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Electrochemical impedance-based DNA sensor using a modified single walled carbon nanotube electrode

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ABSTRACT

Carbon nanotubes have become promising functional materials for the development of advanced electrochemical biosensors with novel features which could promote electron-transfer with various redox active biomolecules. This paper presents the detection of *Salmonella enterica* serovar Typhimurium using chemically modified single walled carbon nanotubes (SWNTs) with single stranded DNA (ssDNA) on a polished glassy carbon electrode. Hybridization with the corresponding complementary ssDNA has shown a shift in the impedance studies due to a higher charge transfer in ssDNA. The developed biosensor has revealed an excellent specificity for the appropriate targeted DNA strand. The methodologies to prepare and functionalize the electrode could be adopted in the development of DNA hybridization biosensor.

1. Introduction

The development of a highly sensitive and miniaturized DNA detection device remains a foremost challenge in biosensor production. The addition of gold nanoparticles [1,2], conducting polymer [3,4], metal oxide [5,6] graphene [7–10] and carbon nanotubes (CNTs) [11–16] to electrode surfaces have been examined in order to best optimize the detection limit and sensitivity of a biosensor. Carbon nanotubes, in particular, are the quintessential electrode material due to their excellent mechanical strength, as well as high electrical and thermal conductivity [17–20]. Recent studies have demonstrated that CNTs can enhance the electrochemical reactivity of important biomolecules [12,13] and can promote the electron-transfer reactions of proteins (including those where the redox center is embedded deep within the glycoprotein shell) [21,22]. Additionally, their large length to diameter aspect ratio along with a high surface area to weight ratio is an ideal combination for providing surface functionalization of biomolecules. However, due to the lack of solubility of CNTs in many solutions, integration of CNTs in biosensor design remains a difficult challenge [23].

While covalent modification on the nanotube surface was originally used to solve solubility issues, this technique is now used extensively for the biological functionalization such as enzyme attachment to the tip of the CNT [21,22]. For example, treatment of CNTs with sulfuric and nitric acid under sonication will shorten and open the ends of the nanotubes while introducing carboxylic acid functional groups [24]. This technique is not only beneficial for nanotubes solubility, but for functional group surface attachment as well. It has been shown previously that proteins will covalently attach to the ends of shortened single walled carbon nanotubes (SWNTs), which behave as molecular wires to allow electrical communication between the underlying electrode and the redox protein [25]. Modification of electrode surfaces with carbon nanotubes has shown to greatly enhance the performance of the sensor for analytes such as lactic acid [26], cinnarizine [27], antibodies [28], and DNA [29]. In particular, carbon nanotube-modified electrodes exhibit superior electrochemical properties, such as a wide working potential window, and demonstrate catalytic activity towards many chemical reactions.

In this article, we report on the use of SWNTs with a diameter range of 20–30 nm as the platform for DNA attachment, and its subsequent hybridization. The carbon nanotubes have been modified with carboxyl groups and the oligonucleotide probes with amino groups on the 3' end were covalently bonded to the functionalized nanotubes via *N*-Ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC). Once hybridization has been achieved,

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electrochemical impedance spectroscopy (EIS) was used to measure the change in capacitance and charge transfer resistance of the electrode to the redox-active compound $Fe(CN)_6^{-3/-4}$. If the single stranded DNA (ssDNA) probe does not hybridize to its complementary strand, then the electrode continues to behave as though it has been blocked, and there will not be a noticeable shift in the charge transfer resistance (R_{ct}). This is due to the inherent nature that ssDNA is more negatively charged with respect to double stranded DNA (dsDNA) and will consequently repel more of the redox-active compound. Under this study, we chose to use *Salmonella* specific probes, because *Salmonella* infections remain one of the most common food borne illnesses in the United States and perhaps even globally [30]. This method of genosensing is a quick, facile approach to detecting DNA without the use of additional labels.

2. Experimental

2.1. Materials

SWNTs were obtained from Cheap Tubes, USA. The electrolyte used in all electrochemical measurements was 0.5 mM $K_3Fe(CN)_6$ in 0.5 M KCl (Sigma-Aldrich). DNA probe attachment took place in an acetate buffer of 0.3 M sodium acetate (Sigma-Aldrich) and 0.3 M acetic acid (Fisher Scientific). DNA hybridization took place in a 0.1 M phosphate buffer solution (PBS), which was made in deionized water with K_2HPO_4 (purity 98%) and KH_2PO_4 (purity 99%), both from Sigma-Aldrich.

2.2. Electrode modification

The label-free impedance based detection method for DNA hybridization is illustrated in Fig. 1. Note that the –COOH attachment takes place wherever there is a broken C–C bond, not just at the ends of tubes.

Single walled carbon nanotubes were chemically shortened and oxidized in a solution of 3:1 sulfuric and nitric acids under ultrasonication for 8 h. This treatment causes carboxylic acid groups to form on the SWNTs. The functionalized SWNTs were then filtered under vacuum and rinsed to clear any excess acid. The SWNTs were collected from the filter paper and sonicated in dimethylformamide (DMF) for an additional 4 h. The new SWNT /DMF solution had a concentration of approximately 0.1 g SWNT in 3.0 mL DMF. A 3-mm diameter glassy carbon disk electrode was polished with 1.0 μm alpha alumina powder, rinsed with deionized water and dried in a N_2 stream. The same electrode was polished again with 0.3 μm alpha alumina powder, rinsed, and dried. A certain amount of the functionalized SWNTs suspension (1–20 μl) was applied with a pipette onto the surface of the polished glassy carbon electrode and allowed to dry overnight at room temperature.

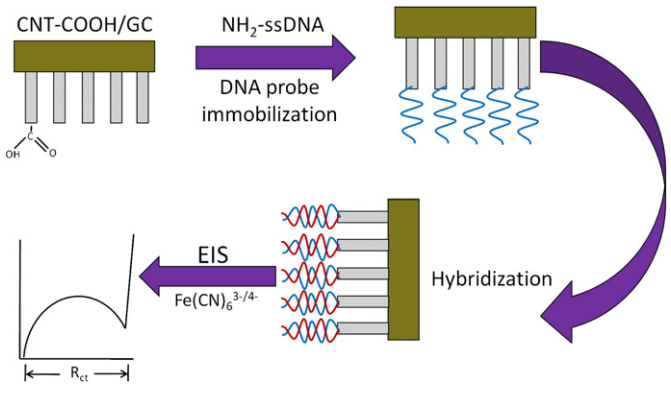

Fig. 1. Schematic diagram of CNT and DNA attachment chemistry process and DNA hybridization measurement in 5 mM $K_3Fe(CN)_6$.

Electrochemical impedance and cyclic voltammetry were measured on the modified glassy carbon electrode with the carboxylated carbon nanotubes (SWNT-COOH) in 0.5 mM $K_3Fe(CN)_6$ in 0.5 M KCl before and after modification. Next, the NH_2-ssDNA probe was attached to the SWNT-COOH electrode using EDAC chemistry. The electrode was incubated in the NH_2-ssDNA probe solution for 2 h at room temperature under gentle agitation. The DNA hybridization took place in complementary probe phosphate buffer solution for 20 min at room temperature under gentle stirring. Nyquist plots and cyclic voltammograms were obtained before and after each modification step.

The *Salmonella* specific probe based on the *wecE* gene was selected from a previous report on detection of *Salmonella* from water [31]. The ssDNA probe, complementary and mismatched ssDNA were purchased from Life Technologies Corporation (Carlsbad, CA). The sequences of the various analytes are provided in Table 1.

2.3. Apparatus

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were performed with a Radiometer Analytical Voltalab 40 potentiostat and the data was analyzed with the corresponding Voltmaster4 software. The standard three-electrode system consisted of a modified glassy carbon electrode with a surface area of 7.07 mm^2, an Ag/AgCl (3 M KCl) reference electrode, and a platinum wire (CH Instruments) as the counter electrode. The electrochemical measurements were carried out in a 10-mL cell at room temperature. The cyclic voltammetry experiments had a potential scan rate of 100 mV s^{-1}, step height of 1.0 mV and applied a potential from +1 V to −0.5 V. The electrochemical impedance experiments applied a frequency range of 50 kHz–100 mHz with an AC sine wave amplitude of 10 mV.

3. Results and discussion

EIS enables the complex electrical resistance of a system to be analyzed. This analytical method is very sensitive to changes on the electrode surface as well as in the bulk solution and therefore is an appropriate application tool for use in DNA detection. The EIS technique is inherently a label-free detection method, which is especially beneficial due to the fact there is no need to additionally modify the biomolecules of interest with markers, such as fluorescent dyes, enzymes, or other redox labels. The electrochemical complex impedance (Z) can be represented as the sum of the real (Z_{re}) and imaginary (Z_{im}) components such that:

$$Z = Z_{re} + jZ_{im} \quad (1)$$

where $j = \sqrt{-1}$.

When taking measurements with surface-modified electrodes, such as DNA attached to modified SWNT electrodes, redox-active compounds are usually added to the electrolyte solution, resulting in a well-defined charge transfer resistance (R_{ct}). If such a redox-active compound is blocked by adding a blocking layer to the electrode, the R_{ct} value will become larger and a more capacitive impedance behavior will be observed. Under this scope, if the ssDNA probe does not hybridize to its complementary strand, then the electrode will continue to behave as though it has been blocked and there will not be a noticeable shift in the R_{ct}.

Table 1
ssDNA probe and complementary strand sequence information.

Sequence	Function
5' TACCGCAGCTAATTGACGTTAC 3' NH	*Salmonella* specific ssDNA probe
5' GTAACGTCAATTAGCTGCGGTA 3'	Complementary ssDNA strand
5' GTAACGTCAATTAGCTGCGGTG 3'	One base-pair mismatched ssDNA strand
5' TAGGGAACGATCGGTCACATCGG 3'	Unrelated ssDNA strand