

DNA-modified Carbon Nanotubes for Self-assembling and Biosensing Applications

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Abstract

We have demonstrated that specific DNA sequences could be covalently immobilized onto acid-oxidized and plasma-activated carbon nanotubes. While various functional supramolecular structures could be prepared by self-assembling of the acid-oxidized carbon nanotubes attached with DNA chains of complementary sequences, the DNA-immobilized aligned carbon nanotubes have been demonstrated to be significant for sensing complementary DNA and/or target DNA chains of specific sequences with a high sensitivity and selectivity.

Keywords: Carbon nanotube, DNA, Self-assembling, Biosensing.

1. Introduction

Since Iijima's discovery in 1991, carbon nanotubes have been shown to possess superior electronic, thermal, and mechanical properties to be attractive for a wide range of potential applications [1]. The use of carbon nanotubes as "building blocks" in microelectronic devices could revolutionize the microelectronic industry in the same way that the microchips have revolutionized computers and electronics. The large surface area, together with good electronic properties, has also made carbon nanotubes and their derivatives very attractive as active sensing materials. However, the efficient integration of the carbon nanotube "building blocks" into functional structures/devices has been one of the long-standing big challenges. Although various synthetic and post-synthesis fabrication methods have been devised for the preparation of ordered (*e.g.* aligned, micropatterned) carbon nanotubes and for functionalization of carbon nanotubes [2], no effective technique has been developed for the controlled connection of pre-formed individual carbon nanotubes into hierarchical structures.

On the other hand, various functional structures and/or devices have been created via the sequence-specific pairing interaction between complementary DNA chains [3]. Examples include the use of DNAs as linkages for self-assembling nanoparticles into multi-dimensional functional structures [4], and the development of miniaturized DNA

chips [5]. Recently, the DNA-based biomolecular recognition principle has also been applied to carbon nanotubes to construct carbon nanotube-DNA electrochemical sensors [6], by chemically attaching a carbon nanotube electrode with single-strand DNA chains (ssDNAs) for hybridization with redox-labeled complementary DNA chains (cDNA), and nanotube field-effect transistors [7] by DNA-directed placements of carbon nanotubes via a region-specific hybridization of ssDNA-attached carbon nanotubes with cDNA chains grafted on a substrate. As far as we are aware, however, the use of DNA pairing interaction for creating hierarchical structures from carbon nanotubes in solution has largely been unrealized. We have chemically grafted ssDNA chains onto *individual* aligned carbon nanotubes for hybridization with redox-labeled cDNA chains for DNA sequence sensing [6]. We have also used the DNA-or-cDNA-attached gold nanoparticles for visualizing the functional DNA sites on the nanotubes through the sequence/region-specific hybridization of DNA chains grafted on the carbon nanotube and gold nanoparticle, respectively, and TEM imaging of the gold nanoparticle [8]. In this paper, we present some results from our recent work on DNA-directed self-assembling of carbon nanotubes and DNA-immobilized carbon nanotube electrochemical biosensors.

2. Experimental

All of the oligonucleotides were purchased from QIAGEN

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Operon. 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), dodecyl sulfate sodium salt (SDS), and tris(hydroxymethyl)aminomethane hydrochloride (Tris) were purchased from Aldrich. The following buffers were used 2×SSC buffer: 0.3 M NaCl + 0.03M sodium citrate (pH=7.0); TE buffer: 10mM Tris-HCl + 1mM EDTA (pH=7.0). Both single wall carbon nanotubes purchased from Rice University and multiwall carbon nanotubes produced from pyrolysis of $\text{FeC}_{32}\text{N}_8\text{H}_{16}$ [2] were used without distinction, unless otherwise stated.

While the acid-oxidation of carbon nanotubes were carried out in HNO_3 according to the published procedure [9], plasma polymerization was performed at 20W and 200KHz under 0.1 Torr pressure of acetic acid vapor. In order to maintain the good electronic properties of the plasma-modified aligned carbon nanotubes, the plasma treatment time has been minimized for a sub-monolayer coverage. H_2N -end-functionalized oligonucleotides were covalently immobilized onto the COOH -containing carbon nanotubes through the amide formation in the presence of EDC. Hybridization was then achieved by immersing the oligonucleotide-immobilized carbon nanotubes into a buffer (2×SSC) solution containing complementary DNA chains or cDNA-attached carbon nanotubes, which was incubated in a water bath at 42°C for 2h.

A PowerLab/4sp Potentiostat with a three-electrode system, consisting of a carbon nanotube working electrode, Ag/AgCl reference electrode, and a platinum counter electrode, was used to record electrochemical response from the hybridization with ferrocene-labelled DNA chains. The measurements were performed in a $0.1\text{H}_2\text{SO}_4$ electrolyte solution by scanning over a potential window was from 0.0V to +0.8V (versus Ag/AgCl). Scanning electron microscopy (SEM) images were taken on a FESEM, Phillips XL-30.

3. Results and Discussion

Fig. 1 shows the reaction steps for the DNA-directed self-assembly of carbon nanotubes. As can be seen in Fig. 1, the reaction sequences involve the acid oxidation of carbon nanotubes to introduce carboxylic end groups, which were used to graft ssDNA chains (Step 1). The ssDNA-attached carbon nanotubes were then subjected to hybridization with cDNA chains grafted on other carbon nanotubes or gold nanoparticles (Step 2). In particular, the acid (HNO_3) oxidation reaction is known to introduce COOH functionalities (mixed with hydroxyl groups) onto the ends of carbon nanotubes [9]. The resultant acid-oxidized nanotubes were redissolved in water for chemical bonding with amino-end-functionalized oligonucleotides (*i.e.* [AmC6]TTGACACCAGACCAACTGGT-3'; **I**) or amino-end-functionalized complementary DNA chains (*i.e.* [AmC6]ACCAGTTGGTCTGGTGTC-3') separately through the amide formation in the presence of EDC

coupling reagent for overnight at room temperature [6]. The products from the above reactions are designated as ssDNA-CNTs and cDNA-CNTs, respectively. The DNA hybridization was then performed by keeping a mixture solution of the ssDNA and cDNA attached carbon nanotubes in a water bath at 42°C for 2h. A droplet of the resultant solution was then deposited on a freshly cleaved mica surface for structural characterization on SEM.

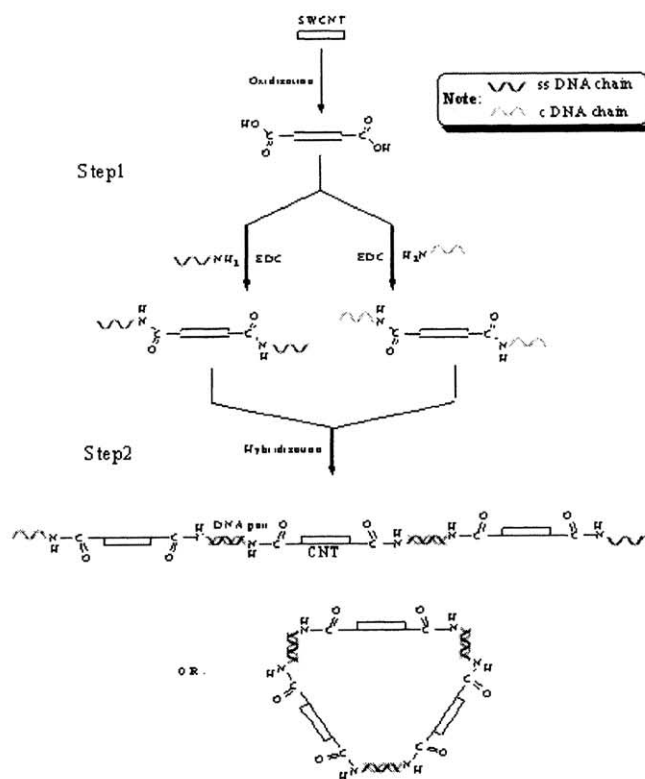


Fig. 1. Procedure to assemble carbon nanotube supramolecular structures through DNA immobilization and hybridization. Both linear and network carbon nanotube structures can be formed.

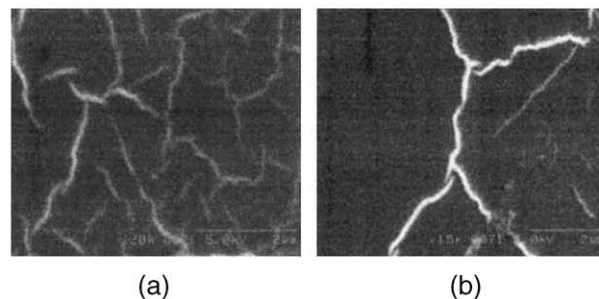


Fig. 2. A typical SEM image of (a) the self-assembly of ssDNA-CNTs and cDNA-CNTs, and (b) as for (a) under a higher magnification.

As can be seen in Fig. 2, individual carbon nanotubes were intimately connected into a network structure, mainly

through the end-to-end attachment. The observed end-to-end connection indicates that carbon nanotubes were linked with each other through the end-attached dsDNA double helix linkages. Closer inspection of Fig. 2a under a higher magnification (Fig. 2b) clearly shows some “gaps” between the connected carbon nanotubes with a separation distance equivalent to the dsDNA length.

Apart from the above mentioned DNA-directed self-assembling, the sequence-specific DNA hybridization has also been applied to the development of biosensors for DNA analysis and diagnosis. In this context, we have developed an approach for grafting single-strand DNA (ssDNA) chains with an amino group at the 5'-phosphate end (*i.e.* I) onto the gold-supported, COOH-containing aligned carbon nanotubes, generated from pyrolysis of iron(II) phthalocyanine [10] and treated with acetic acid plasma [11], through the amide formation in the presence of EDC coupling reagent. Complementary DNA (cDNA) chains pre-labeled with ferrocenecarboxaldehyde, FCA, (*i.e.* [FCA-C6]ACCAGTTGGTCTGGTGTCAA-3', II) were then used for hybridizing with the surface-immobilized oligonucleotides to form double strand DNA (dsDNA) helices on the aligned carbon nanotubes (Fig. 3).

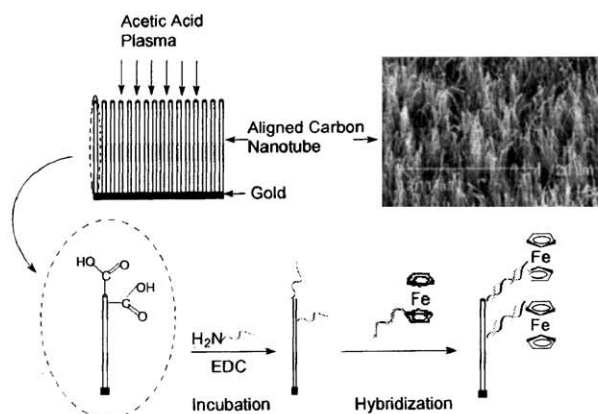


Fig. 3. A schematic illustration of the aligned nanotube-DNA electrochemical sensor. The up-right SEM image shows the aligned carbon nanotubes after having been transferred onto a gold foil. For reasons of clarity, only one of the many carboxyl groups is shown at the nanotube tip and wall, respectively.

Fig. 4A shows the electrochemical characteristics for the pristine aligned carbon nanotubes before (curve a) and after (curve b) the acetic-acid-plasma treatment, along with the ssDNA-immobilized nanotubes (curve c), in 0.1M electrolyte H_2SO_4 solution. Only capacitive current was observed for the pristine aligned carbon nanotubes (curve a of Fig. 4A). The capacitive current increased after treating the nanotube electrode with the acetic acid plasma (curve b of Fig. 4A), presumably because the plasma-induced carboxyl groups facilitated the charge-transfer between the nanotube electrode and H_2SO_4 electrolyte through the enhanced hydrophilic-hydrophilic interaction [11]. Upon grafting the ssDNA chains onto the plasma-induced surface carboxyl groups, a significant decrease in the capacitive

current was observed (curve c in Fig. 4A), indicating the replacement of carboxyl groups by a thin layer of the covalently-grafted DNA chains. The performance of the surface-bound ssDNA(I) chains on the plasma-treated nanotube electrode for sequence-specific DNA diagnoses was demonstrated in Fig. 4B. The strong reduction peak seen at 0.29 V in curve a of Fig. 4B can be attributable to ferrocene [12] and indicates the occurrence of hybridization of FCA-labeled cDNA(II) chains with the nanotube-supported ssDNA(I) chains, leading to a long-range electron transfer from the FCA probe to the nanotube electrode through the DNA duplex [13]. In contrast, the addition of FCA-labeled non-complementary DNA chains (*i.e.* [FCA-C6]GGTGGCGACTCCTGGAG-3', III) under the same conditions did not show any redox response of FCA (curve b of Fig. 4B). This indicates that, as expected, there was no specific DNA pairing interaction with the non-complementary DNA chains, and that physical adsorption of the FCA-labeled DNA chains, if any, was insignificant in this particular case. Subsequent addition of target DNA chains (designated as: 5'-CTCCAGGAGTCGTCGCCACCTTGGTCTGGTGTCAA-3', IV) into the above solution, however, led to a strong redox response from the FCA-labeled DNA (III) chains (curve c of Fig. 4B) because the target DNA (IV) contains complementary sequences for both DNA (I) and DNA(III) chains.

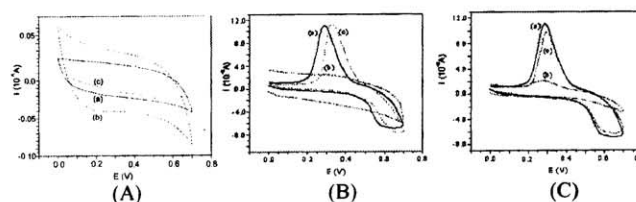


Fig. 4. Cyclic voltammograms of (A) the pristine aligned carbon nanotube electrode (a), the nanotube electrode after the acetic acid plasma treatment (b), and the plasma-treated nanotube electrode after the immobilization of ssDNA (c); (B) the ssDNA (I)-immobilized aligned carbon nanotube electrode after hybridization with FCA-labeled noncomplementary DNA (II) chains (a), in the presence of FCA-labeled noncomplementary DNA (III) chains (b), and after hybridization with target DNA (IV) chains in the presence of the FCA-labeled noncomplementary DNA (III) chains (c); and (C) the ssDNA (I)-immobilized aligned carbon nanotube electrode after the 1st hybridization with FCA-labeled complementary DNA (II) chains (a), after being denatured (b), and after the 2nd hybridization with FCA-labeled complementary DNA (II) chains (c).

More interestingly, the electrochemical responses seen in Fig. 4B were revealed to be highly reversible. As can be seen in Fig. 4C, the electrochemical response of the FCA-labeled cDNA(II) chains (curve a of Fig. 4C) diminished almost completely after being thermally denatured on the aligned carbon nanotube electrode (curve b of Fig. 4C). Thermal denaturing of the cDNA (II) chains after hybridization was achieved by heating the combined cDNA probes on the aligned carbon nanotube electrode in 2xSSC buffer solution (*i.e.* 0.3 M NaCl + 0.03M sodium citrate, pH=7.0) at 100°C for 6 min, followed by subsequent rapid cooling in an salt ice bath for 10 min. Re-hybridization with

fresh FCA-labeled cDNA(II) chains, however, led to a rapid recovery of the electrochemical response characteristic of FCA (curve c of Fig. 4C). All the cyclic voltammograms were recorded in 0.1 M H₂SO₄ solution with a scan rate of 0.1Vs⁻¹. The concentration of the FCA-labeled DNA probes is 0.05μg/ml.

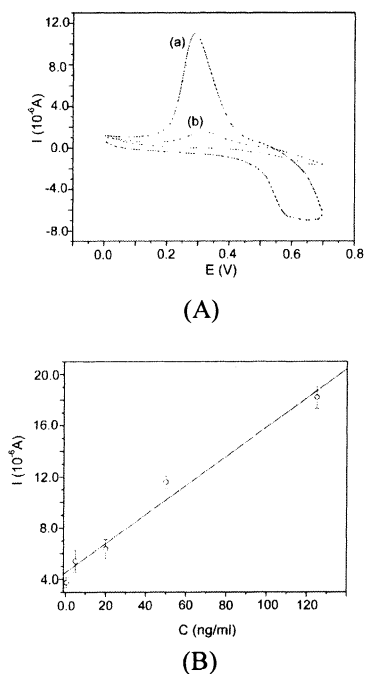


Fig. 5. (A) Cyclic voltammograms of the aligned carbon nanotube electrode immobilized with ssDNA(I) chains followed by hybridization with the FCA-labeled cDNA(II) probes (a) and an Au electrode immobilized with ssDNA(I) chains followed by hybridization with the FCA-labeled cDNA(II) probes under the same conditions (b). Note, the geometric area of the aligned carbon nanotube electrode is 1.5mm × 1.0 mm and the area of the gold is 2.0 mm × 1.5 mm. The electrochemical measurements were carried out in an aqueous solution of 0.1 M H₂SO₄ vs. Ag/AgCl at a scan rate of 0.1Vs⁻¹. The concentration of the FCA-labeled cDNA(II) probe is 0.05μg/ml. (B) The dependence of redox current at the reduction potential of FCA (0.29 V) on the cDNA (II) concentration for the aligned carbon nanotube-DNA sensor.

The above results suggest that the ssDNA immobilized aligned carbon nanotubes can be repeatedly used as a highly-selective electrochemical sensor for sequence-specific DNA diagnoses. Furthermore, the amperometric response from the aligned carbon nanotube-DNA sensors (curve a of Fig. 5A) was found to be much higher (ca. 20 times) than that of more conventional flat electrodes immobilized with the ssDNA(I) chains under the same conditions (curve b of Fig. 5A). The linear dependence of the redox current on the complementary DNA concentration shown in Fig. 5B further ensures practical applications of the aligned carbon nanotube-DNA electrochemical sensors for DNA sensing and/or sequence-specific diagnoses over a wide range of the cDNA concentrations.

3. Conclusion

We have demonstrated that a wide range of hierarchical structures of carbon nanotubes can be constructed by DNA-direct self-assembling of carbon nanotubes. We have also shown that specific DNA sequences could be covalently immobilized onto plasma-activated aligned carbon nanotubes for sensing complementary DNA and/or target DNA chains of specific sequences with a high sensitivity and selectivity. In view of the availability of various carbon nanotubes of different structures and DNA chains of different base sequences, the work presented here should have an important implication to not only the sequence-specific analyses/diagnoses of DNA chains but also advanced device applications of carbon nanotubes.

Acknowledgement

We are grateful for financial support from the American Chemical Society (ACS-PRF 39060-ACSM).

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