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Electrochemical characterization of oligonucleotide-carbon nanotube functionalized using different strategies

P. Cañete-Rosales[a], M. González[c], A. Ansón[c], M.T. Martínez[c], C. Yáñez[a], S. Bollo[a,b,*]

[a] Laboratorio de Bioelectroquimica. Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile
[b] Advanced Center for Chronic Diseases (ACCDiS). Universidad de Chile
[c] Instituto de Carboquímica, CSIC, c/Miguel Luesma, 450018 Zaragoza, Spain

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ABSTRACT

Carbon nanotubes (CNTs) were functionalized with a single-strand deoxyoligonucleotide oligomer (DNO) using two different methods and characterized using different electrochemical techniques to finally evaluate their ability of the DNO to hybridize. The DNO immobilization methods were: direct adsorption (CNT-DNO_{ADS}) and covalent attachment (CNT-DNO_{COV}). The electrochemical behavior of both systems was characterized using cyclic voltammetry, differential pulse voltammetry and scanning electrochemical microscopy. These systems were compared using a bare glassy carbon (GC) electrode and an electrode containing oxidized CNTs without DNO (CNT_{COOH}). Our results indicate that the direct physical adsorption of DNO improves the dispersion of CNTs in aqueous media and therefore, the film generated over the electrode is more homogeneous than the films created with CNT_{COOH} and CNT-DNO_{COV}. The results demonstrated the existence of two different spatial conformations of the oligonucleotide on the CNT depending on the type of functionalization. The modified electrodes to hybridization studies were tested using the well-known DNA intercalation agent methylene blue. Thus, both conformations allowed for the detection of the hybridization process between DNO and its complementary strand; however, using CNT-DNO_{ADS} yielded a higher response.

1. Introduction

Carbon nanotubes (CNTs) have been recognized as a promising nanomaterial for analytical electrodes since their first application to the oxidation of dopamine in 1996 [1]. The reasons for utilizing CNTs are their structural, mechanical and electronic properties, such as thermal stability, elasticity, tensile strength and conductivity [2]. CNT research in electroanalysis is focused on their electrocatalytic behavior through the oxidation or reduction of biomolecules, showing improvements in reaction rates, reversibility and detection limits compared with other carbon based electrodes [3].

In the development of a DNA biosensor, there are a few important aspects to consider, such as the nucleic acid to be immobilized; the interaction between the immobilized DNA and the electrode surface, the nanomaterial being used, among others.

CNT based biosensors have been very helpful in the development of DNA-detecting probes with low detection limits [4,5]. One of the challenges in the development of CNT hybridization biosensors is the immobilization of the oligonucleotide on the CNT structure. In general, the immobilization can be performed using covalent or non-covalent strategies [5,6]. The covalent approach is usually performed after an oxidation step that generates oxygenated functional groups on the CNTs, such as COOH groups that can be easily functionalized with different molecules, for example, amines, thiols, and so on [7]. This type of reaction allows the binding of different molecules to CNTs as polypeptides [8] and nucleic acids [9,10].

The non-covalent functionalization allows for the successful derivatization of CNTs without disturbing their unique electronic characteristics [11]. Nucleic acids are ideal biomolecules to form supramolecular complexes with CNTs. Based on π-π interactions; it is possible to adsorb oligonucleotides onto the side-walls of nanotubes through the interaction of the aromatic bases of the biopolymer with the nanotube. Zeng et al. [12] described an easy way to disperse single-walled carbon nanotubes modified with a single strand of DNA (ssDNA) using ultrasonication. More recently,

* Corresponding author. FAX: +5629782988 PHONE: +5629782896.
E-mail address: sbollo@ciq.uchile.cl (S. Bollo).

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Primo et al. [13] reported the use of a double strand of DNA (dsDNA) to successfully disperse bamboo-like, multi-walled carbon nanotubes, creating a new method of building supramolecular architectures for biosensing.

Additionally, the detection of the hybridization process between complementary strands can be achieved through the direct measurement of the oxidation of guanine bases [14] or through an indirect measurement using an intercalating electroactive mediator capable of distinguishing between a single strand (ss-) and a double strand (ds-) of DNA. The indirect measurement is preferred because it offers a wide variety of ways to detect hybridization with high selectivity and sensitivity [15]. Thus, Erdem et al. [16] reported the detection of DNA sequences of the hepatitis B virus using Methylene Blue (MB) as a redox mediator. MB has a high affinity for ssDNA due to its interactions with free guanines [17–19]. According to Erdem et al., there is a decrease in the MB reduction current value after hybridization because its access to guanine bases becomes blocked. This behavior was also observed by Kerman et al. [18] where DNA was immobilized on gold electrodes and by Kara et al. in carbon paste electrodes [19]. These reports were devoted to the development of the final biosensors; they did not compare the probe immobilization methods or study which transduction method is the best.

Thus, the main goal of the present work was to determine if the method used to immobilize an oligonucleotide (DNO) molecule produces a different spatial conformation and orientation that could restrict the accessibility of the complementary strand. Therefore, an amino-terminated oligonucleotide probe sequence was bound to the CNTs to generate biosensors for hybridization studies. In one case, the DNO was adsorb onto the side-wall of CNTs ($CNT\text{-}DNO_{ADS}$) through π-stacking interactions. In the other case, the DNO was covalently bound to the surfaces of the CNTs via diimide-activated amidation ($CNT\text{-}DNO_{COV}$).

The CNT-DNO/modified electrodes were characterized using electrochemical techniques, namely, cyclic voltammetry, differential pulse voltammetry and scanning electrochemical microscopy. Additionally, the modified electrodes were applied in hybridization studies using the direct measurement of the oxidation of the guanine bases as the analytical parameter. Additionally, the electrochemical response of the intercalative agent MB was used to corroborate the hybridization.

2. Experimental

2.1. Materials

Multi-walled carbon nanotubes (CNTs) (5-20 μm long and 30 ± 15 nm diameter, NanoLab. USA) were oxidized using 3 mol L^{-1} H_2SO_4/HNO_3 (3:1) by refluxing for 6 h. Then, the CNTs were filtered, washed thoroughly with deionized water until the solution reached a neutral pH and dried at 50 °C for 24 h. The resulting product is hereafter written as CNT_{COOH}.

A single-strand deoxyribonucleic oligonucleotide (DNO, 5′-NH_2- *CTC**G**AT**G**ACTCAAT**G**ACTCG*-3′) was obtained from Eurogentec. 1-ethyl-3-3- dimethylaminopropyl)carbodiimide hydrochloride (EDC) and *N*-hydroxy-succinimide (NHS) were purchased from Acrõs Organics and Pierce, respectively. DNO, EDC and NHS solutions were prepared with a 10 mmol L^{-1} phosphate buffer solution (PBS, pH 7.4) for the functionalization of CNTs.

The complementary DNO strand (DNO_C, 5′-*CGAGTCATTGAGTCATCGAG*-3′) and a non-complementary strand (DNO_{NC}, 5′-*TATACATCTACATCTACATAT*-3′) for hybridization studies were purchased from E. Reilley

Ferrocenemethanol (FcOH) was obtained from Sigma Aldrich, and Methylene blue (MB) and sodium chloride were obtained from Merck. All the solutions were prepared with ultrapure water from a Millipore Milli-Q system.

2.2. Apparatus

Cyclic voltammetry (CV), differential pulse voltammetry (DPV) and scanning electrochemical microscopy (SECM) were performed with a CHI 900 setup (CH Instruments Inc., USA). For SECM measurements, a ca. 10-μm diameter home-made carbon fiber electrode served as the SECM tip while a GCE with a 3-mm diameter (Model CHI104, CH Instruments) was used as the SECM substrate. A platinum wire and a 3 mol L^{-1} NaCl Ag/AgCl electrode (BAS, Model RE-5B) were used as the counter and reference electrodes, respectively. All potentials are given relative to the reference electrode. A magnetic stirrer provided convective transport when necessary.

2.3. Procedure

2.3.1. Functionalization of CNT with an oligonucleotide

2.3.1.1. Non-covalent functionalization ($CNT\text{-}DNO_{ADS}$). Non-covalent functionalization of the carbon nanotubes (Fig. 1a) was carried out by stirring 3 mg of the oxidized CNT_{COOH} sample at room temperature in 3 mL of different DNO concentration solutions (3.9, 5.9 and 7.9 μmol L^{-1}) in 10 mmol L^{-1} PBS at pH 7.4. After 24 h, the dispersions were centrifuged (13000 rpm, 60 min), and the supernatant was collected.

2.3.1.2. Covalent functionalization ($CNT\text{-}DNO_{COV}$). Carboxylic groups of CNT_{COOH} (3 mg) were activated via a carbodiimide reaction (Fig. 1b), adding *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC, 0.4 mg mL^{-1}) and N-hydroxysuccinimide (NHS, 0.6 mg mL^{-1}) in 10 mmol L^{-1} PBS (pH 7.4). After 15 minutes of bath sonication, the mixture was centrifuged (13000 rpm, 30 min), and the supernatant was discarded. Then, different DNO solutions (3.9, 5.9 and 7.9 μmol L^{-1}) were added, and the total volume (3 mL) was brought to a concentration of 10 mmol L^{-1} with PBS. The solution was stirred for 24 h at room temperature. The dispersion was centrifuged (13000 rpm, 60 min), and the supernatant was collected.

2.3.2. Quantification of immobilized oligonucleotide onto CNTs

A stock solution of DNO was prepared in 10 mmol L^{-1} PBS (pH 7.4) with a concentration of 100 μmol L^{-1}. A calibration curve was obtained starting with different amounts of the stock solution over a range of 2-32 μmol L^{-1}. A volume of the stock solution was added into a quartz cell filling to a final volume of 400 μL with ultrapure water. Then, the solutions were homogenized, and their absorbance was measured at 260 nm.

Different solutions of DNO were prepared (3.9, 5.9, 7.9, 16.0 and 32.0 μmol L^{-1}). The CNTs-DNO supernatants from ***2.3.1*** were evaluated using UV-visible absorption spectroscopy. The adsorbed amount of DNO was quantified by comparison with the calibration curve; as the initial concentration of DNO in solution was known, we were able to determine the immobilized amount of DNO by the difference.

2.3.3. Electrochemical characterization of the modified electrodes

2.3.3.1. Preparation of the CNT-modified glassy carbon electrode. Prior to surface modification, the GCE was cleaned by polishing with 0.3 and 0.05 μm alumina slurries for 1 min. The functionalized-CNTs suspensions were prepared by ultrasonically dispersing 1 mg of the CNTs (CNT_{COOH}, $CNT\text{-}DNO_{COV}$ and $CNT\text{-}DNO_{ADS}$) in 1 mL of 10 mmol L^{-1} PBS (pH 7.4) for 30 min. Finally, the treated GCE surface was coated with 8 μL of the CNTs dispersion and dried for