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A New Electrochemical Biosensor of Host-guest Recognition Mode for DNA Hybridization Detection

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Abstract: *This paper reports on an electrochemical biosensor to detect the hybridization specificity by using host-guest recognition technique. A hairpin DNA with a dabcyl molecule which is typical guest molecule to β-Cyclodextrins (β-CD) at the 3'-terminus and a NH_2 linked at the 5'-terminus as the probe DNA. The probe DNA was immobilized on the PdS nanoparticle to construct a double-labeled probe (DLP) and could selectively hybridize with its target DNA. A β-CD modified Poly(N-acetylaniline) glassy carbon electrode was used for capturing dabcyl in DLP. Without binding with target DNA, the DLP keep stem-loop structure and block dabcyl enter into the cavity of β-CD on electrode. However, a target-binding DLP is incorporated into double stranded DNA, causing loop-stem structure opened and dabcyl could be easy captured by β-CD which brought DLP on electrode surface. With electrochemical measurement, the signal come from Pd^{2+} be used for target DNA quantitative analysis.*

Keywords: DNA, electrochemical, Cyclodextrins, double-labeled probe, host-guest recognition

1. INSTRUCTION

In recent years electrochemical DNA biosensors have been rapidly developed due to the advantages including low-cost, fast response, good selectivity and miniaturization of instruments [1-3]. Most of the electrochemical DNA biosensors are based on a nucleic acid recognition layer, which is immobilized on the different kinds of working electrodes. The hybridization reaction is further transformed into different electrochemical responses such as the voltammetric results of the electrochemical indicators or the intrinsic signals of DNA, or from the other electrochemical parameters such as capacitance or conductivity [4-6]. This characteristic made detecting DNA in most electrochemical sensors only available under heterogeneous system thus limited the application of electrochemical sensors.

In our previous works [7-9], we have constructed several non-immobilizing electrochemical DNA biosensors for DNA detection based on the host-guest recognition technique. Based on these results, we now attempt to further develop this electrochemical method for DNA sensing with the shorter time of hybridizer capture in the homogeneous solution.

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The molecular recognition technology, defined as the supramolecular noncovalent interaction between the "host" and "guest" molecules, has played an important role in the chemical sensing field [10-11]. Cyclodextrins (CDs) and their derivatives were the typical host molecules to recognize and encapsulate guest partners such as dabcyl [12-13]. They have been applied in electrode modification to detect organic molecules [14-15].

Here, we utilized host-guest recognition between dabcyl with β-CD modified on electrode to construct a double-labeled probe (DLP) for sequence-specific DNA detection. As Figure 1 shows,

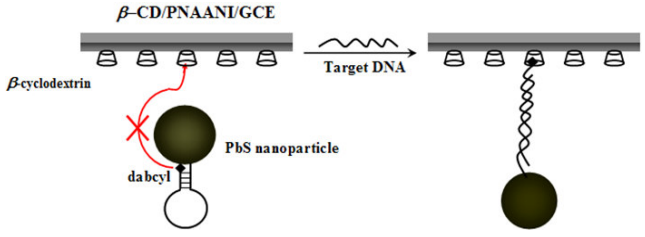

Figure 1. Schematic diagram of the method of monitoring of hybridization process by DLP.

the DLP was PdS nanoparticle conjunct with NH_2 at 5' terminal of a 26-mer hairpin DNA (probe DNA) through imido bond, at which of 3' terminal dabcyl had been labeled. In the absence of target, the DLP is in the folded configuration in which its termini are held in close proximity, and its 3' dabcyl get close enough to PdS nanoparticle. In this state, comparatively huge bulk of PdS nanoparticle and the loop of hairpin DNA would prevent the dabcyl entered the cavity of β-CD on the electrode, which made DLP could not be brought on electrode surface. After the DLP solution were incubated in a buffer solution containing target DNA, complementary sequence of the DLP would combine with target DNA, the stem–loop was converted into a rigid, linear double helix, removing the dabcyl from proximity to the PdS nanoparticle surface. Thus, when β-CD modified electrode immersed in solution after hybridization event happen, the dabcyl of the DLP could enter cavity of β-CD on the electrode and the DLP was captured on the electrode. After a thorough washing procedure, the DLP on the electrode were dissolved by adding 0.10M HNO_3. Identification and quantification of the dissolved metals were performed by anodic stripping voltammetry [16].

2. EXPERIMENTAL SECTION

2.1. Chemicals and DNA sequence

N-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), imidazole and β-CD were provided by Sigma. All other reagents used were of analytical reagent grade. All of the solutions were prepared with ultrapure water from a Millipore Milli-Q system. The DNA in the experiment was obtained from Sangon Biotechology Inc. (Shanghai, China) with HPLC purification.

Hairpin DNA (probe DNA):

5'SH-CACGCTTGTGGGTCAACCCCCGTGG-DABCYL3'

Target DNA: 5'-GGGGTTGAC CCACAAG-3'

One base-mismatched DNA: 5'-GGGGT***C***GACCCACAAG-3'

Three base-mismatched DNA: 5'-GGGGT***CTT***CCCACAAG-3'

Non-complementary DNA: 5'-TTCGGCTCTATCAATC-3'

2.2. Electrochemical Apparatus

All voltammetric experiments were performed using a CHI 660 electrochemical analyzer (CHI Instrument Inc, USA). Electrochemical experiments were carried out in a 5 ml electrochemical cell at room temperature (25°C), using three electrode configuration. A platinum wire served as a counter electrode and an Ag/AgCl with saturated KCl solution as reference electrode. A glassy carbon electrode with diameter 3mm were modified β-CD as capture instrument. The other glassy carbon electrode with diameter 1mm was fabricated mercury-film for Pd^{2+} detection.

2.3. Preparation of nano PdS covered with thioglycollic acid

$Pb(NO_3)_2$ and Na_2S solutions were filtered through a 22 μm microporous membrane filter prior to use. PbS-NPs were prepared according to the literature [17] by using mercaptoacetic acid as the stabilizer. In brief, 9.22μL mercaptoacetic acid was added to 50mL 0.4mM $Pb(NO_3)_2$ solution, then pH was adjusted to 7 with 0.5M NaOH. Then the solution was bubbled with nitrogen for 30min, followed by slowly addition of 1.34mM Na_2S to the mixture solution. The molar ratio of Na_2S to $Pb(NO_3)_2$ was kept for 2.5. The reaction was carried out for 24 h under nitrogen protection and then gradually a brown colloid was obtained. As TEM images showed, the diameter of PdS nanoparticles was about 7 nm. (Fig. 2)

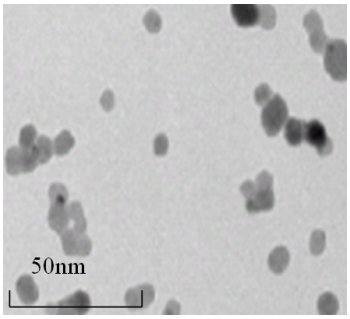

Figure 2. TEM image of the synthesized PdS nanoparticles

2.4. Preparation of the DLP

The hairpin DNA probe was bond with PbS nanoparticle through imido bond was prepared according to the literature [16]. In brief, 200 μl of 0.1M imidazole was added to 2 OD hairpin probe DNA. After stirred for 30 min, 100 μl of 0.1 M EDC and 1 ml of PdS nanoparticle were added to the mixture. The resulting mixture was stirred for 12 h at room temperature and then continued to centrifugate for at least 25 min at 14,000 rpm to remove the excessive hairpin probe DNA. The hairpin probe DNA-PdS precipitate was washed with 0.1 mol/L phosphate buffer and re-dispersed in 0.1 mol/L phosphate buffer (0.1 M containing 0.3 M NaCl and 1 mM Mg^{2+}, pH 7.0).

2.5. Fabrication of the β-CD Modified Poly(*N*-acetylaniline) (PNAANI) glassy carbon electrode (β-CD /PNAANI GCE)

The β-CD modified Poly(*N*-acetylaniline) glassy carbon electrode was obtained according to the reported processes [19]: firstly the glassy carbon electrode modified with PNAANI film was prepared by electrodeposition of PNAANI in a 0.1M *N*-acetylaniline, 1M $HClO_4$ solution. The electrolysis was carried out by potential sweeping from -0.2 to 0.9 V for 20 cycles at 100mV/s. As the electrolysis proceeded, an excellent cohesive brown film was formed on the surface of glassy carbon electrode. After electrooxidation of the PNAANI electrode in a 0.05 M β-CD, 0.1M$LiClO_4$ DMSO solution at a constant potential of 1.2 V for 600s, the β-CD /PNAANI GCE was accomplished.

2.6. Assay Performance

The assay procedure was initiated by adding the 10 ml of 6.4 mM DLP to 90ml of target DNA and incubating for 45 min with stirring at 37°C. After that, the β-CD/PNNAI/GCE was immersed in the solution for capturing DLP-target DNA hybridizer. After incubation for 4h, the electrode washed with a thorough washing procedure, the DLP on the substrate were dissolved by adding

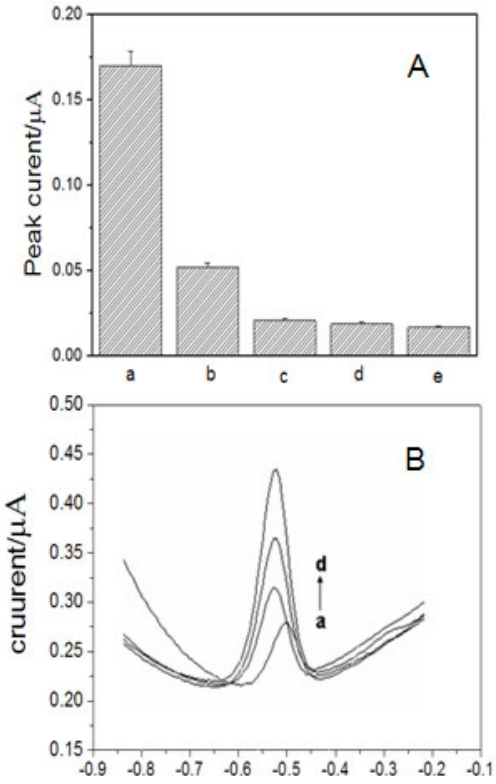

Figure 3. (A) The DPV currents of the DLP challenged with (a) 4.3×10^{-7} M target DNA, (b)4.3×10^{-6} M one base-mismatched DNA, (c) 4.3×10^{-6} M three base-mismatched DNA, (d) 4.3×10^{-6} M non-complementary DNA, (e) only phosphate buffer (0.1M pH 7.0) containing no DNA sequences. (B) The DPV response of difficult target DNA from 8.6×10^{-10} to 8.6×10^{-7} M (a-d).

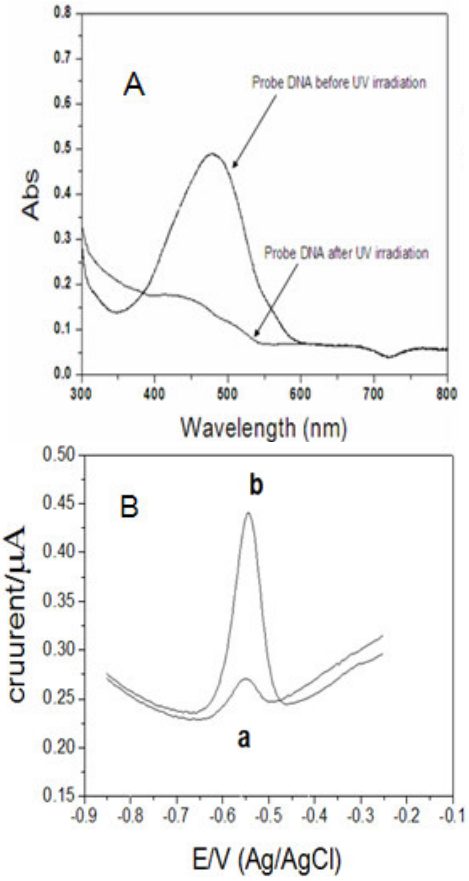

Figure 4. (A) UV-vis spectra of the probe DNA solution before (upper trace) and after (bottom trace) UV irradiation.(B) The DPV response of the DLP for detecting 7.3×10^{-7} M target DNA, in which the probe DNA before hybridization has treated without (a) and with (b) the UV irradiation.

200ul of 0.1 mol/L HNO_3. Then 1.8 ml acetate buffer (0.1 mol/L, pH=5.3) was added into 200ul of HNO_3 solution (containing dissolved Pd^{2+}). Electrochemical detection of the dissolved Pd^{2+} was performed at a mercury-film electrode according to the literature [16]. The DPV peak height at a potential of -0.54 V of the oxidation of Pd was used in all of the measurements.

3. RESULTS AND DISCUSSION

3.1. The selectivity of the DLP for DNA detection in homogenous solution

A series of hybridization experiments were carried out for the different sequence DNA with 0.64 mM DLP, including target DNA, one base mismatched DNA, three base mismatched DNA and non-complementary DNA to evaluate the specificity of the DLP. As Figure 3(A) shows, compared to the background signal (signal (e)), the non-complementary DNA produced an insignificant DPV signal (signal (d)) with approximate value, and the signal produced by three base-mismatched DNA (signal (c)) was less than twice fold. When the DLP challenged with one base-mismatched DNA, the corresponding signal (signal (b)) was about one third of that of target DNA(signal (a)), suggesting that the DLP has high sequence specificity toward even one base-mismatched DNA. The hybridization selectivity of DLP to target sequences was partially due to the intrinsic recognition ability of DNA bases, and also to the reason that even if the probe DNA was "hybridized" with mismatched target and the DLP's hairpin structure was partially opened, a steric effect still existed for such "hybridizer" to contact the electrode. For the non-complementary DNA, the signal was low and neglectable, and such low background signal demonstrated that the DLP could be hardly captured by β-CD in its initial state, might with a very little of adsorption. As shown in Figure 3(B), the corresponding enhancive DPV signals were obtained with concentration of target DNA increasing. They were logarithmically related to the target concentration and the sensitivity tests showed that the linearity range for the sensing protocol covered from 8.6×10^{-7} to 8.6×10^{-10} M and the detection limit was 6.2×10^{-10}M.

3.2. The conformation of the electrochemical DNA sensing based on the host-guest recognition

To confirm that the DLP be captured by the β-CD/PNAANI GCE is due to the host-guest recognition between dabcyl with β-CD, the experiment was carried out to transform the conformation of dabcyl on the probe DNA by UV lighting first and then employ

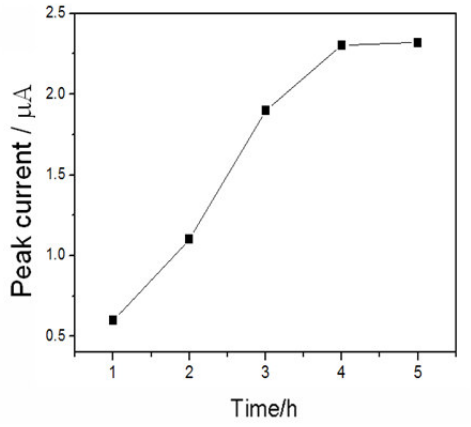

Figure 5. The DPV responses of 8.8×10^{-7} M target DNA detection with different capture time.

it to synthesize DLP for sensing target DNA. In the knowledge that the dabcyl is characterized by reversible transformations from the generally more stable *trans* form to the less stable *cis* form upon irradiation with UV [17]. The host-guest recognition between the dabcyl with *β*-CD in fact depended on the dabcyl's conformation, of which only the *trans* typed one can enter the cavity of *β*-CD, not the *cis* one[21]. As figure 4(A) showed (upper trace) is a UV-vis spectrum of the probe DNA aqueous solution before UV irradiation. Appearance of a peak at 480 nm indicates that the dabcyl molecule on *trans* form. When this solution was irradiated by UV light for 30min, the 480 nm peak in the UV-vis spectrum disappeared, as is shown in Figure 4(A) (bottom trace).This spectral change suggests that the dabcyl were changed to the *cis* form via the photoisomerization of azobenzenes [22]. Then after the hybridization of the synthesized DLP to target DNA, a marked current signal of Pd^{2+} was obtained for the DLP without UV treatment (Figure. 4(B), curve a), whereas the signal of Pd^{2+} could hardly be observed due to the *cis* formation of dabcyl after the UV irradiation process (Figure 4(B), curve b). Therefore, it came to the conclusion that the detection of the DNA hybridizer in aqueous solution was fulfilled by the host-guest recognition of *β*-CD to dabcyl.

3.3. Optimization of the capture time of the *β*-CD/PNAANI/GCE to Dabcyl-labeled hybridization

The capture time of the *β*-CD/PNAANI/GCE in DLP-target DNA hybridization solution was expected to have a direct influence on the DLP amount captured onto the *β*-CD. Therefore, the experiments were preformed for choosing the appropriate capture time by, firstly, preparing a hybridization solution with 0.64 mM DLP and 8.8×10^{-7} M target DNA, and then immersing a series of *β*-CD/PNAANI/GCE separately in such solution with different capture time. After electrochemical measurement, the results showed that the DPV response increased significantly with the capture time increased from 1 to 5 h, and almost reached the platform after 4 h (Fig. 5). It illuminated that with capture of 4 h, majority of the DLP-target DNA hybridization in solution have been captured onto the *β*-CD/PNAANI/GCE, which therefore was chosen as the capture time in experiments.

4. CONCLUSIONS

In this communication, the PbS nanoparticle-based double-labeled DNA probe was designed for DNA detection. Compare to early work, the time of hybridizer capture is shortened. This method provided a foundation for multi-DNA detection by using multi-inorganic nanoparticles in homogenous solution.

5. ACKNOWLEDGEMENTS

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