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A new electrochemical biosensor for DNA detection based on molecular recognition and lead sulfide nanoparticles

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ABSTRACT

In this paper, we constructed a new electrochemical biosensor for DNA detection based on a molecule recognition technique. In this sensing protocol, a novel dual-labeled DNA probe (DLP) in a stem–loop structure was employed, which was designed with dabcyl labeled at the 3′ end as a guest molecule, and with a Pb nanoparticle labeled at the 5′ end as electrochemical tag to indicate hybridization. One α-cyclodextrin-modified electrode (α-CD/MCNT/GCE) was used for capturing the DNA hybridization. Initially, the DLP was in the "closed" state in the absence of the target, which shielded dabcyl from the bulky α-CD/MCNT/GCE conjugate due to a steric effect. After hybridization, the loop sequence (16 bases) formed a rigid duplex with the target, breaking the relatively shorter stem duplex (6 bases). Consequently, dabcyl was forced away from the Pb nanoparticle and became accessible by the electrode. Therefore, the target hybridization event can be sensitively transduced via detecting the electrochemical reduction current signal of Pb. Using this method, as low as 7.1×10^{-10} M DNA target had been detected with excellent differentiation ability for even a single mismatch.

As research has moved into the era of genomics, electrochemical DNA biosensors have been rapidly developed due to the advantages such as low cost, fast response, small size, good selectivity, and miniaturization of instruments [1,2]. Most of the electrochemical DNA biosensors are based on a nucleic acid recognition layer, which is immobilized on different kinds of working electrodes [3–9]. 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 other electrochemical parameters such as capacitance or conductivity.

Electrochemical biosensors need probe DNA preimmobilization on the electrode surface, which causes lower available hybridization efficiency when compared with the sensors when hybridization happens in homogeneous solution, thus difficult for real-time electrochemical monitoring PCR[1] and recognizing specific sequence DNA in living cells. On the other hand, fluorescent molecular beacons have been widely used for real-time monitoring of DNA in PCR and in cells with hybridization in homogeneous solution [10–12]. Therefore, the development of an electrochemical DNA sensing technology with hybridization in homogeneous solution is still a challenge. Herein, a new electrochemical DNA sensing method with hybridization occurring in a homogeneous system was reported based on a molecular recognition technique. The molecular recognition technology, defined as the supramolecular noncovalent interaction between the "host" and the "guest" molecules, has played an important role in chemical sensing research, involving a supramolecular sensor chip for metal ion detection and fluorescent DNA detection [13,14]. Cyclodextrins (CDs) are a type of special oligosaccharide, which consist of six, seven, or eight glucose units (named α, β or γ-CD, respectively), and characterized by a toroidal form with a hydrophobic inner cavity and a hydrophilic outer side [15]. Their unique "cage" structure endows CDs and their derivatives with outstanding recognition and encapsulation abilities to their guest molecules, and consequently they have been employed as the host molecule in organic chemistry and in the pharmaceutical field [16,17]. Recently, CD-modified electrodes have been applied to encapsulate organic molecules selectively and to detect electrochemical molecules, such as thioridazine and aminobiphenyl [18–20].

Here, we utilized molecular recognition between a dual-labeled DNA probe (DLP) and a α-CD-modified electrode for sequence-specific DNA detection in homogeneous solutions. As illustrated in Fig. 1, two key components were employed for this electrochemical biosensor construction. One component is an α-cyclodextrin/multiwalled carbon nanotube-modified glassy carbon electrode (α-CD/MCNT/GCE), which was prepared by a mixture of α-CDs and MCNTs,

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[1] *Abbreviations used:* α-CD/MCNT/GCE, α -cyclodextrin/multiwalled carbon nanotube-modified glassy carbon electrode; CDs, cyclodextrins; DPV, differential pulse voltammetry; EDC, 1-ethyl-(3-dimethylaminopropyl)carbodiimide; DLP, dual-labeled DNA probe; GCE, glassy carbon electrode; PCR, polymerase chain reaction.

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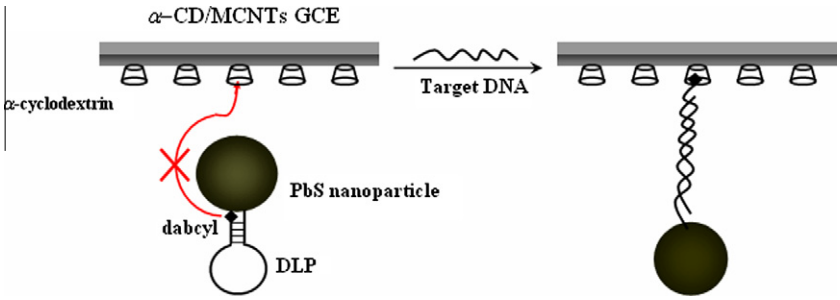

Fig.1. A schematic illustration of electrochemical DNA sensing principle based on the molecular recognition technique.

and possessed special recognition ability to the dabcyl molecule due to the presence of α-CD. It was used to capture and detect the DNA hybridizer of DLP and target DNA. Another one is a novel DLP which was designed based on a hairpin DNA probe with 16 bases in the loop structure complementary to target DNA. Its 3′ terminal had been labeled with one dabcyl molecule, which is α-CD's typical guest, and thus would be captured by the α-CD-modified electrode during the DNA sensing procedure. Its 5′ terminal was labeled with PbS nanoparticles via an imido bond and used as the electrochemical signal producing marker. As shown in Fig. 1, before the hybridization, the DLP remained in the "closed" state in which the probe DNA adopted the stem-loop structure, and thus the dabcyl molecule was forced to be closed to the PbS nanoparticle. Due to the steric effect of the PbS nanoparticle and the stem-loop structure of probe DNA, dabcyl was prevented from entering the cavity of the α-CD on the electrode. Once the DLP was hybridized with the target DNA in a homogeneous aqueous solution, they formed into a rigid double-stranded hybridizer structure, and therefore the dabcyl molecule went away from the PbS nanoparticle. Then, the "opened" DLP-target DNA hybridizer was captured onto the α-CD/MCNT/GCE through the special interaction between dabcyl and α-CD. After a thorough washing procedure, the DLP on the electrode was dissolved by adding 0.10 M HNO_3. Identification and quantification of the dissolved metals were performed by anodic stripping voltammetry [21].

Experimental

Materials

Unless otherwise noted, all chemicals were purchased from Dingguo Biotchnology Inc. (Shanghai, China) and of analytical reagent grade. The α-cyclodextrins were purchased from Sigma–Aldrich Chemical Company. 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.

The dabcyl-labeled hairpin DNA probe consisted of the following: 5′-NH_2-(CH_2)6-GTGAGCCAAGACGGAAAGACCCGCTCAC-$(CH_2)_6$-DABCYL-3′; target DNA, 5′-GGGTCTTTCCGTCTTG-3′; 1-base-mismatched DNA, 5′-GGGTCTTACCGTCTTG-3′; 3-base-mismatched DNA, 5′-GGGGTCAAGCCACAAG-3′; noncomplementary DNA, 5′-CAGGAAAC AGCTATGA-3′.

Electrochemical apparatus

All voltammetric experiments were performed on a CHI 660 electrochemical analyzer (CHI Instrument Inc., USA) in a 5-ml electrochemical cell at room temperature (25 °C) by using three electrode configurations. A platinum wire served as counterelectrode and an Ag/AgCl with saturated KCl solution served as reference electrode. A glassy carbon electrode (GCE) with diameter 3 mm modified with α-CD was used as the DNA capture instrument. The other one GCE with diameter 1 mm was fabricated with a layer of mercury film [22] for the Pd^{2+} electrochemical detection in a 3-ml cell.

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 nanoparticles were prepared according to the literature [23] by using mercaptoacetic acid as the stabilizer. In brief, 9.22 μl mercaptoacetic acid was added to 50 ml 0.4 mM $Pb(NO_3)_2$ solution, and then the pH was adjusted to 7 with 0.5 M NaOH. The solution was bubbled with nitrogen for 30 min, followed by the slow addition of 1.34 mM Na_2S to the mixture solution. The molar ratio of Na_2S to $Pb(NO_3)_2$ was kept at 2.5. The reaction was carried out for 24 h under nitrogen protection and then gradually a brown colloid which is the PdS nanoparticles covered with a carboxyl group was obtained. As TEM images show, the diameter of PdS nanopatricles was about 7 nm (Fig. 2).

Preparation of the double-labeled probe DNA

The hairpin DNA probe was bound with PbS nanoparticles covered with a carboxyl group through imide bonds was prepared according to the literature [22]. In brief, 200 μl of 0.1 M imidazole was added to 2 OD hairpin probe DNA. After being stirred for 30 min, 100 μl of 0.1 M 1-ethyl-(3-dimethylaminopropyl)carbodiimide (EDC) and 1 ml of PdS nanoparticles were added to the mixture. The resulting mixture was stirred for 12 h at room temperature and then continued to centrifuge for at least 25 min at 14,000 rpm to remove the excessive hairpin probe DNA. In this

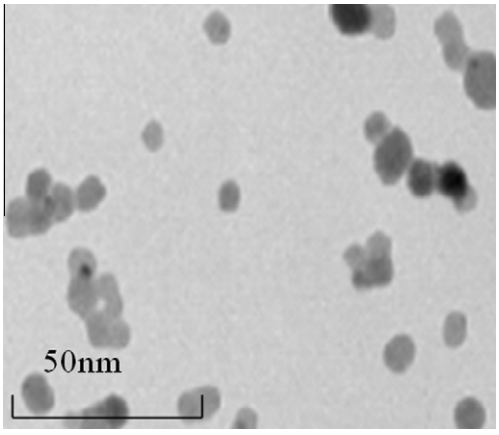

Fig.2. TEM image of the synthesized PdS nanoparticles.

process, the imide bond is synthesized by the condensation reaction between the acids and the amines in the presence of imidazole and EDC. The hairpin probe DNA–PdS precipitate was washed with 0.1 mol/L phosphate buffer and redispersed in 0.1 mol/L phosphate buffer (0.1 M containing 0.3 M NaCl and 1 mM Mg^{2+}, pH 7.0).

Preparation of the α-CD/MCNT/GCE

The α-cyclodextrin/multiwalled carbon nanotube-modified glassy carbon electrode was prepared according to the reported processes [20]. First, a glassy carbon electrode (3 mm in diameter) was carefully polished with emery paper and chamois leather containing Al_2O_3 slurry (0.05 and 0.3 μm) and then sonicated in distilled water. One milligram of MCNTs was dispersed into a 10 ml of 2% α-CD aqueous solution with the aid of ultrasonic agitation to give a 0.1 mg/ml α-CD/MCNT black solution. In this process, α-CDs are adsorbed on the surface of CNT. The α-CD/MCNT complex was precipitated from the solution, washed with distilled water to remove the residual α-CD molecules, dried under a desiccator, and mixed with KBr for the IR slice preparation. With the IR spectra test (Fig. 3), the presence of the functional groups of C–O, C–H, and H–O indicated the successful adsorption of MWCNTs to α-CD. An α-CD/MCNT/GCE was prepared by dropping 7 μl of the α-CD/MCNT solution onto one GCE surface and then drying it under an IR lamp to form a black uniform film.

DNA sequence sensing performance

The DNA assay procedure was initiated in 0.1 M phosphate buffer (PBS) containing 1.0 mM Mg^{2+} by adding 10 μl of 3.3 μM DLP to target DNA and stirring the mixture for 45 min at 37 °C. During the process, the target DNA hybridized with DLP, and then one α-CD/MCNT/GCE was immersed in the solution for capturing DLP-target DNA hybridization onto the signal detecting electrode. After 3 h, the α-CD/MCNT/GCE was washed with a thorough washing procedure and the PdS nanoparticles on the electrode were dissolved by adding 200 μl of 0.1 M HNO_3. Then 1.8 ml of 0.1 M acetate buffer (pH 5.3) was added into the solution. Electrochemical detection of the Pd^{2+} was performed at a mercury-film electrode according to the literature. The differential pulse voltammetry (DPV) peak height of the oxidation of Pd to Pd^{2+} at −0.5 V was used as the DNA sequence detection signal in all of the measurements.

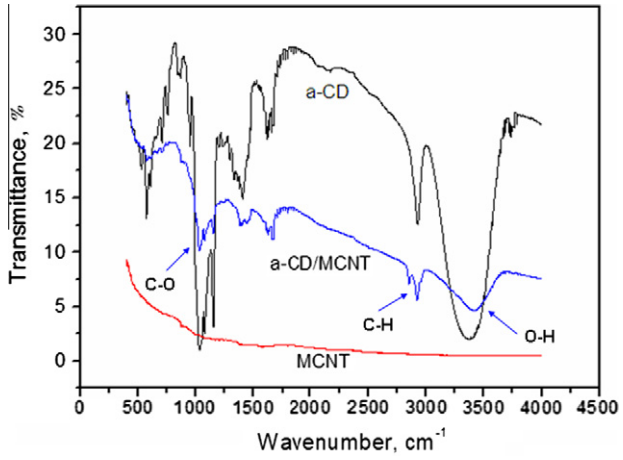

Fig.3. FTIR spectra of α-CD, MCNTs, and α-CD/MCNTs.

Results and discussion

Electrochemical DNA sensing based on molecular recognition

Contrastive experiments were performed to investigate the electrochemical DNA sensing based on the host–guest recognition. First, the α-CD/MCNT/GCE was immersed in 0.33 μM DLP solution for 3 h, and only a relatively small DPV current signal was obtained, as shown in Fig. 4, curve a. On the contrary, a marked electrochemistry signal was obtained (Fig. 4, curve b) after the α-CD/MCNT/GCE was immersed for 3 h in the solution containing the hybridization of 0.33 μM DLP and target DNA. It demonstrated that before the hybridization, the DLP remained in the closed state in which the probe DNA adopted a stem-loop structure, and thus causing the dabcyl molecule to be closed to the PdS nanoparticle. Due to the steric effect of the PdS nanoparticle and the stem-loop structure of probe DNA, dabcyl was prevented from entering the cavity of the α-CD on the electrode. Once the DLP was hybridized with the target DNA in a homogeneous aqueous solution, it formed a rigid double-stranded hybridization structure, and therefore the dabcyl molecule went away from the PdS nanoparticle. Then, the opened DLP-target DNA hybridization was captured onto the α-CD/MCNT/GCE through the special interaction between dabcyl and α-CD.

Effect of the conformation of dabcyl on the DLP

The molecular recognition between dabcyl with α-CD in fact depended on the dabcyl's conformation; that is, only the *trans* type can enter the cavity of α-CD and then be encapsulated by the α-CD-modified electrode, not the *cis* one [24]. The *trans* one can reversibly transform to its *cis* structure upon UV irradiation [25]. To confirm that the capture of DLP-target DNA hybridization onto the α-CD/MCNT/GCE is mainly due to the molecular recognition between the dabcyl guest and the α-CD host but not the nonspecial physical or chemical adsorption, contrastive experiments were carried out as follows. First, the conformation of dabcyl on the original probe DNA was transformed by UV lighting, and such probe DNA was employed to synthesize DLP for target DNA detection. As seen in Fig. 5A, before the UV lighting, the probe DNA displayed a typical UV–Vis spectrum of the *trans* formed dabcyl at 480 nm (upper trace). After the probe DNA solution in a quartz cuvette was placed 40 cm beneath a model mercury vapor UV lamp (220 V, 200 W)

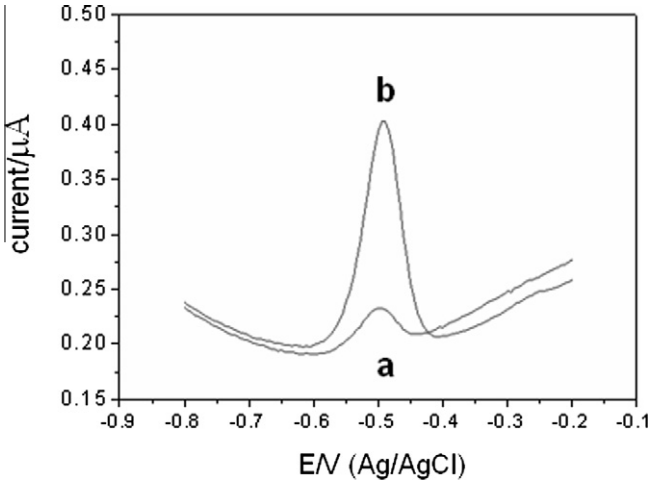

Fig.4. The DPV response of the Pd^{2+} when the α-CD/MCNT/GCE was incubated with phosphate buffer containing DLP (a) in the absence of target DNA and (b) in the presence of 4.8×10^{-7} M target DNA.

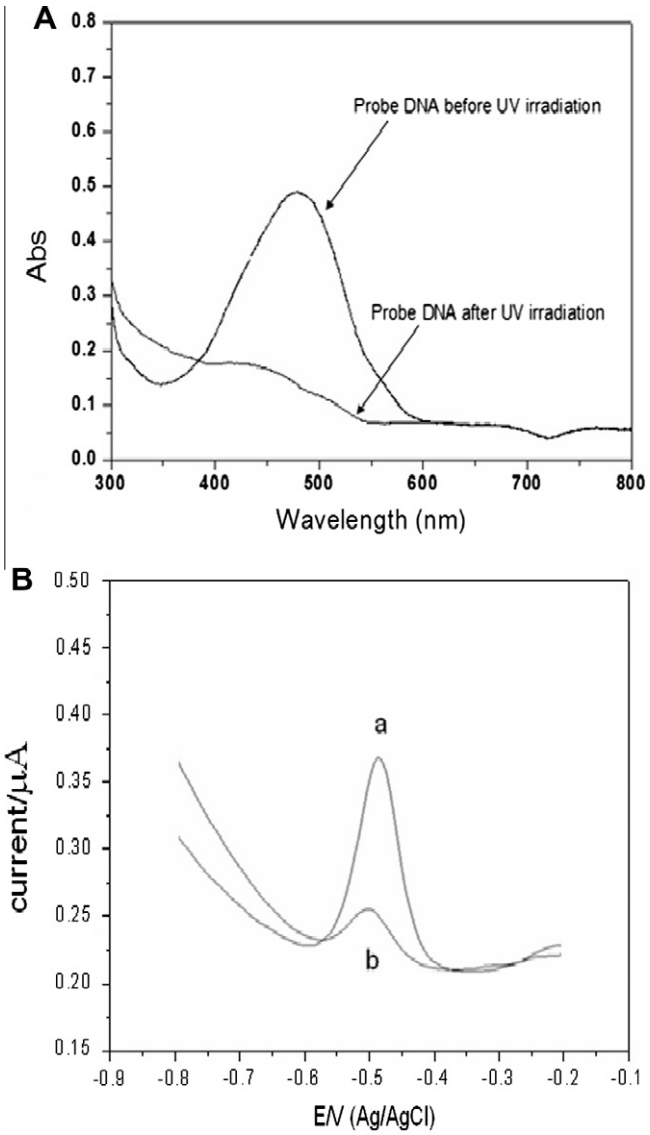

Fig.5. The DPV responses of 4.8×10^{-7} M target DNA detection with different capture times.

and irradiated at 365 nm for 30 min, this peak disappeared as a result (bottom trace), suggesting that the *trans* dabcyl labeled at the 3′ end of probe DNA had transformed to its *cis* conformation via the photoisomerization of azobenzenes [26].

Then after hybridization with 2.3×10^{-8} M target DNA, a marked current signal of Pd^{2+} was obtained for these DLPs without UV treatment (Fig. 5B, curve a), whereas the signal of Pd^{2+} could hardly be observed due to the *cis* formation of dabcyl after the UV irradiation process (Fig. 5B, curve b). Therefore, we concluded that the DLP-target DNA hybridization in aqueous solution that was captured onto α-CD/MCNT/GCE was mainly due to the molecular recognition between α-CD on the electrode and dabcyl on the DLP-target DNA hybridization.

Optimization of the capture time of the α-CD/MCNT/GCE to dabcyl-labeled hybridization

The capture time of the α-CD/MCNT/GCE in DLP-target DNA hybridization solution was expected to have a direct influence on the DLP amount captured onto the α-CD/MCNTs. Therefore, experiments were preformed for choosing the appropriate capture time by, first, preparing a hybridization solution with 0.33 μM DLP and 4.8×10^{-7} M target DNA, and then immersing a series of α-CD/MCNT/GCEs separately in such solution with different capture times. The affinity of host–guest interaction between α-CD and dabcyl is comparatively weak [27]. For this reason, the recognition process usually need several hours [26]. 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 3 h (Fig. 6). It illuminated that with a capture of 3 h, the majority of the DLP-target DNA hybridization in solution were captured onto the α-CD/MCNT/GCE, and therefore was chosen as the capture time in experiments.

Target DNA determination and the DLP specificity study

In the experiments, 0.33 μM DLP was incubated with different concentrations of target DNA, and then the peak current from the reduction of Pd^{2+} was measured as the hybridization signal. As the concentration of the complementary target DNA sequence continuously increased, one continuously enhancing DPV signal was obtained. The DPV signal was logarithmically related to the target DNA concentration from 8.3×10^{-7} to 8.3×10^{-10} M. The equation for the resulting calibration plot was $y = 0.6312 \log x - 1.071$ (x is the concentration of target DNA divide by pM, y is the peak current) with a correlation coefficient of 0.9978 and a detection limit of 7.1×10^{-10} M.

In order to confirm the binding specificity of the DLP to the target DNA, control experiments were performed by using different DNA sequences to hybridize with 0.33 μM DLP, including complementary DNA, 1-base-mismatched DNA, 3-base-mismatched DNA, and noncomplementary DNA. As shown in Fig. 7, the noncomplementary DNA produced a neglectable DPV signal (signal (d)), which was similar to that of the background signal (signal (e)), and when compared to the complementary sequence (signal (a)), the 3-base-mismatched DNA produced a very low insignificant DPV signal (signal (c)), and the 1-base-mismatched DNA generated a one-third signal (signal (b)). The hybridization selectivity of the DLP is partially due to the intrinsic recognition ability of DNA bases, and also for the reason that even if the probe is hybridized with mismatched target, the DLP's hairpin structure is partially opened; an obvious steric effect still exists for such "hybridization" to contact the electrode.

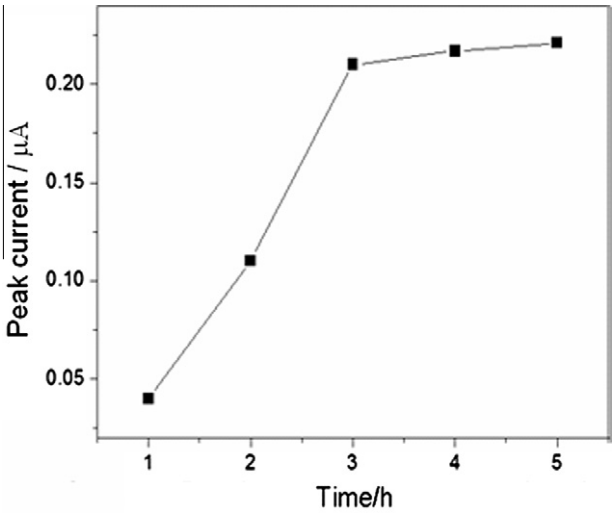

Fig.6. (A) UV–Vis spectra of the probe DNA solution before (upper trace) and after (bottom trace) UV irradiation; (B) target DNA of 2.3×10^{-8} M detection by using (a) probe DNA before UV irradiation and (b) probe DNA after UV irradiation.

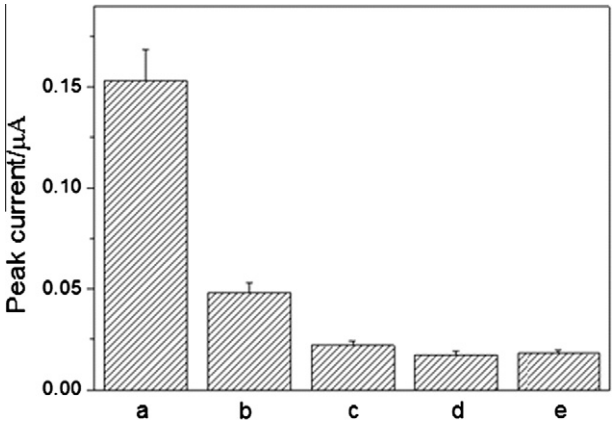

Fig.7. Comparison of the DPV signal of Pd^{2+} intensity for sensor challenged with (a) 4.3×10^{-9} M perfectly matched target DNA sequence; (b) 4.3×10^{-8} M 1-base-mismatched DNA sequence; (c) 4.3×10^{-8} M 3-base-mismatched sequence; (d) 4.3×10^{-8} M noncomplementary DNA sequence; and (e) without DNA sequences.

Conclusions

In this paper, a novel double-labeled DNA probe was designed with PdS nanoparticles and dabcyl-labeled hairpin probe DNA for electrochemical DNA detection based on molecular recognition. One α-CD/MCNT/GCE was fabricated for the hybridizer capture. The method of electrochemical DNA sensing has the advantage that the hybridization event between DLP and target DNA occurs in a homogeneous solution. The results illustrate that the DLP has high hybridization specificity and sensitivity. The proposed method provides a novel approach for the detection of DNA species.

Acknowledgment

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References

[1] J. Wang, Electrochemical nucleic acid biosensors, Anal. Chim. Acta 469 (2002) 63–71.
[2] E. Paleček, Past, present and future of nucleic acids electrochemistry, Talanta 56 (2002) 809–819.
[3] P. Kara, K. Kerman, D. Ozkan, B. Meric, A. Erdem, Z. Ozkan, M. Ozsoz, Electrochemical genosensor for the detection of interaction between methylene blue and DNA, Electrochem. Commun. 4 (2002) 705–709.
[4] A. Erdem, K. Kerman, B. Meric, M. Ozsoz, Methylene blue as a novel electrochemical hybridization indicator, Electroanalysis 13 (2001) 219–223.
[5] J. Wang, R. Polsky, A. Merkocüi, K.L. Turner, "Electroactive beads" for ultrasensitive DNA detection, Langmuir 19 (2003) 989–991.
[6] T. Ihara, M. Nakayama, M. Murata, K. Nakano, M. Maeda, Gene sensor using ferrocenyl oligonucleotide, Chem. Commun. 17 (1997) 1609–1612.
[7] H. Cai, N. Zhu, Y. Jiang, P. He, Y. Fang, Cu@Au alloy nanoparticle as oligonucleotides labels for electrochemical stripping detection of DNA hybridization, Biosens. Bioelectron. 18 (2003) 1311–1319.
[8] A. Jacob Hansen, R. Mukhopadhyay, J. Hansen, V. Kurt Gothelf, Femtomolar electrochemical detection of DNA targets using metal sulfide nanoparticles, J. Am. Chem. Soc. 128 (2006) 3860–3861.
[9] G. Liu, T.M.H. Lee, J. Wang, Nanocrystal-based bioelectronic coding of single nucleotide polymorphisms, J. Am. Chem. Soc. 127 (2005) 38–39.
[10] S. Ram, P. Vajpayee, R. Shanker, Rapid culture independent quantitative detection of enterotoxigenic *Escherichia coli* in surface waters by real-time PCR with molecular beacon, Environ. Sci. Technol. 42 (2008) 4577–4582.
[11] P.J. Santangelo, B. Nix, A. Tsourkas, G. Bao, Dual FRET molecular beacons for mRNA detection in living cells, Nucleic Acids Res. 32 (2004) e57.
[12] N. Nitin, P.J. Santangelo, G. Kim, S. Nie, G. Bao, Peptide-linked molecular beacons for efficient delivery and rapid mRNA detection in living cells, Nucleic Acids Res. 32 (2004) e58.
[13] I. Yoshimura, Y. Miyahara, N. Kasagi, H. Yamane, A. Ojida, I. Hamachi, Molecular recognition in a supramolecular hydrogel to afford a semi-wet sensor chip, J. Am. Chem. Soc. 126 (2004) 12204–12205.
[14] T. Ihara, A. Uemura, A. Futamura, M. Shimizu, N. Baba, S. Nishizawa, N. Teramae, A. Jyo, Cooperative DNA probing using a β-cyclodextrin–DNA conjugate and a nucleobase-specific fluorescent ligand, J. Am. Chem. Soc. 131 (2009) 1386–1387.
[15] M.V. Rekharsky, Y. Inoue, Complexation thermodynamics of cyclodextrins, Chem. Rev. 98 (1998) 1875–1917.
[16] V.T. D'Souza, K.B. Lipkowitz, Cyclodextrins, Chem. Rev. 98 (1998) 1741–2076.
[17] W. Saenger, Cyclodextrin inclusion compounds in research and industry, Angew. Chem., Int. Ed. Engl. 19 (1980) 344–362.
[18] A. Ferancová, J. Labuda, Cyclodextrins as electrode modifiers, J. Anal. Chem. 370 (2001) 1–10.
[19] A. Ferancová, E. Korgová, T. Buzinkaiová, W. Kutner, I. Štepánek, J. Labuda, Electrochemical sensors using screen-printed carbon electrode assemblies modified with the beta-cyclodextrin or carboxymethylated beta-cyclodextrin polymer films for determination of tricyclic antidepressive drugs, Anal. Chim. Acta 447 (2001) 47–54.
[20] Z. Wang, Y. Wang, G. Luo, The electrocatalytic oxidation of thymine at α-cyclodextrin incorporated carbon nanotube-coated electrode, Electroanalysis 15 (2003) 1129–1133.
[21] J. Wang, G. Liu, A. Merkocüi, Electrochemical coding technology for simultaneous detection of multiple DNA targets, J. Am. Chem. Soc. 125 (2003) 3214–3215.
[22] H. Fan, Z. Chang, R. Xing, M. Chen, Q.J. Wang, P.G. He, Y.Z. Fang, An electrochemical aptasensor for detection of thrombin based on target protein-induced strand displacement, Electroanalysis 19 (2008) 2113–2117.
[23] T.N. Milica, I.C. Mirjana, V. Veana, I.M. Olga, Transient bleaching of small lead sulfide colloids: influence of surface properties, J. Phys. Chem. 94 (1990) 6390–6396.
[24] I. Tomatsu, A. Hashidzume, A. Harada, Photoresponsive hydrogel system utilizing molecular recognition of alpha-cyclodextrin, Macromolecules 38 (2005) 5223–5227.
[25] G.S. Kumar, Photochemistry of azobenzene-containing polymers, Chem. Rev. 89 (l989) 1915–1925.
[26] I.A. Banerjee, L. Yu, H. Matsui, Application of host-guest chemistry in nanotube-based device fabrication: photochemically controlled immobilization of azobenzene nanotubes on pattern, J. Am. Chem. Soc. 125 (2003) 9542–9543.
[27] M.T. Khalid, J. Sa'ib Khouri, An equilibrium study of p-methyl red inclusion complexes with β- and α-cyclodextrins, Carbohydr. Res. 245 (1993) 165–173.