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Development of an electrochemical DNA biosensor with the DNA immobilization based on in situ generation of dithiocarbamate ligands

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ABSTRACT

In this article, a simple and effective strategy for DNA immobilization on gold electrode surface is developed. The amine-modified oligonucleotide was firstly reacted with CS_2 and then in situ generated dithiocarbamate group functionalized probe DNA (DTC–DNA) was directly attached onto the gold surface by bidentate anchoring points. The DNA biosensor fabrication process was characterized by cyclic voltammetry and electrochemical impedance spectroscopy with the use of $Fe(CN)_6^{3-/4-}$ as a redox indicator. The hybridization of DTC–DNA with complementary target DNA could be well distinguished with the use of $Co(phen)_3^{3+}$ as an electrochemical indicator. The fabricated electrochemical DNA biosensor could achieve a detection limit of about 0.1 nM toward complementary target DNA. Also, the current strategy is readily operated with less time consumed and lower cost compared with those commonly used strategies.

1. Introduction

The effective immobilization of probe DNA on electrode surface is a primary and essential step in DNA biosensor or biochip fabrication for its amounts of important applications in clinical diagnosis, genetics, pathology, and environmental monitoring [1–6]. Control of the surface density and orientation of the probe DNA is of substantial importance for the achievement of efficient and specific hybridization [7–11]. Improvement of immobilization strength of probe DNA on the substrate would allow us to realize precise and reliable genetic analysis as well as repeated use of the DNA biosensor or chip. Thus, the simple and robust immobilization techniques are always pursued by most researchers working in clinical or laboratory analysis to achieve optimized performance of biosensor or biochip [12,13]. A number of strategies have been explored for DNA immobilization including adsorption [14–17], copolymerization [18,19], complexation [20], covalent attachment [21,22], and avidin–biotin linking [23], etc. Among them, the most widespread immobilization strategy is via a self-assembled monolayer (SAM) of thiolated ssDNA chemisorbed on a gold surface [24–30]. The thiolated ssDNA could be easily oxidized by the oxidizing agents present in solution [31,32]. Thus, the stability and regeneration of fabricated DNA sensing interface is often not satisfactory enough. Also, the optimization of probe DNA immobilization is usually executed with the post-treatment by a diluent of mercapto-hexanol. Recently, ternary mixed monolayers are also developed to control the orientation of probe DNA and enhance the electrochemical DNA biosensor performance [33–35]. Although these strategies are easy to achieve and often advised by many researchers, a large amount of efforts are necessary for the screening of optimum probe DNA density on the DNA biosensor interface. Also, a sole anchoring point is not robust enough for the probe DNA immobilization on metal surface in biosensing events.

In order to enhance the DNA immobilization stability and improve the hybridization efficiency of immobilized ssDNA on electrode surface, a number of strategies have been explored [36–39]. It is especially intriguing for these strategies of DNA immobilization that employ multiple anchoring points with substrate to enhance the stability of DNA immobilization. Furthermore, the probe DNA density on the substrate prepared in these manners could be more efficiently adjusted compared with that by a single-point attachment of thiolated DNA. The Mirkin group [40–42] has applied steroid cyclic disulfide groups, multiple thiol-anchors, and triple cyclic disulfide moieties as anchors to optimize the DNA immobilization on metal surface. Dougan et al. [43] used thioctic acid modified oligonucleotides to realize the stable immobilization of ssDNA on the surface of gold nanoparticle. Sakata et al. [44] achieved a stable immobilization of oligonucleotide probe on a gold substrate using tripodal thiol derivatives. All these strategies resulted in relatively more stable DNA immobilization but involved complicated synthetic procedures. A strategy that is easy to perform with, less time consuming and lower cost is highly desirable.

In this report, a simple and effective strategy for DNA immobilization on gold electrode surface is utilized and the development in electrochemical DNA biosensor is investigated in detail. The probe DNA immobilization was operated with the first generation of a dithiocarbamate group functionalized probe DNA (DTC–DNA) by reaction of

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amine-modified oligonucleotides with CS_2 in situ and then the direct attachment of DTC–DNA onto the gold surface by bidentate anchoring points. Dithiocarbamates (DTC) conjugated with small organic entities have been previously used to stabilize nanomaterials, including gold nanoparticles, quantum dots, etc. [45–47]. The DTC ligands could be easily generated by treating CS_2 with primary or secondary amines in an alkaline media. The small interatomic distance between two sulfur atoms of the DTC ligand confer a stronger affinity between ligands and the metal surface.

2. Experimental

2.1. Materials

All of synthetic oligonucleotides were purchased from SBS Genetech. Co. Ltd. (Beijing, China). Their base sequences are as follows:

Probe DNA sequence (P-DNA): 5′-NH_2-ACGCTACTAATAGTATTTTT-3′;
Complementary target DNA sequence (CT-DNA): 5′-AAAAATACTA-TTAGTAGCGT-3′;
One-base mismatched DNA sequence (MT-DNA): 5′-AAAAATACTAT-TACAAGCGT-3′; and
Non-complementary target DNA (NT-DNA): 5′-CATTAGAGATG-GGGACGA-3′.

The tris(1,10-phenanthroline) Cobalt(III) perchlorate, $Co(phen)_3(ClO4)_3$, was synthesized as that reported in the literature [48]. $K_3Fe(CN)_6$, $K_4Fe(CN)_6$, CS_2 were purchased from Tianjin Ruijinte chemical. Co. Ltd (Tianjin, China). All other chemicals were all of analytical grade and used without further purification. Doubly distilled water was used throughout.

2.2. Apparatus

Cyclic voltammetry (CV) and differential Pulse Voltammetry (DPV) measurements were performed with a CHI832B electrochemical analyzer (Shanghai CH Instrument Company, China). Electrochemical Impedance spectroscopy (EIS) measurements were carried out on a CHI660C electrochemical workstation (Shanghai CH Instrument Company, China). All electrochemical experiments were performed with a conventional three-electrode system comprising a gold working electrode, a platinum wire auxiliary electrode, and an Ag/AgCl reference electrode. The EIS measurements were performed in a solution of 1 mM $K_4Fe(CN)_6/K_3Fe(CN)_6$ in phosphate-buffered saline (PBS buffer, 10 mM, pH 7.0, 0.1 M KCl).

2.3. Preparation of the DNA modified electrode

The functionalization of amine-modified DNA by a dithiocarbamate group (DTC–DNA) was accomplished according to the procedures from literature [49]. Briefly, 100 μM amine-modified probe DNA was reacted with 100 μM CS_2 in 10 mM borate buffer (pH 9) for 1 h under stirring.

The gold working electrode surface was freshly polished prior to use with 0.05 μm alumina powder and then cleaned ultrasonically sequentially in acetone and water for 3 min. The immobilization of DTC–DNA on cleaned gold electrode was achieved with the immersion of electrode in diluted DTC–DNA (1.78 μM) for 36 h. Then the electrodes were taken out and rinsed with borate buffer solution and doubly distilled water.

2.4. The hybridization of DTC–DNA modified electrode with target DNA

The hybridization experiments were carried out by immersing the DTC–DNA modified electrode into different concentration of target DNA (complementary or non-complementary) in 10 mM PBS buffer solution (pH = 7.0) for 40 min at 37 °C. And then, the hybridized electrodes were rinsed with PBS buffer solution (10 mM, pH = 7.0) containing 0.1% SDS buffer solution and water and then dried with nitrogen.

2.5. Electrochemical measurement using $Co(phen)_3^{3+}$ as the electrochemical indicator

The immobilized DTC–DNA or hybridized electrodes were firstly immersed into 0.1 mM $Co(phen)_3^{3+}$ of 0.1 M PBS (pH 7.0) for 30 min, and then the cyclic voltammograms (CV) and differential pulse voltammograms (DPV) were recorded in blank 0.1 M PBS (pH 7.0) solution.

3. Results and discussion

3.1. The fabrication principle of DNA biosensor

The whole fabrication process of DNA biosensor is schematically illustrated in Fig. 1. The amine-modified probe DNA is firstly activated with CS_2 to generate the dithiocarbamate–DNA conjugate (DTC–DNA), which is then directly attached onto the gold electrode surface by bidentate anchoring points. The immobilized DNA could be further hybridized with target DNA. It has been well known that the most commonly employed strategy for the immobilization of amine-modified DNA is via EDC/NHS activation method. Also, the carboxyl acid group should be firstly introduced onto the electrode surface.

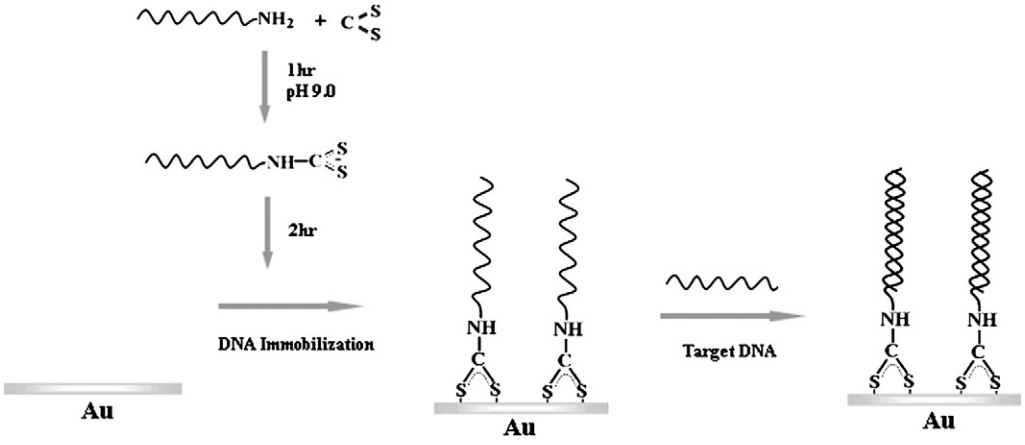

Fig. 1. The schematic illustrations of DNA biosensor fabrication process.

Compared with it, the current strategy by only one-step direct immobilization avoids the use of expensive reagents of EDC/NHS and is easier to operate. Also, the DNA immobilization via bidentate anchoring points is considered to confer a more strong adhesion on electrode surface compared with that of the immobilized probe DNA by a sole anchoring point.

3.2. Electrochemical behaviors of $[Fe(CN)_6^{3-/4-}]$ on the DTC–DNA modified electrode surface

The DNA biosensor fabrication process could be monitored based on the electron transfer of ferricyanide through the modified electrode. Fig. 2 shows the cyclic voltammograms obtained for differently modified electrodes in 1 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ of 10 mM PBS solution (pH 7.0, 0.1 M KCl) at the scan rate of 100 mV s^{-1}. A pair of well-defined redox peaks could be observed at the bare gold electrode with the anodic (E_{pa}) and cathodic (E_{pc}) peak potential of 0.23 V and 0.16 V, respectively, and a peak to peak potential separation of about 70 mV (Curve a). These peaks could be definitely attributed to the redox behaviors of $[Fe(CN)_6^{3-/4-}]$. The direct immobilization of dithiocarbamate–DNA conjugate on electrode surface induced a big decrease of peak current and an increase of peak to peak potential separation (Curve b), indicating that the probe DNA has been successfully immobilized on the electrode surface and the peak current decrease could be well assigned to the repellence of $[Fe(CN)_6^{3-/4-}]$ by the negatively charged phosphate backbone of probe DNA. When the hybridization of DTC–DNA modified electrode with target complementary DNA was proceeded, the peak current of $[Fe(CN)_6^{3-/4-}]$ was observed with a further large decrease (Curve c). The introduction of complementary DNA increases the negative charge responsible for the increased repellence toward redox species.

The cyclic voltammograms for DTC–DNA modified electrode in 10 mM PBS solution containing 1.0 mM $[Fe(CN)_6^{3-/4-}]$ and 0.1 M KCl with the scan rates varied from 0.03 to 0.3 V s^{-1} were investigated. It could be obtained that the peak current were proportional to the square roots of the scan rates (data not shown), which was characteristic for a typical diffusion-controlled electrochemical behavior.

EIS could provide further information on the impedance changes of the electrode surface during the modification process. In EIS, the semicircle diameter could represent the electron transfer resistance, R_{et}, which dominate the electron transfer kinetics of the redox probe at the electrode interface. Fig. 3 shows the Nyquist plot of the differently modified electrodes in 10 mM PBS solution containing 1.0 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) and 0.1 M KCl at open circuit potential with the frequency varied from 0.1 Hz to 10 kHz. An almost straight line was observed for the bare gold electrode (Curve a), which was

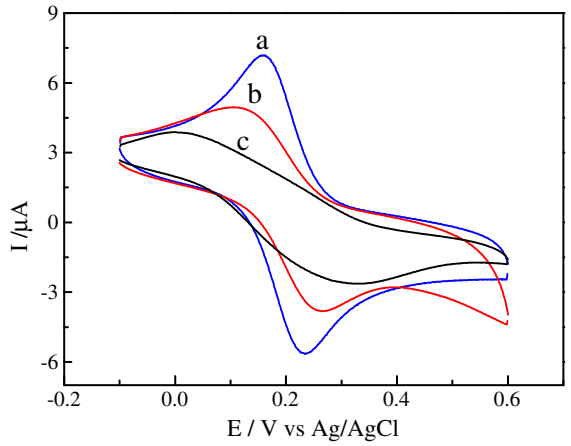

Fig. 2. Cyclic voltammograms in 1 mM $Fe(CN)_6^{3-/4-}$ of 10 mM PBS buffer (pH 7.0, 0.1 M KCl) for bare (a), DTC–DNA modified (b) and hybridized with complementary target DNA (c) at a scan rate of 100 mV s^{-1}.

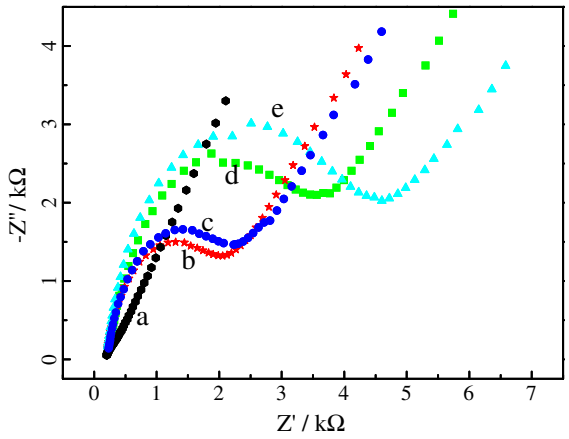

Fig. 3. Ac impedance responses in 10 mM PBS buffer (pH 7.0, 0.1 M KCl) containing 1.0 mM $Fe(CN)_6^{3-/4-}$ for bare gold electrode (a), DTC–DNA modified (b), hybridized electrodes with non-complementary target (c), one-base mismatched DNA (d) and complementary target DNA (e), respectively.

typical characteristics of a mass diffusion-controlled electron transfer process. The immobilization of DTC–DNA monolayer on electrode surface makes an increase of Ret from 10^{-12} Ω for the bare gold electrode to 2532 Ω (Curve b), which could be ascribed to the repellence of redox probe from approaching electrode surface by negative-charged phosphate skeletons of DNA. A further increase of Ret to be 4259 Ω could be obtained after the hybridization of the immobilized DTC–DNA with the complementary target DNA (Curve e). Also, it was observed that the hybridization with non-complementary target DNA yielded almost no resistance change compared with that of the DTC–DNA modified electrode (Curve c). The hybridization with one-base mismatched target DNA induced a Ret value of about 3545 Ω (Curve d). The as-observed results fully indicated that the EIS could be used as a feasible tool to monitor DNA hybridization.

3.3. The selectivity of electrochemical DNA biosensor

Fig. 4 shows the cyclic voltammograms of the differently modified electrodes in blank PBS buffer solution after immersion in 0.1 mM $Co(phen)_3^{3+}$ of 0.1 M PBS (pH 7.0) for 30 min. DTC–DNA immobilization on electrode surface induced a small pair of peaks of $Co(phen)_3^{3+}$ with the anodic and cathodic potential of 0.18 and 0.11 V, respectively (Curve a). This could be ascribed to the non-specific adsorption of $Co(phen)_3^{3+}$ on the DTC–DNA chain by an electrostatic interaction mode. After hybridization with complementary target DNA, the peaks were found to be largely increased from about 1.5 μA to 3.9 μA (Curve d). The binding mode between $Co(phen)_3^{3+}$ and dsDNA could be simply considered as the main intercalated interaction according to Bard [50] and Pang [51]. The recognition of DTC–DNA modified electrode with non-complementary DNA and one-base mismatched DNA has also been characterized. The peak current of $Co(phen)_3^{3+}$ for the hybrid of DTC–DNA with one-base mismatched DNA was found to be about 2.5 μA (Curve c). Whereas the hybridization of DTC–DNA with non-complementary target DNA invoked almost no peak current change of $Co(phen)_3^{3+}$ (Curve b) compared with that of only DTC–DNA modified electrode, indicating that $Co(phen)_3^{3+}$ could be used as a good indicator to selectively detect the target DNA [52].

Fig. 5 shows the responses of different scan rates on the redox peaks of $Co(phen)_3^{3+}$ bound into dsDNA modified electrode in 0.1 M PBS (pH 7.0). It was obvious that the peak current increased with scan rates and was in direct proportion with the scan rates in the tested range from 30 to 300 mV s^{-1} (Inset in Fig. 5), while the peak potential was almost insensitive to the change in scan rate and the

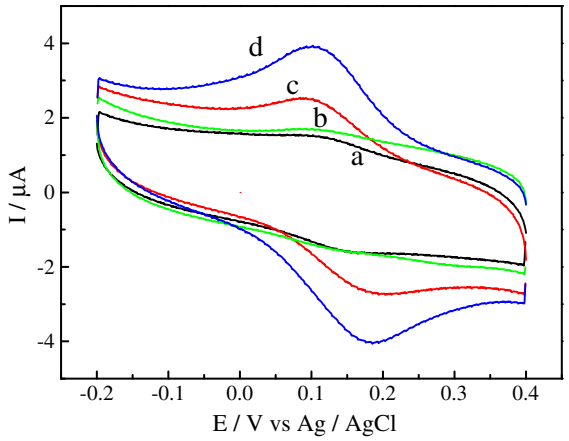

Fig. 4. Cyclic voltammograms of different modified electrodes in blank PBS buffer solution after immersion in $Co(phen)_3^{3+}$ for 30 min. Curves a, b, c, and d were DTC–DNA modified, hybridized with non-complementary target DNA, one-base mismatched DNA, and complementary target DNA, respectively. The scan rates were all 100 mV s^{-1}.

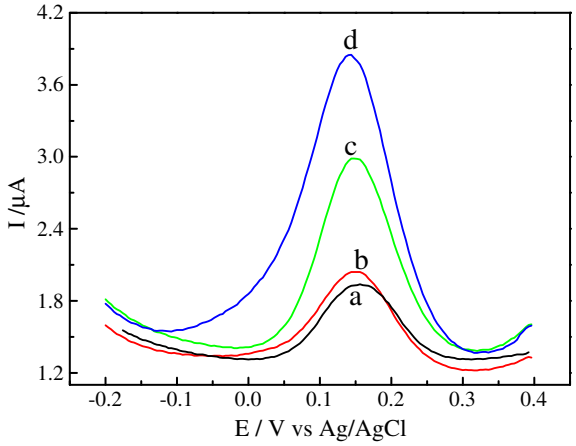

Fig. 6. Differential pulse voltammograms of different modified electrodes in blank PBS buffer solution after immersion in $Co(phen)_3^{3+}$ for 30 min. Curves a, b, c, and d were DTC–DNA modified, hybridized with non-complementary target DNA, one-base mismatched DNA, and complementary target DNA, respectively.

peak potential separation (ΔE_p) was nearly 70 mV. This strongly indicated a surface-controlled electrochemical process.

The DPV experiments using $Co(phen)_3^{3+}$ as an electrochemical hybridization indicator for detection of different DNA sequences were further advised and the results were shown in Fig. 6. DPV was commonly used because it could alleviate the background current effect to a large extent. Curves a, b, c, and d were the representative DPV curves obtained for DTC–DNA modified electrode, the hybrids of DTC–DNA with non-complementary target DNA, one-base mismatched DNA, complementary target DNA sequence, respectively. Each measure was performed in blank PBS buffer solution after immersing in $Co(phen)_3^{3+}$ for 30 min. It could be seen that a small DPV peak current of about 1.9 μA for DTC–DNA modified electrode, demonstrating that $Co(phen)_3^{3+}$ has a little affinity for ssDNA on the modified electrode surface (Curve a). A significant increase of about 2 μA in the DPV signal was observed after hybridization of DTC–DNA modified electrode with fully complementary target DNA (Curve d). An obviously decreased peak current was obtained when the DTC–DNA was exposed to one-base mismatched ssDNA sequence compared with that for the complementary target DNA (Curve c). When the non-complementary target DNA was used for a control experiment, almost the same peak current with the solely probe DNA immobilized electrode was obtained (Curve b). The DPV experiments showed a high selectivity of the constructed DNA biosensor for hybridization detection, which were in good accordance with that of cyclic voltammetry experiments.

3.4. The sensitivity of fabricated DNA biosensor

The sensitivity of the electrochemical hybridization assay was performed by varying the complementary target DNA concentration by DPV experiments with the use of $Co(phen)_3^{3+}$ as an indicator. The response of DPV peak current increase (ΔI) of $Co(phen)_3^{3+}$ with decreasing the concentration of target DNA on DTC–DNA modified electrode is displayed in Fig. 7. Three repetitive experiments were conducted for each concentration of target DNA. It could be found that the target DNA could still be detected by current fabricated strategy at a low concentration of 0.1 nM.

3.5. Regeneration and stability of the fabricated DNA biosensor

The reproducibility and stability of fabricated DNA biosensor is also a very important feature for biosensor. It was found that the fabricated DNA biosensor could be conveniently regenerated by incubation of dsDNA modified electrodes in hot water (80 °C) for 10 min, which could completely remove the hybridized DNA via thermal denaturation. The DPV experiments by $[Co(phen)_3]^{3+}$ indicator showed

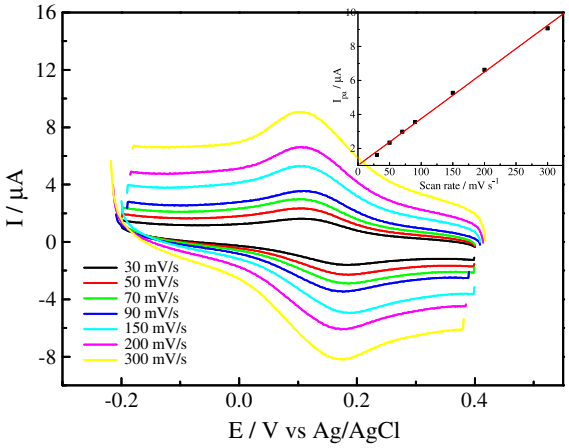

Fig. 5. The responses of cyclic voltammograms of $Co(phen)_3^{3+}$ for double-stranded DNA modified electrode with different scan rates from 30 mV/s to 300 mV s^{-1}. Inset: the linear plot of peak current of $Co(phen)_3^{3+}$ with scan rates.

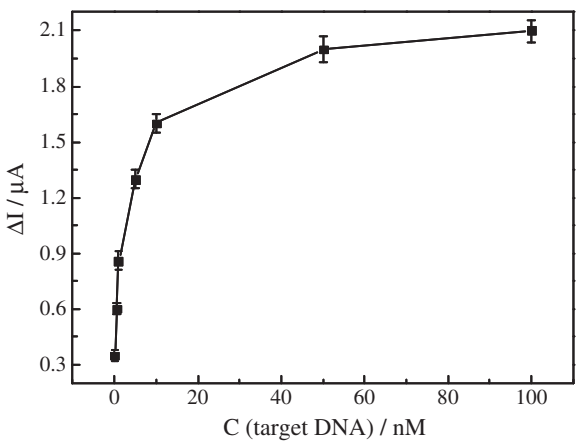

Fig. 7. Relation of the DPV peak current increase (ΔI) of $Co(phen)_3^{3+}$ before and after DNA hybridization with the concentration of target DNA varied from 0.1 nM to 100 nM. Each point was obtained with three repetitive experiments.

almost the original performance after 11 regeneration cycles, with only about 8% loss of the original signal. Compared with it, the hybridization signal for the thiolated probe DNA occurred with 15% loss after only 6 generation cycles. In addition, the DTC–DNA is also fairly robust at normal storage in the refrigerator with minimal loss of DTC–DNA SAMs for at least 1 month. It could be well understood that the DTC–DNA assembled by two anchoring points enhanced the adhesion ability compared with that of the thiolated probe DNA.

4. Conclusions

The current article provides a simple strategy for DNA immobilization on the gold electrode surface. The amine-functionalized probe DNA was firstly activated by CS_2 and then directly immobilized on the gold surface via bidentate anchoring points. With the use of $Co(phen)_3^{3+}$ as an indicator, the fabricated electrochemical DNA biosensor could achieve a low detection limit of 0.1 nM toward the complementary target DNA. Also, the DNA biosensor shows a superior reproducibility and stability. The relatively good selectivity of $Co(phen)_3^{3+}$ toward ssDNA and dsDNA with current fabricated DNA biosensor should be able to satisfy some applications in real samples for example toward the detection of PCR amplified target DNA. The current immobilization method for DNA is also expected to receive further applications in protein, enzyme biosensor fabrication.

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References

[1] K. Hu, D. Lan, X. Li, S. Zhang, Electrochemical DNA biosensor based on nanoporous gold electrode and multifunctional encoded DNA—Au bio bar codes, Anal. Chem. 80 (2008) 9130–9194.
[2] T.G. Drummond, M.G. Hill, J.K. Barton, Electrochemical DNA sensors, Nat. Biotechnol. 21 (2003) 1192–1199.
[3] S.J. Park, T.A. Taton, C.A. Mirkin, Array-based electrical detection of DNA with nanoparticle probes, Science 295 (2002) 1503–1506.
[4] N.C. Seeman, From genes to machines: DNA nanomechanical devices, Trends Biochem. Sci. 30 (2005) 119–125.
[5] T. Garcia, E. Casero, M. Revenga-Parra, F. Pariente, E. Lorenzo, Dual-stage DNA sensing: recognition and detection, Anal. Chem. 80 (2008) 9443–9449.
[6] Y.C. Huang, B. Ge, D. Sen, H.-Z. Yu, Immobilized DNA switches as electronic sensors for picomolar detection of plasma proteins, J. Am. Chem. Soc. 130 (2008) 8023–8029.
[7] K. Suzuki, K. Hosokawa, M. Maeda, Controlling the number and positions of oligonucleotides on gold nanoparticle surfaces, J. Am. Chem. Soc. 131 (2009) 7518–7519.
[8] A.W. Peterson, R.J. Heaton, R.M. Georgiadis, The effect of surface probe density on DNA hybridization, Nucleic Acids Res. 29 (2001) 5163–5168.
[9] P. Gong, C.-Y. Lee, L.J. Gamble, D.G. Castner, D.W. Grainger, Hybridization behavior of mixed DNA/alkylthiol monolayers on gold: characterization by surface plasmon resonance and ^{32}P radiometric assay, Anal. Chem. 78 (2006) 3326–3334.
[10] E. Huang, M. Satjapipat, S. Han, F. Zhou, Surface structure and coverage of an oligonucleotide probe tethered onto a gold substrate and its hybridization efficiency for a polynucleotide target, Langmuir 17 (2001) 1215–1224.
[11] E.L.S. Wong, E. Chow, J.J. Gooding, DNA recognition interfaces: the influence of interfacial design on the efficiency and kinetics of hybridization, Langmuir 21 (2005) 6957–6965.
[12] J. Wang, Nanoparticle-based electrochemical DNA detection, Anal. Chim. Acta 500 (2003) 247–257.
[13] E. Palecek, Surface-attached molecular beacons light the way for DNA sequencing, Trends Biotechnol. 22 (2004) 55–58.
[14] R. Kaufmann, I. Averbukh, R. Naaman, S.S. Daube, Controlling the reactivity of adsorbed DNA on template surfaces, Langmuir 24 (2008) 927–931.
[15] Y. Belosludtsev, B. Iverson, S. Lemeshko, R. Eggers, R. Wiese, S. Lee, T. Powdrill, M. Hogan, DNA microarrays based on noncovalent oligonucleotide attachment and hybridization in two dimensions, Anal. Biochem. 292 (2001) 250–256.
[16] V. Chan, D.J. Graves, P. Fortina, S.E. McKenzie, Adsorption and surface diffusion of DNA oligonucleotides at liquid/solid biosensor interfaces, Langmuir 13 (1997) 320–329.
[17] S.V. Lemeshko, T. Powdrill, Y.Y. Belosludtsev, M. Hogan, Oligonucleotides form a duplex with non-helical properties on a positively charged surface, Nucleic Acids Res. 29 (2001) 3051–3058.
[18] T.T. Jiang, J.B. Chang, C.M. Wang, Z. Ding, J.N. Chen, J.F. Zhang, E.T. Kang, Adsorption of plasmid DNA onto N, N′-(dimethylamino)ethyl-methacrylate graft-polymerized poly-L-lactic acid film surface for promotion of in-situ gene delivery, Biomacromolecules 8 (2007) 1951–1957.
[19] P. Caillat, D. David, M. Belleville, F. Clerc, C. Massit, F. Revol-Cavalier, P. Peltie, T. Livache, G. Bidan, A. Roget, E. Crapez, Biochips on CMOS: an active matrix address array for DNA analysis, Sens. Actuators, B 61 (1999) 154–162.
[20] R. Levicky, T.M. Herne, M.J. Tarlov, S.K. Satija, Using self-assembly to control the structure of DNA monolayers on gold: a neutron reflectivity study, J. Am. Chem. Soc. 120 (1998) 9787–9792.
[21] M. Beier, J.D. Hoheisel, Versatile derivatisation of solid support media for covalent bonding on DNA-microchips, Nucleic Acids Res. 27 (1999) 1970–1977.
[22] K.M. Millan, S.R. Mikkelsen, Sequence-selective biosensor for DNA based on electroactive hybridization indicators, Anal. Chem. 65 (1993) 2317–2323.
[23] S. Ghafouri, M. Thompson, Interfacial properties of biotin conjugate–avidin complexes studied by acoustic wave sensor, Langmuir 15 (1999) 564–572.
[24] M.R. Vilar, A.M. Botelho do Rego, A.M. Ferraria, Y. Jugnet, C. Nogues, D. Peled, R. Naaman, Interaction of self-assembled monolayers of DNA with electrons: HREELS and XPS studies, J. Phys. Chem. B 112 (2008) 6957–6964.
[25] T.M. Herne, M.J. Tarlov, Characterization of DNA probes immobilized on gold surfaces, J. Am. Chem. Soc. 119 (1997) 8916–8920.
[26] D.A. Giljohann, D.S. Seferos, P.C. Patel, J.E. Millstone, N.L. Rosi, C.A. Mirkin, Oligonucleotide loading determines cellular uptake of DNA-modified gold nanoparticles, Nano Lett. 7 (2007) 3818–3821.
[27] D. Peled, S.S. Daube, R. Naaman, Selective enzymatic labeling to detect packing-induced denaturation of double-stranded DNA at interfaces, Langmuir 24 (2008) 11842–11846.
[28] S. Moses, S.H. Brewer, L.B. Lowe, S.E. Lappi, M. Sauthier, R.C. Tenent, D.L. Feldheim, S. Franzen, Characterization of single- and double-stranded DNA on gold surfaces, Langmuir 20 (2004) 11134–11140.
[29] S. Imabayashi, D. Hobara, T. Kakiuchi, Voltammetric detection of the surface diffusion of adsorbed thiolate molecules in artificially phase-separated binary self-assembled monolayers on a Au(111) Surface, Langmuir 17 (2001) 2560–2563.
[30] N. Garg, E. Carrasquillo-Molina, T.R. Lee, Self-assembled monolayers composed of aromatic thiols on gold: structural characterization and thermal stability in solution, Langmuir 18 (2002) 2717–2726.
[31] K. Heister, D.L. Allara, K. Bahnck, S. Frey, M. Zharnikov, M. Grunze, Deviations from 1:1 compositions in self-assembled monolayers formed from adsorption of asymmetric dialkyl disulfides on gold, Langmuir 15 (1999) 5440–5443.
[32] M.H. Schoenfisch, J.E. Pemberton, Air stability of alkanethiol self-assembled monolayers on silver and gold surfaces, J. Am. Chem. Soc. 120 (1998) 4502–4513.
[33] J. Wu, S. Campuzano, C. Halford, D.A. Haake, J. Wang, Ternary surface monolayers for ultrasensitive (zeptomole) amperometric detection of nucleic acid hybridization without signal amplification, Anal. Chem. 82 (2010) 8830–8837.
[34] V. Dharuman, B.-Y. Chang, S.-M. Park, J.H. Hahn, Ternary mixed monolayers for simultaneous DNA orientation control and surface passivation for label free DNA hybridization electrochemical sensing, Biosens. Bioelectron. 25 (2010) 2129–2134.
[35] S. Campuzano, F. Kuralay, M. Jesús Lobo-Castañón, M. Bartošík, K. Vyavahare, E. Paleček, D.A. Haake, J. Wang, Ternary monolayers as DNA recognition interfaces for direct and sensitive electrochemical detection in untreated clinical samples, Biosens. Bioelectron. 26 (2011) 3577–3583.
[36] Y.L. Zhang, Y. Wang, H.B. Wang, J.H. Jiang, G.L. Shen, R.Q. Yu, J.H. Li, Electrochemical DNA biosensor based on the proximity-dependent surface hybridization assay, Anal. Chem. 81 (2009) 1982–1987.
[37] J. Zhang, H.L. Qi, Y. Li, J. Yang, Q. Gao, C.X. Zhang, Electrogenerated chemiluminescence DNA biosensor based on hairpin DNA probe labeled with ruthenium complex, Anal. Chem. 80 (2008) 2888–2894.
[38] Z.Q. Gao, A. Agarwal, A.D. Trigg, N. Singh, C. Fang, C.H. Tung, Y. Fan, K.D. Buddharaju, J.M. Kong, Silicon nanowire arrays for label-free detection of DNA, Anal. Chem. 79 (2007) 3291–3297.
[39] C.Y. Lee, P.T. Nguyen, D.W. Grainger, L.J. Gamble, D.G. Castner, Structure and DNA hybridization properties of mixed nucleic acid/maleimide-ethylene glycol monolayers, Anal. Chem. 79 (2007) 4390–4400.
[40] J.-S. Lee, A.K.R. Lytton-Jean, S.J. Hurst, C.A. Mirkin, Silver nanoparticle—oligonucleotide conjugates based on DNA with triple cyclic disulfide moieties, Nano Lett. 7 (2007) 2112–2115.
[41] R.L. Letsinger, R. Elghanian, G. Viswanadham, C.A. Mirkin, Use of a steroid cyclic disulfide anchor in constructing gold nanoparticle—oligonucleotide conjugates, Bioconjug. Chem. 11 (2000) 289–291.
[42] Z. Li, R. Jin, C.A. Mirkin, R.L. Letsinger, Multiple thiol-anchor capped DNA–gold nanoparticle conjugates, Nucleic Acids Res. 30 (2002) 1558–1562.
[43] J.A. Dougan, C. Karlsson, W.E. Smith, D. Graham, Enhanced oligonucleotide–nanoparticle conjugate stability using thioctic acid modified oligonucleotides, Nucleic Acids Res. 35 (2007) 3668–3675.
[44] T. Sakata, S. Maruyama, A. Ueda, H. Otsuka, Y. Miyahara, Stable immobilization of an oligonucleotide probe on a gold substrate using tripodal thiol derivatives, Langmuir 23 (2007) 2269–2272.
[45] Y. Zhao, W.P. Segarra, Q.C. Shi, A. Wei, Dithiocarbamate assembly on gold, J. Am. Chem. Soc. 127 (2005) 7328–7329.
[46] F. Dubois, B. Mahler, B. Dubertret, E. Doris, C. Mioskowski, A versatile strategy for quantum dot ligand exchange, J. Am. Chem. Soc. 129 (2007) 482–483.
[47] T.B. Huff, L. Tong, Y. Zhao, M.N. Hansen, J.-X. Cheng, A. Wei, Hyperthermic effects of gold nanorods on tumor cells, Nanomedicine 2 (2007) 125–132.

[48] R.D. Gillard, R.J. Lancashire, P.A. Williams, A convenient preparation of salts containing the bis-2,2′-bipyridylcobalt(III) group, and analogues, Transit. Met. Chem. 8 (1983) 188–189.
[49] J. Sharma, R. Chhabra, H. Yan, Y. Liu, A facile in situ generation of dithiocarbamate ligands for stable gold nanoparticle–oligonucleotide conjugates, Chem. Commun. (2008) 2140–2142.
[50] M.T. Carter, M. Rodriguez, A.J. Bard, Voltammetric studies of the interaction of metal chelates with DNA. 2. Tris-chelated complexes of cobalt(III) and iron(II) with 1,10-phenanthroline and 2,2′-bipyridine, J. Am. Chem. Soc. 111 (1989) 8901–8911.
[51] D.W. Pang, H.D. Abruna, Interactions of benzyl viologen with surface-bound single- and double-stranded DNA, Anal. Chem. 72 (2000) 4700–4706.
[52] J. Wang, E. Palecek, P.E. Nielsen, G. Rivas, X. Cai, H. Shiraishi, N. Dontha, D. Luo, P.A.M. Farias, Peptide nucleic acid probes for sequence-specific DNA biosensors, J. Am. Chem. Soc. 118 (1996) 7667–7670.