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Sequence-specific detection of trace DNA via a junction-probe electrochemical sensor employed template-enhanced hybridization strategy

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ABSTRACT

A novel junction-probe electrochemical biosensor for the sequence-specific detection of DNA with higher sensitivity and higher discrimination ability was described in here. This DNA biosensor is based on "junction-probe" detection strategy, which operates via a concept called template-enhanced hybridization processes (TeHyP). TeHyP encompasses a design strategy whereby two probes that do not hybridize to each other at a specific temperature can be made to anneal to each other in the presence of a template (target) via the formation of a ternary complex ("Y" junction structure). The resulting structure that forms after the template-enhanced hybridization then was detected by electrochemical method with $[Ru(NH_3)_6]^{3+}$ as signal molecule. We demonstrated that the formation of "Y" junction structure brings more $[Ru(NH_3)_6]^{3+}$ to the electrode surface via electrostatic interaction and results in an increasing electrochemical signal. The increasing electrochemical signal sensitively reflects the concentration of target DNA and shows a good linear relationship with the concentration of target DNA. By employing above strategy, this DNA biosensor could detect as low as 7.6×10^{-13} M target DNA and exhibited high discrimination ability even against single-base mismatch. In addition, this novel DNA biosensor is easy to fabricate and convenient to operate, and shows good stability, reproducibility and reusability.

1. Introduction

Many studies have reported that the individual differences in drug metabolism or susceptibility to diseases such as cancer and some genetic diseases are caused by single nucleotide polymorphisms (SNPs) and short insertion/deletion variations (InDels) (Sachidanandam et al., 2001). These studies have led to an increasing demand to detect specific genes in clinical samples for diagnostic purposes. So far, the main DNA detection methods are based on the preamplification of polymerase chain reaction (PCR) or ligase chain reaction (LCR) (Saiki et al., 1988; Barany, 1991). PCR is extremely sensitive, however, PCR or LCR based methods have some disadvantages including relatively longer assay time, higher assay cost and certain alleles amplify better than others under PCR conditions leading to over- or underestimation of the concentration of target DNA in a sample. Spured by the aforementioned limitations of PCR or LCR based methods, some alternative DNA detection methods such as hybridization-based biosensors have been developed (Abe and Kool, 2004; Grossmann and Seitz, 2006). Especially, the DNA biosensor developed recently takes the advantage of hybridization and modern optoelectronics, has shown great promise for rapid, sensitive, reliable and cost-effective DNA detection in clinical diagnostics (Jorge et al., 1996; Fan et al., 2005).

A variety of signal transduction techniques have been incorporated in the biosensor design, such as optical (Broude, 2002; Christel et al., 2002) and electrochemical transducer (Willner et al., 1999; Drummond et al., 2003; Fan et al., 2003; Boal and Barton, 2005; Wong and Gooding, 2006). Owing to the fact that electrochemical detectors are simple, portable, sensitive, fast response and low cost, electrochemical DNA biosensors have been recognized to be a promising way for diagnostic purpose as well as environmental and antibioterrorism monitoring (Miao and Bard, 2003, 2004). A typical electrochemical DNA biosensor can be prepared by immobilizing DNA probes on an electrode and using electroactive indicators to measure the hybridization events between the DNA probes and target DNA. Up to date, various sensing strategies were developed to improve the sensitivity and selectivity of electrochemical DNA biosensor (Wang et al., 2002, 2003; Kim et al., 2003; Liu and Anzai, 2004; Zhang et al., 2004, 2006; Niu et al., 2006; Li et al., 2007; Chen et al., 2008a,b; Liu et al., 2008). However, commercialization of these sensors might be hampered by one or more of following shortages: relatively poor stability of enzyme, com-

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plexity of fabrication, relatively low sensitivity or relatively poor specificity.

Recently, a novel nucleic acid detection technique called as junction-probe, which operates via a concept called template-enhanced hybridization processes (TeHyP), has been reported by Nakayama et al. (2008). As a new nucleic acid detection technique, junction-probe strategy was reported to have much better specificity ability for the detection of single-base mismatch. However, the method previously reported needs fluorescent labeling and restriction endonuclease, has obvious limitations such as time-consuming, complexity of fabrication and higher cost (Nakayama et al., 2008). In order to overcome above limitations, we herein firstly reported a junction-probes electrochemical DNA biosensor, which is simple, reproducible, sensitive, reusable, and has excellent differentiation ability for single-base mismatch.

2. Experimental

2.1. Reagents

All oligonucleotides were synthesized by TaKaRa biotechnology Co., Ltd. (Dalian, China), and their base sequences are as follows: target DNA: 5′-AGA GTC CAC CAA GCT TCA GAT TAG-3′; capture probe (probe A): 5′HS-AAA AGA TCA AAT TGG TGG ACT CTA AAA-3′; reporter probe (probe B): 5′-TCT GAA GCT TTG ATC-3′; single-base mismatched probes: S1-5′-AGA GTC CAC CAA GCT TGA GAT TAG-3′; S2-5′-AGA GTC CAC CAA GCT TAA GAT TAG-3′; S3-5′-AGA GTC CAC CAA GCT TTA GAT TAG-3′; non-complementary probe: 5′-CCC ACA TGA GTG CGA AGT TCG CGA-3′. Tris-(hydroxymethyl) aminomethane was purchased from Cxbio Biotechnology Co. Ltd. (Denmark). Ethylenediaminetetraacetic acid (EDTA), mercaptohexanol (MCH) and tris (2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sigma–Aldrich (USA). The buffer solutions are as follows: hybridization buffer solution was 1 M NaCl–10 mM TE solution (pH 8.0), electrochemical measurement buffer solution was 10 mM Tris–HCl solutions (pH 7.0), DNA immobilization buffer solution was 10 mM TE–10 mM TCEP–1 M NaCl solution (pH 7.4), and washing buffer solution was 0.1 M NaCl–10 mM PB solution (pH 7.4). All solutions were prepared with MilliQ water (18.2 MΩ).

2.2. Electrochemical measurements

All electrochemical measurements were performed by using CHI 660C Electrochemical Workstation (CH Instrument, USA). The electrochemical system consisted of a working electrode (a gold disk electrode modified with DNA probe), a platinum wire as the auxiliary electrode, and a reference electrode (Ag/AgCl). Cyclic voltammetry (CV) and linear sweep voltammetry (LSV) was carried out in 10 mM Tris–HCl–5 μM RuHex solution (pH 7.0) at a scan rate of 100 mV/s in the potential range between −0.5 and +0.1 V. Electrochemical impedance spectra (EIS) was measured in 10 mM Tris–HCl–10 mM $[Fe(CN)6]^{4-/3-}$ solution (pH 7.0) in the frequency range of 0.1–10^5 Hz.

2.3. DNA self-assembly and hybridization at gold electrode

A gold disk electrode (2 mm diameter) was firstly polished to obtain mirror surface with 0.05 μm alumina powder, followed by sonication in ethanol and water for 5 min, respectively. Then, the gold electrode (GE) was electrochemically cleaned to remove any remaining impurities (Fan et al., 2003). After drying with nitrogen gas, the GE was immediately used for DNA immobilization. Firstly, GE was modified with 3 μL of 2 μM probe A solution (Steel et al., 1998; Lao et al., 2005). Then, the DNA-modified GE was further treated with 1 mM MCH for 2 h to cover the spare room of electrode surface and to optimize the orientation of the capture probes to make hybridization easier (Miao and Bard, 2004). The DNA surface density as well as DNA hybridization efficiency was measured with chronocoulometry (CC) as previously described (Steel et al., 1998; Lao et al., 2005).

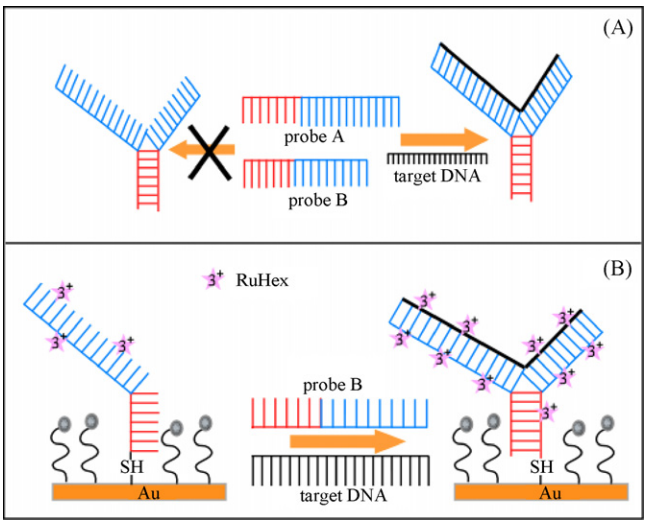

Fig. 1. The sketching of TeHyP concept (A) and the experimental principle of junction-probe electrochemical DNA sensor (B).

2.4. Determination of target DNA

Target DNA was pre-annealed with probe B at 30 °C for 30 min in hybridization buffer solution, and then 5 μL of the hybridization solution was placed on the DNA SAM (self-assembly monolayer) of probe A modified GE for 1 h at room temperature. After hybridization, the GE was extensively rinsed with washing buffer solution and dried under a stream of nitrogen gas, and was used for electrochemical measurement. After once measurement, the GE was then denatured in ultrapure water at 65 °C for 5 min (the melting temperature of "Y" junction-type complex was about 47 °C) in order to revive the sensor (probe A modified GE), and then the probe A modified GE was used for the next cycle of hybridization and detection.

3. Results and discussion

3.1. Experimental principle of the junction-probes electrochemical DNA sensor

As we mentioned above, junction-probe operates via a concept called TeHyP, which encompasses a design strategy whereby two probes that do not hybridize to each other at a specific temperature can be made to anneal to each other in the presence of a template (target) via the formation of a ternary complex. The detailed principle of our work is illustrated in Fig. 1. As shown in Fig. 1A, probe A contains two regions (colored blue and red) that are complementary to part of target DNA (colored blue) and probe B (colored red), respectively. Probe B also contains two regions: a 7-mer region (colored red) that is complementary to part of probe A and a region that is complementary to part of target DNA (colored blue). (For interpretation of the references to color in this sentence, the reader is referred to the web version of the article.) In the absence of target DNA, probes A and B do not hybridize to each other; whereas, in the presence of target DNA, probes A and B and target DNA will hybridize to form a ternary "Y" junction structure. The resulting structure that forms after the template-enhanced

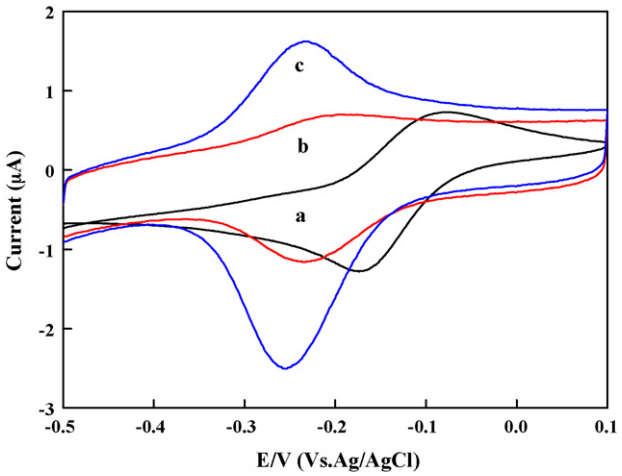

Fig. 2. CVs of RuHex on pure MCH modified GE (a), probe A/MCH modified GE (b) and probe A/MCH/target/probe B modified GE (c). The concentration of RuHex was 20 μM for curve a, was 5 μM for others, data was obtained in 10 mM Tris–HCl buffer solution (pH 7.0), scan rates = 100 mV/s.

hybridization then was detected by electrochemical method with $[Ru(NH_3)_6]^{3+}$ (RuHex) as signal molecule (see Fig. 1B). Previous studies have demonstrated that RuHex can be bound to DNA by electrostatic interaction since DNA backbone has negative charge (Ho et al., 1987; Steel et al., 1998). Therefore, if we expose the probe A modified GE in an RuHex solution at a low ionic strength, the electrochemical signals responding to the redox behavior of RuHex reflect the amounts of DNA on the GE surfaces. As shown in Fig. 1B, when probe A modified GE is incubated in the solution containing target DNA and probe B, probe A would hybridize with target DNA and probe B to form ternary "Y" junction structure. As a result of the formation of ternary "Y" junction structure, the amount of the RuHex electrostatically binding to the surface of GE increases, and results in a bigger redox signal in electrochemical measurement. In contrast, in the absence of target DNA, the "Y" junction structure cannot be formed, and as a result, the amount of the RuHex electrostatically binding to the surface of GE decreases and results in a lower redox signal in electrochemical measurement. By employing this strategy, we demonstrate that the DNA biosensor has a higher sensitively and better discrimination ability for the detection of target DNA.

3.2. Characterization of the different stage of the electrochemical DNA biosensor preparation

In order to test if indeed the DNA biosensor works as our expected, the CV behavior of RuHex was investigated at different stages of the electrochemical DNA biosensor preparation, and the cyclic voltammograms (CVs) of RuHex at different GE surfaces was shown in Fig. 2. As shown in Fig. 2, a pair of peaks corresponding to the reduction and oxidation of RuHex can only be observed at a relatively higher concentration (20 μM) of RuHex solution at MCH modified GE (curve a), and the peak currents are linearly proportional to the square root of scan rates (V) [I (μA) = 0.4183 + 0.0788$\nu^{1/2}$, r = 0.9959], suggesting that the electron-transfer reaction of RuHex is diffusion-controlled at pure MCH surface (Nielsen et al., 2004; Lao et al., 2005). Whereas, on the probe A/MCH modified GE, a pair of peaks at −0.26 V can be observed in a more dilute RuHex solution (5 μM) (curve b), indicating that the electron-transfer reaction of RuHex at probe A/MCH surface is a surface-confined redox process since the peak separation was close to zero and peak currents are linearly proportional to scan rates [I (μA) = 1.8875 + 0.0203ν, r = 0.9978]. This pair of peaks was ascribed to RuHex electrostatically binding to DNA surface, and reflects the amount of DNA on the electrode surface.

In Fig. 2, an obvious difference of the redox potentials between curves a and b was also observed. The difference of the redox potentials between curves a and b provides the possibility to distinguish the background current caused from the RuHex diffusively accessing to the electrode and the current caused from the RuHex electrostatically binding to the DNA surface. This is important because RuHex in solution can still diffusively access to electrode despite of the presence of the MCH layer, since the short alkanethiol of MCH provides an insufficient barrier to block electron transfer. Thus, the rather large difference in redox potential offers the opportunity to separate signal current from background current, and provides a basis to obtain high sensitivity.

When probe A/MCH modified GE is incubated in the solutions containing target DNA and probe B, the capture probe hybridized with target DNA and probe B to form a ternary "Y" junction structure. As a result, the amount of the RuHex electrostatically binding to the GE surface increases and finally results in a much higher current signals (curve c), which forms the basis of electrochemical quantification of target DNA.

The DNA-modified GE was also characterized by EIS to demonstrate the modification process further, and the EIS results are shown in Fig. 3. Compared with bare GE (curve a), probe A modified GE showed a larger R_{et} (resistance of electron-transfer) (curve c) since the electrostatic repulsion between negative charges of DNA backbone and $Fe(CN)_6^{3-/4-}$. In comparison with probe A modified GE, probe A/MCH modified GE showed a little smaller R_{et} (curve b), indicting that the treatment of MCH has removed the nonspecifically adsorbed probe from GE surface. When probe A/MCH modified GE is incubated in the solutions containing target DNA and probe B, probe A/MCH/target DNA/probe B modified GE showed a much bigger R_{et} (curve d). This is due to that probe A hybridized with target DNA and probe B to form "Y" junction structure, and the formation of "Y" junction structure resulted in a much denser negative charges on the GE surface and generated a much stronger electrostatic repulsion between GE surface and $Fe(CN)_6^{3-/4-}$. The results of EIS are consistent with that of CVs, indicated that our biosensor indeed works as our expected.

3.3. Effect of surface density of probe A on the sensor's sensitivity

Generally, DNA hybridization efficiency at electrode surfaces is sensitively dependent on the surface density of DNA. We thus pre-

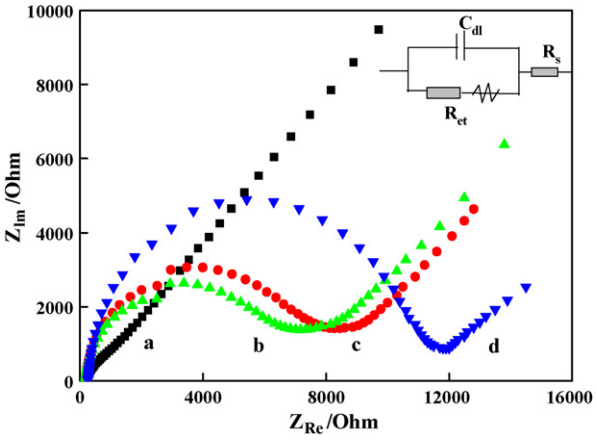

Fig. 3. EIS (Nyquist plot) of a bare GE (a), a probe A/MCH modified GE (b), a probe A modified GE (c), and a probe A/MCH/target/probe B modified GE (d). Data was obtained in 10 mM Tris–HCl–10 mM $[Fe(CN)6]^{4-/3-}$ solution (pH 7.0), the biased potential was 0.18 V (versus Ag/AgCl), the frequency range is 0.1–10^5 Hz and the amplitude was 5.0 mV.

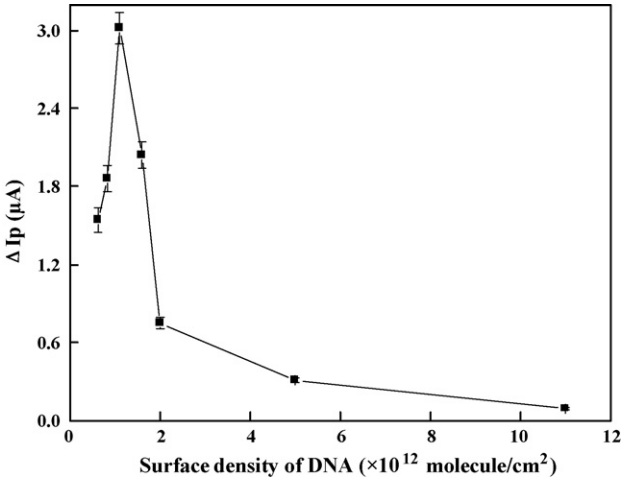

Fig. 4. The relationship between sensor signal and surface density of capture probe. The concentration of RuHex was 5 μM, data was obtained in 10 mM Tris–HCl buffer solution (pH 7.0), scan rates = 100 mV/s.

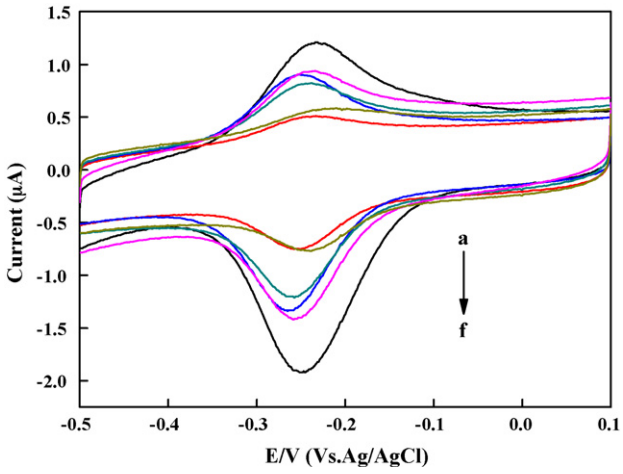

Fig. 5. CVs of 5 μM RuHex at probe A/MCH modified GE (a) and at probe A/MCH modified GE after it hybridized with non-complementary sequence and probe B (b), S2 one-base mismatch sequence and probe B (c), S1 one-base mismatch sequence and probe B (d), S3 one-base mismatch sequence and probe B (e) and complementary target sequence and probe B (f). Other experimental conditions are the same as that in Fig. 4.

pared a series of probe A SAM with different surface density by varying probe A concentration and self-assembly time, and measured the peak currents of $[Ru(NH_3)_6]^{3+}$ at each probe A SAM. Fig. 4 shows the correlation between the sensor signal ΔIp (μA, $\Delta Ip = I_0 - I_{after}$, where I_0 and I_{after} refer to the current obtained before and after the probe A/MCH modified GE was incubated in 50 pM of target DNA and probe B, respectively) and the surface density of the probe A. Fig. 4 clearly indicates that the control of DNA assembly was essential for the sensitivity. From Fig. 4, it was clearly observed that the ΔIp increased with the increase of surface density when the surface density is smaller than 1.3×10^{12} molecules/cm^2, then ΔIp decreased with the increase of surface density. This is because that the hybridization of target DNA, probe B and probe A is hindered due to spatial restriction in higher surface density. Our results showed that the ideal surface density for the sensor is 1.1×10^{12}–1.6×10^{12} molecules/cm^2. In above range, there is a largest magnitude in the sensor signal (ΔIp).

3.4. Hybridization specificity of the DNA biosensor

The selectivity of the sensor was investigated by using capture probe/MCH modified GE to hybridize with various DNA sequences (complementary DNA, non-complementary DNA sequence and three kinds of one-base mismatch DNA mentioned in reagents). Fig. 5 shows the CVs of RuHex at probe A/MCH modified GE (a) and at probe A/MCH modified GE after it hybridized with the same amount of complementary DNA sequence (f), non-complementary DNA sequence (b) and one-base mismatch DNA sequence (c, d, and e). From Fig. 5, we clearly observed that: (1) There is a lowest signal on probe A/MCH modified GE (curve a). This is due to that relatively smaller quantity of RuHex was adsorbed on the probe A/MCH modified GE surface. (2) A highest signal was observed on probe A/MCH modified GE after it hybridized with complementary DNA sequence. This is because that the probe A hybridized with complementary DNA sequence and probe B to form "Y" junction structure, and increased the density of DNA and negative charges of the electrode surface. As a result of above effect, the amount of RuHex electrostatically binding to the electrode's surface increased, and resulted in a much bigger current signal (curve f). (3) Compared with curve a, the peak current almost did not increase when the probe A/MCH modified GE was exposed to the non-complementary DNA (curve b), implied that there was no change occurring at probe A/MCH modified electrode surface and the hybridization or the "Y" junction structure formation had not occurred. The result demonstrated that only a complementary DNA sequence could form a "Y" junction structure on probe A/MCH modified electrode surface and resulted in a significant increase on the signal. No significant change in signal was observed for non-complementary DNA sequence, indicating the good sequence-specific detection of our sensor. (4) For one-base mismatch DNA, the signals (curves c, d, and e) were much lower than that of fully complementary DNA sequences (curve f), but higher than that of non-complementary DNA sequences (curve b), indicating that the complete hybridization had not been accomplished. All above results indicating that our sensor has good sequence specificity for one-base mismatch DNA.

3.5. The sensitivity of the sensor

The sensitivity of the electrochemical DNA biosensor for the target DNA was investigated by varying the target DNA concentration. The currents response to 5 μM RuHex after the sensor hybridized with different concentration of target DNA was measured with LSV. The average current shows a good linear correlation with the

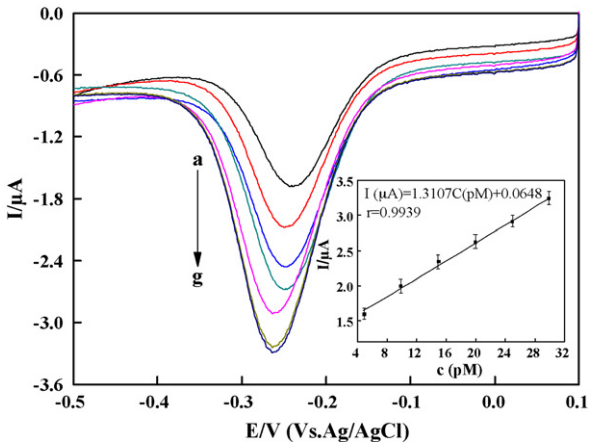

Fig. 6. LSV of 5 μM RuHex on the probe A/MCH modified GE after it hybridized with probe B and different concentration of the target DNA. Target DNA concentration is ($\times 10^{-12}$ M): (a) 5.0; (b) 10; (c) 15; (d) 20; (e) 25; (f) 30; (g) 35. Inset shows the plot of the peak current of RuHex as a function of the target DNA concentration. Other experimental conditions are the same as that in Fig. 4.

concentration of the complementary target DNA in the range of 5.0×10^{-12}–3.0×10^{-11} M (see Fig. 6). The regression equation is

$$I\ (\mu A) = 1.3107C\ (pM) + 0.0648, 44, \quad r = 0.9939$$

The detection limit ($3\sigma/S$, where σ is the standard deviation of the blank solution, $n = 8$) was calculated to be 7.6×10^{-13} M for complementary target DNA. The relative standard deviations (RSD) of the sensor for the detection of 2.0×10^{-11} M target DNA is 6.8% ($n = 8$).

The electrochemical DNA biosensor based on the electrostatic interaction of DNA and RuHex is easy to fabricate, convenient to operate and has a good stability, however, this type of sensor has been reported to have a relatively lower sensitivity even if it coupled with some complicated techniques such as nanoelectrodes, etc. (Gasparac et al., 2004). In this study, by employing the strategy of junction-probe, the sensitivity and the discrimination ability of the sensor have been greatly improved. The detection limit of 7.6×10^{-13} M of our sensor is much lower than that of the same-type sensor previously reported (Steel et al., 1998; Gasparac et al., 2004), is just a little higher than that of other DNA biosensors, which used complicated sensing strategy such as signal amplification of enzyme-based incorporation (Zhang et al., 2004) or nanoparticles-mediated amplification (Zhang et al., 2006; Li et al., 2007). However, considering the easiness of fabrication, our sensor is still of analytical advantage.

4. Conclusions

A novel electrochemical DNA biosensor for the sequence-specific detection of DNA targets based on junction-probe was reported in this study. The sensor exhibited very high sensibility and discrimination ability even for the detection of single-base mismatch. These features, as well as its fabrication easiness, operation convenience, stability and reusability, make it a promising alternative to conventional DNA detection methods. Since the sensor reported in this work is an electrochemical biosensor, one might expect the design of an integrated, portable and low cost device for DNA detection based on junction-probe described in this study.

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References

Abe, H., Kool, E.T., 2004. J. Am. Chem. Soc. 126, 13980–13986.
Barany, F., 1991. Proc. Natl. Acad. Sci. U.S.A. 88, 189–193.
Boal, A.K., Barton, J.K., 2005. Bioconjugate Chem. 16, 312–321.
Broude, N.E., 2002. Trends Biotechnol. 20, 249–256.
Chen, J.H., Zhang, J., Huang, L.Y., Lin, X.H., Chen, G.N., 2008a. Biosens. Bioelectron. 24, 349–355.
Chen, J.H., Zhang, J., Wang, K., Lin, X.H., Huang, L.Y., Chen, G.N., 2008b. Anal. Chem. 80, 8028–8034.
Christel, M., Evelin, H., Christina, F., 2002. Cancer Genet. Cytogenet. 136, 149–150.
Drummond, T.G., Hill, M.G., Barton, J.K., 2003. Nat. Biotechnol. 21, 1192–1199.
Fan, C.H., Plaxco, K.W., Heeger, A., 2003. Proc. Natl. Acad. Sci. U.S.A. 100, 9134–9137.
Fan, C.H., Plaxco, K.W., Heeger, A., 2005. Trends Biotechnol. 23, 186–192.
Gasparac, R., Taft, B.T., Lapicrre-Devlin, M.A., Lazareck, A.D., Xu, J.M., Kelley, S.O., 2004. J. Am. Chem. Soc. 126, 12270–12271.
Grossmann, T.N., Seitz, O., 2006. J. Am. Chem. Soc. 128, 15596–15597.
Ho, P.S., Frederick, C.A., Saal, D., Wang, A.H., Rich, A.J., 1987. Biomol. Struct. Dyn. 4, 521–534.
Jorge, E.C., Moshe, T., Hagop, K., 1996. Am. J. Med. 100, 555–570.
Kim, E., Kim, K., Yang, H., Kim, Y.T., Kwak, J., 2003. Anal. Chem. 75, 5665–5672.
Lao, R., Song, S., Wu, H., Wang, L., Zhang, Z., He, L., Fan, C.H., 2005. Anal. Chem. 77, 6475–6480.
Li, D., Yan, Y., Wieckowska, A., Willner, I., 2007. Chem. Commun. 34, 3544–3546.
Liu, A., Anzai, J.-i., 2004. Anal. Chem. 76, 2975–2980.
Liu, G., Wan, Y., Vincent, G., Zhang, J., Wang, L.H., Song, S.P., Fan, C.H., 2008. J. Am. Chem. Soc. 130, 6820–6825.
Miao, W., Bard, A.J., 2003. Anal. Chem. 75, 5825–5834.
Miao, W., Bard, A.J., 2004. Anal. Chem. 76, 5379–5386.
Nakayama, S., Yan, L., Sintim, H.O., 2008. J. Am. Chem. Soc. 130, 12560–12561.
Nielsen, M., Larsen, L.H., Jetten, M.S., Revsbech, N.P., 2004. Appl. Environ. Microbiol. 70, 6551–6558.
Niu, S.Y., Zhang, S.S., Wang, L., Li, X.M., 2006. J. Electroanal. Chem. 597, 111–118.
Sachidanandam, R., Weissman, D., Schmidt, S.C., Kakol, J.M., Stein, L.D., Marth, G., 2001. Nature 409, 928–933.
Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B., Erlich, H.A., 1988. Science 239, 487–491.
Steel, A.B., Herne, T.M., Tarlov, M.J., 1998. Anal. Chem. 70, 4670–4677.
Wang, J., Liu, G., Polsky, R., Merkoçi, A., 2002. Electrochem. Commun. 4, 722–726.
Wang, J., Rincon, O., Polsky, R., Dominguez, E., 2003. Electrochem. Commun. 5, 83–86.
Willner, I., Katz, E., Willner, B., 1999. Sensors Update 5, 45–102.
Wong, E.L.S., Gooding, J.J., 2006. Anal. Chem. 78, 2138–2144.
Zhang, Y., Pothukuchy, A., Shin, W., Kim, Y., Heller, A., 2004. Anal. Chem. 76, 4093–4097.
Zhang, J., Song, S.P., Zhang, L.Y., Wang, L.H., Wu, H.P., Pan, D., Fan, C.H., 2006. J. Am. Chem. Soc. 128, 8575–8580.