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STUDY OF SELF-ASSEMBLED MONOLAYERS OF DNA AND DNA-CARBON NANOTUBE HYBRIDS

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Abstract. This report presents the study of the supramolecular complex formed by the non-covalent functionalization of carbon nanotubes (CNTs) by single-stranded DNA. The properties of the DNA-CNT hybrids in aqueous solution were determined and self-assembled monolayers (SAMs) of DNA-CNTs hybrids were formed on gold substrates. The formation of DNA-CNT hybrids SAMs on gold presents a fast and simple alternative to the conventional methods for the attachment of CNTs to solid substrates such as chemical vapor deposition. In addition, DNA-CNT SAMs might substitute traditional DNA SAMs in the design of enhanced DNA sensors.

1. INTRODUCTION

The amount of studies focused on the development of DNA sensors that employ DNA probes immobilized on solid substrates has increased over the past few years [1]. Among the methods used for DNA immobilization, self-assembled monolayers (SAMs) immobilization is gaining broad acceptance, as it provides advantages in terms of simplicity, efficiency, and cost. SAMs are molecular layers formed on a surface when it is immersed in a solution containing molecules that specifically interact with this surface [2].

The most commonly used system for SAMs-based DNA sensors is thiol-modified-single-stranded-DNA (HS-ssDNA) in combination with gold. Gold substrates offer several attractive properties for SAMs experiments, including their high chemical inertness (i.e. only a few compounds can bind covalently to it), a very plane surface, high conductivity- which is perfect for electrochemical experiments-, and a very strong interaction with sulfur. Nevertheless, immobilization of thiol-modified DNA strands on gold is not a trivial matter. Herne and coworkers have shown that non-specific adsorption from nitrogen groups in the DNA can occur on the gold surface, and these interactions can affect the efficiency of the DNA sensor [3].

Different substrates have been studied for DNA adsorption, including highly orientated pyrolytic graphite [4], glassy carbon [5] and diamond [6]. Recently, several groups have proposed the use of carbon nanotubes (CNTs) for DNA immobilization via covalent functionalization of the tubes [7-9]. On the other hand, Zheng *et al*. immobilized DNA

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strands on CNTs by non-covalent functionalization, specifically by means of DNA wrapping of the tubes [10]. In this report, we propose the use of disulfide-modified DNA strands to non-covalently functionalize CNTs and the subsequent modification of gold substrates with self-assembled monolayers of these DNA-CNT complexes.

2. MATERIALS AND METHODS

2.1. Materials

All chemicals were of analytical grade and were used as received. Aqueous solutions were made with nanopure water and degassed with nitrogen prior to any electrochemical measurement. Single-stranded DNA (ssDNA) solutions were prepared by dissolving as-received (i.e., without deprotecting the S-S bond) 5' HO-$(CH_2)_6$-S-S-$(CH_2)_6$-AGAAGGCCTAGA 3' (referred to as RS-ssDNA) from Synthegen, in 0.05 M Na_2HPO_4/ 0.1 M NaCl buffer (pH 7.00). Quartz crystal microbalance polycrystalline gold sensors from MAXTEK were employed as the working electrodes for the experiments. A three-electrode cell was used to carry out electrochemical measurements. The reference and auxiliary electrodes were Ag/AgCl and a platinum mesh, respectively. The working electrodes were cleaned with nanopure water and any excess was removed with nitrogen.

2.2. Substrate pre-treatment and roughness determination

Cyclic voltammetry (CV) is a commonly used technique to study the electrode surface. CV in 1M H_2SO_4, from 200 to 1500 mV at a scan rate of 100 mV/s, was used to determine the cleanliness and roughness factor of the bare gold electrodes and as a polishing pretreatment to control the roughness of the surface. The typical cyclic voltammogram in sulfuric acid for a gold electrode shows a single cathodic peak, which corresponds to the desorption of the oxygen monolayer on the surface.

For the determination of the surface roughness (R_f), we used Eq. 1.

$$R_f = \frac{Q_e}{Q_l A_e}. \quad (1)$$

The roughness factor is obtained from the ratio between the experimental (Q_e) and theoretical (Q_l) charge associated with the gold oxide reduction peak and the geometrical area of the electrode (A_e), where Q_l has a value of 400 $\mu C \cdot cm^{-2}$ [11].

2.3. DNA-CNT hybrids preparation

DNA-CNT hybrids were prepared by a procedure similar to the one published by Zheng *et al.* [10], in which 1 mg of as-received single-walled carbon nanotubes (SWNTs from Carbon Nanotechnologies) was immersed in 1 mL of a 0.5 μM solution of as-received RS-ssDNA , and sonicated for 1 hour in an ice-bath. Subsequent centrifugation for 30 minutes was used to remove insoluble material from the dispersion. Finally, the dispersion was stored at 4 °C and no precipitation occurred even after months of storage.

2.4. SAMs formation

Formation of self-assembled monolayers on the Au surface was achieved by immersing a clean Au electrode in a vial containing a solution of the desired species to be immobilized. The solution was degassed with nitrogen and the system was sealed with paraffin and left unperturbed for a period of 24 hours. At the end of the modification period, the electrodes were cleaned with ethanol and nanopure water and dried with a nitrogen stream.

3. RESULTS AND DISCUSSION

3.1. Aqueous solution of DNA-CNT hybrids

Atomic force microscopy (AFM) in tapping-mode was used to determine the length and diameter distribution of the RS-ssDNA-CNT hybrids and the results are shown in Table 1. The lengths of the RS-ssDNA-CNT ranged approximately from 40 to 100 nm with an average of (70 ± 20) nm. The diameters of the complexes were estimated from AFM height measurements and varied from approximately 2.2 nm to 2.8 nm, with an average diameter of (2.5 ± 0.2) nm, which is in good agreement with previously reported values for DNA-wrapped SWNTs [10,12].

3.2. DNA and DNA-CNT SAMs

3.2.1. Bare gold

The working gold electrodes had roughness factor values that varied from approximately 1.1 to 1.5, with an average of 1.3 ± 0.2. These values were estimated from the reduction peak at (936 ± 5) mV obtained from the CVs in H_2SO_4.

3.2.2. Description of the SAMs

DNA SAMs were prepared by using a solution of HO-$(CH_2)_6$-S-S-$(CH_2)_6$-ssDNA (i.e. with a protecting

Table 1. Length and diameter distribution of the RS-ssDNA-CNT hybrids on mica.

Length (nm)	Diameter (nm)
70.8	2.28
81.2	2.71
64.5	2.42
48.7	2.65
66.6	2.47
69.1	2.51
46.1	2.16
84.5	2.52
39.5	2.69
99.6	2.81
(70 ± 20)	(2.5 ± 0.2)

group; in disulfide form). Experimental results by several groups strongly suggest that the S-S bond is cleaved upon chemisorption [13,14]. As a result, our so-called DNA SAM will be a mixed monolayer having DNA-thiolate- and hydroxyalkylthiolate-gold linkages. DNA-CNT SAMs, on the other hand, were prepared by immersing the gold electrode in a solution of RS-ssDNA-CNT hybrids with RS-ssDNA in excess. Consequently, the DNA-CNT SAMs are mixed monolayers that contain DNA-thiolate-, CNT-DNA-thiolate- and hydroxyalkylthiolate-gold linkages.

3.2.3. AFM results

Tapping-mode AFM images of the DNA and DNA-CNT SAMs on gold were obtained and are shown in Fig. 1. AFM was also used to determine the height of the substrate, and a maximum height of 9 nm was estimated for the bare gold electrode. The AFM image for DNA SAM on gold shows an aggregate with a maximum height of 15 nm, which corresponds to 6 nm after subtracting the substrate height. This value is in excellent agreement with the 5.9 nm height predicted for a fully extended conformation of the DNA sequence immobilized on Au [15]. Additionally, the formation of aggregates identified as DNA is in good agreement with previously reported results that showed that disulfide-modified-ssDNA binds to the gold forming islands on the surface [13].

The AFM images of DNA-CNT SAMs on gold show areas with a maximum height of 15 nm, which corresponds to the height of fully-extended DNA plus the substrate. In addition, the images show aggregates with a maximum height (after subtracting the

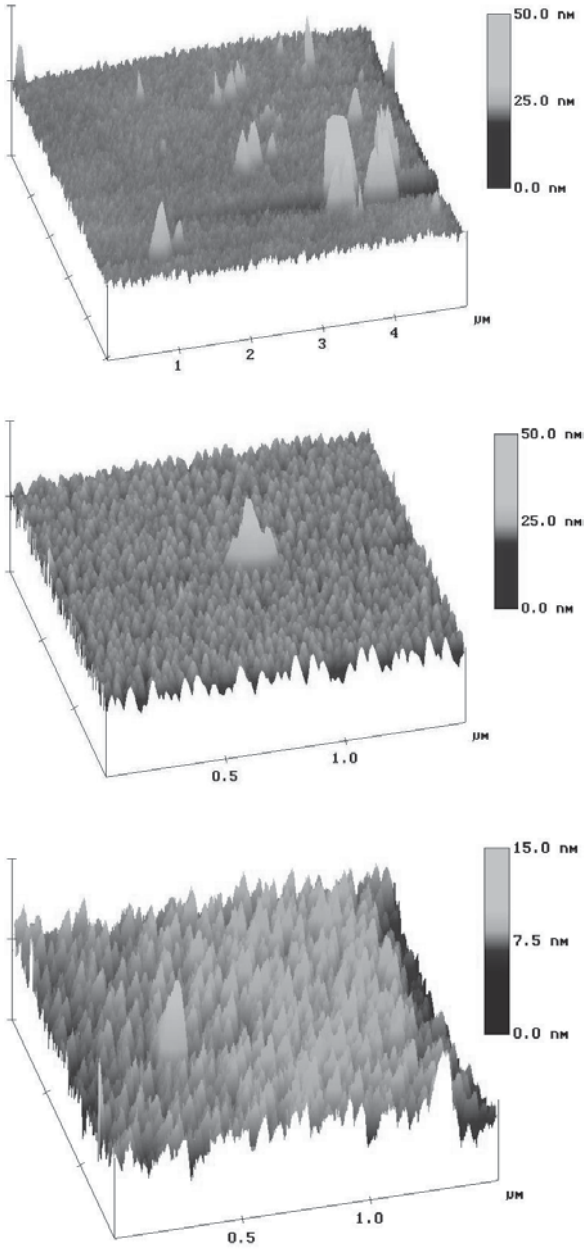

Fig. 1. Tapping mode atomic force microscopy images of DNA and DNA-CNT SAMs on gold. (a) 5 μm × 5 μm × 50 nm image of a DNA-CNT SAM; (b) 1.5 μm × 1.5 μm × 50 nm image of a DNA-CNT SAM; (c) 1.5 μm × 1.5 μm × 15 nm image of a DNA SAM.

substrate height) of 95 nm and an average of 60 nm. These values agree, within experimental error, to the lengths obtained for the DNA-CNT complexes in a horizontal conformation on mica (maximum: 100 nm; average: 70 nm), which might suggest that

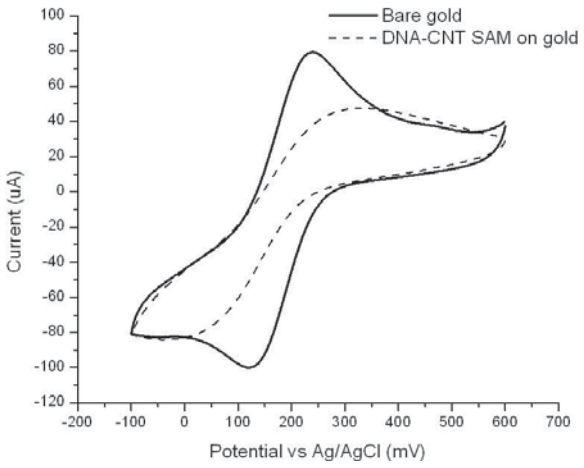

Fig. 2. Cyclic voltammograms (in 1 mM $K_3Fe(CN)_6$ in 0.1 M KCl; scan rate: 50 mV/s) of bare and DNA-CNT-modified gold electrodes.

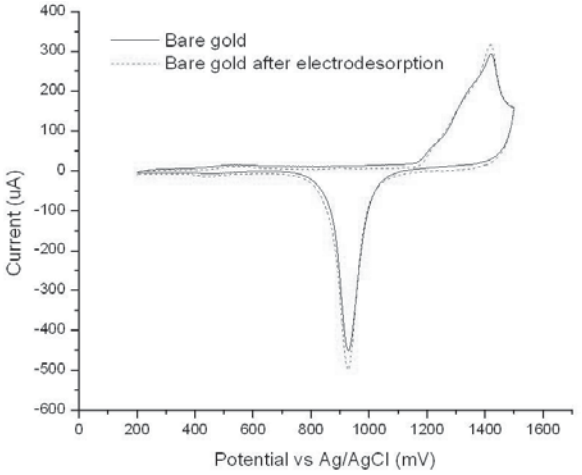

Fig. 3. Cyclic voltammograms (in 1 M H_2SO_4; scan rate: 100 mV/s) of gold electrodes before and after electrochemical desorption.

the nanotubes are aligned nearly perpendicular to the gold surface.

3.2.4. Electrochemical results

Immobilization Efficiency. Cyclic voltammetry in $K_3Fe(CN)_6$ (1 mM in 0.1 M KCl) was used to study the efficiency of electrode modification with the DNA-CNT complexes. A comparison between bare and modified electrodes is shown in Fig. 2. The peak separation values were 99 mV for bare gold and 310 mV for DNA-CNT-modified gold. The voltammograms for the modified electrodes exhibited larger peak separation, a decrease in the peak current, and changes towards a more sigmoidal shape. These differences are consistent with those reported in the literature for SAM-modified electrodes, since SAMs provide an effective barrier for the electron transfer of the $Fe(CN)_6^{4-/3-}$ redox couple at the electrode surface, which slows the kinetics of electron-transfer [14].

Effect of Electrochemical Desorption. Electrochemical desorption of the SAMs was performed to remove all the immobilized species and to analyze the feasibility of reusing the gold electrodes. Desorption of the SAMs in 0.1 M KOH was performed by two methods: 20 cycles of voltammetry from 0 to -1300 mV, at a scan rate of 50 mV/s; and application of a –1300 mV potential for five minutes. The results for both procedures were similar. After the electrochemical desorption, cyclic voltammetry in 1 M H_2SO_4 was performed to determine the cleanliness and roughness factor of the electrodes. Fig. 3 shows the voltammograms for the electrodes before and after the desorption. The voltammograms for the electrodes after the desorption, showed a reduction peak at (935 ± 3) mV and a roughness factor of 1.4 ± 0.2, compared to the (936 ± 5) mV and 1.3 ± 0.2 values for the electrodes before the procedure. These results indicate that electrochemical desorption (by either one of the methods presented here) is a feasible option for cleaning and reusing the electrodes after analysis without the need to use additional polishing treatments, which represents a reduction in time and costs.

4. CONCLUSIONS

We have showed that the use of a short DNA sequence appears to favor the functionalization of short nanotubes (lengths < 100 nm). Additional DNA sequences with variations in length will be studied in an attempt to optimize the functionalization and subsequent immobilization of the nanotubes. In addition, we have demonstrated the feasibility of the DNA-mediated immobilization of CNTs on gold by the self-assembly technique. The procedure is not time-consuming, is straightforward, and represents a simple and fast route to the attachment of SWNTs to gold surfaces. This methodology, in combination with electrochemical desorption can serve as the basis for the design and fabrication of innovative DNA sensors.

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