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Aligned carbon nanotube–DNA electrochemical sensors

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Single-strand DNA chains were chemically grafted onto aligned carbon nanotube electrodes, leading to novel aligned carbon nanotube–DNA sensors of a high sensitivity and selectivity for probing complementary DNA and target DNA chains of specific sequences.

A major feature of the Watson–Crick model of DNA is that it provides a vision of how a base sequence of one strand of the double helix can precisely determine the base sequence of the partner strand for passing the genetic information in all living species. The principle learned from this breakthrough has now been applied to the development of biosensors for DNA analysis and diagnosis through the very specific DNA pairing interaction.[1] Owing to their high sensitivity, low cost, and good compatibility with optical detection technologies, DNA biosensors with optical transducers are currently under intense investigation.[2] In comparison with those optical transduction methods, electronic transduction has many advantages including easier data processing, greater simplicity, and broader applicability. Consequently, a few DNA electrochemical biosensors have recently been reported.[1]

On the other hand, carbon nanotubes are ideal electrodes for constructing advanced biosensors.[3] In this regard, both protein and DNA chains have been chemically attached onto either nonaligned or aligned carbon nanotubes.[4,5] The use of aligned carbon nanotubes provides additional advantages for a maximized access of the nanotube electrode surface and an efficient device construction. However, it is not straightforward to retain the alignment structure of the carbon nanotubes after grafting with protein or DNA chains. Recently, Meyyappan and co-workers have developed a very useful method to protect the alignment structure by filling the gaps between the aligned nanotubes with a spin-on glass (SOG) prior to the oxidative reaction and subsequent immobilization of DNA chains[5] while Gooding and co-workers[6] reported self-assembling-induced alignment of the oxidized carbon nanotubes for protein immobilization. In both cases, however, rather tedious procedures were involved and the DNA/protein attachments were limited to the nanotube tip only. Here, we report a simple, but effective, method for preparing aligned carbon nanotube–DNA sensors by chemically coupling DNA probes on both the tip and wall of plasma-activated aligned carbon nanotubes. The nanotube–DNA electrochemical sensors thus produced are highly sensitive and selective even in the absence of any electron mediator,[5] as we shall see later.

In particular, we carried out acetic acid-plasma treatment[7] on gold-supported aligned carbon nanotubes generated from pyrolysis of iron(II) phthalocyanine,[8] followed by grafting single-strand DNA (ssDNA) chains with an amino group at the 5′-phosphate end (*i.e.* [AmC6]TTGACACCAGACCAACTGGT-3′, **I**) onto the plasma-induced –COOH group through the amide formation in the presence of EDC [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride] coupling reagent. Complementary DNA (cDNA) chains pre-labeled with ferrocenecarboxaldehyde, FCA, (designated as: [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 nanotube electrodes (Fig. 1).

Figure 2A 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 0.1 M electrolyte H_2SO_4 solution. Only capacitive current was observed for the pristine aligned carbon nanotubes (curve a of Fig. 2A). The capacitive current increased after treating the nanotube electrode with the acetic acid plasma (curve b of Fig. 2A), 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.[7] 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. 2A), 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. 2B. The strong oxidation peak seen at 0.29 V in curve a of Fig. 2B can be attributable to ferrocene[9] 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.[10] The apparent irreversible redox response arises probably from the nature of carbon electrodes[11] interplayed with the electric-field effect on the carbon nanotube resistance.[12] In contrast, the addition of FCA-labeled non-complementary DNA chains (*i.e.* [FCA-C6]CTCCAGGAGTCGTCGCCACC-3′, **III**) under the same conditions did not show any redox response of FCA (curve b of Fig. 2B). 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

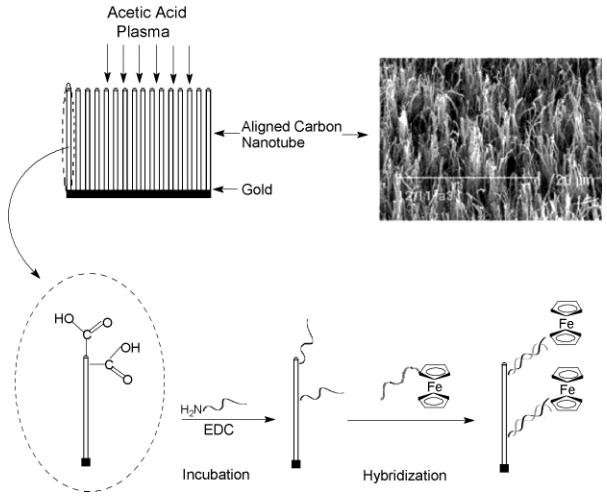

Fig. 1 A schematic illustration of the aligned nanotube–DNA electrochemical sensor. The upper right SEM image shows the aligned carbon nanotubes after having been transferred onto a gold foil.[8] For reasons of clarity, only one of the many carboxyl groups is shown at the nanotube tip and wall, respectively.

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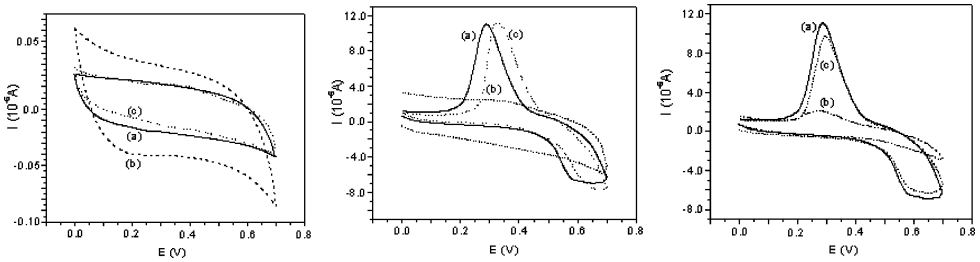

Fig. 2 Cyclic voltammograms of (A) the pristine aligned carbon nanotube electrode (a), the nanotube electrode after the acetic acid-plasma treatment (b), and the plasma-treated nanotube electrode after the immobilization of ssDNA (c); (B) the ssDNA (**I**)-immobilized aligned carbon nanotube electrode after hybridization with FCA-labeled complementary 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). All the cyclic voltammograms were recorded in 0.1 M H_2SO_4 solution with a scan rate of 0.1 V s^{-1}. The concentration of the FCA-labeled DNA probes is 0.05 μg ml^{-1}. Thermal denaturing of the cDNA (**II**) chains after hybridization was achieved by heating the combined cDNA probes on the aligned carbon nanotube electrode in 2 × SSC buffer solution (*i.e.* 0.3 M NaCl + 0.03 M sodium citrate, pH = 7.0) at 100 °C for 6 min, followed by subsequent rapid cooling in a salt ice bath for 10 min.

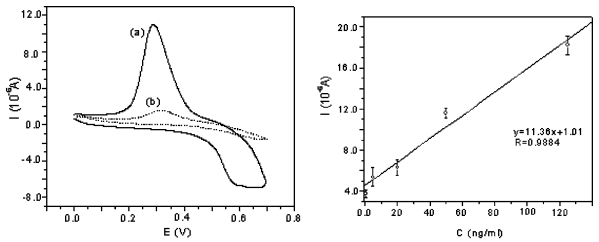

Fig. 3 (A) Cyclic voltammograms of the aligned carbon nanotube electrode immobilized with ssDNA (**I**) chains followed by hybridization with the FCA-labeled cDNA (**II**) probes (a) and an Au electrode immobilized with ssDNA (**I**) chains followed by hybridization with the FCA-labeled cDNA (**II**) probes under the same conditions (b). Note, the geometric area of the aligned carbon nanotube electrode is 1.5 mm × 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 H_2SO_4 *vs.* Ag/AgCl at a scan rate of 0.1 V s^{-1}. The concentration of the FCA-labeled cDNA (**II**) probe is 0.05 μg ml^{-1}. (B) The dependence of redox current at the oxidation potential of FCA (0.29 V) on the cDNA (**II**) concentration for the aligned carbon nanotube–DNA sensor.

insignificant in this particular case. Subsequent addition of target DNA chains (*i.e.* 5′-GAGGTCCTCAGCAGCGGTGGAC-CAGTTGGTCTGGTGTCAA-3′, **IV**) into the above solution, however, led to a strong redox response from the FCA-labeled DNA (**III**) chains (curve c of Fig. 2B) because the target DNA (**IV**) contains complementary sequences for both DNA (**I**) and DNA (**III**) chains.

More interestingly, the electrochemical responses seen in Fig. 2B were revealed to be highly reversible. For instance, the electrochemical response of the FCA-labeled cDNA (**II**) chains (curve a of Fig. 2C) diminished almost completely after they were thermally denatured from the nanotube electrode (curve b of Fig. 2C). 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. 2C).

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 amperiometric response from the aligned carbon nanotube–DNA sensors (curve a of Fig. 3A) 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. 3A). The linear dependence of the redox current on the complementary DNA concentration shown in Fig. 3B further ensures the use of the aligned carbon nanotube–DNA sensors for DNA sensing and/or sequence-specific diagnoses over a wide range of the cDNA concentrations.

In summary, we have demonstrated 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, though the aligned nanotube–DNA sensor has not been optimized yet. Along with the techniques for micropatterning aligned carbon nanotubes,[8] these results should have important implications not only for the sequence-specific analyses/diagnoses of DNA chains but also for advanced device applications of carbon nanotubes (*e.g.* in DNA chips).

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