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A new DNA-nanobiosensor based on G-quadruplex immobilized on carbon nanotubes modified glassy carbon electrode

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ABSTRACT

The G-quadruplex (GDNA) was immobilized on multi-walled carbon nanotubes (MWCNTs) modified glassy carbon electrode (CNT/GC). Prior to use, MWCNTs were treated with nitric acid and sulfuric acid to introduce the carboxylic acid groups. Then, the carboxylate-terminated MWCNTs covalently bound to the amino groups of GDNA (GDNA/CNT/GC), with the aid of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide. Circular dichroism was used, to confirm the formation and stability of parallel form of GDNA in the solution using the named oligonucleotide and in the presence of the mentioned ligands. Morphologies of the modified electrodes in each step were studied by scanning electron microscopy. Characteristics of the modified electrodes and interaction of the immobilized GDNA with ethidium bromide/polyamines (spermine or spermidine) were investigated by different techniques. The cyclic voltammetry peak currents of $[Ru(NH_3)_6]^{3+}$ decreased with increasing concentration of the employed ligands. The electrochemical impedance spectroscopy studies showed the increase of charge resistance after addition of each of these ligands, indicating their interaction with GDNA. The binding constant, the linear dynamic range and the limit of detection of this sensor was determined for each ligand. This modified electrode may be used as a label free nanobiosensor for detection of ligand–GDNA interaction, with respect to the binding mechanism.

1. Introduction

Chromosomes are compact form of double stranded DNA that has turned around the core histones; then the higher order of chromatin structure is formed in the presence of linker histone, scaffold proteins, etc. [1]. However, at the end of chromosomes specific sequences of DNA with particular structure, which has linked to specific proteins, exist to protect the chromosomes from degradation, recombination, and end to end fusion during cell cycle. These parts which are named the telomeres are consisting of tandem repeats of guanine rich sequences. Each time a cell divides, telomeres become shorter. The specific enzyme named telomerase is responsible to maintain the length of telomere within cells, thereby permitting cellular replication. The Nobel prize on 2009 in physiology or medicine was awarded to the scientists[2] who discovered the telomere and telomerase [2].

The human telomeric DNA that is the G-rich sequences are capable of forming a four stranded helical structure, known as the guanine-quadruplex, G-quadruplex or GDNA. The GDNA is an unusual DNA secondary structure, based on the Hoogesteen G–G pairing, which gives rise to hydrogen binding of four guanines [3,4]. The stabilization of the quadruplex structure at the telomeric repeats is an effective way to inhibit telomerase activity. Telomerase activity is high in 85% of cancer cells. So, GDNA have recently received a great attention because of its potential links to mechanisms that relate to cancer, HIV, and some other diseases [5–11]. These structures are also stabilized in the presence of certain metal cations, mainly alkali ions such as K^+ coordinated by the carbonyl groups of guanines at the center of helical core of quadruplex [12,13]. However, some studies were done using other multivalent cations such as Tb^{3+} to form and stabilize this structure [14,15].

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Nowadays different techniques are employing to investigate the formation of GDNA and its properties; they are including NMR spectroscopy, circular dichroism (CD), and fluorescence spectroscopy [16–18]. When we began to study the GDNA using electrochemical method, there was no report about GDNA immobilization on the electrode surface, but at present a few paper was published in this regard [18,19].

Carbon nanotubes (CNT) thin films are a 2D structure with a mixture of semiconducting and metallic tubes. They are being extensively studied in different fields of science due to their unique structural and physicochemical properties such as high electrical conductivity, physical and thermal stability, nanometer size and so on [19–22]. CNT not only enhance kinetics of electrode reactions but also prepare biocompatible medium for immobilization of biomolecules. For the preparation of a biosensor, the first step is immobilization of biological molecules on the surface of carbon nanotubes through covalent or non-covalent bonds [23–28]. Nucleic acids are the important component for the preparation of electrochemical biosensors and capable of recognizing biological samples [26,29,30]. The covalent immobilization on the CNT is usually performed by reacting amino-terminated DNA with the functional groups at the ends and sidewalls of CNT (especially carboxylic groups) via the cross linkers such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) [31,32]. The covalent immobilization has some advantages including stability, simplicity, accessibility, efficiency, selectivity, and low cost.

Ethidium bromide (2,7-diamino-10-ethyl-9-phenylphenanthridinium bromide; EtBr) is a dye and a powerful mutagen widely used in biochemical research for *in vitro* visualization of nucleic acids. Among the numerous binding studies of small molecules with DNA, EtBr is one of the most frequently studied intercalators. The EtBr binding to DNA was studied using various spectroscopic (e.g. absorption, fluorescent and NMR spectroscopy) [33–37] and electrochemical methods [34,38–40]. Generally, EtBr can be interacted with DNA in different modes including electrostatic binding, and intercalation [41]. The type of interaction depends on the concentration of EtBr and ionic strength of the solution [39,42]. There are some investigation about interaction of EtBr and its derivatives with G-quadruplex DNA by spectroscopic methods [33,43,44]. Previous studies indicated that 1 mol of EtBr could bind to one mole of GDNA [43,44] and stabilizes it.

Spermine (Spm) and spermidine (Spd) also bind to DNA through both electrostatic interaction and groove binding. Our research group previously investigated their interaction with double stranded DNA using electrochemical techniques [45,46]. Interaction of these ligands with G-quadruplex was also shown through electrostatic interaction [47,48]. Although, according to our search and literature survey, their interaction with GDNA was not investigated by electrochemical methods.

In continue to our previous works on the electrochemical investigation of DNA–ligand interaction [49–51], recently some nanostructured materials were prepared and applied for studying the interaction of drug [52], and some biologically important molecules [45,46] with double stranded DNA. In the present study a new nanobiosensor using G-quadruplex is designed for electrochemical investigation of some ligands that can interact with it.

2. Experimental

2.1. Chemicals

The DNA oligonucleotide was purchased from FAZA Biotech (Tehran, Iran). This 30-mer DNA sequence (as follows) was synthesized as lyophilized powder by ALFA Company (Canada):

5′-NH2-GGGAGGGAGGGAAGGAGGGAGGGAGGGAGC

The oligonucleotide stock solution was prepared by addition of buffer (10 mM HEPES buffer, 50 mM KCl, 20 mM CsCl, pH 7.5) to its powder to generate G-Quadruplex. Then it was stored at −20 °C. EtBr, Spm and Spd were obtained from Sigma Chemical Co. MWNTs purchased from DropSense Co. (average diameter 10 nm, average length 1.5 μm). All other reagents including 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), Ruthenium hexamine chloride (RuHex) (98%), $K_3[Fe(CN_6)]$ and $K_4[Fe(CN_6)]$ were of analytical grade from Sigma–Aldrich Co., and were used directly without further purification. A 10 mM HEPES buffer containing 50 mM KCl (pH 7.5) was used as the supporting electrolyte. All other reagents were of analytical grade from Fluka or Merck. All solutions were prepared using distilled deionized water and stored at 4 °C.

2.2. Apparatus

All electrochemical experiments were performed in a conventional three-electrode cell, powered by an electrochemical system comprised of an Autolab PGSTAT30 Potentiostat/Galvanostat and FRA2 boards (Eco Chemie, Utrecht, The Netherlands). For impedance measurements, we used a frequency range of 100 kHz to 10 mHz, an AC voltage amplitude of 10 mV and an equilibrium time of 5 s. The system was run by a PC through FRA and GPES 4.9 software. Scanning electron microscopy (SEM) imaging was carried out with a Philips model X-30 electron microscope. CD measurements were made on a JASCO Model J-810 spectropolarimeter. A glassy carbon electrode (Metrohm, Herisau, Switzerland) 2 mm in diameter (modified or otherwise), an Ag/AgCl saturated KCl electrode and a Pt electrode were used as the working, reference and counter electrodes, respectively. All experiments were carried out at room temperature.

2.3. Preparation of functionalized MWCNT

The acid treatments of CNT were done for oxidation and carboxylic acid formation at the defect site of the end of CNT by a mixture of HNO_3 and H_2SO_4. MWNTs were heated to reflux in mixture of the named acidic solution (3:1, v/v), for about 24 h and 200 °C. The solution containing dispersed powder of CNT was sonicated for 1 h, then centrifuged and washed with double-distilled water several times to become neutral, and was dried at 50 °C.

2.4. Preparation of CNT-modified glassy carbon (GC) electrode

The surface of the GC electrode was polished using a polishing microcloth with 0.05 μm alumina powder and then rinsed with distilled water several times. After that, it was sonicated in ethanol and then in distilled water solutions for 15 s. Then, it was dried at room temperature prior to modification. The electrodes were prepared by polishing GC electrode on the some CNT. The prepared electrode was introduced to a cell containing 1 M HNO_3 solution potentially cycled between −600 and 2000 mV vs. Ag/AgCl about 30 cycles. Then electrode was cycled in 10 mM of HEPES + 50 mM KCl solution until a reproducible cyclic voltammogram was obtained (about 20 cycles).

2.5. GDNA immobilization and the interaction between GDNA and ligands

The electrode immersed in the 10 mM EDC solution for 1 h for activation of the carboxylic acid groups on the carbon nanotubes.