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Simple detection of nucleic acids with a single-walled carbon-nanotube-based electrochemical biosensor

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ABSTRACT

We report for the first time a simple approach to fabricate an electrochemical DNA (E-DNA) biosensor by introducing the single-walled carbon nanotubes (SWNTs). The SWNTs combine with the electrochemical label (methyl blue, MB)-modified single-stranded DNA (ssDNA) probes to generate a nanomaterial–biomolecule composite, which functions as a signal amplification platform to facilitate the electron-transfer between the electrochemical label and the electrode. This SWNT-based E-DNA biosensor produces a high square wave voltammetry (SWV) signal in the absence of target DNA. In the presence of target DNA, the MB-labeled ssDNA probes are removed from the SWNT-modified electrode due to the formation of a double-stranded DNA (dsDNA), generating a relatively low SWV signal. This signal-off SWNT-based E-DNA biosensor exhibits improved sensitivity and large linear dynamic range with low detection limit; it can even distinguish 1-base mismatched target DNA. Further experiments demonstrate that the SWNT-based E-DNA biosensor is superior to the multi-walled carbon nanotube (MWNT)-based one for DNA detection. Moreover, the introduction of aptamer into the SWNT-based biosensor might be further extended to detect small biomolecules such as adenosine.

1. Introduction

The cost-effective, sensitive DNA biosensors offer great promise for rapid diagnosis of pathogenic and genetic diseases as well as monitoring of environmental and food safety (Wu et al., 2010; Stern et al., 2010; Xiao et al., 2006; Sharon et al., 2010). So far, the development of DNA biosensors has been focused on the sensitivity enhancement, the facile fabricating process and the sequence-specificity (Yang and Zhang, 2010; Slinker et al., 2010; Toubanaki et al., 2009; Ren et al., 2000; Yang et al., 2009). Various approaches have been tried to improve the performance of such biosensors, including fluorescence (Mathias et al., 2010; Zhang et al., 2005; Zhang and Hu, 2010; Agnes et al., 2010), surface-plasmon resonance (Stenlund et al., 2006; Yoshida et al., 1999), electronic transduction-based analysis system (Taton et al., 2000; Minunni et al., 2005; Patolsky et al., 2001) and surface-enhanced Raman scattering spectroscopy (Hu and Zhang, 2010). In comparison with the above methods, electrochemical DNA (E-DNA) biosensors display distinguishing advantages of simplicity, cost-effective, and high sensitivity (Li et al., 2010; Wang, 2006; Fan et al., 2003). To further improve the sensitivity of the E-DNA biosensors, different strategies have been developed, such as combination with electrochemical enzyme-linked immunoassay (ELISA) (Ju et al., 1999), introduction of magnetic beads (Wang et al., 2003; Zhang et al., 2009a) and adjunct probes (Yang and Zhang, 2010) into the E-DNA biosensors. Especially, the use of nanomaterials (such as gold nanoparticles (Tokonami et al., 2008; Zhang et al., 2010), quantum dots (Pinwattana et al., 2010; Hansen et al., 2006; Zhou et al., 2010; Song et al., 2010), and conducting polymer nanomaterials (Chang et al., 2007)) as signal amplifiers in the E-DNA biosensors has greatly improved the detection sensitivity. However, few reports are available about the development of carbon nanotube-based electrochemical biosensors for DNA analysis (Zhang et al., 2009b; Zhu et al., 2009; Santiago-Rodriguez et al., 2009).

Carbon nanotubes have promising applications in immobilizing biomolecules (such as DNA, proteins, and small biomolecules) (Zheng et al., 2003a; Müller et al., 2010; Yang et al., 2008) and facilitating the electron-transfer between the biomolecules and substrate electrode (Munge et al., 2005; Hu et al., 2005). The unique properties of the single-walled carbon nanotubes (SWNTs) such as vast surface-to-bulk ratio make them extremely attractive amplification platforms for the signal enhancement in a variety of research fields, including DNA-directed self-assembling of carbon nanotubes (Li et al., 2005; Chen et al., 2007), carbon nanotube as a carrier for single-stranded DNA (ssDNA) probe delivery (Wu et al., 2008), photoinduced charge transfer mediated by DNA-wrapped carbon nanotubes (Zheng and Rostovtsev, 2006), and DNA-wrapped SWNT-network thin-film transistors (Asada et al., 2011). Previous researches also demonstrate that carbon nanotubes exhibit strong

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electrocatalytic activity and might minimize the surface fouling in the electrochemical devices (Wang and Musameh, 2003). Recently, two interesting findings related to the SWNTs and DNA get our attentions: (1) the ssDNA might wrap around the SWNTs to form tight helices in spite of ssDNA sequence length (Zhang et al., 2009b; Gigliotti et al., 2006) and (2) the double-stranded DNA (dsDNA) is incapable of binding to either charged or uncharged SWNTs (Zhao and Johnson, 2007; Chen and Zhang, 2006). Inspired by the above discoveries, we propose a simple approach to fabricate an E-DNA biosensor by introducing the single-walled carbon nanotubes (SWNTs). In this SWNT-based E-DNA biosensor, the SWNTs wrapped by methylene blue (MB)-labeled ssDNA probes function as a signal amplification platform (Millan and Mikkelsen, 1993) to obtain a high SWV signal in the absence of target DNA. In the presence of target DNA, the hybridization of the MB-labeled ssDNA probe with target DNA forms a dsDNA which is incapable of binding to SWNTs (Zhao and Johnson, 2007; Chen and Zhang, 2006), making MB remove from the electrode surface and consequently leading to a low SWV signal. This signal-off E-DNA biosensor has significant advantages of improved sensitivity, sequence-specificity, and large linear dynamic range with low detection limit. Importantly, compared with the chemical-modification-based methods (Li et al., 2005; Peng et al., 2009), the SWNT-based E-DNA biosensor avoids the interference from the complicated chemical-modification, and prevents the denaturation of the ssDNA probes.

2. Materials and methods

2.1. Materials

All the probes and target oligonucleotides were purchased from Takara Biotechnology Company. The oligonucleotides were purified via C18 reversed-phase HPLC and polyacrylamide gel electrophoresis (PAGE). The sequence of probes and target oligonucleotides used were as follows: capture probe, 5′-T GAA GTG GGT CGA AAA-$(CH_2)_7$-MB-3′; perfect matched target DNA, 5′-TTT TCG ACC CAC TTC A-3′; 1-base mismatched target DNA, 5′-TTT TCC ACC CAC TTC A-3′; MB-labeled adenosine aptamer, 5′-AGA GAA CCT GGG GGA GTA TTG CGG AGG AAG GT-$(CH_2)_7$-MB-3′. Single-walled carbon nanotubes (SWNTs, diameter of ~2 nm, length of 5–15 μm) and multi-walled carbon nanotubes (MWNTs, diameter of ~10 nm, length of 5–15 μm) were purchased from Shenzhen Nanotech Port Co. Ltd. (Shenzhen, China). Adenosine, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (DEC), N-hydroxysulfosuccinimide sodium salt (sulfo-NHS), and N,N-dimethylformamide (DMF) were purchased from Sigma–Aldrich, Inc. (U.S.A) and used as received without further purification. The buffer solutions were as follows: the DNA immobilization buffer containing 100 mM phosphate buffer solution (PBS, pH 6.9, 25 °C); the DNA hybridization buffer containing 50 mM Tris–HCl, 100 mM NaCl, and 0.1% Tween 20 (pH 7.4, 25 °C); the washing buffer containing 20 mM phosphate buffer solution (PBS, pH 7.4, 25 °C) and 0.5% Tween 20; and the buffer for electrochemical measurements containing 10 mM PBS (pH 7.4, 25 °C) and 100 mM NaCl. All above solutions were prepared with Milli-Q water (18.2 MΩ cm).

2.2. Preparation of SWNT-based E-DNA biosensors

The SWNT-based E-DNA biosensor was fabricated via the surface modification of the glassy carbon electrode (GCE, 2.0 mm in diameter, CHI Co. Ltd., Shanghai, China). Before the immobilization of the SWNTs, the GCE was cleaned following the reported protocol (Zhao and Johnson, 2007). Briefly, the GCE was first polished by a microcloth with the alumina suspensions (1.0, 0.3, 0.05 μm in diameter), and the residual alumina powder was removed by sonicating the electrode sequentially in Milli-Q water, ethanol, acetone, Milli-Q water. The electrode was further activated by oxidation at +1.5 V for 15 s in an aqueous solution containing 2.5% $K_2Cr_2O_7$ and 10% HNO_3, followed by rinsing with the solution containing 5 mM DEC and 8 mM sulfo-NHS in 0.02 M phosphate buffer (pH 7.4) at room temperature (Millan and Mikkelsen, 1993).

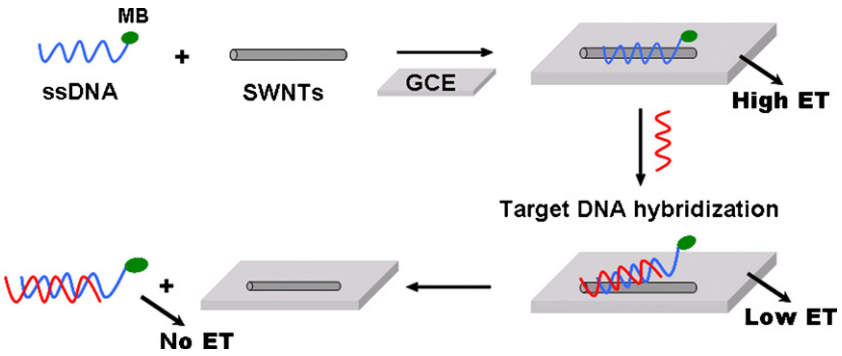
SWNTs were pretreated according to the methods described elsewhere (Munge et al., 2005). In a typical experiment, the purification of SWNTs (200 mg) was carried out in an aqueous solution of 2 M HNO_3 under ultrasonification at room temperature for 8 h, followed by ultracentrifugation (9000 rpm)/wash/redispersion cycle for three times and drying in a vacuum. The SWNTs were then shortened and oxidized by ultrasonicating in a mixture of HNO_3 and H_2SO_4 (v/v, 1:3) for 5 h, followed by extensive washing in Milli-Q water until the filtrate was neutral and the pH of solution was over 6. The dried SWNTs were further dispersed in 1 mL DMF with ultrasonication for 1 h to generate a black suspension, followed by transferring into 100 mM phosphate buffer solution (PBS, pH 6.9, 25 °C) containing 0.5 μM capture probes (ssDNA probes) and sonicated for 2 h. The DMF in buffer solution was beneficial to the dispersion of SWNTs in the aqueous solution (Kerman et al., 2004). Owing to their unique electronic properties, the ssDNA probes wrapped on the surface of the SWNTs (Zheng et al., 2003b).

The modification of GCE was performed with a protocol similar to Millan et al. (Millan et al., 1994). In brief, the pretreated GCE was incubated with the ssDNA-probe-wrapped-SWNTs in 100 mM PBS (pH 6.9, 25 °C) containing 8 mM sulfo-NHS and 5 mM DEC for 12 h to fabricate a SWNT-based E-DNA biosensor. This SWNT-based E-DNA biosensor was then extensively rinsed with ice-cold washing buffer (20 mM PBS, pH 7.4, 25 °C, 0.5% Tween 20) for at least three times. For the preparation of the MWNT-based E-DNA biosensor, the similar protocol was employed.

For the fabrication of the electrodes without SWNT modification, the GCE was first polished, ultrasonicated, and oxidized as described above, and was then modified with the ssDNA probes following previously reported approach (Millan and Mikkelsen, 1993). The cleaned electrode was immersed in a solution containing 8 mM sulfo-NHS and 5 mM DEC in 100 mM PBS (pH 6.9, 25 °C) for 12 h. After rinsing, the electrode was transferred into 100 mM PBS (pH 6.9, 25 °C) containing 0.5 μM ssDNA probe for 2 h with gentle shaking at room temperature. This functionalized electrode was rinsed with ice-cold washing buffer (20 mM PBS, pH 7.4, 25 °C, 0.5% Tween 20) for at least three times.

2.3. DNA hybridization and electrochemical measurement

Square wave voltammetry (SWV) at an alternative frequency of 15 Hz was performed on the electrochemical workstation (CHI 660D, CH Instruments, Chenhua Corp., Shanghai, China) in a typical three-electrode system with a platinum electrode as the counter electrode, an Ag/AgCl electrode (saturated with KCl) as the reference electrode, and the CNT (SWNT and MWNT)-based electrodes as the working electrode. The solution containing 10 mM PBS (pH 7.4) and 100 mM NaCl was used as the supporting electrolyte medium. For the DNA hybridization, the SWNT-based electrodes were incubated with target DNA (125 μL in volume) in the hybridization buffer (50 mM Tris–HCl, 100 mM NaCl, and 0.1% Tween 20, pH 7.4, 25 °C) at 47 °C or 5 h. For the control experiments, the electrode without SWNT modification was used as working electrode instead of the SWNT-based electrode in the three-electrode system. All potentials were measured with respect to the reference electrode (Ag/AgCl, +0.226 V, 25 °C).



Scheme 1. Scheme of the SWNT-based E-DNA biosensor for nucleic acids detection.

2.4. Preparation of aptameric SWNT-based electrochemical biosensor for adenosine detection

The fabricating process of the aptameric biosensor was similar to that of the SWNT-based E-DNA biosensor. Briefly, the pretreated SWNT suspension in DMF was transferred into 100 mM phosphate buffer solution (PBS, pH 6.9, 25 °C) containing 0.5 μM MB-labeled adenosine aptamer and sonicated for 2 h. These aptamer-wrapped-SWNTs were then incubated with the pretreated GCE in 100 mM PBS (pH 6.9, 25 °C) containing 8 mM sulfo-NHS and 5 mM DEC for 12 h to fabricate an aptameric biosensor, followed by extensively rinsed with ice-cold washing buffer (20 mM PBS, pH 7.4, 25 °C, 0.5% Tween 20) for at least three times.

For the detection of adenosine, the aptameric SWNT-based electrodes were first incubated with various concentration of adenosine (100 μL in volume) in the hybridization buffer (50 mM Tris–HCl, 100 mM NaCl, and 0.1% Tween 20, pH 7.4, 25 °C) at 37 °C for 1 h, and were then subjected to the measurement on the electrochemical workstation after rinsing with the washing buffer.

3. Results and discussion

3.1. Improved sensitivity for the SWNT-based E-DNA biosensor

As shown in Scheme 1, the pretreated SWNTs were wrapped by MB-labeled ssDNA probes owing to the non-covalent binding (π-stacking) of ssDNA to the side walls of SWNTs (Zheng et al., 2003a). The SWNTs wrapped with ssDNA probes were immobilized on the surface of substrate electrode with the assistance of the sulfo-NHS and DEC (Millan and Mikkelsen, 1993; Yim et al., 2005). The MB in the ssDNA probe functioned as a labeling agent to generate a high SWV signal in the absence of target DNA (Fig. 1A-a). In the presence of 16-base complementary target DNA, the hybridization of ssDNA probe with the target DNA formed a dsDNA. This dsDNA separated from the surface of the SWNTs (Chen and Zhang, 2006), making MB remove from the electrode surface and consequently generating a relatively low SWV signal. Fig. 1A-b shows the SWV signal obtained from the SWNT-based E-DNA biosensor in the presence of 0.8 nM perfect matched target DNA. The SWV signal had decreased by as much as 53% in comparison with that observed in the absence of target DNA (Fig. 1A-a), indicating the high sensitivity of this E-DNA biosensor for target DNA detection. The relative standard deviation (RSD) was determined to be 2.3% for 10 repetitive experiments, suggesting good reproducibility of the SWNT-based E-DNA biosensor. In contrast, the E-DNA biosensor without SWNT modification displayed a relatively poor sensitivity. Fig. 1B-b shows the SWV signal obtained from the E-DNA biosensor without SWNT modification in the presence of 0.8 nM perfect matched target DNA. The SWV signal decreased only by as much as 17% in comparison with that in the absence of target DNA (Fig. 1B-a). These results demonstrated that E-DNA biosensor modified with the SWNTs was more sensitive than that without SWNTs modification. It should be noted that even in the absence of target DNA, the SWV signal from the SWNT-based E-DNA biosensor (Fig. 1A-a) was much larger than that from the E-DNA biosensor without SWNT modification (Fig. 1B-a). This was attributed to the introduction of the SWNTs which allowed a greater amount of MB-labeled ssDNA to bind on their surfaces.

The improved sensitivity of the SWNT-based E-DNA biosensor was attributed to the unique properties of the SWNTs: (1) The vast surface-to-bulk ratio of the SWNTs allowed plenitudinous MB-labeled ssDNA probe to immobilize on their surface; this made more MB collide with the electrode, and also increased the chance of ssDNA probes to hybridize with the target DNA. (2) The SWNTs imparted strong electrocatalytic activity and promoted the electron-transfer reaction (Wang and Musameh, 2003). (3) The non-covalent binding (π-stacking) of ssDNA probes on the

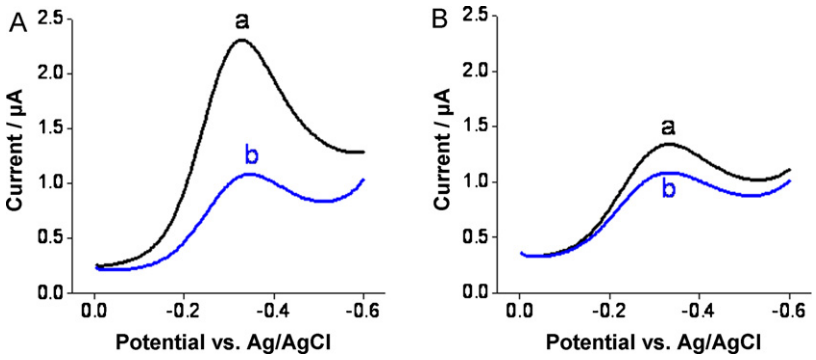


Fig. 1. Improved sensitivity for the SWNT-based E-DNA biosensor. (A) Square wave voltammetry (SWV) signals obtained from the SWNT-based E-DNA biosensor in response to the buffer (a) and 0.8 nM target DNA (b). (B) SWV signals obtained from the E-DNA biosensor without SWNT modification in response to the buffer (a) and 0.8 nM target DNA (b).

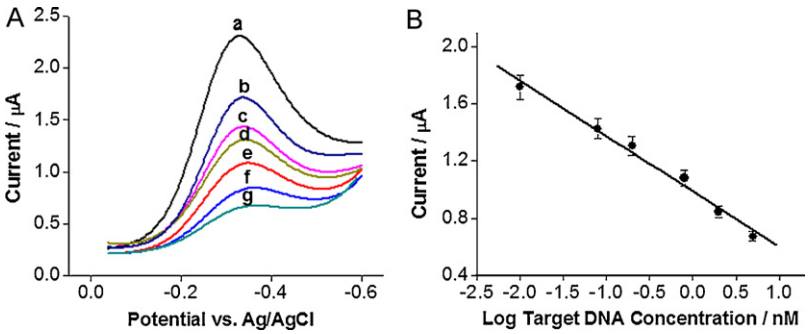


Fig. 2. (A) SWV signals obtained from the SWNT-based E-DNA biosensor in response to different concentration of target DNA. The target concentration shown was 0 (a), 0.01 nM (b), 0.08 nM (c), 0.2 nM (d), 0.8 nM (e), 2.0 nM (f), and 5.0 nM (g). (B) Variation of the SWV signal as a function of target DNA concentration, measured by the SWNT-based E-DNA biosensor. Error bars showed the standard deviation of three experiments.

SWNTs had some distinct advantages over other immobilization methods. Conventional approaches for the immobilization of electrochemical active substrate on the surface of the SWNTs included covalent binding, direct physical adsorption and entrapment in different substrate materials (Li et al., 2005; Peng et al., 2009). The direct physical adsorption and entrapment made the distribution of ssDNA probes on the surface of SWNTs non-uniform and easily leaching with time. Conversely, chemical reaction (covalent binding) tended to partially denature the activity of the biomolecules. Unlike the above approaches, non-covalent binding (π-stacking) of ssDNA probes on the surface of SWNTs generated a stable composite without either denaturation of the ssDNA probes or the involvement of complicated chemical modification procedures (Zheng et al., 2003a).

3.2. Low detection limit for the SWNT-based E-DNA biosensor

The introduction of SWNTs in the E-DNA biosensor led to a large linear dynamic range and low detection limit. Fig. 2 shows the SWV signals in response to different concentration of target DNA. The SWV signal decreased with the increase of target DNA concentration (Fig. 2A), and the peak current of SWV signal had a good linear fit to the logarithm of target DNA concentration in the range from 0.01 nM to 5.0 nM. The regression equation was $i_{pa} = 0.9874–0.3871 \log C$, where C was the concentration of target DNA, and the correlation coefficient was 0.9931. The detection limit was calculated to be 1.0 pM based on the method of 3 s, where s was the standard deviation for 10 parallel measurements of blank solution. This detection limit had improved by as much as 4 orders of magnitudes than the reported E-DNA biosensors (Hu et al., 2005; Immoos et al., 2004). The large linear dynamic range and low detection limit were attributed to the introduction of SWNTs in the E-DNA biosensor, which made more MB-labeled ssDNA probes immobilize onto the electrode surface and facilitated the electron-transfer from the electrochemical label to the electrode (Zheng et al., 2003a; Müller et al., 2010; Yang et al., 2008; Munge et al., 2005; Hu et al., 2005).

Even in comparison with the reported SWNT-based E-DNA biosensor which was designed to monitor the electrooxidation of guanine bases in ssDNA (Zhang et al., 2009b), the detection limit of current SWNT-based E-DNA biosensor had improved by as much as 4 orders of magnitudes. More importantly, current SWNT-based E-DNA biosensor was much simple without the involvement of complicated chemical and electrochemical procedures.

3.3. Sequence specificity for the target DNA detection

Differentiation of 1-mismatched base was very important for the nucleic acids detection. To investigate the sequence specificity of the SWNT-based E-DNA biosensor, the differentiation of perfect matched target DNA from 1-base mismatched one was tested. As shown in Fig. 3, the SWV signal obtained from 0.8 nM 1-base mismatched target DNA decreased by as much as 10% compared to that obtained from only buffer; while in the presence of 0.8 nM perfect matched target DNA, the SWV signal decreased by as much as 53% compared to that obtained from only buffer. There was over 5 times difference in the SWV signal response between perfect matched target DNA and 1-base mismatched one. This result demonstrated that the SWNT-based E-DNA biosensor possessed good sequence specificity toward even 1-base mismatched target DNA.

3.4. Comparison of SWNT-based E-DNA biosensor with MWNT-based E-DNA biosensor

To further investigate the mechanism for the improved sensitivity in the SWNT-based E-DNA biosensor, the MWNT-based biosensor was constructed for comparison. As shown in Fig. 4, the

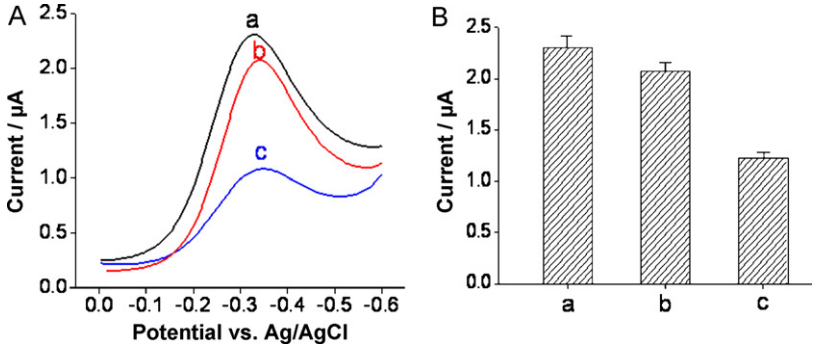


Fig. 3. (A) SWV signals obtained from the SWNT-based E-DNA biosensor in response to buffer (a), 0.8 nM 1-base mismatched target DNA (b), and 0.8 nM perfect matched target DNA (c). (B) Comparison of the SWV signal obtained from the SWNT-based E-DNA biosensor in response to buffer (a), 0.8 nM 1-base mismatched target DNA (b), and 0.8 nM perfect matched target DNA (c). Error bars showed the standard deviation of three experiments.

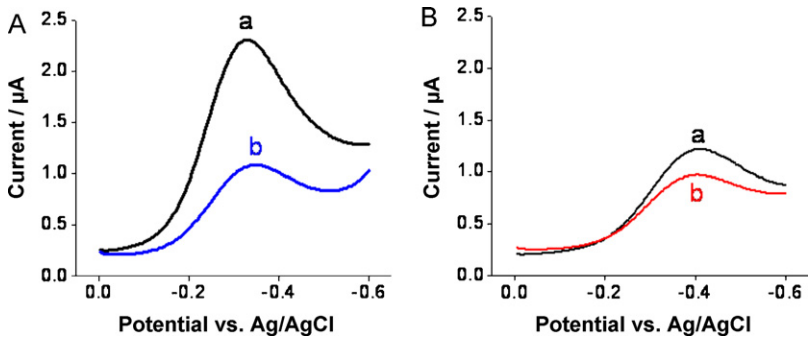


Fig. 4. (A) SWV signals obtained from the SWNT-based E-DNA biosensor in response to buffer (a) and 0.8 nM perfect matched target DNA (b). (B) SWV signals obtained from the MWNT-based E-DNA biosensor in response to buffer (a) and 0.8 nM perfect matched target DNA (b).

MWNT-based biosensor exhibited poor sensitivity for DNA detection. In the presence of 0.8 nM target DNA, the SWV signal obtained from the MWNT-based E-DNA biosensor decreased by as much as 19% compared to that in the presence of only buffer (Fig. 4B), while the SWNT-based E-DNA biosensor decreased by as much as 53% compared to that in the presence of only buffer (Fig. 4A). This result suggested the SWV signal obtained from the modified electrode was strongly dependent on the type of carbon nanotubes used (Peng et al., 2009). It was well known that the surface-to-bulk ratio decreased linearly with increasing size for a given shape, the larger the diameter, the smaller the surface-to-bulk ratio (Bhushan, 2007). The MWNTs had a larger diameter (~10 nm) than SWNTs (~2 nm), and correspondingly the MWNTs possessed a smaller surface-to-bulk ratio. As a result, fewer MB-labeled ssDNA probes immobilized on the surface of MWNTs to form CNT-DNA composite, making fewer MB collide with the electrode and also adversely affecting the chance of ssDNA probes to hybridize with the target DNA. Moreover, MWNTs had poor electrical conductivity (Ajayan, 1999), which limited the electron-transfer between the electrochemical label and substrate electrode. In the contrast, the SWNTs had vast surface-to-bulk ratio and good electrical conductivity, and functioned as signal amplifiers to obtain improved sensitivity in the E-DNA biosensor. Even in the absence of target DNA, the SWV signal from the SWNT-based E-DNA biosensor (Fig. 4A-a) was much larger than that from the MWNT-based biosensor (Fig. 4B-a), further confirming the signal-amplification function of SWNTs in the SWNT-based E-DNA biosensor.

3.5. *Aptameric SWNT-based electrochemical biosensor for adenosine detection*

The SWNT-based E-DNA biosensor could be further extended to the construction of the aptameric electrochemical biosensor for small biomolecule detection. To demonstrate the principle-of-the-concept, we fabricated an aptameric SWNT-based electrochemical biosensor for adenosine detection (Fig. 5A). The MB-labeled adenosine aptamer functioned as both a capture probe and a reporter probe. In the absence of adenosine, the aptamer-wrapped SWNTs

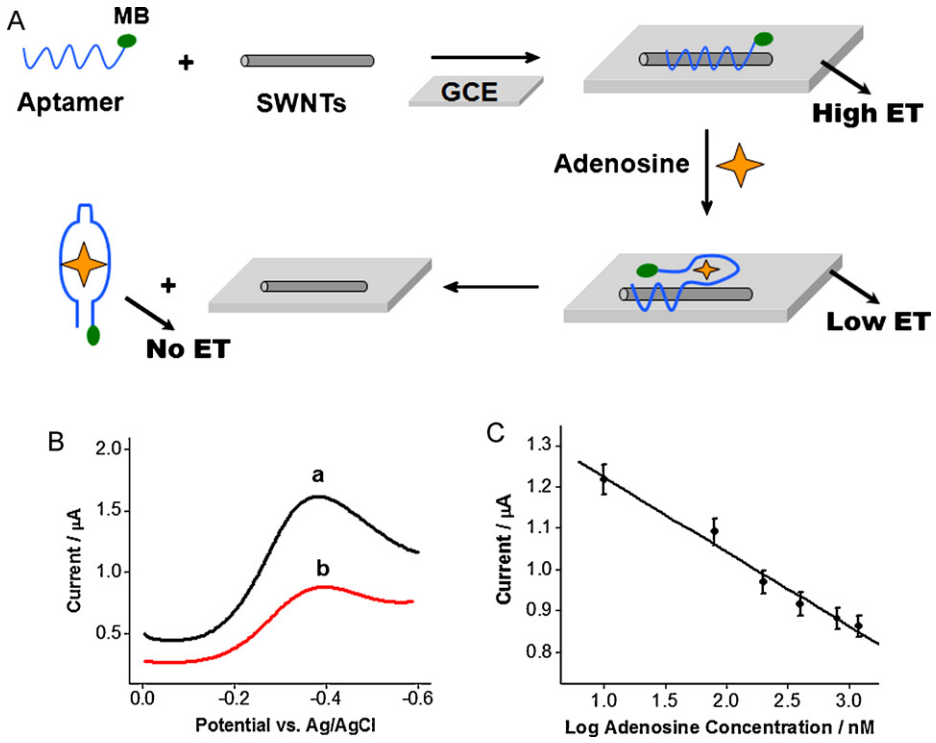


Fig. 5. (A) Scheme of aptameric SWNT-based electrochemical biosensor for adenosine detection. (B) SWV signals obtained from the aptameric SWNT-based electrochemical biosensor in response to the buffer (a) and 0.8 mM adenosine (b). (C) Variation of the SWV signal as a function of adenosine concentration. Error bars showed the standard deviation of three experiments.

were immobilized on the electrode surface (Fig. 5A), generating a high SWV signal (Fig. 5B-a). While in the presence of 0.8 mM adenosine, the MB-labeled aptamer was removed from the SWNTs surface due to the formation of a stable aptamer-target complex (Fig. 5A) (Zhang et al., 2008; Ouyang et al., 2011), leading to a low SWV signal (Fig. 5B-b). The decrease of SWV signal was proportional to the increase of adenosine concentration (Fig. 5C). Based on above method of 3 *s*, the detection limit was calculated to be 0.2 nM, which had improved by 25 times than the aptameric biosensor reported previously (Sun et al., 2010).

4. Conclusions

We have demonstrated a simple approach to fabricate an E-DNA biosensor by introducing the SWNTs. Due to the unique properties of vast surface-to-bulk ratio and good electrical conductivity, SWNTs function as signal amplifiers to facilitate both the immobilization of the MB-labeled ssDNA probes on their surface and the electron-transfer between the electrochemical label and the electrode. This signal-off E-DNA biosensor has significant advantages of improved sensitivity, sequence-specificity, and large linear dynamic range with low detection limit. Moreover, the introduction of aptamer into the SWNT-based biosensor might be further extended to detect small biomolecules such as adenosine. The high sensitivity and selectivity of SWNT-based E-DNA biosensor makes it a potential tool for early diagnosis of gene-related disease and also offers great promise for sensitive detection of numerous biomolecules such as thrombin and cocaine (Yang and Zhang, 2010; Zhang and Johnson, 2009; Swensen et al., 2009).

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