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Universal DNA biosensing based on instantaneously electrostatic attraction between hexaammineruthenium (III) and DNA molecules

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ABSTRACT

Despite rapid progress in DNA biosensors by employing various materials as well as techniques, most of the reported sensors are based on specific recognition of a DNA fragment, however can not perform universal measurement of DNA molecules (i.e. genomic DNA). In this work, we proposed a novel DNA biosensing method based on instantaneously electrostatic attraction (IEA) between hexaammineruthenium (III) and DNA molecules. The current variation of freely diffused $Ru(NH_3)_6^{3+}$ caused by its quick and strong static interaction with phosphate backbones was employed as a universal probe to detect DNA molecules in solution, with no need for immobilization of capture probes on the electrode. After optimization, 30 μL of 300 μM $Ru(NH_3)_6^{3+}$ solution was added onto the gold electrode with a working electrode diameter of 2 mm, and a detection limit of 3.8 ng/μL was achieved, which is equivalent to NanoDrop™ One spectrometer, the commonly used instrument for DNA quantification. Using reusable and inexpensive gold electrode, the approach provided an easy-operated sequence-independent DNA detection method, and was proved to be able to detect genomic and plasmid DNA directly.

1. Introduction

Nucleic acids are the carriers of genetic information, which determine the structure of proteins, biomolecules and cellular components. Quantitative detection of nucleic acid is critical in many fields such as tumour screening, molecular diagnosis, forensic identification, food safety and environmental monitoring. In order to meet the expanding demand, considerable research effort has been focused on sensitive and selective detection of DNA molecules. Kinds of methods including southern blot (Wahl et al., 1987), gene chip (Schena et al., 1995), DNA biosensor (Kirsch et al., 2013) and real-time quantitative PCR (Bustin and Mueller, 2005) are introduced and widely used for DNA quantification. However, sequence-independent techniques for DNA quantification were seldom reported except spectrophotometry (Vahakangas et al., 1985), fluorometry (Hierholzer et al., 1993) and some methods based on magnetic aggregation and colorimetry (Leslie et al., 2012; Lin et al., 2013).

Among all these methods, electrochemical techniques are especially popular for its simplicity and low cost (Barton et al., 2003; Liu et al., 2012; Wang, 2006). Moreover, electrochemical measurements are suitable for fabrication of miniaturized devices. A variety of DNA biosensors have been proposed for electrochemical detection of DNA molecules, for example, enzymatic reaction (Ji et al., 2012; Wang et al., 2013; Wu et al., 2012) and gold nano-particle-based signal amplification (Authier et al., 2001; Das et al., 2014; Zhang et al., 2006). In addition, an alternate way is to increase the amount of immobilization and accessibility of DNA probes (Li et al., 2011; Liu et al., 2005).

However, most of the reported DNA biosensors recognize and capture target molecules by hybridization with DNA probes immobilized on electrode surface (Zhu et al., 2015). Generally high sensitivity can be obtained through signal or genetic amplification which employs enzymes or nanomaterials (Wu et al., 2014). However, since the efficiency of enzymatic reactions on solid surface is far below that in solution, cascade amplification methods are sometimes employed to achieve high sensitivity (Long et al., 2013; Yang et al., 2016a; Zhao et al., 2013), which however easily leads to complicated reaction and poor reproducibility. Alternately, researchers proposed some methods for DNA biosensing based on measuring the electro-active molecules in solution, such as methylene blue (MB), which was free of immobilization of capture probe. MBs can bond to DNA molecules through intercalating into the grooves of double strand which would prevent them from participating redox reactions on electrode. Therefore, it is possible to manipulate the enzyme reactions (DNA products) by quantification of free MBs in solution, even real time genetic quantification was achieved (Deféver et al., 2011; Fang et al., 2009; Won et al., 2011).

Hexaammineruthenium ($Ru(NH_3)_6^{3+}$) is also a popular electro-

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active molecule for DNA biosensors (Hu et al., 2008; Sato and Takenaka, 2012; Steel et al., 1998). $Ru(NH_3)_6^{3+}$ molecules bound to DNA backbones through electrostatic interaction. Then they participate redox reactions on the surface of electrode and induce electron transportation (ET). The according electrochemical signals reflect the amount of DNA molecules absorbing on the surface of electrode (Steel et al., 1998). However, $Ru(NH_3)_6^{3+}$ is at present limited to detect DNA molecules absorbed on the surface of electrode, but could not recognize free nucleic acids in solution.

In this work, a new DNA biosensing method based on instantaneously electrostatic attraction (IEA) between hexaammineruthenium (III) and DNA molecules was developed for nucleic acids detection directly, which was free of immobilization of capture probe. The strategy was derived from the significant difference of peak potential between $Ru(NH_3)_6^{3+}$ bound to DNA backbones and freely diffused in solution, leading to an instantaneous change of amperometric current at the present of DNA molecules. The instantaneous change of current was employed as a probe for quantification of total DNA molecules in solution. The proposed dynamic process was demonstrated by monitoring the electrochemical signal in real time. The effects of working electrode area, Ruhex concentration, the volume of fluid, and length of DNA strands for IEA signal were investigated. The limit of detection (LOD) of IEA assay was explored and the detection performance was compared with NanoDrop™ One spectrometer from Thermo scientific™. The IEA assay was applied for detection of genomic DNA extracted from mouse tissues and plasmids extracted from E. coli.

2. Experimental

2.1. Reagents and materials

All oligonucleotides (Tab. S1) used in the experiment were purchased from Sangong (Shanghai, China). All of those oligonucleotides were purified by Ultra-PAGE or HPLC and quantified with NanoDrop™ One spectrophotometer from Thermo scientific™.

All solutions were prepared with milli-Q water (Millipore, 18 mΩ cm at 25 °C). The buffers involved in this work were as follows: buffer for electrochemical measurement consisted of 10 mM Tris-HCl buffer (pH 7.4) and 50/100/200/300/400/500 μM $[Ru(NH_3)_6]^{3+}$. Buffer for immobilization (I buffer) contained 10 mM Tris-HCl, 1.0 mM EDTA, 1.0 M NaCl, and 1.0 mM TCEP (pH 7.4). Hexaammineruthenium (III) chloride ($[Ru(NH_3)_6]^{3+}$) and tris(2-carboxyethyl)phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich.

2.2. Fabrication of PGE by chemical plating

Three types of integrated gold three-electrode biosensors with working electrode diameters of 1 mm, 2 mm and 4 mm were fabricated on polystyrene (PS) substrate through UV directed chemical plating technique that previously reported by our group (Yao et al., 2014). In brief, a piece of PS sheet with a thickness of 1 mm was exposed to UV-lights (254 nm) emitted by a low-pressure mercury lamp for 4 h at 8 mW/cm^2. The exposed PS sheet was then immersed into 100 mM of phosphate buffer (pH 7.0) containing 360 mM of ethylenediamine (Sigma, China) and 50 mM of N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC, Fluka, China) at room temperature for three hours. After rinsing with deionized water, the selectively aminated substrate was sequentially treated with 1 mM of $HAuCl_4$ (Sinopharm, China) aqueous solution for 2.5 h and 0.1 M of $NaBH_4$ aqueous solution for 20 min. Then the substrate was sonicated in 0.5 M KSCN solution for 20 min to remove non-specifically adsorbed gold species on the surface, so as to prevent non-specific over-plating in the following plating process. Finally, the activated PS was placed into a gold plating bath containing 0.125 M of Na_2SO_3 (Sinopharm, China), 0.6 M of methanal (Sinopharm, China) and 8 mM of $Na_3Au(SO_3)_2$ (Changzhou Institute of Chemical Research, Changzhou, China) for about three hours. After rinsing with deionized water, the PGE substrate was baked at 80 °C for 1 h and ready for usage.

2.3. Electrode modification and IEA assay

Before use, gold electrodes were cleaned by electrochemical oxidation and reduction. One hundred cycles of cyclic voltammetry in 50 μL of 0.1 M H_2SO_4 solution were applied on the electrode using the following parameters: potential range, −0.5 to 1.0 V; scan rate, 1 V/s. After being rinsed with Milli-Q water, the electrodes were dried with nitrogen. Then the electrodes were immersed in 1 mM of 6-Mercapto-1-hexanol (MCH, Aldrich, China) solution for at least 2 h. Excess MCH was removed by the rinsing with Milli-Q water and the electrodes were dried under nitrogen flow for usage.

For IEA assay, 30 μL 10 mM Tris-HCl buffer (pH 7.4) containing $[Ru(NH_3)_6]^{3+}$ was added and the initial curent signal was recorded. Then, 1 μL of concentrated DNA aqueous solution or genomic DNA sample containing 50% formamide(Sangon, Shanghai) was instilled and electrochemical measurement was performed every several minutes.

2.4. Electrochemical measurement

Cyclic voltammetry (CV), amperometric i-t curve and differential-pulse voltammetry (DPV) were performed with an electrochemical station (CHI830D, Chenhua, Shanghai, China) at room temperature. The following parameters were employed. For DPV measurement, parameters from 0 to −0.5 V with a pulse amplitude of 0.05 V and a pulse width of 0.5 s were employed. Amperometric i-t curve was operated in −0.3 V and a sample interval of 0.1 s for 600 s. For measuring the oxidation and reduction peaks of Ruhex, cyclic voltammetry was performed from 0.05 V to −0.4 V with a scan rate of 0.1 V/s and a sample interval of 0.001 s. We have to admit that there is sometimes a drift of peak potential because that an integrated three-electrode system instead of traditional Ag/AgCl reference electrode is employed in this experiment.

3. Results and discussion

3.1. Design of the biosensing strategy based on IEA

Recently, we have developed a novel polystyrene based gold electrode (PGE) containing unique nano-scaled structure surface which was found having superior electrochemical properties and higher capacity of DNA immobilization compared with gold electrodes fabricated by standard sputtering based photolithography (Yang et al., 2016b; Yao et al., 2014). Accidently, we recognized that DNA molecules adding into the test solution could induce significant change of amperometric current instantaneously at the presence of Hexaammineruthenium (III) as electrochemical redox (Fig. S1, S2). When a drop of complementary DNA molecules were added to the electrode modified with capture probes, the DPV currents of Ruhex, both adsorption (peak I, Fig. S2a) and free diffusion (peak II, Fig. S2a) declined significantly, which caused by strong static interaction between DNA and Ruhex. Replacing the targeted DNA molecules with non-complementary sequences, similar results were observed, proving that this interaction was not related to DNA sequence.

Based on the advantageous electrochemical properties of PGEs and rapid interaction between DNA molecules and Ruhex, herein a novel method for DNA sensing was proposed (Fig. 1). Different with most of the existing biosensors, peak II (Fig. S2) which corresponds to the redox reaction of $Ru(NH_3)_6^{3+}$ diffused to electrode, was studied and employed to recognize and detect DNA molecules quantitatively in this work. Since $Ru(NH_3)_6^{3+}$ bonds to DNA phosphate backbones via electrostatic attraction, when a drop of concentrated DNA aqueous solution was instilled, $Ru(NH_3)_6^{3+}$ molecules freely in solution as well as those bonded to immobilized DNA strands promptly gathered around

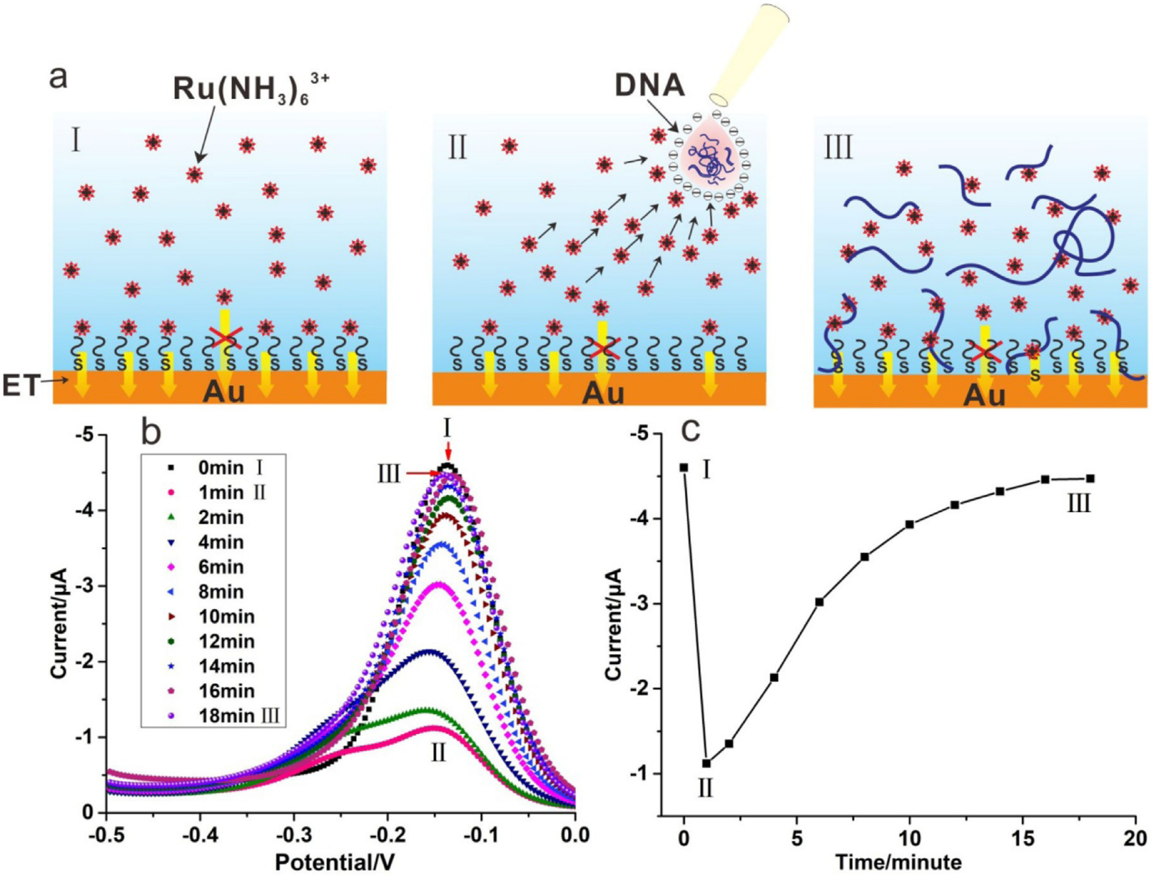

Fig. 1. Electrochemical detection for DNA molecules based on instantaneously electrostatic attraction between $Ru(NH_3)_6^{3+}$ and DNA molecules on the gold electrode without capture probe. Schematic diagram for the dynamic procedure(a), DPV (b) and variation of peak current caused by electrostatic attraction between $Ru(NH_3)_6^{3+}$ and DNA molecules (c). I, II and III refer to three stages of this procedure. ET means electron transfer.

this negatively charged molecule (Fig. 1a I, II and III).

Fig. 1b and c demonstrated this dynamic procedure in real time. The results showed that peak current related to freely diffused Ruhex in solution decreased sharply, then approached to the original signal gradually. The bounce of current is probably because after few minutes DNA molecules dispersed uniformly in solution and the strong electrostatic attraction disappeared. Free ruhex molecules returned back to the surface of electrode and participated electrochemical reaction again. However, it is observed that the resumed current (stage III) was slightly lower than the original level (stage I) due to the adsorption of some ruhex molecules to DNA backbones. It was found from Fig. 1c that the most obvious variation occurred between stage I and the dynamic stage II. Therefore, current variation of stage II (defined as IEA signal) was employed in the following work for sequence-independent detection of DNA molecules, such as genomic DNA.

3.2. Optimization of conditions

The DPV peak current is not only proportional to the concentration of electroactive material, but also related to the effective electrode area. Therefore, size of working electrodes would impact the peak current of DPV dramatically. Three types of integrated gold three-electrode biosensors were prepared and tested. It was proved that using the chip with a working electrode diameter of 2 mm obtained the maximum IEA signal (reduction of the peak current) when other conditions were controlled. It was mainly because both the volume of $Ru(NH_3)_6^{3+}$ solution and the total amount of $Ru(NH_3)_6^{3+}$ ions on the electrode surface were positively correlated with the electrode area, resulting in a large difference between the initial as well as the reduction values of peak current after the addition of DNA. Due to the large amount of $Ru(NH_3)_6^{3+}$ on the electrode surface with a working electrode diameter of 4 mm, only part of those ions would be attracted by the negative charges carried by the DNA droplet through electrostatic attraction. Therefore, the change of Ruhex concentration on the electrode surface was relatively small and the IEA signal was accordingly low. Thus, 2 mm diameter of PGE were employed in subsequent experiments for its maximum sensitivity. (Fig. 2).

Since detected current was produced by the redox reaction of Ru

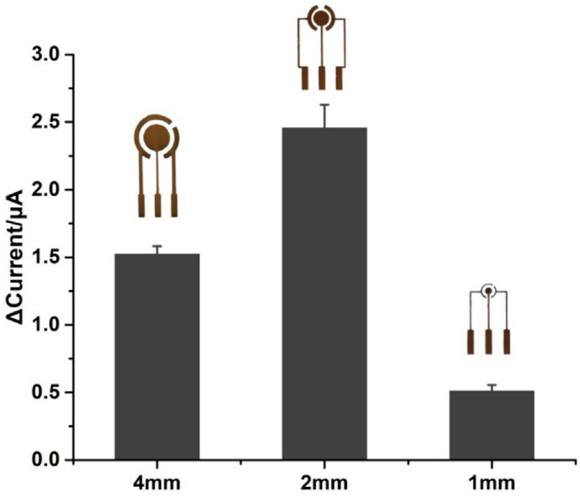

Fig. 2. Influence of working area of electrode for IEA signal: current variation during the first minute.

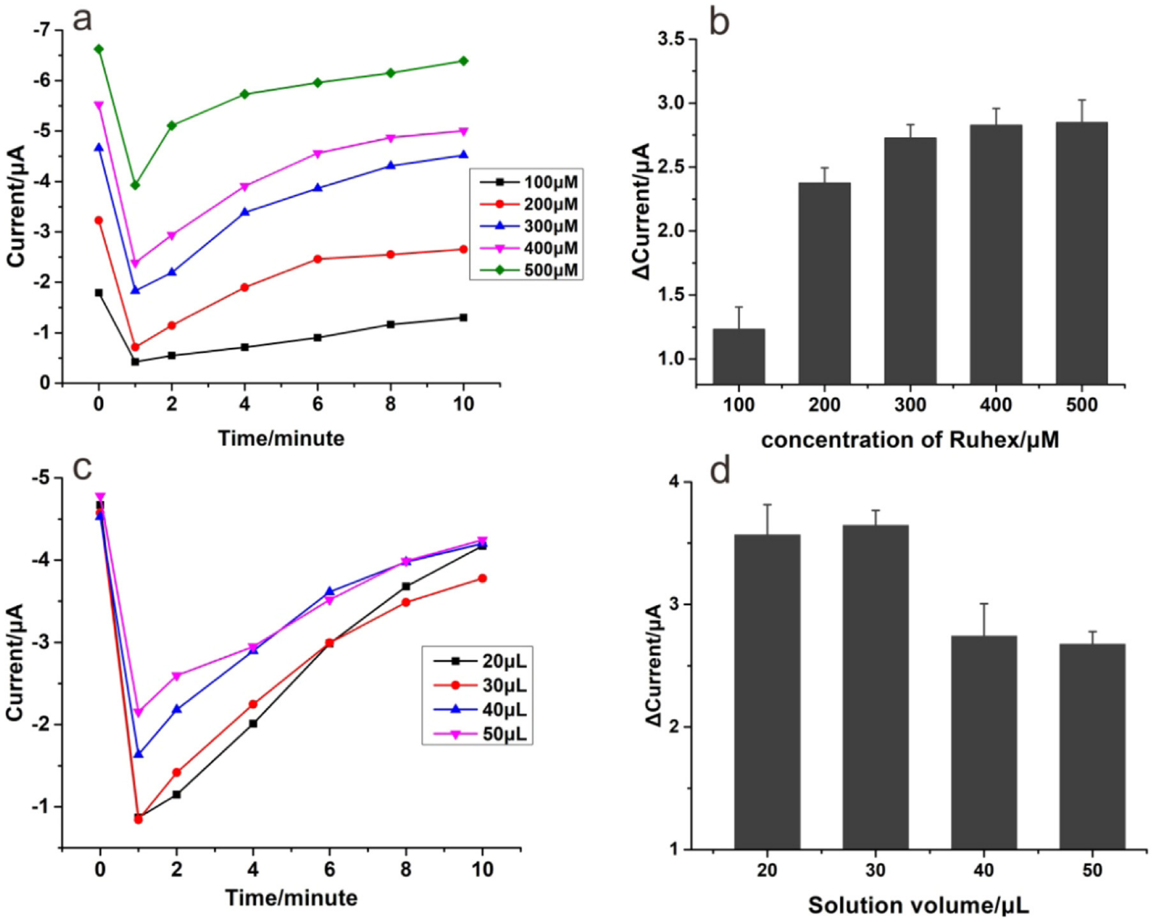

Fig. 3. Influence of the $Ru(NH_3)_6^{3+}$ concentration for IEA signal: peak current recorded at 1/2/4/6/8/10 min (a) and current variation during the first minute (b). Investigation of relationship between IEA signal and the volume of fluid: peak current recorded at 1/2/4/6/8/10 min (c) and current variation during the first minute (d).

$(NH_3)_6^{3+}$ freely diffused at the electrode surface, the concentration of $Ru(NH_3)_6^{3+}$ in solution would have an effect on current of DPV, as well as the amplitude of IEA signal. Therefore, subsequently concentration of Ruhex was optimized in Fig. 3a and b. It was observed that the signal of IEA assay was positively correlated with the concentration of Ruhex in the solution, but this effect became smaller as the concentration continuously increased. It was mainly because when above 300 μM, the concentration of Ruhex on the electrode surface was relatively high and less influenced by the instilled DNA solution, resulting in the reduction of sensitivity. It suggested that applying a test solution with excess Ruhex concentration could not produce an apparent response to thinner DNA samples. Based on the considerations above, 300 μM of $Ru(NH_3)_6^{3+}$ was selected.

Similarly, volume of fluid would also impact the sensitivity of IEA assay by affecting the total amount of Ruhex in the solution. Subsequently volume of fluid was optimized in Fig. 3c and d. It was observed that the initial peak current was independent of the solution volume while the signal of IEA assay was positively related to the volume of fluid ranging from 20 to 30 μL and then decreased sharply with its increasement. This is due to the fact that with the same Ruhex concentration, the influence of the applied DNA solution becomes smaller as the volume of fluid increases, which would raise the total amount of Ruhex in the solution, resulting in a smaller change in the Ruhex concentration on the electrode surface. Therefore, a 30 μL solution was chosen.

3.3. Length of DNA strand

Moreover, length of DNA strands is essential for IEA assay, because the amount of negative charge carried by DNA backbones is theoretically proportional to the length of DNA strands due to the accumulation of phosphate anions. Accordingly, the force of IEA for long DNA strands would be stronger than short ones at same molarity. That means, for instance, 1 nmol/L of DNA molecules with 20 nucleotides (20 phosphate anions) bring less negative charges than same concentration of DNA molecules with 100 nucleotides (100 phosphate anions), thus attract less $Ru(NH_3)_6^{3+}$ molecules and induce lower current change during IEA assay. Fig. 4b demonstrated approximatively linear trend when the length of DNA strands ranged from 15 to 59 nucleotides at the same concentration of 50 μM/L. It indicated that this IEA assay would be sensitive for long DNA strands and the signal of IEA assay was irreverent to DNA sequence.

3.4. The relationship between IEA curve and DNA concentration

From previous work, we noticed that the value of current was related to the time elapsed after DNA solution was added (Fig. 1c), which indicated that the IEA process was affected by both the instantaneous electrostatic attraction and diffusion process. In order to better understand this process, serially diluted DNA solution was instilled and amperometric i-t curve was performed. (Fig. S3 and Fig. S4). It was obvious that the higher concentration of DNA solution added, the shorter time required for the current to reach the minimum, thus the higher IEA signal achieved. And the variation of current was roughly proportional

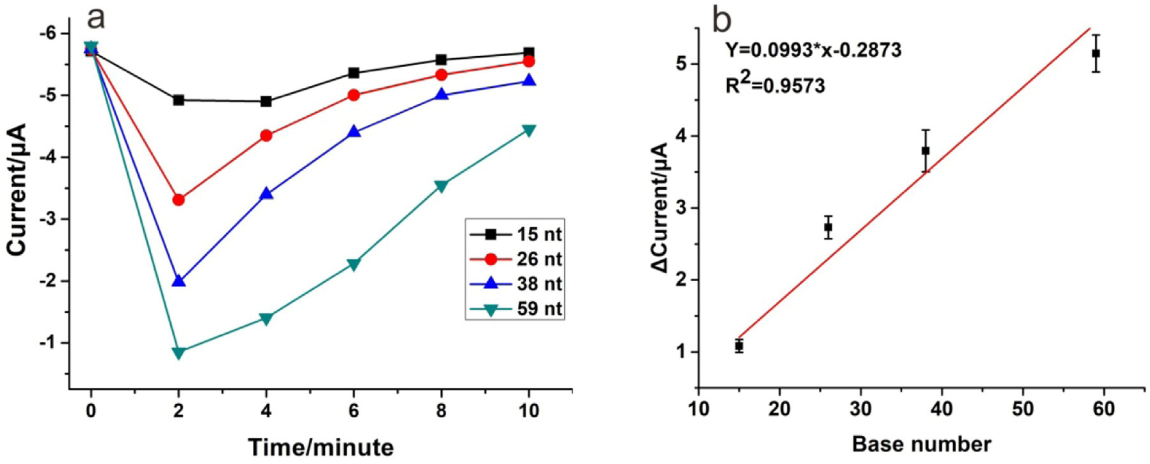

Fig. 4. Investigation of relationship between IEA signal and the length of DNA strands: peak current recorded at 1/2/4/6/8/10 min (a) and the calibration curve between IEA signal and the length of DNA strands (b). DNA concentration was 50 μM/L.

to the DNA concentration while it was in the range of 20–50 μM. However, with a DNA concentration of 10 μM or below, the IEA signal was too small to be recognized, which suggested that the amperometric i-t curve investigation was less sensitive.

Performing DPV investigation, similar results were obtained (Fig. 5). With the increasement of DNA concentration, the time reaching the lowest peak current decreased accordingly. To accurately quantify DNA samples, the DPV investigation must be performed continuously to record the highest IEA signal. Despite its low sensitivity, the amperometric i-t curve can be used to roughly manipulate the time for the lowest peak current before performing IEA assay on a new sample, which could improve the accuracy of determination.

3.5. Analytical performance of the IEA assay

Sensitivity of IEA assay was investigated by directly instilling a droplet (1 μL) of sample solution containing serially diluted synthesized DNA molecules (~78nt length, Fig. 6). When the concentration of DNA droplet reduced, IEA signal was observed significantly decreasing for losing of the surface charges contained by DNA droplet. Thus, the force induced by instantaneously electrostatic attraction between positive-charged Ruhex and negative-charged DNA droplet declined sharply. A linear relationship between IEA signal and DNA concentration ranged from 0.1 μM to 50 μM with correlation coefficient of 0.9974 was achieved. Same examples were also detected by NanoDrop™ One (Fig. S5). Referring to the test results of Nanodrop, the unit of DNA concentration in the linear fit was replaced with ng/μL, the formula changed into $y = 0.00341*x + 0.0933$ and the correlation coefficient was 0.9963. Based on the slop of linear fit, the sensitivity of our DNA biosensor was calculated as $3.41\ \text{nA}\ \mu\text{L}\ \mu\text{g}^{-1}$ and the detection limit was 3.8 ng/μL ($S/N = 3$), which was basically equivalent to NanoDrop™ One according to the instructions. Moreover, the volume of DNA sample that can be measured with IEA assay is also a crucial performance because DNA samples isolated from tissues or other biomaterials are always in small amount ranging from tens to hundreds micro litres which is too little to use the cuvettes of traditional spectrophotometers. That is why micro volume spectrophotometers like NanoDrop™ which usually take 1–2 micro litre of samples are more popular for DNA quantification. In the IEA assay, 30 μL of working buffer was preloaded on the surface of electrode, then micro litre or nano litre of DNA sample was dropped and measured. From Fig. S6, we observed that as low as 200 nL of DNA sample (30 μM) had been detected, indicating the IEA assay is also a micro- or nano- DNA sensor.

Therefore, though slightly less-stable than NanoDrop™ One, the IEA assay still showed great potential as the first micro volume and universal electrochemical DNA biosensor. Moreover, the sensitivity of IEA could be improved by detecting long DNA molecules such as genomic DNA and DNA product of amplification reaction based on its detection

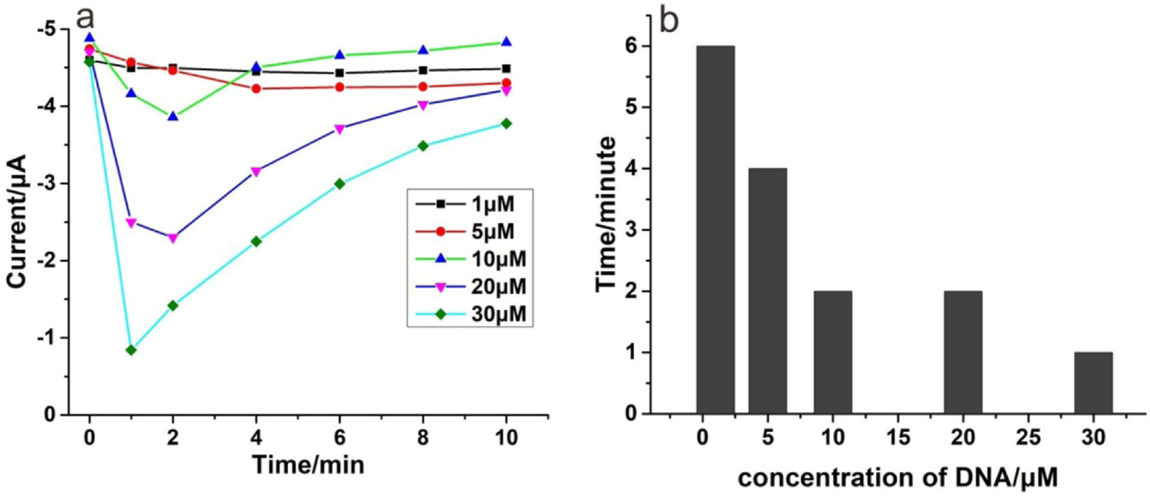

Fig. 5. Investigation of relationship between DPV curve and DNA concentration: peak current recorded at 1/2/4/6/8/10 min (a), the concentration of DNA and the time for the lowest peak current (b).

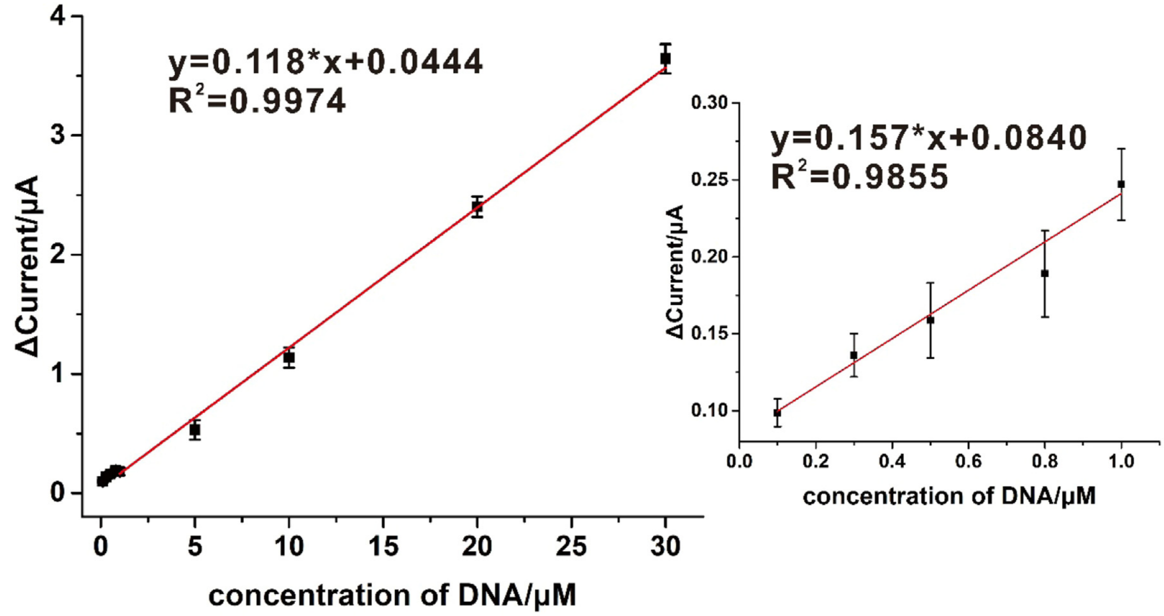

Fig. 6. The calibration curve between IEA signal and the concentration of sample DNA solution.

principle.

3.6. Application of the IEA strategy for genome DNA

Genome is extremely long and double-stranded DNA molecule which composes of billions of nucleotide pairs. Up to date, microspectrophotometer (i.e. NanoDrop™ One) is the usually the authoritative tool for quantification of genomic DNA, which is sensitive and universal, measuring DNA molecules sequence independently. Similar with spectrophotometer, IEA assay was a universal detection strategy. It is able to detect total amount of DNA molecules in sample regardless of their sequences. Fig. 7 demonstrated the detection of genomic DNA extracted from mouse tissue and plasmids extracted from E. coli by IEA assay. The concentration of genomic DNA was measured by spectrophotometer. To the best of our knowledge, it is the first electrochemical biosensor for quantification of genomic DNA directly.

To be noted that IEA assay was a universal strategy and no hybridization or enzymatic reaction occurred on surface of electrode. Sensors could be refreshed within several minutes and detected next sample, which was more convenient and economical than existing biosensors.

4. Conclusions

Despite numerous biosensors employing various materials as well as techniques have been developed for sensitive detection of DNA, RNA and proteins,(Rosi and Mirkin, 2005; Wang et al., 2009) techniques for sequence-independently sensing of genomic DNA were seldom reported. In this work we developed a novel DNA biosensing strategy based on instantaneously electrostatic attraction (IEA) between hexaammineruthenium (III) and DNA molecules. This method benefited from the superior electrochemical characteristics of our previously developed polystyrene gold electrode (PGE). The current produced by free diffusion of $Ru(NH_3)_6^{3+}$ was employed as a probe to detect DNA molecules dropped on the surface of PGE. IEA assay was a universal strategy which was proved capable of performing detection of plasmids and genomic DNA. By comparing with NanoDrop™ One, our IEA assay obtained comparative sensitivity and linear range, showing great potential as a universal biosensor for quantification of isolated DNA molecules, sequence-independently.

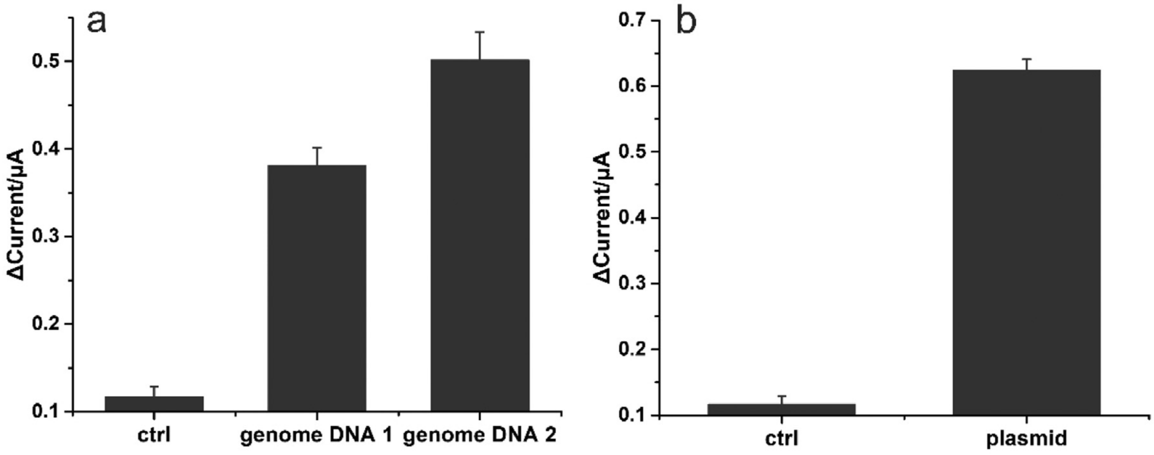

Fig. 7. Detection of genomic DNA extracted from mouse tissue (a) and plasmids extracted from E. coli by IEA assay. The concentration of genomic DNA was 241 ng/μL (1), 328 ng/μL (2) respectively and the concentration of plasmids was 351 ng/μL.

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Declaration of interests

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2018.12.019.

References

Authier, L., Grossiord, C., Brossier, P., Limoges, B., 2001. Anal. Chem. 73 (18), 4450–4456.
Barton, J.K., Hill, M.G., Drummond, T.G., 2003. Nat. Biotechnol. 21 (10), 1192–1199.
Bustin, S.A., Mueller, R., 2005. Clin. Sci. 109 (4), 365–379.
Das, R., Sharma, M.K., Rao, V.K., Bhattacharya, B.K., Garga, I., Venkatesh, V., Upadhyay, S., 2014. J. Biotechnol. 188, 9–16.
Deféver, T., Druet, M., Evrard, D., Marchal, D., Limoges, B., 2011. Anal. Chem. 83 (5), 1815–1821.
Fang, T.H., Ramalingam, N., Dong, X., Ngin, T.S., Zeng, X., Kuan, A.T.L., Huat, E.Y.P., Gong, H., 2009. Biosens. Bioelectron. 24 (7), 2131–2136.
Hierholzer, J.C., Halonen, P.E., Dahlen, P.O., Bingham, P.G., McDonough, M.M., 1993. J. Clin. Microbiol. 31 (7), 1886–1891.
Hu, K., Lan, D., Li, X., Zhang, S., 2008. Anal. Chem. 80 (23), 9124–9130.
Ji, H., Yan, F., Lei, J., Ju, H., 2012. Anal. Chem. 84 (16), 7166–7171.
Kirsch, J., Siltanen, C., Zhou, Q., Revzin, A., Simonian, A., 2013. Chem. Soc. Rev. 42 (22), 8733–8768.
Leslie, D.C., Li, J., Strachan, B.C., Begley, M.R., Finkler, D., Bazydlo, L.A., Barker, N.S., Haverstick, D.M., Utz, M., Landers, J.P., 2012. J. Am. Chem. Soc. 134 (12), 5689–5696.
Li, F., Han, X., Liu, S., 2011. Biosens. Bioelectron. 26 (5), 2619–2625.
Lin, C., Zhang, Y., Zhou, X., Yao, B., Fang, Q., 2013. Biosens. Bioelectron. 47, 515–519.
Liu, A., Wang, K., Weng, S., Lei, Y., Lin, L., Chen, W., Lin, X., Chen, Y., 2012. TRAC-Trends Anal. Chem. 37, 101–111.
Liu, S., Li, Y., Li, J., Jiang, L., 2005. Biosens. Bioelectron. 21 (5), 789–795.
Long, Y., Zhou, X., Xing, D., 2013. Biosens. Bioelectron. 46, 102–107.
Rosi, N.L., Mirkin, C.A., 2005. Chem. Rev. 105 (4), 1547–1562.
Sato, S., Takenaka, S., 2012. Anal. Chem. 84 (3), 1772–1775.
Schena, M., Shalon, D., Davis, R.W., Brown, P.O., 1995. Science 270 (5235), 467–470.
Steel, A.B., Herne, T.M., Tarlov, M.J., 1998. Anal. Chem. 70 (22), 4670–4677.
Vahakangas, K., Trivers, G., Rowe, M., Harris, C.C., 1985. Environ. Health Perspect. 62 (OCT), 101–104.
Wahl, G.M., Meinkoth, J.L., Kimmel, A.R., 1987. Methods Enzymol. 152, 572–581.
Wang, C., Zhou, H., Zhu, W., Li, H., Jiang, J., Shen, G., Yu, R., 2013. Biosens. Bioelectron. 47, 324–328.
Wang, H., Yang, R., Yang, L., Tan, W., 2009. ACS Nano 3 (9), 2451–2460.
Wang, J., 2006. Biosens. Bioelectron. 21 (10), 1887–1892.
Won, B.Y., Shin, S., Baek, S., Jung, Y.L., Li, T., Shin, S.C., Cho, D.-Y., Lee, S.B., Park, H.G., 2011. Analyst 136 (8), 1573–1579.
Wu, H., Liu, S., Jiang, J., Shen, G., Yu, R., 2012. Chem. Commun. 48 (50), 6280–6282.
Wu, L., Xiong, E.H., Zhang, X., Zhang, X.H., Chen, J.H., 2014. Nano Today 9 (2), 197–211.
Yang, L., Tao, Y., Yue, G., Li, R., Qiu, B., Guo, L., Lin, Z., Yang, H.-H., 2016a. Anal. Chem. 88 (10), 5097–5103.
Yang, Z., Liu, Y., Lu, W., Yuan, Q., Wang, W., Pu, Q., Yao, B., 2016b. Talanta 152, 301–307.
Yao, B., Liu, Y., Tabata, M., Zhu, H., Miyahara, Y., 2014. Chem. Commun. 50 (68), 9704–9706.
Zhang, J., Song, S., Zhang, L., Wang, L., Wu, H., Pan, D., Fan, C., 2006. J. Am. Chem. Soc. 128 (26), 8575–8580.
Zhao, Y., Qi, L., Chen, F., Zhao, Y., Fan, C., 2013. Biosens. Bioelectron. 41 (1), 764–770.
Zhu, C.Z., Yang, G.H., Li, H., Du, D., Lin, Y.H., 2015. Anal. Chem. 87 (1), 230–249.