAcore 1000 instrument (Biosensor AB, Uppsala, Sweden) SPR system with BIAcore SA (research grade) sensor chip, precoated with approximately 4000 RU [1000 RU corresponds to approximately 1 ng/mm^2] of streptavidin, in the measurements. Before use in experiments, the sensor chips were treated with several (five to six) pulses of 50 mmol/L sodium hydroxide to precondition the surface (corresponding to a decrease of approximately 600 RU). Then 20 μL, 1 μg/mL oligonucleotide S5 in HBS was injected across the sensor chip. After that, 25 μL, 10 μg/mL biotin was injected and repeated three times to block the free biotin-binding site.

Analysis of DNA hybridization and amplification was carried out by injecting 20 μL, 1 μg/mL analyte DNA S2 and then 20 μL, 1 μg/mL S3. After that 20 μL amplify complex was injected. After each measurement, the sensor chip was regenerated with 10 μL, 50 mmol/L NaOH. All the hybridized oligonucleotides were removed and the sensor chip could be used for the next cycle.

The experiments were performed at temperature of 25 ℃. The running buffer was HBS-EP (BIA Certified). The flow rate was 5 μL/min.

Results and discussion

Immobilization of thiol-terminated DNA *onto gold surface*

Several different methods for preparing DNA modified electrodes, other than simple physisorption on electrode surface, were reported previously.[13] These included attaching a thiol-modified DNA to a gold electrode, electrochemical deposition of oligonucleotide-functionalized polypyrrole, using the strong electrostatic interaction between DNA and aluminum (III) alkanebisphosphonate films, and linking the DNA to a dicarboxylate self-assembled monolayer formed previously on tin-doped indium oxide (ITO) electrode.[15] Among these techniques, the end-tagged thiol oligonucleotides modified electrode has been widely used. This immobilization between the sulfur atom and gold is very strong, and the formed self-assembled DNA monolayers are stable in air, water and organic solvents at room temperature. In the present work, this method was adopted to immobilize S1 on gold electrode.

Fig. 1 shows the CV curves of electrode. The average of the redox peak potentials is 0.24 V. So in impedance measurements we chose the d.c. potential as 0.24 V. Fig. 2 shows the results of Faradic impedance spectroscopy presented as Nyquist plots (Z_{im} versus Z_{re}) on a bare gold electrode (curve a), and DNA (S1) molecule immobilized electrode (curve b) in the presence of redox probe $Fe(CN)_6^{4-/3-}$ measured at their formal potential. The impedance spectra follow the theoretical shapes and include a semicircle portion, observed at higher frequencies, which corresponds to the electron transfer controlled process, followed by a linear part characteristic of the lower frequency attributable to a process controlled by mass transfer. The respective semicircle diameters correspond to the electron transfer resistances at the electrode surface (R_{ct}). It can be seen that the diameter of semicircle at the high frequency increases upon the immobilization of S1 to the electrode surface. Fox example, for the bare electrode R_{ct} = 1613 Ω whereas R_{ct} increases to 2877 Ω upon the immobilization of S1. Two factors resulted in this effect: one is the assembled DNA layer that decreases electroactivity of redox probe in solution, the other is the surface of immobilization of S1 that is negatively charged to repulse the negatively chrged redox probe.

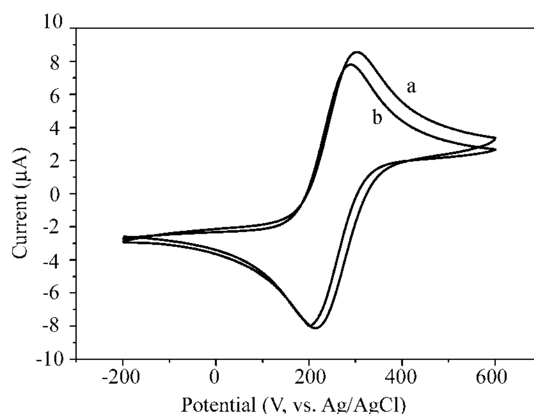


Fig. 1 Cyclic voltammogram recorded in an 5 mmol/L $Fe(CN)_6^{4-/3-}$ and 0.1 mol/L KCl solution when the measuring electrode was (a) bare gold and (b) gold modified with DNA S1. Scan rate was 100 mV/s.

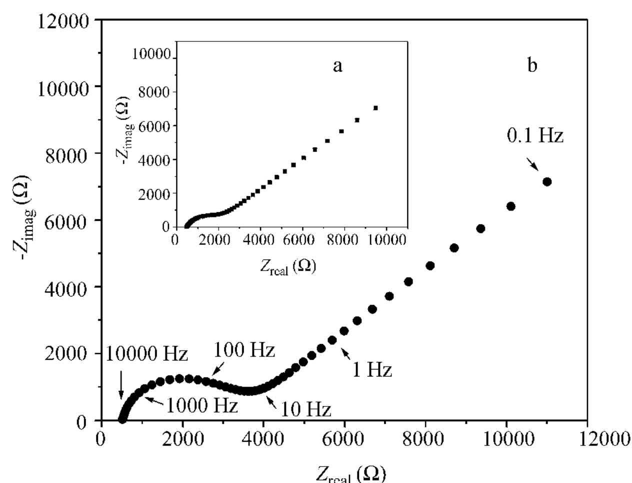


Fig. 2 Nyquist diagram for the Faradaic impedance measurement in 5 mmol/L $Fe(CN)_6^{4-/3-}$ and 0.1 mol/L KCl solution at DNA (S1) immobilized gold electrode. Inset: nyquist diagram for bare gold electrode.

From above, it could be suggested that the thiol-terminated DNA (S1) was successfully assembled on gold surface.

Amplified DNA *hybridization detected by impedance spectroscopy*

Although the impedance spectra could change upon DNA hybridization, the signal is small. In this work, a new amplification strategy was introduced for improving the sensitivity of impedance measurements using biotinylated protein-streptavidin network complex. After the DNA (S1) immobilized-electrode was immersed in the complementary DNA (S2) solution, the DNA hybridization between the immobilized DNA (S1) and the complementary DNA (S2) led to the binding of DNA (S2) on the sensing surface. Then, the biotinylated DNA (S3) bonded to the S2 in proportion. Finally, the biotinylated protein streptavidin network complex (S4) was introduced to increase the mass on the surface considerably. The binding amount of this complex was related to the amount of the biotin molecules on the surface, then also related to the amount of the analyte. This amplification strategy was based on the high affinity interaction of biotin and streptavidin. Each streptavidin has four equivalent sites for biotin. Each biotin-BSA has six biotin moieties. The tetravalency of streptavidin for biotin allows the construction of a molecular complex between streptavidin and biotin labeled protein. The complex can be formed in a cross-linking network of molecules so that only a few binding events of the analyte at the sensing surface may lead to a detectable surface mass due to the big molecular size of complex. The process of amplified DNA hybridization of the analyte is represented in Scheme 1.

Fig. 3 shows the impedance curves of amplification DNA hybridization. 0.1 μg/mL S2 reacted with the S1 immobilized gold surface (curve b), a 1 μg/mL biotinylated DNA (S3) was further introduced to the surface (curve c). A substantial increase in the diameter of the semicircle could be observed when biotin labeled protein-streptavidin network complex interacted with biotinylated antibody (curve d).

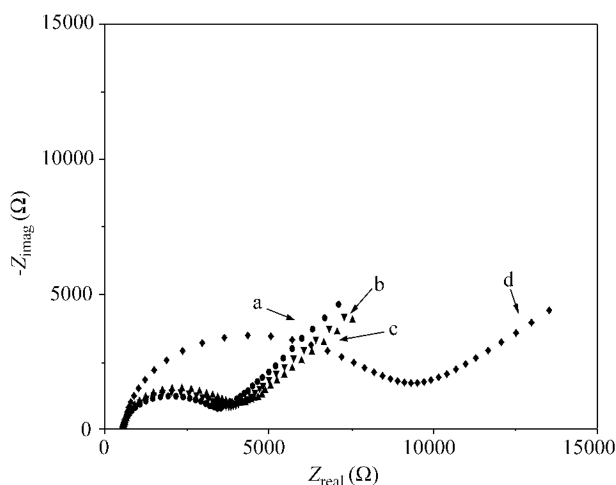


Fig. 3 Nyquist diagram for the Faradaic impedance measurement in 5 mmol/L $Fe(CN)_6^{4-/3-}$ and 0.1 mol/L KCl solution at (a) Au/S1; (b) Au/S1 + S2; (c) Au/S1 + S2 + S3 and (d) Au/S1 + S2 + S3 + S4.

In order to view the amplification effect clearly, the values of electron-transfer resistance for each step were calculated, as shown in Fig. 4. The DNA hybridization between the immobilized DNA (S1) and the analyte DNA (S2) led to 122 Ω response change. Then, 1 μg/mL biotinylated DNA (S3) was introduced and led to 155 Ω response change. Finally, after adding the streptavidin-biotinylated protein complex, the response signal was about 4866 Ω. The total response signal of enhanced DNA hybridization based on the specific binding of DNA (S2) was 5143 Ω, about 42 times higher than the primary response (122 Ω).

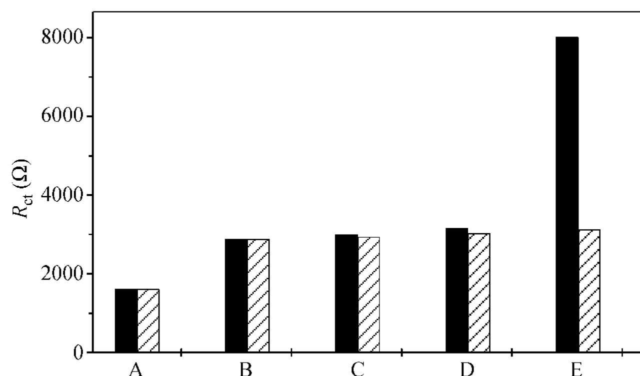


Fig. 4 Electron transfer resistance, R_{ct}, in the procedure of DNA immobilization and amplified hybridization. The columns with slant line represent the control experiments. The solid columns represent the experiment with analyte S2. The values were extracted from the impedance spectra in Fig. 1 and Fig. 2. (A) bare gold electrode; (B) S1 immobilized electrode; (C) after reaction with 0.1 μg/mL S2; (D) after the following incubation with 1 μg/mL biotinylated S3; (E) after the final incubation with biotinylated protein-streptavidin network complex. The values were extracted from the impedance spectra in Fig. 1 and Fig. 2.

To verify that binding of amplified complex to the surface was due to hybridization of complementary strands, control experiments were performed to reveal high selectivity and specificity of enhanced DNA hybridization detection. It was started with reaction of 1 mg/mL oligonucleotide which is non complementary to DNA (S1). The same enhanced operations were applied in following steps. No obvious changes occurred in the impedance response of the S1 immobilized surface with protein-streptavidin network complex (Fig. 4). This result indicates that the change in impedance spectra upon adding amplified complex resulted from a specific hybridization to surface-confined S2, rather than nonspecific adsorption.

Amplified DNA *hybridization detected by surface plasmon resonance*

The BIAcore instrument based on SPR principle was

able to measure the mass change on the sensor chip in real time. The sensorgram of DNA hybridization and signal amplification is shown in Fig. 5. The immobilized DNA S5 has the same sequence as that of DNA S1 used in electrochemical experiments. The DNA hybridization between the immobilized DNA (S5) and the analyte DNA (S2) led to 26 RU response change. Then, biotinylated DNA (S3) was introduced and led to 50 RU response increase. Finally, after adding the amplify complex, the response signal was about 580 RU. The total response signal of enhanced DNA hybridization based on the specific binding of DNA (S2) was 660 RU, about 25 times higher than the primary response (26 RU).

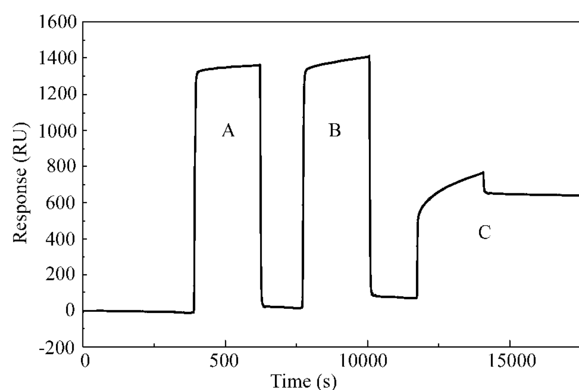


Fig. 5 Real time sensorgram of DNA hybridization and amplification detected by BIAcore 1000. The running buffer was HBS-EP(BIA Certified). The flow rate was 5 μL/min. Part A is the injection of sample S2. Part B is the injection of S3. Part C is the injection of amply complex.

Conclusion

In conclusion, we have demonstrated a novel enhanced DNA hybridization method by electrochemical impedance spectroscopy and surface plasmon resonance. It is indicated that the amplification strategy using streptavidin-biotinylated protein complex causes a dramatic improvement of the reaction signal compared to normal reaction signal. The sensing process also showed specificity. The method can be generally applicable to enhanced assay of other biomolecules and other transduction means.

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