A NOVEL BIOSENSOR OF DNA IMMOBILIZATION ON NANO-GOLD MODIFIED ITO FOR THE DETERMINATION OF MIPEPRISTONE

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A novel DNA modified indium tin oxide (ITO) electrode has been prepared. (3-Aminopropyl)Trimethoxysilane, gold nanoparticles and DNA molecules are modified on the ITO electrode surface by self-assembly and electrochemical techniques, respectively. This is a simple, stable, repeatable approach. The modified electrode can be used to detect mifepristone. A linear dependence of the peak currents on mifepristone concentrations is observed in the range $4 \times 10^{-7}$-$6 \times 10^{-6}$ mol/L. The relative standard deviation is 4.5% for six successive determinations at $1 \times 10^{-6}$ mol/L solution. The detection limit is $2 \times 10^{-7}$ mol/L.
**INTRODUCTION**

It is of great interest to research the interaction between DNA and other molecules in biology and biochemistry, because such research will aid the progress of society in the new century. As an important fundamental issue in life sciences, it is related to the replication and transcription of DNA in vivo, mutation of genes and related variations of species in character, action mechanisms of some DNA-targeted drugs, origins of some diseases, and action mechanisms of some synthetic chemical nucleases etc. An investigation of DNA modified electrodes capable of molecular recognition is essential in order to design electrochemical DNA biosensors for gene detection. DNA-modified electrodes are also being used to study the interactions of DNA with other molecules. DNA-modified electrodes have been applied to analytical purposes over the last decade. Wang and coworkers have prepared a DNA biosensor for the detection of hydrazines. Pang and coworkers have developed a method to determine the trace Co$^{2+}$ ion by using the highly sensitive response of the DNA-modified gold electrode to the Co(bpy)$_3^{3+}$ ion and studied the interaction under an antitumor drug by using DNA-modified glassy carbon electrode. However, it is also an interesting field to prepare novel DNA-modified electrodes in a simple, stable and repeatable approach.

The properties of nano-scale gold and bulk exhibit several differences. More noteworthy are the surface charge, high surface area and biocompatibility of nano-gold (NG). The formation of self-assembled monolayers on the electrode surface has enabled the design of new interfaces for the study of specific redox-active analysis, solar energy conversion, and fundamental electrochemistry.

In this work, a novel approach has been developed to prepare a DNA modified electrode. (3-Aminopropyl) trimethoxysilane is primarily modified on the electrode surface by a self-assembly technique, then gold nanoparticles are absorbed on the surface by static electricity interaction: finally the DNA molecules are immobilized on nano-gold modified ITO electrode by electrochemical technique. This modified electrode can be used to detect mifepristone. A linear dependence of the peak currents on mifepristone concentrations is observed in the range of $4 \times 10^{-7}$-6$ \times 10^{-6}$ mol/L by differential pulse voltammetry (DPV). The relative standard derivation is 4.5% for six successive determinations at $1 \times 10^{-6}$ mol/L. The detection limit is $2 \times 10^{-7}$ mol/L.
EXPERIMENTAL

Reagent and Solution

Calf thymus DNA (CT-DNA) and HAuCl$_4$ were obtained from Sigma Company, and were used as received. Native double stranded DNA (dsDNA) was dissolved in 0.02 mol/L phosphate buffer (pH 7.0) prior to use. Tris(2,2'-bipyridyl)-Cobalt(III) perchlorate [Co(bpy)$_3$(ClO$_4$)$_3$] was prepared as reported(10). (3-Aminopropyl) trimethoxysilane was obtained from Nanjing Shuguang Chemical Factory (China). Mifepristone was obtained from Shanghai Hualian Pharmaceutical Company (Shanghai, China), and its stock solution was prepared in 0.05mol/L phosphate buffer solution containing 30% ethanol (pH7.0). Other chemicals were of analytical reagent grade. Water used was twice distilled.

Apparatus

Differential pulse voltammetry (DPV) was performed with a CHI660 Instrument (CHI Co. USA). ITO (one side only) glass slides (Delta Technologies) had a resistance negative 20Ω. Three-electrode system was employed with saturated calomel reference electrode (SCE), ITO working electrode and platinum auxiliary electrode. Transmission electron micrographs were obtained with a JEOL-JEM 200CX electron microscope (TEM).

Preparation of the Au Colloid

All glassware used in the preparations was thoroughly cleaned in aqua regia (HCl: HNO$_3$ = 3:1), rinsed in twice distilled H$_2$O. In a 1 L round-bottom flask equipped with a condenser, 500mL of 0.01% H AuCl$_4$ was brought to a boil with vigorous stirring. 7.5mL of 1% sodium citrate was added into this solution. The solution turned blue in 25s; the final color change to red-violet occurred after 70s. Boiling continued for an additional 10 min, the heating source was removed, and the colloid was stirred for another 15 min(11). TEM data indicated that the gold particles were monodisperpersed with an average diameter of ca.18nm.
Preparation of the Modified Electrode

Electrode ITO-coated sheets were cut into 0.5cm×1.0cm squares. The ITO electrodes were cleaned using the following procedure: sonication in acetone for 20 min, sonication in soap solution for 15 min, and sonication twice in water for 15 min each cycle. The electrodes were then treated with 5M NaOH solution for 5 h prior to rinsing with water and derivatization in an aqueous solution of 1% (3-Aminopropyl) trimethoxysilane for 15 min to form a self-assembled monolayer. Electrodes were rinsed in water and allowed to dry in air. The electrodes were incubated in Au colloids solution 10 h to form a gold nanoparticle layer. Then the electrode was immersed in the DNA solution for accumulation of CT-DNA for 10 min at 1.5 V. Finally, the electrode was rinsed with water to remove unabsorbed molecules and a DNA immobilization on nano-gold modified ITO electrode (DNA/NG/ITO) was prepared successfully.

Procedure

The DPV curves were recorded from −0.2 V to 0.4 V in [Co(bpy)₃³⁺] solution with a pulse width of 0.06 s and a pulse amplitude of 50 mV. Others were recorded from 0.0 V to 0.9 V in mifepristone solution with the same conditions. After each measurement, the modified electrode was cleaned by thoroughly scanning the electrode in the bare solution from 0.0 V to 0.9 V.

RESULTS AND DISCUSSION

Electrochemical Behavior of DNA/NG/ITO Electrode

Because [Co(bpy)₃³⁺] is able to interact strongly with DNA at low ionic strengths, it can be used to characterize DNA-modified ITO electrodes(1). The differential pulse voltammetry (DPV) of 50 umol/L [Co(bpy)₃³⁺] with the DNA/NG/ITO and NG/ITO electrode are displayed in Fig.1(a) and Fig.1(b), respectively. It can be found that the response of [Co(bpy)₃³⁺] at the DNA/NG/ITO electrode is well defined and much higher than that obtained at the NG/ITO electrode. Such a response enhancement corresponds to [Co(bpy)₃³⁺] preconcentrated at the surface-bonded dsDNA, which is in good agreement with the reference(12). In order to examine the stability of the DNA modified electrode, the peak current only decreases 10% after a modified electrode has been kept in the refrigerator for 12 h. This shows that the modified electrode can be used for analytical purposes.
We have investigated the influence of the accumulation potential on a DNA modified electrode. The result is displayed in Fig. 2. We find that the DNA cannot be accumulated on the nano-gold when the potential is under 1.2 V. When the potential reaches 1.5 V, the peak current increases quickly, which shows the DNA has been modified at the ITO electrode(9).

In order to obtain the best accumulation time, the DPV graphs of the different time are displayed in Fig. 3, which shows that the peak current increases step by step accompanied by accumulation times up to 10 min, and the peak current achieved a stable-state current after 10 min. The peak potential shifts negative from +0.068 mV to 0.048 mV in this course. According to the experimental results, 10 min is selected as the best accumulation time.

**Figure 1.** Differential pulse voltammetry of 50 μmol/L Co(bpy)$_3^{3+}$ on DNA modified electrode in pH7.0 phosphate buffer solution. Pulse width: 0.06s; pulse amplitude: 50mV. (a): DNA/NG/ITO; (b): NG/ITO.

Determination of Mifepristone

Mifepristone is a kind of progesterone receptor compound, mainly used in anti-pregnancy. Its analytical methods include HPLC(13), radioreceptor assay(14) and adsorptive stripping voltammetry at a mercury electrode(15). It has been reported that mifepristone can be intercalated into a DNA double helix(16).

As a kind of sensitive electrochemical technique, differential pulse voltammetry is employed to determine trace mifepristone in the range of...
Figure 2. Differential pulse voltammetry of 50 μmol/L Co(bpy)$_3^{3+}$ on DNA modified electrode in different accumulation potential. Other conditions as in Fig.1. (a): 1.0V; (b): 1.2V (c): 1.5V.

Figure 3. Differential pulse voltammetry of 50 μmol/L Co(bpy)$_3^{3+}$ on DNA modified electrode in different accumulation time. Other conditions as in Fig.1. (a): 3min; (b): 6min; (c): 9min; (d): 12min; (e): 15min.
0.0-0.9 V. Fig.4 shows the difference between the NG/ITO electrode and DNA/NG/ITO electrode. The peak current of curve(b) is obviously larger than the curve(a). At a DNA/NG/ITO electrode, mifepristone accumulates onto the surface through interaction with immobilized DNA and causes an increased oxide peak.

Fig.5 shows the plot of the anodic peak current on mifepristone concentrations observed in the range 4×10^{-7}-6×10^{-6} mol/L. The relative standard deviation is 4.5% for six successive determinations at 1×10^{-6} mol/L. The detection limit is 2×10^{-7} mol/L. Because this modified electrode is easy-made, stable, sensitive, reproducible and does not require deoxygenation before electrochemical tests, it is suitable for the determination of mifepristone.

CONCLUSION

In this paper, a novel DNA modified ITO electrode is prepared. The character of nano-scale gold is the surface charge, high surface area and biocompatibility, and the merit of self-assembled monolayers is that the new
interface can be designed for the study of specific redox-active analytes. It is a simple and reproducible method to design a new kind of biosensor. We use the DNA modified electrode as a sensitive biosensor for the detection of mifepristone. Satisfactory results are obtained.

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