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Electrochemical DNA biosensor based on silver nanoparticles/poly(3-(3-pyridyl) acrylic acid)/carbon nanotubes modified electrode

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ABSTRACT

In this work, we present an electrochemical DNA sensor based on silver nanoparticles/poly(*trans*-3-(3-pyridyl) acrylic acid) (PPAA)/multiwalled carbon nanotubes with carboxyl groups (MWCNTs–COOH) modified glassy carbon electrode (GCE). The polymer film was electropolymerized onto MWCNTs–COOH modified electrode by cyclic voltammetry (CV), and then silver nanoparticles were electrodeposited on the surface of PPAA/MWCNTs–COOH composite film. Thiol group end single-stranded DNA (HS–ssDNA) probe was easily covalently linked onto the surface of silver nanoparticles through a 5′ thiol linker. The DNA hybridization events were monitored based on the signal of the intercalated adriamycin by differential pulse voltammetry (DPV). Based on the response of adriamycin, only the complementary oligonucleotides gave an obvious current signal compared with the three-base mismatched and noncomplementary oligonucleotides. Under the optimal conditions, the increase of reduction peak current of adriamycin was linear with the logarithm of the concentration of the complementary oligonucleotides from 9.0×10^{-12} to 9.0×10^{-9} M with a detection limit of 3.2×10^{-12} M. In addition, this DNA sensor exhibited an excellent reproducibility and stability during DNA hybridization assay.

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Nowadays, specific sequences DNA detection has become a most important research field due to its application in disease diagnosis, drug screening, epidemic prevention, and environmental protection. A variety of techniques have been developed for the detection of DNA hybridization, including fluorescent [1,2], radiochemical [3,4], piezoelectric [5], surface plasmon resonance spectroscopy [6,7], quartz crystal microbalance [8,9], and electrochemical [10–13] methods. Among these, the electrochemical method has many advantages compared with other existing technologies. First, it does not require the use of hazardous radioactive labels that are indispensable in traditional hybridization-based isotopic detection methods such as Southern blot (DNA blot). Second, electrochemical detection is usually more specific than label-free methods (e.g., surface plasmon resonance spectroscopy, piezoelectric technology). Third, unlike fluorescent dyes, electroactive (redox) labels are relatively stable and usually insensitive to photobleaching. Therefore, the electrochemical technique is very attractive in DNA hybridization detections. To date, a variety of approaches have been explored in electrochemical detection of DNA hybridization. Some reviews about electrochemical DNA sensors have been reported recently [14–20].

Electrochemical DNA sensors generally contain three components: (i) a solid electrode (e.g., gold, glassy carbon electrode [GCE]), (ii) capture DNA probes, and (iii) electroactive (redox) labels. The sensitivity and lifetime of DNA sensors depend on the immobilization of DNA probes onto electrode surfaces. Several methods for immobilizing DNA probes onto electrode surfaces have been reported, including physical adsorption, entrapment in a gel or polymer, covalent binding, cross-linking, and electrochemical polymerization. One of the promising approaches is electrochemical polymerization to use conducting polymers, such as polypyrrole [21,22], and this kind of DNA biosensor can give good sensitivity and stability.

Carbon nanotubes (CNTs),[1] discovered in 1991 by Iijima [23], represent an important group of nanoscale materials. They have been widely recognized as an ideal support for fabricating electrochemical sensors [24–27]. Among them, the biosensors based on hybrid composite of CNTs and conducting polymers have received significant interest because the composite materials possess the properties of each component with a synergistic effect [28,29]. Another novel approach is to employ hybrid composite film of metal nanoparticles and CNTs such as copper, gold, platinum, silver, and

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[1] *Abbreviations used:* CNT, carbon nanotube; GCE, glassy carbon electrode; PPAA, poly(*trans*-3-(3-pyridyl) acrylic acid); MWCNTs–COOH, multiwalled carbon nanotubes with carboxyl groups; DPV, differential pulse voltammetry; PAA, *trans*-3-(3-pyridyl) acrylic acid; SDS. sodium dodecyl sulfate; CV, cyclic voltammetry; SCE, saturated calomel electrode; ss-DNA, single-stranded DNA; ds-DNA, double-stranded DNA; SEM, scanning electron microscope; *S*/*N*, signal/noise; RSD, relative standard deviation.

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palladium nanoparticles that have been deposited on the CNTs [30–34]. These kinds of biosensors can remarkably improve the sensitivity of DNA sensors. For example, Fang and coworkers have used Pd_{nano}–CNTs [35] and Pt_{nano}–CNTs [36] composite film to fabricate DNA sensors that show a higher sensitivity.

Here we combine the feature of metal nanoparticles and composite film of CNTs–conductive polymeric film and hope to fabricate a more sensitive DNA sensor. In this work, we have fabricated an Ag_{nano}/poly(*trans*-3-(3-pyridyl) acrylic acid) (PPAA)/multiwalled carbon nanotubes with carboxyl groups (MWCNTs–COOH) modified electrode. Probe DNA was immobilized on Ag_{nano}–composite film through a 5′ thiol linker. Differential pulse voltammetry (DPV) was used to detect hybridization events using adriamycin as an electroactive indicator. The experiment results showed that the DNA sensor was of good reproducibility. The approach to probe DNA immobilization and hybridization with target oligonucleotides is illustrated in Scheme 1.

Materials and methods

Reagents

Adriamycin, *trans*-3-(3-pyridyl) acrylic acid (PAA), and sodium dodecyl sulfate (SDS) were purchased from Alfa Aesar (Tianjing, China). $AgNO_3$ was purchased from Aldrich. NaOH, HNO_3, $NaNO_3$, and H_3PO_4 were obtained from Nanjing Chemical Reagent (Nanjing, China). MWCNTs–COOH (diameter 20–30 nm, length 30 mm, purity >95%) were obtained from Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences, and used without further purification. The various oligonucleotides were purchased from Shanghai Sangon Bioengineering (Shanghai, China), and their sequences are as follows:

Probe DNA: SH-$(CH_2)_6$-5′-AAG CGG AGG ATT GAC GAC TA-3′
Complementary oligonucleotides: 5′-TAG TCG TCA ATC CTC CGC TT-3′
Noncomplementary oligonucleotides: 5′-AAG CGG AGG ATT GAC GAC TA-3′
Three-base mismatched oligonucleotides: 5′-TAG ACG TCA TTC CTC CCC TT-3′.

Stock solutions of oligonucleotides were prepared with PBS solution (pH 7.0) and stored in a freezer. The following buffer solutions were used: 0.01 M PBS (0.1 M NaCl + 0.01 M phosphate buffer solution, pH 7.0) and 0.1 M PBS (pH 7.0). All chemicals were of analytical grade and used without further purification. All solutions were prepared with doubly distilled water.

Apparatus

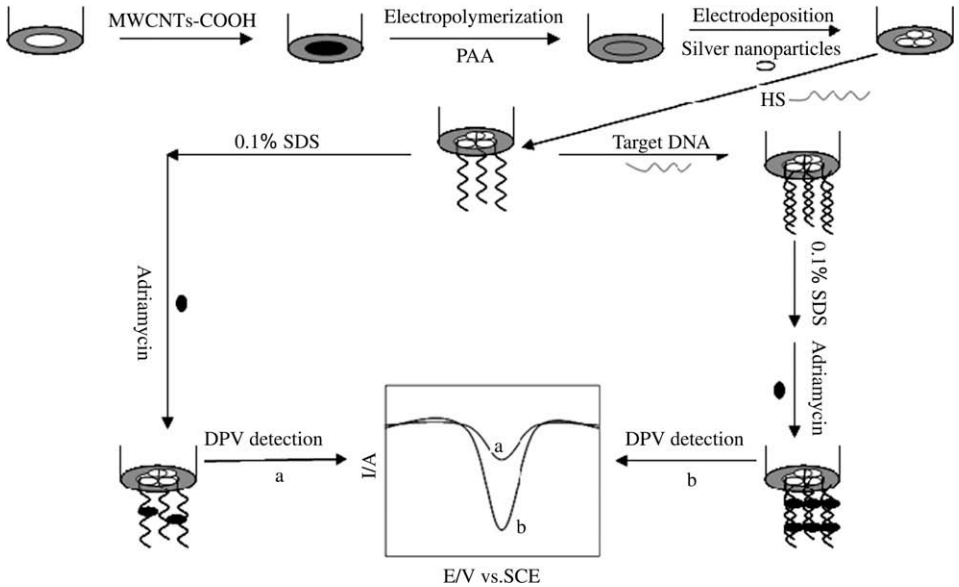
Cyclic voltammetry (CV) and DPV were performed on a CHI 650 C electrochemical workstation (Shanghai Chenhua Instruments, China). The three-electrode system was used in the experiment with bare GCE or modified electrode as working electrode, a saturated calomel electrode (SCE) as reference electrode, and a platinum wire as counter electrode. All electrochemical measurements were carried out in a 10-ml electrochemical cell, where oxygen was removed with high-purity nitrogen for 30 min and a blanket of nitrogen was maintained over the solution during the measurements. All potentials given in this article are referred to SCE.

Fabrication of ss-DNA/Ag_{nano}/PPAA/MWCNTs–COOH modified electrode

Prior to modification, the bare GCE was orderly polished to a mirror-like surface with 1.0, 0.3, and 0.05 μm α-Al_2O_3. Then the electrode was successively washed ultrasonically with anhydrous ethanol and doubly distilled water for 3 min.

MWCNTs–COOH (1.0 mg) were dispersed in 10 ml of anhydrous ethanol with the aid of ultrasonic agitation to give a black suspension. Then 10 μl of MWCNTs–COOH suspensions was dropped onto the fresh GCE surface and dried naturally at room temperature to form MWCNTs–COOH film. After that, it was immersed into doubly distilled water for 5 min to remove loosely adsorbed CNTs. The electropolymerization of PAA on modified electrode with MWCNTs–COOH was performed in 0.1 M PBS solution containing 1.0×10^{-3} PAA by cyclic potential scanning from −1.2 to +2.2 V for 15 cycles with a scan rate of 50 mV/s. The obtained electrode was donated as PPAA/MWCNTs–COOH/GCE.

Silver electrochemical deposition was performed in 3.5×10^{-3} M $AgNO_3$/0.1 M $NaNO_3$ solution and electrodeposition



Scheme 1. Schematic representation of the immobilization and hybridization detection of probe DNA.

300 s at 0.25 V (vs. SCE). The fabricated electrode was donated as Ag_{nano}/PPAA/MWCNTs–COOH/GCE.

Immobilization of the probe DNA on the electrode surface is described as follows. After 10 μl of 1.0×10^{-6} M probe DNA was dropped on the surface of Ag_{nano}/PPAA/MWCNTs–COOH/GCE and it was dried at 4 °C in a refrigerator, the electrode was immersed into 0.1% SDS solution for 10 min to wash out the unimmobilized probe DNA. The probe modified electrode was donated as single-stranded DNA (ss-DNA)/Ag_{nano}/PPAA/MWCNTs–COOH/GCE.

Electrochemical detection of DNA hybridization

DNA hybridization was performed by immersing probe modified electrode into 0.01 M PBS solution containing different concentrations of complementary oligonucleotides for 60 min at 40 °C. The hybridized double-stranded DNA (ds-DNA)/Ag_{nano}/PPAA/MWCNTs–COOH modified electrode was then rinsed with 0.01 M PBS solution for 10 s to remove the unhybridized oligonucleotides. After that, the hybridized ds-DNA/Ag_{nano}/PPAA/MWCNTs–COOH modified electrode was immersed into 0.1% SDS for 10 min. Then the hybridized electrode was immersed into 0.01 M PBS solution containing 1.0×10^{-6} M adriamycin for 45 min, followed by rinsing with doubly distilled water and 0.01 M PBS three times to remove uninteracted adriamycin.

The DNA hybridization was assessed with the DPV peak current of adriamycin in 0.1 M PBS (pH 7.0). The concentration of target oligonucleotides was quantified by the increase of reduction peak current (ΔI) of adriamycin, which was subtracted from the reduction peak current generated at ss-DNA/Ag_{nano} /PPAA /MWCNTs–COOH modified electrode.

Regeneration of the electrochemical DNA sensor

Regeneration of the DNA sensor was performed by immersing the sensor into 1:1 HNO_3 solution for 15 min, and then it was washed with doubly distilled water. After that, the electrode was characterized in 0.5 M $NaNO_3$ solution by CV; if no oxidation/reduction peaks were observed on CVs, this showed that the silver nanoparticles thin film was fully removed. New ss-DNA/Ag_{nano}/PPAA/MWCNTs–COOH modified electrode was fabricated according to the above description.

Results and discussion

Fabrication of Ag_{nano}/PPAA/MWCNTs–COOH modified electrode

Electropolymerization of PAA and preparation of silver nanoparticles

CV was used to form the polymerization film. Fig. 1 shows the repetitive cyclic voltammograms for PAA in 0.1 M PBS containing 1.0×10^{-3} M PAA at the MWCNTs–COOH modified electrode. A pair of redox peaks was obviously observed at +1.315 V (trace a) and −0.695 V (trace b) at the first cyclic scan. Then larger peaks were observed on continuous scanning (this can be clearly observed at −0.695 V), reflecting the continuous growth of the film. When the cyclic potential was scanned up to 15 cycles, the peak currents hardly grew. If the potential scan was further performed from −1.2 to +2.2 V, the polymer reaction did not occur further. After the electropolymerization process ended, a uniform adherent blue polymer film was observed on the MWCNTs–COOH surface. These facts indicated that PAA was successfully deposited on the surface of MWCNTs–COOH through the electropolymerization mode.

In this work, we used the electrochemical deposition method for preparation of silver nanoparticles. Deposition potential was

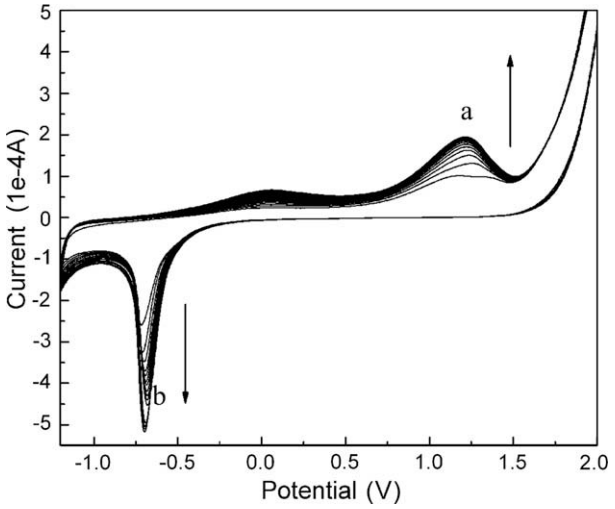


Fig. 1. Repetitive cyclic voltammograms of 1.0×10^{-3} M PAA in 0.1 M PBS solution (pH 7.0) at MWCNTs–COOH modified electrode: (a) oxidation peak of PAA; (b) reduction peak of PAA. Terminal potential: +2.2 V; initial potential: −1.2 V; sensitivity: 1.0×10^{-4} A/V; scan rate: 50 mV/s.

selected through the CV method. In cyclic voltammograms, a pair of redox peaks was observed with a reduction peak at +0.251 V and an oxidation peak at +0.455 V in 3.5×10^{-3} M $AgNO_3$/0.1 M $NaNO_3$ solution (not shown). Therefore, +0.25 V was selected as the deposition potential of silver to prepare silver nanoparticles.

Evaluation of Ag_{nano}/PPAA/MWCNTs–COOH

Fig. 2 displays the scanning electron microscope (SEM) pictures of the MWCNTs–COOH, PPAA/MWCNTs–COOH, and Ag_{nano}/PPAA/MWCNTs–COOH on GCE surface. From Fig. 2A, it can be seen that the MWCNTs–COOH were distributed very homogeneously on the GCE. Because the PAA was electropolymerized on the surface of electrode modified with MWCNTs–COOH, the amount of CNTs was less and a thin layer of PPAA was covered on the surface (Fig. 2B). When silver nanoparticles were electrodeposited on PPAA/MWCNTs–COOH, apparently a mount of silver nanoparticles was observed on PPAA/MWCNTs–COOH film surface (Fig. 2C), providing an easier way to immobilize the probe DNA with thiol groups at the 5′ end.

Electrochemical characterization of the different modified electrodes

Fig. 3A compares cyclic voltammograms of 1.0×10^{-6} M adriamycin at different electrodes. Fig. 3B shows corresponding histograms of the reduction peak currents. It can be seen from Fig. 3A that a pair of weak redox peaks of adriamycin was observed at the bare GCE (trace a) and Ag_{nano}/GCE (trace b). For Ag_{nano}/PPAA/GCE (trace c), the redox peak currents of adriamycin were greatly enhanced. Importantly, when MWCNTs–COOH were present, the peak response of adriamycin was remarkably improved (trace d). In addition, the immobilization of probe DNA on the Ag_{nano}/PPAA/MWCNTs–COOH modified electrode surface resulted in an increase in the peak currents of adriamycin that was attributed to the electrostatic interaction between the negatively charged phosphate backbone of DNA and adriamycin (trace e). This result also indicated the silver nanoparticles thin film could provide a well platform for probe DNA immobilization. The quantities of probe DNA immobilized on the electrode surface can be greatly improved.

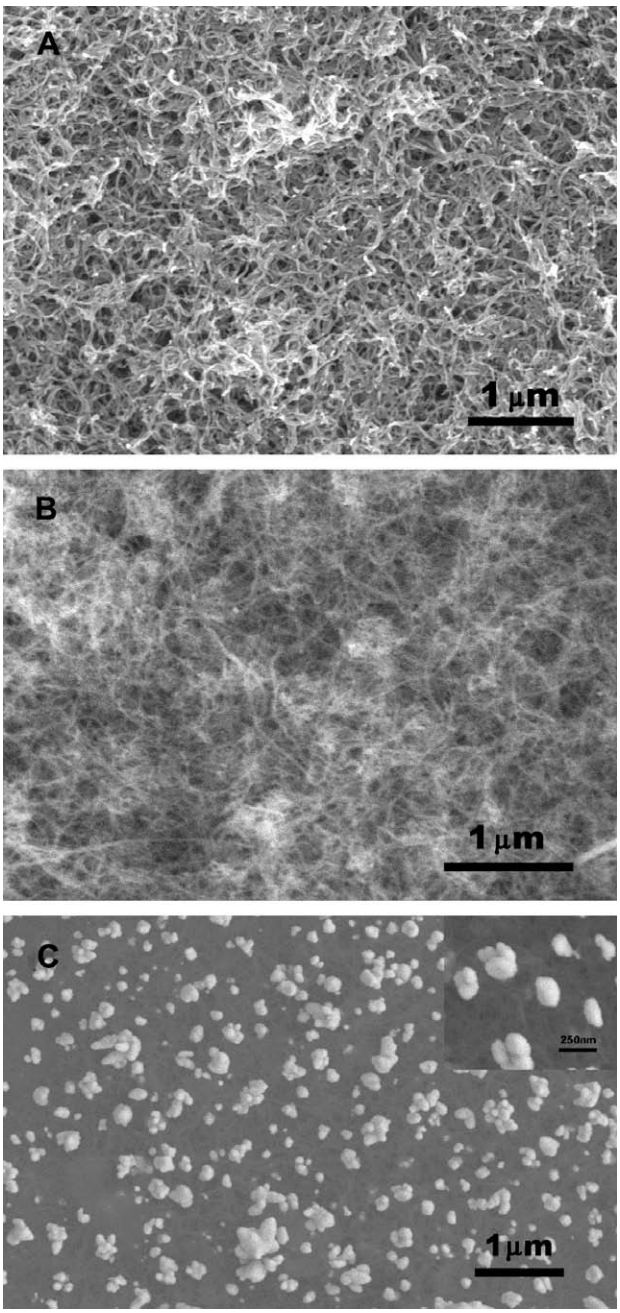


Fig. 2. SEM pictures of MWCNTs–COOH (A), PPAA/MWCNTs–COOH (B), and Ag_{nano}/PPAA/MWCNTs–COOH (C) on GCE surface. A higher magnification SEM picture of Ag_{nano}/PPAA/MWCNTs–COOH is shown in the inset of panel C. Silver deposition time: 300 s.

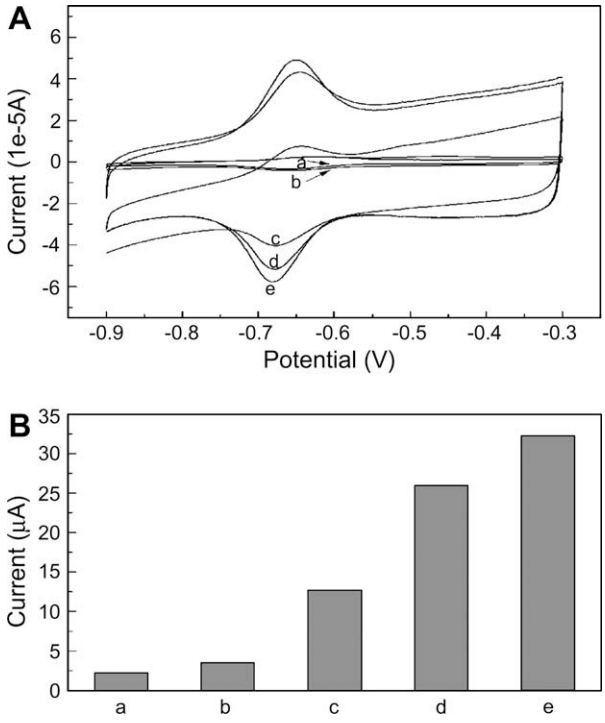


Fig. 3. (A) Cyclic voltammograms of 1.0×10^{-6} M adriamycin at the bare GCE (a), the Ag_{nano} (b), the Ag_{nano}/PPAA (c), the Ag_{nano}/PPAA/MWCNTs–COOH (d), and the ss-DNA/Ag_{nano}/PPAA/MWCNTs–COOH (e) modified GCE. Support electrolyte: 0.1 M PBS (pH 7.0); scan rate: 100 mV/s. (B) Corresponding histograms of the reduction peak currents.

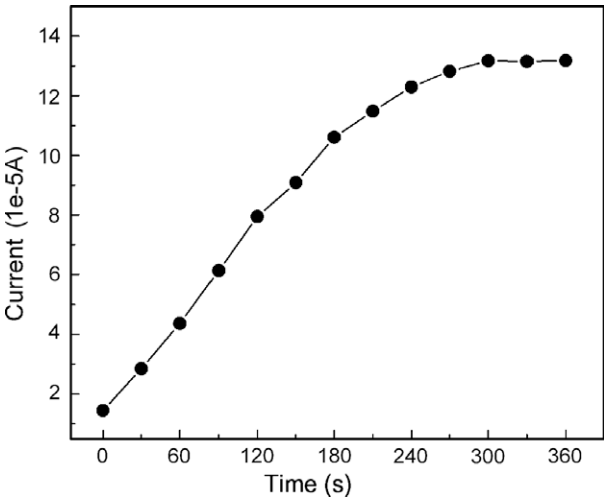


Fig. 4. Effect of Ag deposition time on DPV signals of adriamycin. Silver electrochemical deposition was performed in 3.5×10^{-3} M $AgNO_3$/0.1 M $NaNO_3$ solution at 0.25 V (vs. SCE) at PPAA/MWCNTs–COOH/GCE at various times (t = 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, and 360 s). DPV measurement was performed at an applied potential from −0.3 to −0.9 V in 0.1 M PBS (pH 7.0) containing 4.0×10^{-6} M adriamycin. Experiment condition: Ag_{nano}/PPAA/MWCNTs–COOH/GCE as for working electrode; pulse amplitude: 50 mV; pulse period: 0.2 s; pulse width: 50 ms.

Optimization of experiment conditions

Optimization of silver deposition time

To obtain a much larger effective surface area of electrode and provide a good environment for probe DNA immobilization and hybridization, silver deposition time was optimized by using CV in 0.1 M PBS solution containing 4.0×10^{-6} M adriamycin. Fig. 4 shows the relationship between the reduction peak current of adriamycin and deposition time. From this figure, it can be observed that the reduction peak current of adriamycin increased as deposition time increased from 0 to 300 s. When the deposition time was 300 s, the reduction peak current of adriamycin reached the maximum value. When the deposition time exceeded 300 s, the reduction peak current of adriamycin decreased slightly. So, 300 s was selected as the optimal deposition time to deposition Ag.

Optimization of probe DNA immobilization

In the process of electrochemical DNA sensor preparation, the immobilization of probe DNA on the electrode surface is a crucial step because densities of probe DNA directly affect the perfor-

mance of the sensor. In this work, the DPV technique was used to investigate the effect of immobilization amount of the probe DNA on DNA hybridization, and the difference of the reduction peak current of adriamycin before and after hybridization was used as analytical signal. Different volumes of 1.0×10^{-6} M probe DNA (4, 7, 10, and 13 μl) were dropped onto the surface of Ag_{nano}/PPAA/MWCNTs–COOH/GCE for electrochemical detection of DNA hybridization according to the above description (hybridization time was 20 min, and accumulation time of adriamycin was 5 min). The DPV signals for 4-, 7-, 10-, and 13-μl volumes were 1.843×10^{-5}, 2.037×10^{-5}, 2.163×10^{-5}, and 1.867×10^{-5} A, respectively. It can be seen that the peak current value was maximal at 10 μl. Thus, 10 μl of probe solution was used to fabricate probe modified electrode in experiments.

Accumulation time of adriamycin

The accumulation time of electroactive indicator was an important effect factor for sensitivity of DNA sensors. As is known, adriamycin is an effective antitumor agent that has a strong affinity to the DNA double helix by intercalating CG–GC steps [37]. Therefore, there is a maximum accumulation amount of adriamycin for a constant ds-DNA. The accumulation time of adriamycin was investigated by incubating hybridized electrode in 0.01 M PBS (pH 7.0) containing 1.0×10^{-6} M adriamycin. As shown in Fig. 5, with increasing accumulation times from 10 to 45 min, the peak current of adriamycin increased significantly. The response signal tended to stabilize when the accumulation time reached 45 min. Therefore, the optimal accumulation time was 45 min.

Optimization of hybridization time

Fig. 6 shows the influence of the hybridization time on the peak current of adriamycin. From this figure, it can be seen that the reduction current initially increased significantly with increasing hybridization times from 10 to 60 min and hardly changed at all after approximately 60 min. This indicated that the hybridization reaction was dominantly completed after 60 min. Considering the sensitivity and assay time, therefore, 60 min was chosen as the hybridization time in this work.

Selectivity of DNA sensor

The selectivity of this sensor was investigated by measuring the response to several kinds of target oligonucleotides. Fig. 7 shows

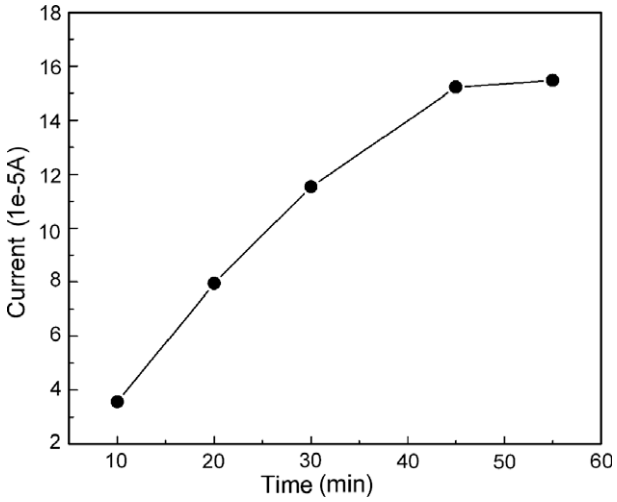


Fig. 5. Effect of accumulation time of adriamycin on DPV signals of adriamycin. The ds-DNA electrode was incubated in 0.01 M PBS (pH 7.0) containing 1.0×10^{-6} M adriamycin for various times (10, 20, 30, 45, and 55 min). The DPV measurement was performed at an applied potential from −0.3 to −0.9 V in 0.1 M PBS (pH 7.0). The DPV parameters were the same as in Fig. 4.

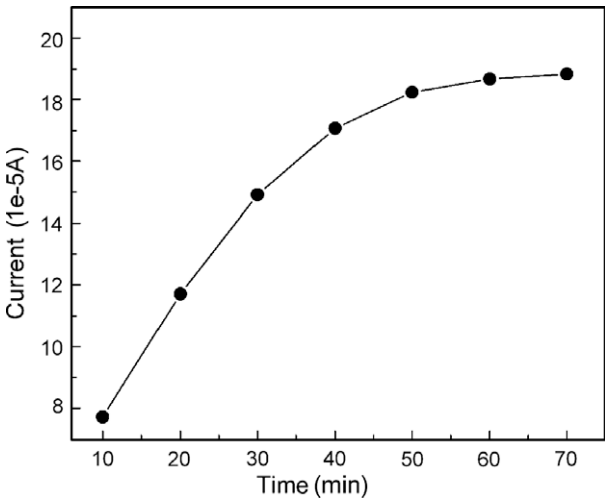


Fig. 6. Effect of DNA hybridization time on DPV signals of adriamycin. The ss-DNA electrode was incubated in 0.01 M PBS (pH 7.0) containing 1.0×10^{-8} M complementary oligonucleotides at 40 °C for various times, and then the hybridized ds-DNA electrode was incubated in 0.01 M PBS (pH 7.0) containing 1.0×10^{-6} M adriamycin for 45 min under laboratory temperature. The DPV measurement was performed at an applied potential from −0.3 to −0.9 V in 0.1 M PBS (pH 7.0). The DPV parameters were the same as in Fig. 4.

the DPV peak currents of adriamycin for DNA probe hybridization with noncomplementary, three-base mismatched, and complementary oligonucleotides. The peak currents of adriamycin were 8.883×10^{-5} A (Fig. 7B) and 9.844×10^{-5} A (Fig. 7C), corresponding to 48.8 and 54.1% of the signal for complementary oligonucleotides (1.82×10^{-4} A). This result suggested that the fabricated DNA sensor could be used to selectively detect different sequences of target oligonucleotides.

Analytical performance

Under the optimal conditions, the analytical performance of the DNA hybridization assay was investigated using an ss-DNA/Ag_{nano}/PPAA/MWCNTs–COOH modified electrode. Fig. 8 displays DPVs of adriamycin for the immobilized probe hybridized with different concentrations of the complementary oligonucleotides. The peak currents of adriamycin increased as the concentrations of the complementary oligonucleotides increased, and the increase of peak

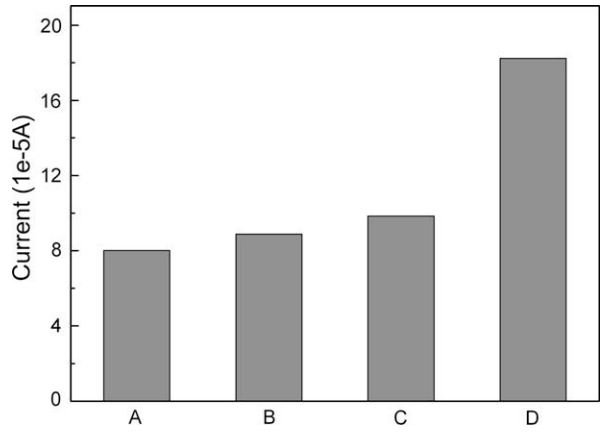


Fig. 7. Column graph of DPV signals of adriamycin recorded at ss-DNA/Ag_{nano}/PPAA/MWCNTs–COOH/GCE: (A) blank solution; (B) hybridized with noncomplementary oligonucleotides; (C) hybridized with three-base mismatch oligonucleotides; (D) hybridized with complementary oligonucleotides. The concentration of various oligonucleotides was 1.0×10^{-8} M. The DPV parameters were the same as in Fig. 4.

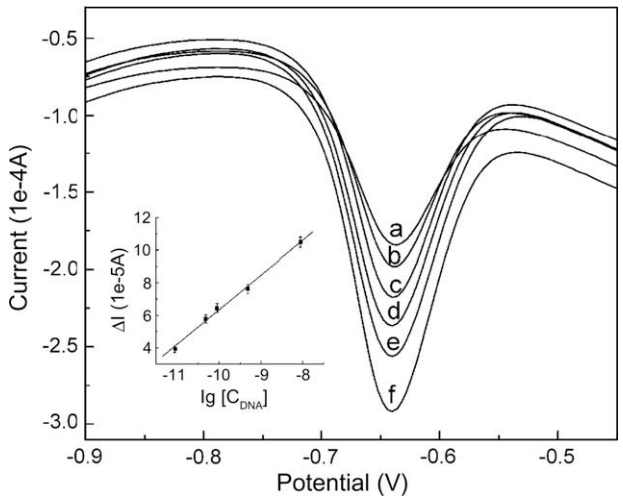


Fig. 8. The DPV response of the intercalated adriamycin recorded for the ss-DNA/Ag_{nano}/PPAA/MWCNTs–COOH/GCE that hybridized with various concentrations of complementary oligonucleotides: (a) 0 M; (b) 9.0×10^{-12} M; (c) 4.8×10^{-11} M; (d) 9.0×10^{-11} M; (e) 4.8×10^{-10} M; (f) 9.0×10^{-9} M. Inset: increase of peak current (ΔI) versus logarithm of concentration of complementary oligonucleotides. The DPV parameters were the same as in Fig. 4.

current (ΔI) was linear with the logarithm of the concentration of the complementary oligonucleotides in the range from 9.0×10^{-12} to 9.0×10^{-9} M (inset of Fig. 8) (the peak current values were recorded with each measurement repeated eight times). The regression equation was ΔI (10 μA) = 27.67 + 2.133 $\lg C_{DNA}$ (unit of C is M), and the regression coefficient (R) of the linear curve was 0.9975. The detection limit of complementary oligonucleotides was 3.2×10^{-12} M (signal/noise [S/N] = 3).

The linear range and detection limit of various electrochemical DNA sensors for detecting the specific sequences of DNA are compared with our analytical data in Table 1. From the data shown, a lower limit of detection and a wide linear range [21,38–40] can be obtained using the proposed sensor. In a word, the proposed DNA sensor has good analytical performances for the specific sequences of DNA detection.

Stability, reproducibility, and regeneration of DNA sensor

The stability of the DNA sensor was investigated by monitoring its CV response in 0.1 M PBS solution containing 1.0×10^{-6} M adriamycin. The experiments indicated that little decrease in the peak currents of adriamycin was observed after continuous scan for 30 cycles. Also, after the DNA sensor was stored in the dry state at 4 °C for 2 weeks, no apparent change in the peak currents of adriamycin was observed. The above results illuminated that the DNA sensor was of high stability and could be applied for the DNA assay.

Table 1
Comparison of linear ranges and detection limits of various electrochemical DNA sensors.

DNA sensor	Electrochemical technique used	Linear range (nM)	Detection limit (nM)	Reference
ss-DNA/polypyrrole/AuE	EIS	3.7–370	1	[38]
PNA/poly(JUG-co-JUGA)/GCE	SWV	10–100	10	[39]
ss-DNA/NG/PDC/GCE	EIS	0.1–10^4	2.4×10^{-2}	[40]
Polypyrrole/ss-DNA/MWCNTs paste electrode	DPV	0.1–10	8.5×10^{-2}	[21]
ss-DNA/Ag_{nano}/PPAA/MWCNTs-COOH/GCE	DPV	0.009–9.0	3.2×10^{-3}	This work

Note. EIS, electrochemical impedance spectroscopy; SWV, square wave voltammetry.

The reproducibility of the DNA sensor was also studied. Five DNA sensors were fabricated independently under the same conditions and used to detect 1.0×10^{-8} M complementary oligonucleotides. Five independent measurements of the reduction peak current of adriamycin gave an average value of 8.37×10^{-5} A with a relative standard deviation (RSD) of 2.2% for probe modified electrode and an average value of 1.746×10^{-4} A with an RSD of 6.3% for hybridized electrode, The results suggested that the DNA sensor was of good reproducibility.

In this work, regeneration of the DNA sensor was investigated by measuring the DPV response of the intercalated adriamycin after one DNA sensor hybridized with 1.0×10^{-8} M complementary oligonucleotides. After the first electrochemical measurement, the reduction peak current of adriamycin was 1.868×10^{-4} A. The DNA sensor was regenerated according to the above description. The reduction peak currents of adriamycin of four times successive DNA sensor regeneration were 1.856×10^{-4}, 1.823×10^{-4}, 1.69×10^{-4}, and 1.666×10^{-4} A, indicating that the proposed DNA sensor was of good reusability.

Conclusions

A novel electrochemical detection method of DNA hybridization-based on ss-DNA/Ag_{nano}/PPAA/MWCNTs–COOH modified electrode has been developed with high sensitivity and selectivity. In addition, a novel regeneration method of DNA sensor has been proposed based on using 1:1 HNO_3 solution to remove silver nanoparticles thin film. The experiment results indicated that the DNA sensor is of excellent reusability. Adriamycin is a promising alternative intercalator for electrochemical detection of DNA hybridization.

Acknowledgments

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References

[1] V. Benoit, A. Steel, M. Torres, Y.Y. Yu, H. Yang, J. Cooper, Evaluation of three-dimensional microchannel glass biochips for multiplexed nucleic acid fluorescence hybridization assays, Anal. Chem. 73 (2001) 2412–2420.
[2] Y. Dharmadi, R. Gonzales, DNA microarrays: experimental issues, data analysis, and application to bacterial systems, Biotechnol. Prog. 20 (2004) 1309–1324.
[3] A.R. Jilbert, In situ hybridization protocols for detection of viral DNA using radioactive and nonradioactive DNA probes, Methods Mol. Biol. 123 (2000) 177–193.
[4] V. Barlet, M. Cohard, M.A. Thelu, M.J. Chaix, C. Baccard, J.P. Zarski, J.M. Seigneurin, Quantitative detection of hepatitis B virus DNA in serum using chemiluminescence: comparison with radioactive solution hybridization assay, J. Virol. Methods 49 (1994) 141–151.
[5] Q. Wang, X.H. Yang, K.M. Wang, Enhanced surface plasmon resonance for detection of DNA hybridization based on layer-by-layer assembly films, Sens. Actuat. B 123 (2007) 227–232.
[6] X.H. Yang, Q. Wang, K.M. Wang, W.H. Tan, H.M. Li, Enhanced surface plasmon resonance with the modified catalytic growth of Au nanoparticles, Biosens. Bioelectron. 22 (2007) 1106–1110.
[7] L.L. Pang, J.S. Li, J.H. Jiang, G.L. Shen, R.Q. Yu, DNA point mutation detection based on DNA ligase reaction and nano-Au amplification: a piezoelectric approach, Anal. Biochem. 358 (2006) 99–103.
[8] T. Liu, J.A. Tang, L. Jiang, The enhancement effect of gold nanoparticles as a surface modifier on DNA sensor sensitivity, Biochem. Biophys. Res. Commun. 313 (2004) 3–7.
[9] F. Patolsky, A. Lichtenstein, I. Willner, Amplified microgravimetric quartz-crystal microbalance assay of DNA using oligonucleotides-functionalized liposomes or biotinylated liposomes, J. Am. Chem. Soc. 122 (2000) 418–419.
[10] J.W. Kang, X.N. Li, G.F. Wu, Z.H. Wang, X.Q. Lu, A new scheme of hybridization based on the Au_{nano}–DNA modified glassy carbon electrode, Anal. Biochem. 364 (2007) 165–170.
[11] J. Wang, R. Polsky, D. Xu, Silver-enhanced colloidal gold electrochemical stripping detection of DNA hybridization, Langmuir 17 (2001) 5739–5741.

[12] S.L. Pan, L. Rothberg, Chemical control of electrode functionalization for detection of DNA hybridization by electrochemical impedance spectroscopy, Langmuir 21 (2005) 1022–1027.
[13] W. Sun, J.H. Zhong, P. Qin, K. Jiao, Electrochemical biosensor for the detection of cauliflower mosaic virus 35S gene sequences using lead sulfide nanoparticles as oligonucleotide labels, Anal. Biochem. 377 (2008) 115–119.
[14] A. Erdem, Nanomaterial-based electrochemical DNA sensing strategies, Talanta 74 (2007) 318–325.
[15] K.J. Odenthal, J.J. Gooding, An introduction to electrochemical DNA biosensors, Analyst 132 (2007) 603–610.
[16] A. Erdem, M. Ozsoz, Electrochemical DNA biosensors based on DNA–drug interactions, Electroanalysis 14 (2002) 965–974.
[17] J. Wang, Nanoparticle-based electrochemical DNA detection, Anal. Chim. Acta 500 (2003) 247–257.
[18] H.X. Ju, H.T. Zhao, Electrochemical biosensors for DNA analysis, Front. Biosci. 10 (2005) 37–46.
[19] M.T. Castaneda, S. Alegret, A. Merkoci, Electrochemical sensing of DNA using gold nanoparticles, Electroanalysis 19 (2007) 743–753.
[20] J.J. Gooding, Electrochemical DNA hybridization biosensors, Electroanalysis 14 (2002) 1149–1156.
[21] H.L. Qi, X.X. Li, P. Chen, C.X. Zhang, Electrochemical detection of DNA hybridization based on polypyrrole/ss-DNA/multi-wall carbon nanotubes paste electrode, Talanta 72 (2007) 1030–1035.
[22] Y. Xu, X.Y. Ye, L. Yang, P.G. He, Y.Z. Fang, Impedance DNA biosensor using electropolymerized polypyrrole/multiwalled carbon nanotubes modified electrode, Electroanalysis 18 (2006) 1471–1478.
[23] S. Iijima, Helical microtubules of graphite carbon, Nature 354 (1991) 56–58.
[24] X.W. Tang, S. Bansaruntip, N. Nakayama, E. Yenilmez, Y.L. Chang, Q. Wang, Carbon nanotube DNA sensor and sensing mechanism, Nano Lett. 6 (2006) 1632–1636.
[25] Y.F. Ma, S.R. Ali, A.S. Dodoo, H.X. He, Enhanced sensitivity for biosensors: multiple functions of DNA-wrapped single-walled carbon nanotubes in self-doped polyaniline nanocomposites, J. Phys. Chem. B 110 (2006) 16359–16365.
[26] J. Yang, K. Jiao, T. Yang, A DNA electrochemical sensor prepared by electrodepositing zirconia on composite films of single-walled carbon nanotubes and poly(2,6-pyridinedicarboxylic acid), and its application to detection of the PAT gene fragment, Anal. Bioanal. Chem. 389 (2007) 913–921.
[27] K. Kerman, Y. Morita, Y. Takamura, M. Ozsoz, E. Tamiya, DNA-directed attachment of carbon nanotubes for enhanced label-free electrochemical detection of DNA hybridization, Electroanalysis 16 (2004) 1667–1672.
[28] E. Granot, B. Basnar, Z. Cheglakov, E. Katz, I. Willner, Enhanced bioelectrocatalysis using single-walled carbon nanotubes (SWCNTs)/polyaniline hybrid systems in thin-film and microrod structures associated with electrodes, Electroanalysis 18 (2006) 26–34.
[29] T. Yang, W. Zhang, M. Du, K. Jiao, A PDDA/poly(2,6-pyridinedicarboxylic acid)–CNTs composite film DNA electrochemical sensor and its application for the detection of specific sequences related to PAT gene and NOS gene, Talanta 75 (2008) 987–994.
[30] X.H. Kang, Z.B. Mai, X.Y. Zou, P.X. Cai, J.Y. Mo, A sensitive nonenzymatic glucose sensor in alkaline media with a copper nanocluster/multiwall carbon nanotube-modified glassy carbon electrode, Anal. Biochem. 363 (2007) 143–150.
[31] S.H. Chen, R. Yuan, Y.Q. Chai, L.Y. Zhang, N. Wang, X.L. Li, Amperometric third-generation hydrogen peroxide biosensor based on the immobilization of hemoglobin on multiwall carbon nanotubes and gold colloidal nanoparticles, Biosens. Bioelectron. 22 (2007) 1268–1274.
[32] H.X. Ma, L.C. Wang, L.Y. Chen, C. Dong, W.C. Yu, T. Huang, Y.T. Qian, Pt nanoparticles deposited over carbon nanotubes for selective hydrogenation of cinnamaldehyde, Catal. Commun. 8 (2007) 452–456.
[33] G.W. Yang, G.Y. Gao, C. Wang, C.L. Xu, H.L. Li, Controllable deposition of Ag nanoparticles on carbon nanotubes as a catalyst for hydrazine oxidation, Carbon 46 (2008) 747–752.
[34] S.D. Yang, X.G. Zhang, H.Y. Mi, X.G. Ye, Pd nanoparticles supported on functionalized multi-walled carbon nanotubes (MWCNTs) and electrooxidation for formic acid, J. Power Sources 175 (2008) 26–32.
[35] Z. Chang, H. Fan, K. Zhao, M. Chen, P.G. He, Y.Z. Fang, Electrochemical DNA biosensors based on palladium nanoparticles combined with carbon nanotubes, Electroanalysis 20 (2008) 131–136.
[36] N.N. Zhu, Z. Chang, P.G. He, Y.Z. Fang, Electrochemical DNA biosensors based on platinum nanoparticles combined carbon nanotubes, Anal. Chim. Acta 545 (2005) 21–26.
[37] H. Beg, G. Horn, U. Luthardt, Interaction of anthracycline antibiotics with biopolymers: V. Polarographic behavior and complexes with DNA, Bioelectrochem. Bioenerg. 8 (1981) 537–553.
[38] H. Peng, C. Soeller, M.B. Cannell, G.A. Bowmaker, R.P. Cooney, J.T. Sejdic, Electrochemical detection of DNA hybridization amplified by nanoparticles, Biosens. Bioelectron. 21 (2006) 1727–1736.
[39] S. Reisberg, L.A. Dang, Q.A. Nguyen, B. Piro, V. Noel, P.E. Nielsen, L.A. Le, M.C. Pham, Label-free DNA electrochemical sensor based on a PNA-functionalized conductive polymer, Talanta 76 (2008) 206–210.
[40] J. Yang, T. Yang, Y.Y. Feng, K. Jiao, A DNA electrochemical sensor based on nanogold-modified poly-2,6-pyridinedicarboxylic acid film and detection of PAT gene fragment, Anal. Biochem. 365 (2007) 24–30.