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Readily Reusable Electrochemical DNA Hybridization Biosensor Based on the Interaction of DNA with Single-Walled Carbon Nanotubes

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Carboxylic group-functionalized single-walled carbon nanotubes (SWNTs) were assembled vertically on the glassy carbon electrode using ethylenediamine as linking agent to fabricate an aligned electrode (SWNTE). Single-stranded DNA (ssDNA) wrapped around the SWNTs to form ssDNA-wrapped SWNTE structures based on the interaction between ssDNA and SWNT. A sensitive differential pulse voltammetric (DPV) response was obtained at the ssDNA-wrapped SWNTE owing to the electrooxidation of guanine bases. Double-stranded DNA (dsDNA) was formed when ssDNA on the ssDNA-wrapped SWNTE was hybridized with complementary ssDNA (cDNA). The dsDNA was removed from the SWNTs by undergoing a process of preconditioning at −0.6 V. Consequentially, the DPV response of guanine bases decreased. The used SWNTE could be renewed easily via ultrasonically rinsing. On the basis of this mechanism, a label-free and readily reusable electrochemical DNA hybridization biosensor was designed by directly monitoring the current change of guanine bases. Under optimum conditions, the plot of the measurement signal of guanine bases versus the cDNA concentrations was a good straight line in the range of 40−110 nM with a detection limit of 20 nM (3s). The biosensor can be switched to detect different target DNAs easily.

Over the past decades, the need for simple portable devices of sequence-specific DNA detection has resulted in considerable research efforts into developing hybridization biosensors. A lot of achievements have been obtained in both label-free approaches[1−8] and labeled approaches.[9−12] Especially, the label-free approaches are prevalent because they greatly simplify the sensing protocol by eliminating the need for the indicator addition/association step. This field has been reviewed several times so far.[13−15]

These technologies commonly rely on the immobilization of a single-stranded DNA (ssDNA) probe onto the electrochemical transducer for subsequent recognition of target complementary ssDNA in sample solution. For the sake of high degree of hybridization efficiency, end-point attachment of the DNA probe is desired.[14] Commonly, formation of a chemical bond with some compounds, such as amide[1,3,7,16,17] and thiol,[2,5,14] is exploited. The merit of such a strategy is its stability. However, it is obvious that once a transducer is immobilized with a kind of DNA probe, it is not easy to be switched to detect another kind of target DNA.

The mechanism of interaction between DNA and carbon nanotubes (CNTs) is interesting and important for fundamental and applied science, and a lot of efforts have been made to this field.[18−27] Up to now, more and more essential contents have been

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realized. It is found that ssDNA of different lengths, either small oligonucleotides consisting of tens of bases[18] or completely random sequence of long genomic strands,[20] can wrap around single-walled carbon nanotubes (SWNTs) to form tight helices. Molecular dynamics simulations show that there is no evidence for canonical double-stranded (dsDNA) wrapping around either charged or uncharged SWNTs.[23] Meng et al.[25] proved that ssDNA and CNTs had complementary structural features, which made it possible to assemble them into a stable structure. DNA can be removed from SWNTs via hybridization,[27] indicating that this offers a huge opportunity for establishing a novel kind of DNA hybridization biosensor.

In this paper, we settled on a solution to the problem of quick renewal by developing a simple immobilization method for fabricating a DNA hybridization biosensor based on the mechanism of interaction between DNA and SWNT. ssDNA probe wrapped onto the SWNTs array electrode (SWNTE) without any aid of auxiliary reagents. The hybridization result was detected by directly monitoring the differential pulse voltammetric (DPV) response of the guanine bases. Unlike the previous reports,[28,29] in the current work guanine bases in the probe sequence did not have to be substituted by hypoxanthine residues (pairing with cytosines). The performance of the biosensor was studied and discussed in terms of optimum analytical conditions. In comparison to existing techniques, this novel approach has two obvious advantages: it is readily reusable and can be switched to detect different target DNAs easily.

EXPERIMENTAL SECTION

Apparatus and Chemicals. Cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and DPV were performed using a CHI 660B electrochemical analyzer (CH Instruments, Shanghai, China) with a three-electrode arrangement, consisting of a glassy carbon electrode (GCE) or SWNTE (Φ = 2 mm) working electrode, a saturated calomel reference electrode (SCE), and a platinum wire auxiliary electrode. Scanning electron microscopy (SEM) measurements were carried out on a JSM-6700F scanning electron microscope (Japan Electron Company). A PicoForce Multimode atomic force microscope (AFM, Veeco Ltd. U.S.A.) was used for the force spectroscopy.

SWNTs (main range of diameter <2 nm; length: 1 to ~2 μm) were purchased from Shenzhen Nanotech Port Ltd. Co. (China). *N*-Hydroxysuccinimide (NHS) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were purchased from Sigma-Aldrich (U.S.A.). The other usual reagents were purchased from Shanghai Chemical Reagent Co. (Shanghai, China) and were all of analytical reagent grade. Solutions were all prepared with Aquapro ultrapure water (resistivity: 18 MΩ cm, Aquaplus AWL-1002-P, Ever Young Enterprises Development Co., Ltd., China).

Synthetic oligonucleotides were purchased from Beijing SBS Genetech Co., Ltd. (Beijing, China). They had the following sequences: probe ssDNA (pDNA), 5′-CGA CAG TGG TCC CAA AGA-3′; complementary ssDNA (cDNA), 5′-TCT TTG GGA CCA CTG TCG-3′; one-end-base mismatch ssDNA, 5′-ACT TTG GGA CCA CTG TCG-3′; one-mid-base mismatch ssDNA, 5′-TCT TTG GAA CCA CTG TCG-3′; two-base mismatch at the same end ssDNA, 5′-AGT TTG GGA CCA CTG TCG-3′; two-base mismatch at two ends ssDNA, 5′-ACT TTG GGA CCA CTG TCC-3′; two-mid-base mismatch ssDNA, 5′-TCT TTG GTG CCA CTG TCG-3′; noncomplementary ssDNA (ncDNA), 5′-ATC GTT CAA ACA TTT GGC-3′; dsDNA was obtained by hybridizing pDNA with cDNA at 42 °C for 40 min with gentle stirring.

Preparation of the SWNTE. SWNTs were pretreated with a previously reported protocol.[30] In brief, 2 g of SWNTs was sonicated for 6 h in 36% HCl. Then they were rinsed with water until the pH of the solution was over 6. They were leached and sonicated for another 1 h in 2.2 M HNO_3, followed by being incubated in an oven at 90 °C overnight. The mixture was sonicated again for 1 h at room temperature. At last the shortened and oxidized SWNTs were rinsed with water until the filtrate was neutral.

An amount of 1 mg of as-pretreated SWNTs was dispersed into 100 mL of water containing 1 mg *N*,*N*-dimethylformamide (DMF)[3] with sonication for 1 h to generate a black suspension. Prior to the surface modification, the GCE was sequentially polished with 0.3 and 0.05 μm alumina slurries and rinsed with acetone and water for 3 min, respectively. Then, the pretreated GCE was oxidized at +1.5 V for 15 s in an aqueous solution containing 2.5% $K_2Cr_2O_7$ and 10% HNO_3.[1] After being rinsed, the electrode was immersed in 10 mM ethylenediamine solution containing 5 mM EDC and 8 mM NHS for 5 h with gentle shaking. After being rinsed again, it was transferred into the SWNTs suspension containing 5 mM EDC and 8 mM NHS for another 12 h to prepare the SWNTE. Prior to use, the SWNTE was ultrasonically rinsed with water.

Immobilization and Hybridization of DNA. The SWNTE was immersed in a 2.0 mL of HAc−NaAc buffer solution (pH 5.8, 0.2 M) containing 1.0 μM pDNA with gentle shaking for a desired time (it was 30 min unless otherwise indicated). Then it was washed with 0.2 M Britton−Robinson buffer (B−R buffer, which consists of a mixture of 0.04 M H_3BO_3, 0.04 M H_3PO_4, and 0.04 M CH_3COOH) solution of pH 7.0 containing 0.5% sodium dodecyl sulfate (SDS), followed by being rinsed with water carefully. The as-prepared electrode was denoted as ssDNA-wrapped SWNTE.

The ssDNA-wrapped SWNTE was immersed in a HAc−NaAc buffer solution (pH 5.8) containing cDNA with gentle agitation for a desired time (it was 45 min unless otherwise indicated) at 42 °C for hybridization. Control experiments were performed with the same process by using ncDNA or other kinds of ssDNA instead of cDNA.

Renewal of the SWNTE. The used SWNTE (the ssDNA-wrapped SWNTE or the hybridized SWNTE) was renewed with the following processes. It was sonicated in B−R buffer solution of pH 7.0 containing 0.5% SDS for 5 min and then rinsed with water carefully.

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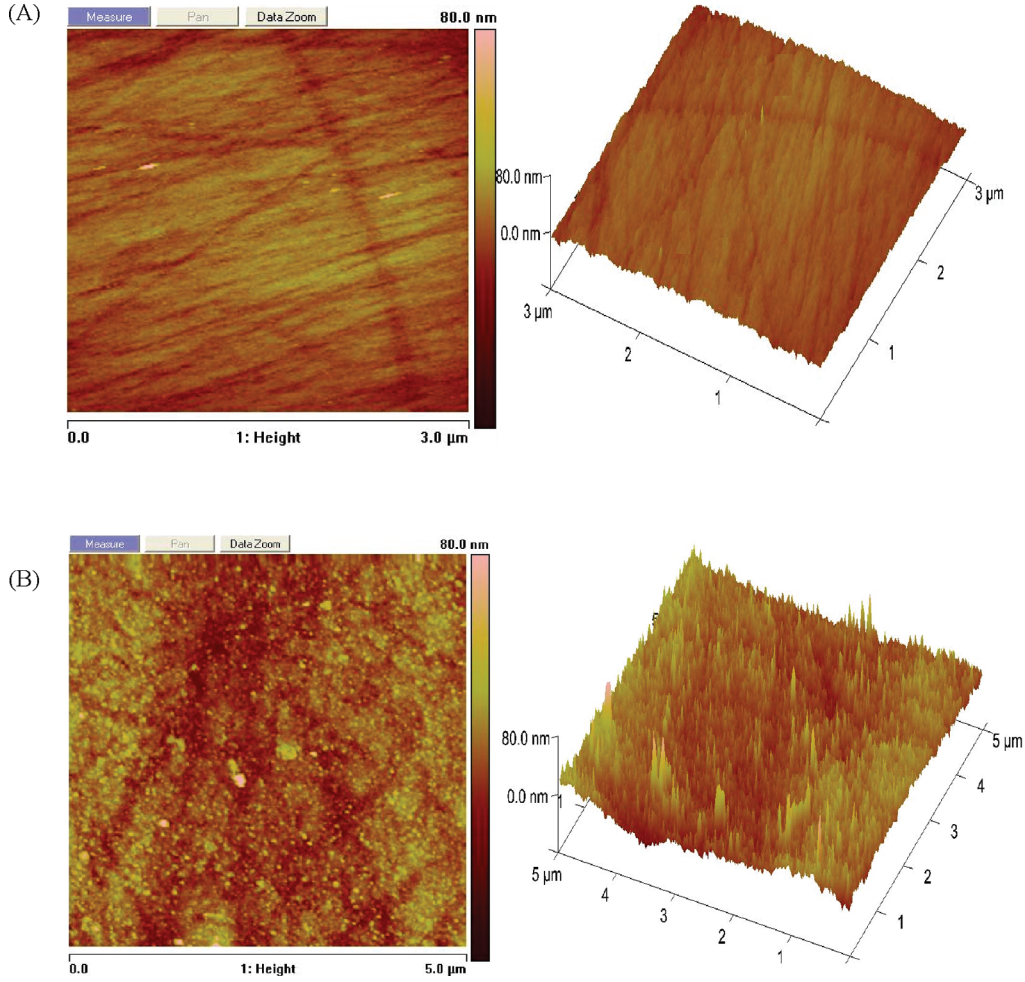

Figure 1. Tapping mode AFM of the surface of the glassy carbon electrode (A) and SWNTs array electrode (B): left, height images; right, 3D images.

CV, DPV, and EIS Experiments. Unless otherwise indicated, CV experiments were performed at a scan rate of 0.10 V/s; DPV experiments were performed at a pulse amplitude of 0.05 V, a pulse width of 0.05 s, and a pulse period of 0.2 s. Before DPV scanning the electrode underwent a process of preconditioning at −0.6 V for 60 s with gentle agitation and quiet for 2 s.

The EIS measurements were carried out in a 1.0 mM $K_3[Fe(CN)_6]$ solution containing 0.10 M KCl. The ac voltage amplitude was 5 mV, and the voltage frequencies were ranged from 10 kHz to 0.05 Hz. The applied potential was selected as 199 mV (vs SCE) from the formal potential of the redox probe $[Fe(CN)_6]^{3-/4-}$. All experiments were conducted at room temperature (25 ± 0.5 °C).

RESULTS AND DISCUSSION

Fabrication and Morphological Characteristics of the SWNTE. The SWNTs sample was pretreated with HCl and HNO_3 to enable the purity of SWNTs.[31] At the same time the treatment introduced carboxylic acid groups onto the SWNTs.[30] As had been reported,[32] the majority of the tubes were individual existence though they tangled randomly in the SEM image of the treated SWNTs. SWNTs are known to aggregate because of the intertube van der Waals attraction, and a high shear force is necessary to exfoliate the nanotube bundles.[29] The exfoliation of SWNT bundles observed in the present study was probably due to the electrostatic repulsion between the SWNTs for the charge of COO^-. At the same time, it was believed that the DMF could help the disperse of SWNTs.[3]

For the fabrication of SWNTE, ethylenediamine was first bound to the pretreated GCE surface via stable amide linkage in the presence of EDC and NHS.[1,17,33] After the removal of the unreacted ethylenediamine from the electrode surface, carboxyl-terminated SWNTs were then assembled covalently onto the electrode via amide formation also with the aid of EDC and NHS. Figure 1 shows the AFM images of the surface of GCE before (A) and after (B) the assembly of SWNTs. The AFM height cross-sectional analysis indicated that the diameters of the majority of the tubes were shorter than 2 nm. In most cases, the aligned SWNTs were observed to occur vertically as individual needles with a length less than 80 nm. The result indicated that carboxylic acid groups were only formed at the tip of the SWNTs, and therefore the SWNTs could be accomplished to align vertically on the GCE surface. This was different from the previous report.[17] Besides, it was impossible for both ends of the SWNT to be coupled onto the GCE owing to the steric hindrance. It was also

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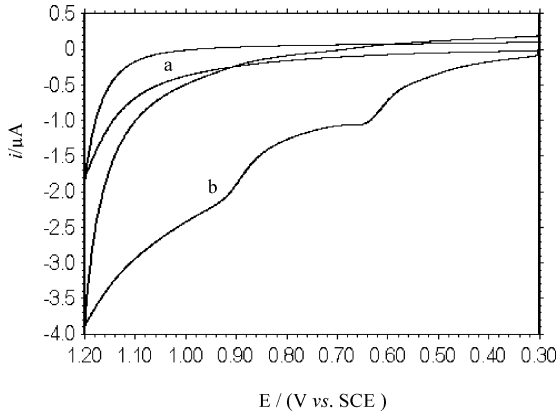

Figure 2. Cyclic voltammograms of 1.0 μM pDNA at the glassy carbon electrode (a) and the SWNTs array electrode (b) in a 0.2 M B−R buffer solution of pH 7.0. Scan rate: 0.10 V/s. Accumulation time: 600 s.

interesting to find that no SWNT longer than 80 nm was linked, though such long nanotubes could be observed from the SEM images of treated SWNTs. The length of most of the assembled SWNTs (about 80%) was no more than 20 nm. Only a few SWNTs in bundles were assembled, the length of which was longer than 30 nm. These results suggested that the smaller the tube in the suspension, the easier the assembly, owing to the better dispersion.

CV Responses of DNA at the SWNTE. There was no visible peak on the CV curve of 1.0 μM pDNA in a 0.2 M B−R buffer solution of pH 7.0 at the GCE working electrode (Figure 2a), whereas at the SWNTE, two well-defined oxidation peaks, which corresponded to the electrochemical oxidation of guanine bases and adenine bases in DNA, respectively, were observed at 0.64 and 0.92 V (Figure 2b). Both the oxidation peak potentials (E_{pa}'s) of guanine bases and adenine bases were lower than those in previous reports,[4,29,34−36] indicating that the SWNTE showed higher catalytic ability toward DNA.

Immobilization and Hybridization of DNA. In the range of 0.48−0.78 V, there was no oxidation peak at the SWNTE in the 0.2 M B−R buffer solution of pH 7.0 (Figure 3a). The SWNTE was immersed in HAc−NaAc buffer solution of pH 5.8 containing 1.0 μM pDNA for 30 min to prepare a ssDNA-wrapped SWNTE, and then the DPV curve of the electrode was recorded in the 0.2 M B−R buffer solution of pH 7.0. A well-defined DPV peak appeared at about 0.63 V, which was ascribed to the electrooxidation of guanine bases (Figure 3d). It indicated that pDNA was immobilized on the surface of the electrode. A ssDNA-wrapped SWNTE was immersed in HAc−NaAc buffer solution of pH 5.8 containing 0.1 μM cDNA for 45 min for hybridization. After being rinsed with 0.5% SDS solution and water for 2 min, respectively, the hybridized electrode (without the process of being preconditioned at −0.6 V) also presented a relatively smaller DPV peak (Figure 3c). If the hybridized electrode underwent the preconditioning at −0.6 V for 60 s and then was rinsed with 0.5% SDS solution and water, the DPV peak decreased further (Figure 3b). The result indicated that DNA had left the electrode almost

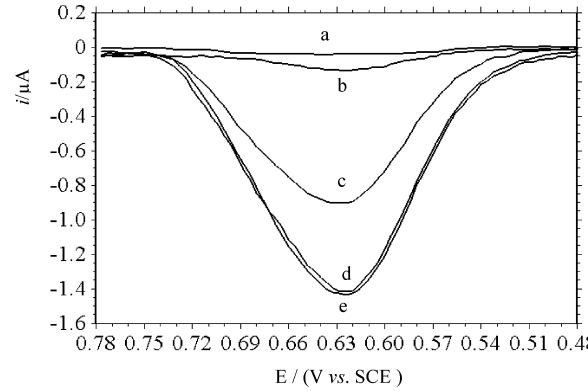

Figure 3. Differential pulse voltammograms of SWNTE (a), ssDNA-wrapped SWNTE hybridized to cDNA with preconditioning at −0.6 V for 60 s (b) and without preconditioning at −0.6 V (c), ssDNA-wrapped SWNTE (d) and ssDNA-wrapped SWNTE hybridized to ncDNA (e) in 0.2 M B−R buffer solution of pH 7.0. The ssDNA-wrapped SWNTE was prepared by immersing a SWNTE in a 1.0 μM pDNA solution for 30 min. Concentration of cDNA or ncDNA was 0.1 μM. Hybridization time was 45 min. Pulse amplitude: 0.05 V. Pulse width: 0.05 s. Pulse period: 0.2 s. Background currents were subtracted.

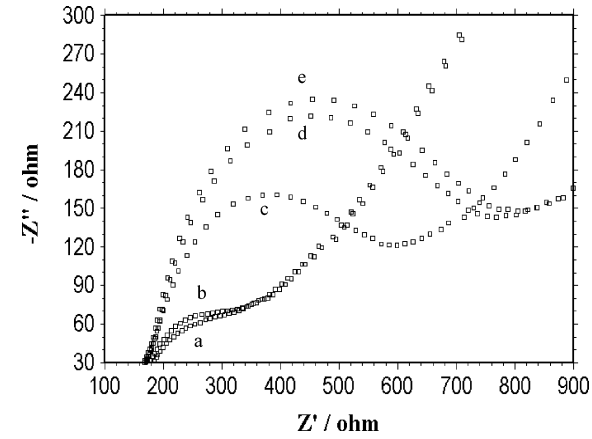

Figure 4. Nyquist diagrams of 1.0 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) at SWNTE (a), ssDNA-wrapped SWNTE hybridized to cDNA with preconditioning at −0.6 V for 60 s (b) and without preconditioning at −0.6 V (c), ssDNA-wrapped SWNTE (d) and ssDNA-wrapped SWNTE hybridized to ncDNA (e). The ssDNA-wrapped SWNTE was prepared by immersing a SWNTE in a 1.0 μM pDNA solution for 30 min. Concentration of cDNA or ncDNA was 0.1 μM. Hybridization time was 45 min. Supporting electrolyte: 0.1 M KCl.

entirely. As a control, ncDNA was utilized instead of cDNA for the experiment. The hybridized electrode displayed still a well-defined DPV peak (Figure 3e) almost the same as Figure 3d whether the electrode had underwent the preconditioning at −0.6 V or not. The results showed that the pDNA wrapped on the SWNTE had negligible change. Namely, they were not removed from the SWNTs during these processes.

The EIS was also utilized to monitor the hybridization recognition of DNA.[37] Curve a in Figure 4 is the Nyquist diagram of 1.0 mM $[Fe(CN)_6]^{3-/4-}$ at the SWNTE. The high-frequency section of the curve showed an arc, the diameter of which displayed a rather low interface electron-transfer resistance (R_{et}) owing to the attractive electrical conductivity of SWNTs, even though

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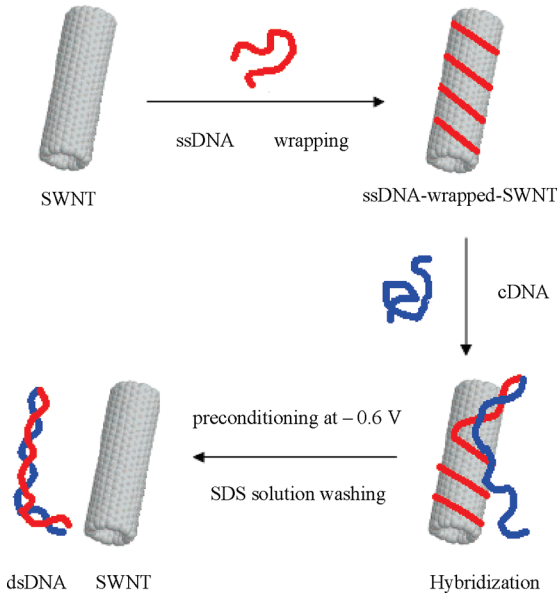

Figure 5. Schematic diagrams illustrating the interaction between SWNT and DNA.

the negative charge of carboxylic acid groups at the tip of the SWNTs could produce repulsion to $[Fe(CN)_6]^{3-/4-}$. Curve d is the Nyquist diagram at the ssDNA-wrapped SWNTE. From this curve a much larger R_{et} value than that from curve a was obtained owing to much more negative charges of the backbone of pDNA, which blocked the interfacial electron transfer. After the hybridization of the ssDNA-wrapped SWNTE with cDNA, and then rinsing the hybridized electrode with 0.5% SDS solution and water but without preconditioning it at −0.6 V, the Nyquist diagram is shown as curve c. The R_{et} value from this curve decreased dramatically compared with that from curve d, indicating that some of the formed dsDNA had been removed from the SWNTE. After the hybridization of the ssDNA-wrapped SWNTE with cDNA as the same processes as above, while preconditioning the electrode at −0.6 V for 60 s, the Nyquist diagram is shown as curve b. The R_{et} value from this curve was almost equal to that from curve a, indicating that the formed dsDNA had been almost completely removed from the SWNTE. If ncDNA was used instead of cDNA in the above experiment, the Nyquist diagram is shown as curve e. It can be seen that the R_{et} value did not decrease compared with that from curve d. The small difference between curve d and curve e originated probably from the experimental error or adsorption of trace ncDNA. This result illustrated that the hybridization reaction between pDNA on the electrode and ncDNA could not carry out, and therefore pDNA was still wrapped on the SWNTs. The EIS experiments were in good accordance with the DPV experiments.

The above phenomena could be illustrated with the schematic diagram shown in Figure 5. The SWNTs aligned on the GCE could be wrapped by ssDNA through π-stacking,[24,27] hydrophobic interaction,[23] and van der Waals force.[21] Hybridization of pDNA wrapped on the SWNTs with cDNA could peel pDNA off the SWNTs. The driving force for this action was the formation of the corresponding dsDNA, a thermodynamically favored product because of the formation of hydrogen bonds between the bases of two ssDNAs. This hydrogen bonding provided more than enough energetic motivation to overcome the comparatively weaker π-stacking interactions between the bases and SWNTs surface.[27] Furthermore, the structure of the double helix was more rigid than that of the single helix,[28] which made it hard wrap around SWNTs. However, it was obvious that dsDNA could be adsorbed onto SWNTs, which was in agreement with a previous report.[36] The mechanism of the adsorption was not clear though Lu et al.[38] considered that SWNTs arranged periodically to fit into the major groove of dsDNA. After the process of preconditioning at −0.6 V, dsDNA was removed from SWNTs with the aid of the rinse. The negatively charged electrode surface was favorable to the removal of dsDNA with negatively charged backbones from the electrode. The electrostatic repulsion between dsDNA and the carboxyl-functionalized SWNTE was also one of the reasons to peel DNA off, as described in a previous report.[22] At the same time, another factor that should be taken into account was the utilization of SDS. By performing a control experiment, we proved that dsDNA could not be removed entirely without the rinsing process with SDS solution. This may be explained by that SDS, which also has π electrons, could displace dsDNA from SWNTs surface through a competitive binding mechanism.[27] However, the rinsing with SDS solution had no obvious effect on the ssDNA-wrapped SWNTE, which confirmed that the stability of the ssDNA/SWNT complex structure was much higher than that of the dsDNA/SWNT complex structure.

Renewal of the SWNTE. The used SWNTE could be renewed to be a fresh SWNTE easily with sonication and rinsing. EIS experiments displayed that all kinds of DNA, whether it was electrooxidized or not, could be removed from the SWNTE, leaving the platform fresh (without memory effect). An as-prepared SWNTE had been used to immobilize pDNA again and again with renewal processes to demonstrate its reusability. Every time the DPV response was recorded, and the representative DPV curves are shown in Figure 6A. There was no obvious decrease of i_{pa} even after the 3000th detection (Figure 6B). In addition, after immersing the SWNTE in a B−R buffer solution of pH 7.0 for 2 months, its ability for immobilizing ssDNA probe had no obvious decrease. These indicated that the reusability and the stability of the SWNTE were both satisfactory.

Optimization of Analytical Performance. After a successful hybridization, the ssDNA wrapped on the SWNTE could be removed from the electrode. On the basis of this, a readily reusable label-free electrochemical DNA hybridization biosensor was developed by monitoring the DPV response of guanine bases. The decrease in the magnitude of the DPV peak of guanine bases reflected the extent of the hybrid formation. In order to obtain high sensitivity and good reproducibility, the effect of experimental parameters on both the immobilization and hybridization was investigated.

The immobilization time of pDNA on the SWNTE was optimized. The result showed that the DPV peak current increased with the increase of immobilization time within the first 30 min and then leveled when the concentration of pDNA was 1.0 μM. The saturated immobilization indicated that the valid SWNTs assembled on the GCE were stable. A 30 min immobilization time was used in the experiments. The effect of pDNA concentration on the immobilization was studied as well. The time for the

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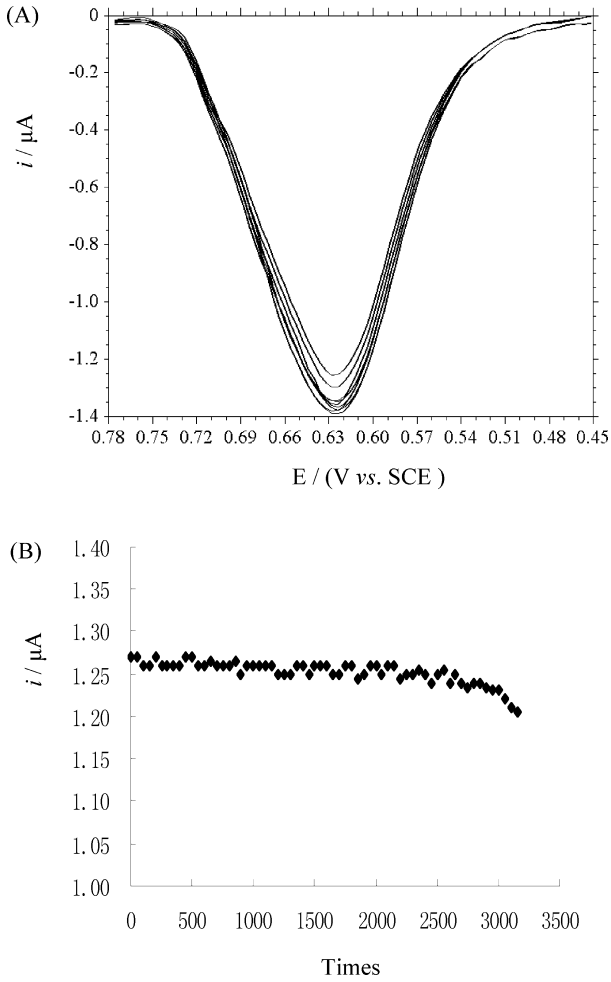

Figure 6. Representative DPV curves of ssDNA-wrapped SWNTEs fabricated repetitively with the same SWNTE. From bottom to top: the 1st, 50th, 100th, 1000th, 500th, 2000th, 3000th, 3200th, and 3400th time (A). The plot of i_{pa} of the ssDNA-wrapped SWNTEs against the immobilization number (B). The preparation of the ssDNA-wrapped SWNTEs and DPV parameters are the same as in Figure 3.

saturated immobilization increased with the diluting of the pDNA solution, whereas the maximum value of the current response was stable. We selected 1.0 μM pDNA solution as the immobilization solution. The influence of dsDNA on the immobilization of pDNA was tested by the following experiments. An amount of 1.0 μM dsDNA was added into 1.0 μM pDNA solution, and the immobilization was checked. The result showed that the DPV peak had almost no change, indicating that dsDNA had no influence on the immobilization of pDNA. The relative standard deviation (RSD) of the DPV response for 11 parallel immobilizations of pDNA was 1.6%, showing that the reproducibility of the immobilization of pDNA was good.

The effect of the hybridization time on the DPV response obtained from hybridization events between pDNA and cDNA was evaluated with different cDNA concentrations. As shown in Figure 7, two segments were observed on all the three curves. As expected, the DPV peak current decreased with the increase of hybridization time between 0 and 45 min (the initial portion). However, prolonged exposure caused an increase of the current signal. It may be ascribed to the rewrapping of cDNA in solution onto the peeled SWNTs. It is an interesting phenomenon that the

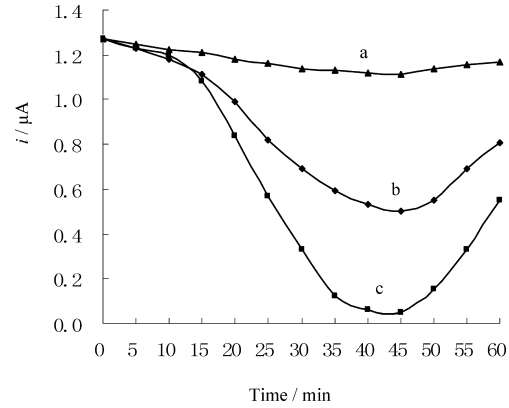

Figure 7. Dependence of DPV currents on the hybridization time. The ssDNA-wrapped SWNTE hybridized to cDNA of 40 (a), 80, (b) and 110 nM (c), respectively. Experimental conditions are the same as in Figure 3.

Table 1. DPV Responses of Hybridization Events between the ssDNA-Wrapped SWNTEs and 0.1 μM Different Sequence-Specific Target DNAs

target DNA	i_{pa} of guanine (μA)	decrease rate of the current (%)
no	1.26	0
cDNA	0.06	95
one-base mismatch (end)	0.09	93
one-base mismatch (mid)	0.17	87
two-base mismatch (one end)	0.31	75
two-base mismatch (two ends)	1.19	5
two-base mismatch (mid)	0.34	73
ncDNA	1.22	3

optimal hybridization time for different cDNA concentrations was all 45 min. In addition, the difference based on the hybridization between pDNA/cDNA and pDNA/ncDNA was also reached to maximum at 45 min. Thus, 45 min was chosen in all the other experiments.

The preconditioning potential and time for the removal of dsDNA after hybridization detection were selected carefully. In the range from 0 to −1.2 V, the removal of formed dsDNA from the electrode became easier with the negative shift of the preconditioning potential. However, if the preconditioning potential was more negative than −0.6 V, the background current was larger. A potential of −0.6 V and a time of 60 s were applied for the preconditioning in the experiments.

Detection of Sequence-Specific DNA. The ssDNA-wrapped SWNTE was used as a label-free biosensor to detect sequence-specific DNA based on the DPV current change of guanine bases before and after hybridization. The processes were summarized as the following: first, a SWNTE was immersed into pDNA solution for immobilization; then the DPV response was recorded; after renewal of the SWNTE, pDNA was immobilized again, followed by hybridizing with cDNA at 42 °C[39] for 45 min; subsequently, the DPV response was recorded again. The difference of the DPV peak currents between the two records was used to be the measurement signal to determine cDNA.

The selectivity of the assay was investigated by using the ssDNA-wrapped SWNTE to hybridize with different target ssDNA sequences. The results are summarized in Table 1. The reported

(39) Yang, T.; Zhang, W.; Du, M.; Jiao, K. *Talanta* **2008**, *75*, 987–994.

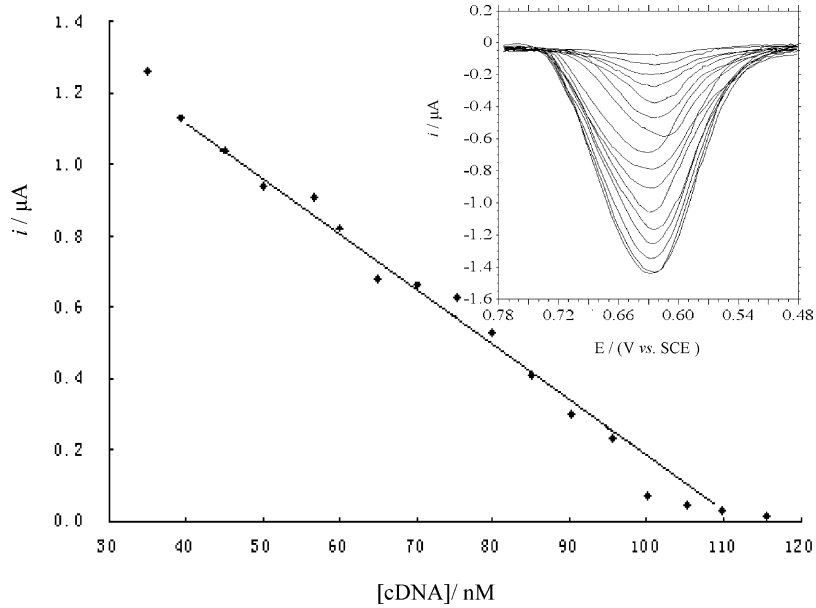

Figure 8. Plot of the DPV current vs the cDNA concentration. Inset: DPV responses of the ssDNA-wrapped SWNTE after hybridization with cDNA of 0, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, and 110 nM (from bottom to top) for 45 min. Experimental conditions are the same as in Figure 3.

result for every target DNA in this table was the mean value of three parallel measurements. A significant decrease of the i_{pa} of guanine bases was observed after the ssDNA-wrapped SWNTE hybridized with cDNA, which was in distinct contrast to the hybridization responses with ncDNA (about 3%). Besides, some interesting results were found when mismatched bases were evaluated. The decrease of i_{pa} was about 93% with the single mismatched base at the end of the sequence. The decrease rate was almost the same as that of cDNA. The results indicated that the protocol was difficult to discriminate one-end-base mismatch. On the other hand, when the single mismatched base was in the middle of the sequence, the current decrease rate was 87%. Similarly, the current decrease rate was evaluated with the two mismatched bases at different points in the DNA sequence. It was especially worthy of note that both of the two end bases were mismatched, the current decrease rate of which was 5%, indicating the unsuccessful hybridization reaction. The value was almost the same as that using ncDNA, indicating that in our studied system the bases located at the end of the target sequence endowed a predominant control for DNA hybridization process. Namely, only if the recognition of the end base (no matter which one) was accomplished, the subsequent base hybridization could be invoked to further execute.

As shown in Figure 8, when the hybridization time was 45 min, the plot of the measurement signal (i_{pa}) versus the cDNA concentrations was a straight line in the range of 40−110 nM with the regression equation of $i_{pa} = -0.0177c + 1.903$ (μA, $r = 0.9906$), where c was the concentration of cDNA (nM). The detection limit was 20 nM using 3s, where s was the standard deviation of the blank solution with 11 parallel measurements. The inset of Figure 8 shows the DPV curves of the ssDNA-wrapped SWNTE after hybridization with 0−110 nM cDNA concentrations. Eight repetitive measurements were performed using 100 nM cDNA, and the RSD of the measurement value was 2.0%, indicating that the reproducibility of hybridization was satisfying.

CONCLUSIONS

The aligned SWNTs array electrode, SWNTE, was suitable for fabricating a DNA hybridization biosensor. On the basis of the special interaction between ssDNA and SWNT, ssDNA probe could be immobilized onto the electrode easily. After the process of hybridization recognition of target DNA, the formed dsDNA would peel off the SWNTs, and hence the current response of guanine bases changed clearly. Ready renewal was the outstanding merit of this label-free biosensor. In our laboratory, one biosensor has been reused for more than 3000 times. Such a biosensor could be utilized to detect different target DNA sequences; thus, tedious modification of the electrode could be avoided.

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