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Sensitive impedimetric DNA biosensor with poly(amidoamine) dendrimer covalently attached onto carbon nanotube electronic transducers as the tether for surface confinement of probe DNA

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ABSTRACT

This study demonstrates a new impedimetric DNA biosensor with second-generation poly(amidoamine) dendrimer (G2-PAMAM) covalently functionalized onto multi-walled carbon nanotube (MWNT) electronic transducers as the tether for surface confinement of probe DNA. G2-PAMAM dendrimer was covalently functionalized onto purified MWNTs and the as-formed G2-PAMAM-functionalized MWNT composite (i.e., G2-PAMAM/MWNT) was used both as the support to confine the single-stranded DNA (ssDNA) probe and as the electronic transducer to form the DNA biosensors. Upon the occurrence of hybridization events between surface-confined ssDNA probe with target DNA in solution to form a double-stranded DNA (dsDNA) at electrode surface, the negative charge in the electrode/electrolyte interface and, as such, the interfacial charge-transfer resistance of the electrodes towards the $Fe(CN)_6^{3-/4-}$ redox couple were changed. Such a change was used for the impedimetric DNA biosensing. The use of G2-PAMAM dendrimer attached onto MWNT electronic transducer as the tether for probe DNA provides a large number of amino groups to increase the surface binding of probe DNA, results in the increase the sensitivity of the impedimetric biosensor for the target DNA. Under the conditions employed here, the change in the interfacial charge-transfer resistance was linear with the logarithm of the concentration of the target DNA within a concentration range from 0.5 to 500 pM with a detection limit of 0.1 pM ($S/N = 3$). The excellent analytical properties of the impedimetric DNA biosensors developed here substantially makes them potentially useful for practical applications.

1. Introduction

Sensitive detection of DNA is of great significance in the analysis of pathogens, single-base mismatches, tissue matching, and environmental and forensic applications (Service, 1998; Debouck and Goodfellow, 1999). So far, various methods including radioactivity (Woff et al., 1987), fluorescence (Gerion et al., 2002), electrochemistry (Sassolas et al., 2008), surface plasma resonance (McDonnell, 2001), and quartz crystal microbalance (Wang et al., 1999) have been used for DNA biosensing. Among those methods, electrochemical method has received extensive attention due to its high sensitivity and selectivity, simple instrumentation and low production cost (Park et al., 2002; Miao and Bard, 2003; Drummond et al., 2003; Fan et al., 2005).

Due to the close association with the sensitivity and selectivity of DNA biosensors, surface confinement of probe DNA and readout of the DNA hybridization have constituted two of the most important factors in the development of electrochemical DNA biosensors (Sassolas et al., 2008). To date, some methods have been developed for probe DNA immobilization. For instance, surface confinement of single-stranded DNA (ssDNA) has been accomplished through weak interactions, such as electrostatic or hydrophobic interactions between the probe DNA and functionalized electrode surfaces (Lemeshko et al., 2001), or through the reactions between DNA sequence having amino moieties and electrode surface modification with aldehyde or epoxide groups (Peng et al., 2005; Marquette et al., 2006). In addition, self-assembled monolayers formed on electrode surface (Dharuman et al., 2006) or avidin (or streptavidin)–biotin interactions (Pan and Rothberg, 2005) have also been used for probe DNA confinement. For the readout of DNA hybridization, direct electrochemical detection of DNA hybridization reaction by monitoring the changes in electronic or interfacial properties accompanying the hybridization events has been demonstrated to be very attractive since those methods abrogated the indicator addition/association steps and greatly simplified the biosensing protocol (Darain et al., 2004).

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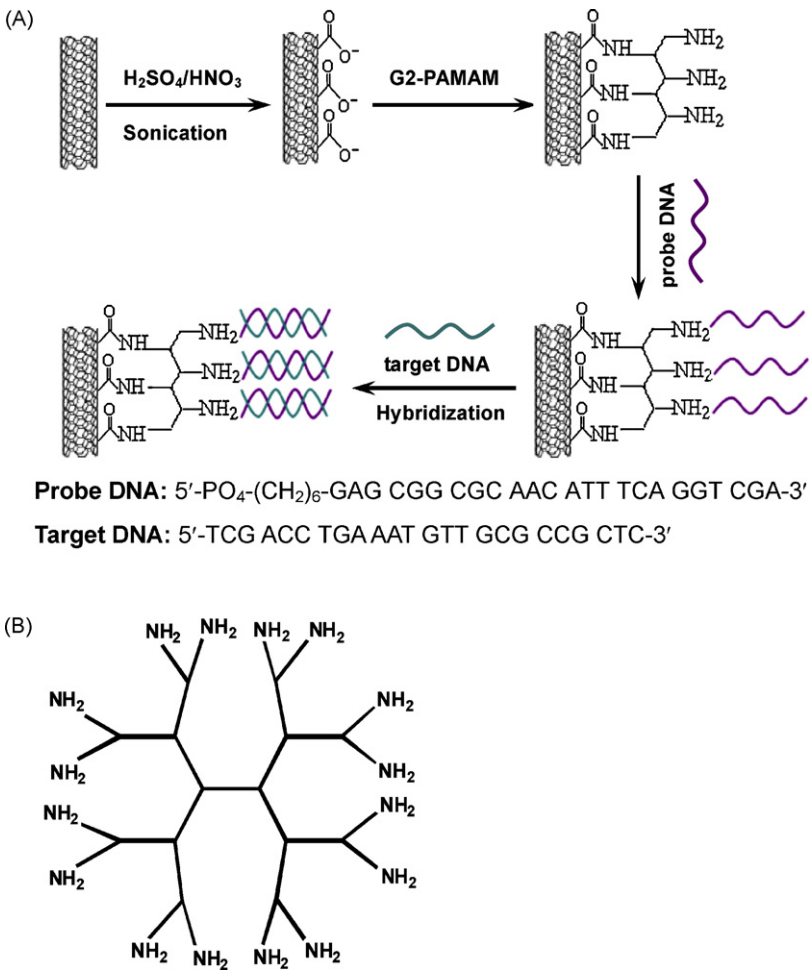

Scheme 1. (A) Schematic illustration of the processes for preparing impedimetric DNA biosensor with G2-PAMAM attached onto MWNT electronic transducer as the tether for probe DNA. (B) Structure of G2-PAMAM.

In our earlier studies, we have demonstrated new strategies to increase the sensitivity and lower the detection limit of the electrochemical DNA biosensors by using carbon nanotubes (CNTs) (Zhu et al., 2009) or poly(amidoamine) dendrimers (Zhu et al., 2006) as the support for surface confinement of the probe DNA. Those strategies have been based on the enriched surface chemistry, high-surface area, good conductivity and excellent electrochemical properties of CNTs (Wong et al., 1998; Kong et al., 2000; Niyogi et al., 2002; Zhang et al., 2007) and the perfectly branched repetitive units and density of functionalities of poly(amidoamine) dendrimers (Newkome et al., 2002; Bosman et al., 1999; Grayson and Frechet, 2001). The early uses of structurally unique nanostructures and functional reagents have well enabled the as-prepared electrochemical biosensors to be sensitive with a low detection limit. Based on our previous efforts, this study demonstrates a new and sensitive impedimetric DNA biosensor with poly(amidoamine) dendrimer attached onto carbon nanotube electronic transducer as the tether for surface confinement of probe DNA. The strategy demonstrated here is based on the combination of the advantages benefited from both CNTs and poly(amidoamine) dendrimers. In this context, NH_2-riched second-generation poly(amidoamine) (G2-PAMAM) dendrimer is covalently attached onto carbon nanotube electronic transducers to serve as the tether for surface confinement of a large amount of probe DNA, as shown in Scheme 1. Upon the hybridization of surface-tethered probe DNA with its complementary target DNA in solution, the interfacial charge-transfer resistance for the negatively charged $Fe(CN)_6^{3-/4-}$ redox probe at the electrochemical biosensor based on the ssDNA/G2-PAMAM/MWNT/-modified electrodes is remarkably increased due to the enhanced electrostatic repulsion between the dsDNA duplex and the $Fe(CN)_6^{3-/4-}$ redox couple. The increase in the charge-transfer resistance could consequently be used as the readout of the biosensor. The electrochemical DNA biosensors demonstrated here with G2-PAMAM as the tether for probe DNA, MWNTs as the electronic transducer, and interfacial charge-transfer resistance as the hybridization readout are sensitive and selective and could thus be useful for practical applications.

2. Experimental

2.1. Chemicals and reagents

Multi-walled carbon nanotubes (MWNTs) with a diameter of about 10–30 nm were obtained from Shenzhen Nanotech Co. Ltd. (Shenzhen, China). Prior to use, the MWNTs were purified by chemical oxidation of the as-received MWNTs in a mixture of H_2SO_4 and HNO_3 (3:1, v/v) under ultrasonication for approximately 4 h. Such a purification procedure also produces carboxyl groups at MWNTs. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was purchased from Sigma. Sodium dodecyl sulfate (SDS) was purchased from Merck. G2-PAMAM dendrimer with an ethylenediamine core contains 30 amine groups, with 16 being the surface primary amine groups and the other 14 being the inner tertiary amine groups were purchased from Aldrich. 0.30 M PBS buffer (0.30 M NaCl, 10 mM sodium phosphate buffer, pH 7.4) and TE buffer (10 mM Tris–HCl and 1 mM EDTA, pH 8.0) were used. All other chemicals were of

at least analytical reagent grade and used without further purification. Aqueous solutions were prepared with doubly distilled water.

The oligonucleotide DNA sequences were all purchased from Shanghai Sangon Bioengineering Ltd. Co. (Shanghai, China). A 24-mer 5′-PO_4-$(CH_2)_6$-GAG CGG CGC AAC ATT TCA GGT CGA-3′ was used as the probe DNA, and the oligonucleotide DNA with the sequence of 5′-TCG ACC TGA AAT GTT GCG CCG CTC-3′ was used as the completely complementary target DNA. The oligonucleotide DNA with a sequence of 5′-GAG CGG CGC AAC ATT TCA GGT CGA-3′ was used as the non-complementary DNA. The oligonucleotide DNA stock solutions were prepared with TE buffer (10 mM Tris–HCl and 1 mM EDTA, pH 8.0) and stored in a −20 °C freezer while not use.

2.2. Preparation of G2-PAMAM/MWNT-modified electrodes and probe ssDNA confinement

MWNT suspension was prepared by dispersing 1 mg purified MWNTs into 1 mL N,N-dimethylformamide with the aid of ultrasonic agitation. A 5.0 μL of the MWNT dispersion was dip-coated onto glassy carbon (GC) electrodes and the electrodes (MWNT-modified GC) were dried under an infrared lamp. The G2-PAMAM/MWNT-modified electrodes were prepared by immersing the MWNT-modified electrodes first into 10 mM EDC for 1 h and then into a 2 mg mL^{-1} G2-PAMAM aqueous solution for 8 h. After being rinsed with phosphate buffer and distilled water, the G2-PAMAM/MWNT-modified electrodes were immersed into 22.5 μM probe ssDNA solution containing 10 mM EDC for 8 h at room temperature (Chu et al., 1983; Yang et al., 2002). The electrodes (ssDNA/G2-PAMAM/MWNT-modified electrodes) were finally rinsed with 10 mM phosphate buffer and distilled water to remove the physically adsorbed DNA.

For X-ray photoelectron spectra (XPS) analysis, G2-PAMAM/MWNT composite was prepared as follows: MWNT dispersion (1 mg mL^{-1}) was firstly mixed with 2 mg mL^{-1} G2-PAMAM solution containing 10 mM EDC for 8 h under a gentle stirring. The mixture was filtered through a filter (0.22 μm) by vacuum filtration to give G2-PAMAM/MWNT composite. The composite was washed with distilled water and re-filtered several times to remove the unreacted G2-PAMAM molecules. ssDNA/G2-PAMAM/MWNT composite was prepared by mixing the obtained G2-PAMAM/MWNT composite into 22.5 μM probe ssDNA solution containing 10 mM EDC for 8 h. The mixture was then filtered with the same procedure as that for G2-PAMAM/MWNT composite to give the ssDNA/G2-PAMAM/MWNT composites. The composites were dried at 105 °C for 4 h before XPS analysis.

2.3. Apparatus and measurements

Hybridization reaction between the ssDNA confined onto MWNT electronic transducer and the target DNA in solution was conducted by immersing the ssDNA/G2-PAMAM/MWNT-modified electrodes into 0.3 M PBS buffer (0.3 M NaCl, 10 mM sodium phosphate buffer, pH 7.4) containing different concentrations of target DNA with a gentle stirring. The temperature and time employed for the hybridization reaction were optimized to be 45 °C and 60 min, respectively, in terms of the change in the charge-transfer resistance obtained at the ssDNA/G2-PAMAM/MWNT-modified electrodes for the $Fe(CN)_6^{3-/4-}$ redox couple after the hybridization with the target DNA. The electrodes (dsDNA/G2-PAMAM/MWNT-modified electrodes) were sequentially rinsed with 0.1% SDS, 0.3 M PBS buffer (pH 7.4) and distilled water to remove the nonspecifically adsorbed target DNA molecules.

Cyclic voltammograms (CV) and electrochemical impedance spectroscopy (EIS) measurements were performed with an electrochemical analyzer (CHI 660B, Chenhua, Shanghai, China). The ssDNA/G2-PAMAM/MWNT-modified GC electrodes were used as a working electrode, a saturated calomel electrode (SCE) as reference electrode, and Pt wire as an auxiliary electrode. For EIS measurements, 100 mM KCl solution buffered with 10 mM phosphate (pH 7.0) was used as the electrolyte and 10 mM $Fe(CN)_6^{3-/4-}$ (1:1) was used as the redox probe. EIS measurements were conducted within a frequency range from 2×10^4 to 0.1 Hz at the open circuit potential. Unless stated otherwise, all electrochemical measurements were performed at ambient temperature.

Thermal gravimetric (TG) analysis was conducted on a PETGA-7 instrument with a heating rate of 10 °C/min. XPS measurement was carried out with PHI 5000 VersaProbe instrument (ULVAC-PHI).

3. Results and discussion

3.1. Attachment of G2-PAMAM and ssDNA probe onto MWNTs

The covalent attachment of G2-PAMAM onto MWNTs to form G2-PAMAM/MWNT composite and the subsequent surface confinement of ssDNA probe with G2-PAMAM as the tether were verified with XPS spectra of MWNTs (black curves) and G2-PAMAM/MWNT composite before (red curves) and after (blue curves) ssDNA linkage, as displayed in Fig. 1. A small N 1s peak was observed at about 402 eV (panel A, black curve) for MWNTs after

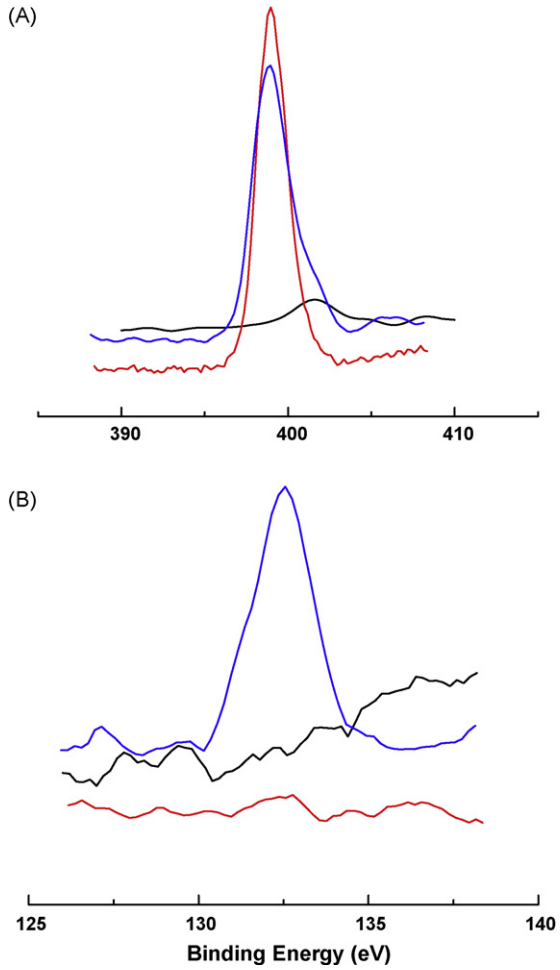

Fig. 1. High-resolution XPS spectra in the N (1s) region (A) and P (2p) region (B) for the purified MWNTs (black curves) and G2-PAMAM/MWNT composite before (red curves) and after (blue curves) ssDNA linkage. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

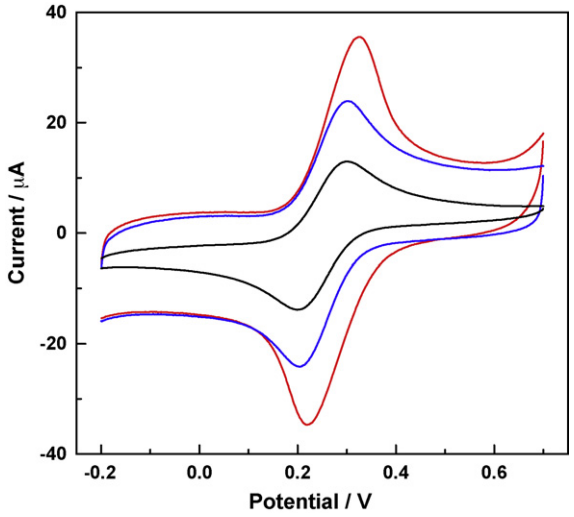

Fig. 2. CVs obtained at bare (black curve), MWNT-modified (blue curve) and G2-PAMAM/MWNT-modified (red curve) GC electrodes in 0.10 M KCl solution containing 1 mM $K_3Fe(CN)_6$. Scan rate, 100 mV/s. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

MWNTs were pretreated in the mixed acidic solution, suggesting that nitrogen-containing groups were also produced at MWNTs in such pretreatment procedure, which was consistent with the literature (Xia et al., 2007; Raymundo-Pinero et al., 2002; Ozensoy et al., 2006). A new peak in the N (1s) region at 398.9 eV (Moulder et al., 1992) was observed at the G2-PAMAM/MWNT composite (panel A, red curve), which was not present for the purified MWNTs (panel A, black curve), indicating that G2-PAMAM was successfully attached onto MWNTs. In addition, a new peak corresponding to the P (2p) region at 132.5 eV (Moulder et al., 1992) emerged at the G2-PAMAM/MWNT composite after ssDNA linkage (panel B, blue curve) and such a peak was not observed at the composite before ssDNA linkage (panel B, red curve) or at the purified MWNTs (panel B, black curve), suggesting that ssDNA probe was confined on the G2-PAMAM/MWNT composite. TG analysis with the G2-PAMAM/MWNT composite (data not shown) indicates that the G2-PAMAM dendrimer was burnt up to 400 °C and the weight content of the dendrimer attached onto MWNTs was evaluated to be about 25%.

G2-PAMAM attachment onto MWNT electronic transducers was also verified by the changes in cyclic voltammetric (CV) response of $Fe(CN)_6^{3-}$ at the bare, MWNT-modified and G2-PAMAM/MWNT-modified GC electrodes under the same experimental conditions, as displayed in Fig. 2. The background and the peak currents obtained at the MWNT-modified electrode (blue curve) in the aqueous solution of $Fe(CN)_6^{3-}$ were obviously larger than those at the bare GC electrode (black curve), which were attributed to the fact that the MWNT modification significantly increases the active electrode area. In addition, the peak current at the G2-PAMAM/MWNT-modified electrode in 1 mM $Fe(CN)_6^{3-}$ solution (red curve) was clearly larger than that at the MWNT-modified electrode (blue curve). Such an increase could be presumably explained in terms of the enhanced electrostatic adsorption of the negatively charged $Fe(CN)_6^{3-}$ redox probe at the positively charged G2-PAMAM-modified electrode surface. These results suggest that G2-PAMAM was covalently attached onto MWNTs. To further prove the covalent attachment of G2-PAMAM onto MWNTs, we also conduct the control voltammetric experiments with the G2-PAMAM/MWNT-modified electrodes prepared by simply immersing the MWNT-modified electrodes into G2-PAMAM solution in the absence of EDC. In 0.10 M KCl solution containing 1 mM $K_3Fe(CN)_6$, the peak currents at the as-prepared G2-PAMAM/MWNT-modified electrodes were larger than those at the bare and MWNT-modified GC electrodes. However, the peak currents were largely decreased after the electrodes were immersed in pure supporting electrolyte solution under stirring conditions for about 30 min and then transferred into 0.10 M KCl solution containing 1 mM $K_3Fe(CN)_6$. On contrast, the same procedures applied to the G2-PAMAM/MWNT-modified electrodes prepared with EDC linkage did not result in an obvious change in the peak currents for the $Fe(CN)_6^{3-/4-}$ redox couple, suggesting the G2-PAMAM dendrimer was stably attached onto MWNT electronic transducers with EDC as the covalent linkage.

3.2. DNA hybridization at the ssDNA/G2-PAMAM/MWNT-modified electrodes

Fig. 3 compares CV and EIS responses at the G2-PAMAM/MWNT-modified electrode and the ssDNA/G2-PAMAM/MWNT-modified electrodes before and after hybridized with the target DNA. As

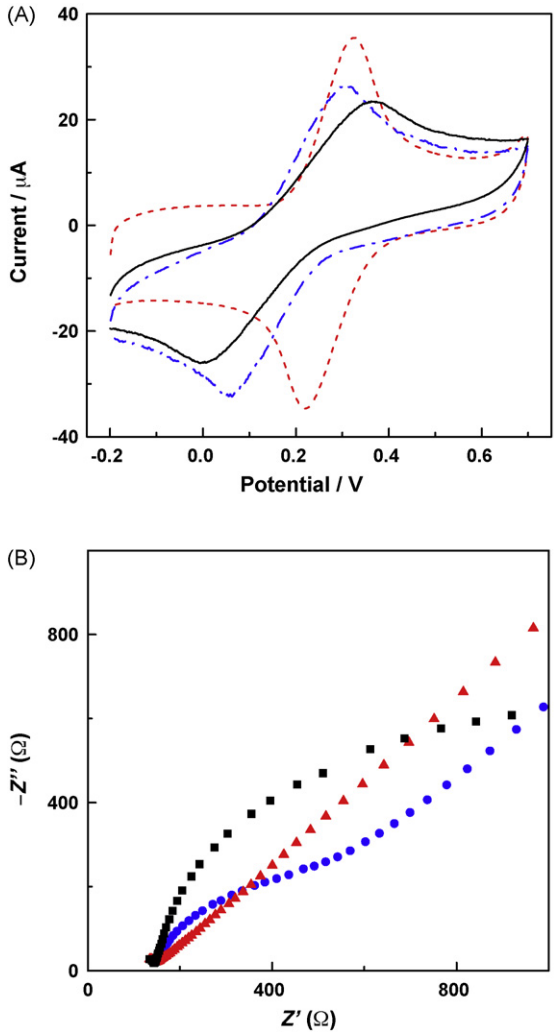

Fig. 3. CVs (A) and Nyquist plots (B) of the G2-PAMAM/MWNT-modified electrodes (short-dashed red curve, red ▲), and the ssDNA/G2-PAMAM/MWNT-modified electrodes before (dash-dotted blue curve, blue ●) and after (solid black curve, black ■) hybridized with the complementary target DNA (1 pM). CV measurements were performed in 0.10 M KCl solution containing 1 mM $Fe(CN)_6^{3-}$ at a scan rate of 100 mV/s. EIS measurements were performed in 0.10 M KCl solution containing 1 mM $Fe(CN)_6^{3-/4-}$ (1:1) within a frequency range from 2×10^4 to 0.1 Hz. Hybridization time: 60 min; hybridization temperature: 45 °C. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

displayed in Fig. 3A, the immersion of the G2-PAMAM/MWNT-modified electrodes into the solution of probe ssDNA results in a decrease in the peak currents and an increase in the cathodic-to-anodic peak separation (ΔE_p) at the electrodes for the $Fe(CN)_6^{3-/4-}$ redox couple (dash-dotted blue curve), as compared with those at the G2-PAMAM/MWNT-modified electrodes (short-dashed red curve). These changes were elucidated with the electrostatic repulsion between the polyanionic ssDNA and the anionic $Fe(CN)_6^{3-/4-}$ redox couple, suggesting that the ssDNA probe was successfully linked onto the G2-PAMAM/MWNT-modified electrodes to form ssDNA/G2-PAMAM/MWNT-modified electrodes that were used as electrochemical DNA biosensors. Also, as shown in Fig. 3A, after the ssDNA/G2-PAMAM/MWNT-modified electrodes were incubated into 0.30 M PBS buffer (pH 7.4) containing the target DNA for 60 min at 45 °C, the peak currents for the $Fe(CN)_6^{3-/4-}$ redox couple were further decreased and the ΔE_p was further enlarged (solid black curve), implying the formation of the dsDNA structure on the electrode surface through the hybridization reaction. This is because the formation of the dsDNA substantially increases the negative charges at the electrode surface and enhances electrostatic repelling interaction between the dsDNA duplex and the $Fe(CN)_6^{3-/4-}$ redox couple.

Fig. 3B depicts Nyquist plots obtained in 0.10 M KCl containing 1 mM $Fe(CN)_6^{3-/4-}$ redox couple with the same electrodes used in Fig. 3A. At the G2-PAMAM/MWNT-modified electrode, a very small R_{ct}, as determined with the diameter of the Nyquist semicircle, was recorded for the $Fe(CN)_6^{3-/4-}$ redox couple (red ▲), suggesting a fast electron-transfer process at such electrode. Primarily, this was due to the confinement of the positively charged NH_2-terminated PAMAM on the electrode and the electrostatic attraction between the $Fe(CN)_6^{3-/4-}$ redox couple. Compared with the G2-PAMAM/MWNT-modified electrode, the ssDNA/G2-PAMAM/MWNT-modified electrode shows a larger R_{ct} (blue ●), indicative of the enlarged charge-transfer resistance caused by the electrostatic repulsion between the negatively charged ssDNA confined onto the electrode and the $Fe(CN)_6^{3-/4-}$ redox couple. The R_{ct} was further enlarged after the ssDNA/G2-PAMAM/MWNTs-modified electrode was incubated in the buffer containing the complementary target DNA (black ■), which was understood from the increase in the negative charge at the electrode surface and the enhanced electrostatic repulsion interaction between the dsDNA and the $Fe(CN)_6^{3-/4-}$ redox couple. The change in the R_{ct} values, on one hand, verifies the hybridization of the ssDNA probe confined onto MWNT electronic transducer with the target DNA in solution and, on the other hand, forms a straightforward basis for impedimetric DNA biosensing, as describe below.

3.3. Analytical properties of the impedimetric DNA biosensor

Electrochemical impedance spectroscopy is an effective technique both for understanding the nature of surface-modified electrodes and for analytical applications. Generally, the equivalent circuit includes: (1) ohmic resistance (R_s) of the electrolyte solution, (2) Warburg impedance (Z_w) from ion diffusion from the bulk electrolyte to electrode interface, (3) interfacial double layer capacitance (C_{dl}) between electrode and solution, which is closely related to the surface condition of the electrode, and (4) charge-transfer resistance (R_{ct}) from the redox probe in the electrolyte. The parallel elements (C_{dl} and $Z_w + R_{ct}$) of the equivalent circuit have been normally considered since the total current through the electrode/electrolyte interface was the sum of respective contributions from the double layer charging and the Faradaic process. According to the circuit, electrochemical impedance spectra generally include a semicircle portion and a linear portion. The former portion at higher frequencies corresponds to the electron-transfer-dominated process, while and the latter one at lower frequencies represents the diffusion-limited process. The diameter of the semicircle equals to the charge-transfer resistance, R_{ct}. Fig. 4 displays the Nyquist plots obtained with the ssDNA/G2-PAMAM/MWNT -modified electrodes in 0.10 M KCl solution containing 1 mM $Fe(CN)_6^{3-/4-}$ after the electrodes were immersed into the hybridization buffer containing various concentrations of the complementary target DNA. The R_{ct} value increases with increasing the concentration of the target DNA in the hybridization reaction. The change in the R_{ct} (ΔR_{ct}, $\Delta R_{ct} = R_{ct,dsDNA} - R_{ct,ssDNA}$) was linear with the logarithm of the concentration of target DNA within a concentration range from 0.5 to 500 pM. The linear regression equation was $\Delta R_{ct} = 652.3 \log C - 872.9$ (C was the concentration of target DNA, fM) with a correlation coefficient $\gamma = 0.9976$. $R_{ct,ssDNA}$ and $R_{ct,dsDNA}$ represent the change-transfer resistance at the ssDNA/G2-PAMAM/MWNT-modified and dsDNA/G2-PAMAM/MWNT-modified electrodes in 0.10 M KCl solution containing 1 mM $Fe(CN)_6^{3-/4-}$, respectively. The detection limit of the impedimetric DNA biosensor was estimated to be 0.1 pM, based on $S/N = 3$. This value is much comparable to those of most kinds of the electrochemical DNA biosensors (Li et al., 2007; Wang et al., 2008) and remains to be much lower than those reported in our earlier studies with CNTs alone as the support for probe DNA with the ΔR_{ct} as the readout (Zhu et al., 2009) and with the G4-PAMAM dendrimer assembled onto Au substrate as the tether for probe DNA with amperometric response for intercalating agent as the readout (Zhu et al., 2006).

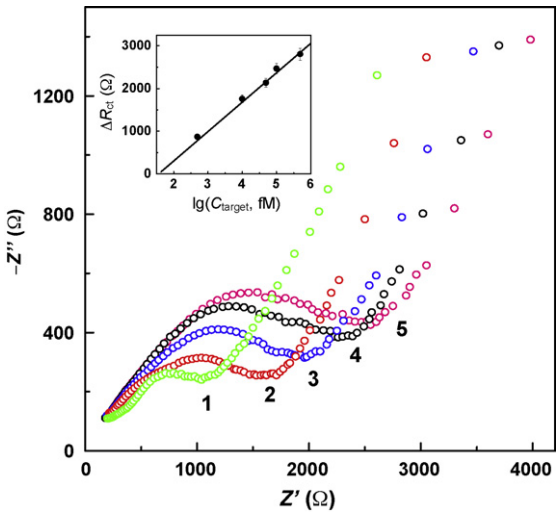

Fig. 4. Nyquist plots obtained at the ssDNA/G2-PAMAM/MWNT-modified electrodes after the electrodes were incubated with different concentrations of target DNA in 0.3 M PBS hybridization buffer. The concentrations of the complementary target DNA were (1) 0.5, (2) 10.0, (3) 50.0, (4) 100 and (5) 500 M, respectively. Inset: calibration curve for the DNA biosensors. Other conditions were the same as those in Fig. 3.

We also investigated the responses of the impedimetric DNA biosensors by incubating the ssDNA/G2-PAMAM/MWNT-modified electrodes in the target-free hybridization buffer and the hybridization buffer containing different kinds of DNA including non-complementary DNA and complementary target DNA. As shown in Fig. 5, a negligible ΔR_{ct} was observed after the ssDNA/PAMAM/MWNT-modified electrodes were incubated in the target-free hybridization buffer, implying the ssDNA/PAMAM/MWNT-modified electrodes were stable under the present conditions. Also, a slight ΔR_{ct} was obtained at the ssDNA/PAMAM/MWNT-modified electrodes after the electrodes were incubated in the hybridization buffer containing the non-complementary DNA, revealing that the ΔR_{ct} caused by the nonspecific adsorption of non-complementary DNA was negligi-

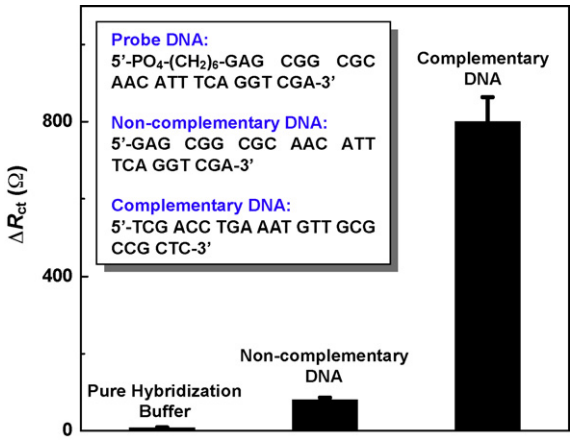

Fig. 5. The $\triangle R_{ct}$ obtained with the ssDNA/G2-PAMAM/MWNT-modified electrodes after the electrodes were incubated into target-free hybridization buffer or 0.30 M PBS buffer (pH 7.4) containing 1 pM of non-complementary DNA, or complementary DNA for 60 min at 45 °C with a gentle stirring. Three electrodes were tested in each group.

ble. On contrast, the ssDNA/PAMAM/MWNT-modified electrodes show a large ΔR_{ct} after the electrodes were incubated in the hybridization buffer containing complementary DNA. These results demonstrate that the changes of the charge-transfer resistance at the electrochemical DNA biosensors were induced by the specific hybridization reaction between the probe DNA confined onto electrode surface and the target DNA in solution. The reproducibility of the prepared DNA biosensor was estimated by making repetitive hybridizations with the same concentration of the complementary DNA according to the procedures described above. The relative standard deviation (RSD) based on 5 measurements for the complementary DNA with the same ssDNA/G2-PAMAM/MWNT-modified electrode was about 8.6%. In addition, the impedance signal almost remained unchanged after the biosensors were stored in the PBS buffer for at least 7 days, demonstrating that the present method for DNA determination has a good reproducibility and stability. The impedimetric DNA biosensor demonstrated here were reusable for repeated detection of DNA hybridization. The regeneration could be simply performed by first immersing the dsDNA/G2-PAMAM/MWNT-modified electrodes into 0.1 M NaOH solution for 120 s and then challenging the electrodes in the hybridization buffer containing complete complementary targets. By doing so, the sensor signal was almost recovered to the original value.

4. Conclusions

By taking advantages of PAMAM dendrimer with abundant amine functional groups to tether ssDNA probe onto MWNT electronic transducers and the good electrochemical properties and high-surface area of MWNTs, we have developed a new impedimetric DNA biosensor. The PAMAM/MWNT-based DNA biosensor is sensitive and selective for target DNA with a low detection limit down to 0.1 pM. Compared with the existing methods for DNA detection, the method demonstrated here is simple, sensitive and reliable and could thus be reasonably envisaged to be useful for practical applications.

Acknowledgments

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