Colloids and Surfaces B: Biointerfaces 121 (2014) 99–105

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Redox targeting of DNA anchored to MWCNTs and TiO_2 nanoparticles dispersed in poly dialyldimethylammonium chloride and chitosan

Ali A. Ensafi[a,*], Parisa Nasr-Esfahani[a], Esmaeil Heydari-Bafrooei[b], B. Rezaei[a]

[a] Department of Chemistry, Isfahan University of Technology, Isfahan 84156–83111, Iran
[b] Department of Chemistry, Faculty of Science, Vali-e-Asr University of Rafsanjan, P.O. Box 518, Rafsanjan, Iran

ARTICLE INFO

Article history:
Received 24 January 2014
Received in revised form 24 May 2014
Accepted 28 May 2014
Available online 4 June 2014

Keywords:
DNA-based biosensors
Multiwall carbon nanotubes
TiO_2 nanoparticles
Methylene blue
Electrochemical method

ABSTRACT

A key issue associated with electrochemical DNA-based biosensors is how to enhance DNA immobilization on the substrates. In order to improve the immobilization of DNA and to optimize DNA interaction efficiency, different kinds of strategies have been developed. In this regard, nanomaterials have attracted a great deal of attention in electrode surface modification for DNA biosensor fabrication. In this study, nanostructured films were deposited at the surface of a pencil graphite electrode (PGE) as a working electrode. For the present purpose, common polyelectrolytes are used for surface modification with double-stranded DNA. Two positively charged polyelectrolyte, namely poly dialyldimethylammonium chloride (PDDA) and chitosan, are initially compared for DNA immobilization at the surface of MWCNTs and TiO_2 nanoparticles (TiO_2NPs). In a second step, the basic electrochemical properties of the sensors are investigated using voltammetric methods. The modified electrodes are also characterized by scanning electron microscopy and electrochemical impedance measurements. It will be shown that electrode modification with DNA and the nanostructure that disperses in PDDA leads to an enhanced sensitivity of the DNA voltammetric detection mechanism. In a previous study, a comparison was done between MWCNTs and TiO_2NPs for determining the effect of nanoparticle effect on DNA immobilization on the electrode surface. In order to compare the efficiency of the prepared DNA-based biosensors, methylene blue is chosen as an electroactive probe. It will be shown that the stability of the immobilized DNA within several days will be much higher when MWCNTs rather than TiO_2NPs are used.

1. Introduction

Sequence-specific detection of nucleic acid targets has become increasingly important in disease diagnostics, drug screening, epidemic prevention, and environmental protection [1]. So far, many techniques including spectrophotometry [2], chemiluminescence [3], phosphorescence [4], fluorescence [5], chromatography [6], and electrochemical methods [7,8] have been developed for DNA determination. In recent years, the development of DNA biosensors has received great attention due to its extreme importance in many fields, such as clinical diagnostics [9], gene therapy [10], environmental monitoring [11], food safety [12], and biological research [13]. DNA recognition layers can be combined with electrochemical transducers to form new and important types of biosensors [14]. Electrochemical DNA biosensor technologies have been developed due to their several advantages, such as high sensitivity, good selectivity, low cost, rapid diagnosis, and the possibility of miniaturization [15].

DNA-based electrochemical sensors exploit a range of different chemistries, but all take advantage of nanoscale interactions between the target in solution, the recognition layer, and a solid electrode surface. Numerous approaches to electrochemical detection have been developed, including direct electrochemistry of DNA, electrochemistry at polymer-modified electrodes, electrochemistry of DNA-specific redox reporters, electrochemical amplifications with nanoparticles, and electrochemical devices based on DNA-mediated charge transport chemistry [16]. Recently, an impressive number of inventive designs have appeared for DNA-based electrochemical sensing. These types of sensors combine the nucleic acid layer with electrochemical transducers to produce a biosensor that expectedly provide a simple, accurate, and inexpensive platform for clinical diagnosis [17]. Electrochemical methods are well suited for DNA diagnostics. Because electrochemical reactions give an electronic signal directly, there is no need for inexpensive signal transduction equipment. Moreover, because immobilized probe sequences can be readily confined to a variety

* Corresponding author. Tel.: +98 311 3912351; fax: +98 311 3912350.
E-mail addresses: aaensafi@gmail.com, Ensafi@cc.iut.ac.ir (A.A. Ensafi).

http://dx.doi.org/10.1016/j.colsurfb.2014.05.040
0927-7765/

of electrode substrates, detection can be accomplished with an inexpensive electrochemical analyzer. Sensitive electrochemical signaling strategies based on the direct or catalyzed oxidation of DNA bases, as well as the redox reactions of reporter molecules or enzyme recruited to the electrode surface by specific DNA probe target interactions and by charge transport reactions mediated by the π-stacked base pairs have all been demonstrated [18].

Surface immobilization of DNA plays an important role in the performance of DNA biosensors. The amount of immobilized DNA probe will directly influence the accuracy, sensitivity, selectivity, and life of a DNA biosensor [19]. The development of nanomaterials for the immobilization of DNA has attracted great attention over the past decades in fabricating electrochemical DNA biosensors [20,21]. Because of their high surface-to-volume ratio and excellent biological compatibility, nanomaterials can enlarge the sensing surface area that greatly increases the amount of immobilized DNA, while the DNA mixed with nanomaterials can satisfactorily maintain its biological activity. The nanomaterials used in DNA biosensors include nanoparticles, nanowires, and nanotubes like carbon nanotubes (CNTs), among others [19].

As one of the most promising nanomaterials in photochemistry and biochemistry, nano-titanium dioxide (TiO_2) acts as an efficient agent in biosensing applications due to its good intrinsic biocompatibility, specific affinity to biomolecules, and high reactivity [22,23]. Owing to its high conductivity and low cost, TiO_2 has become an attractive electrode material in its different forms such as nanoparticles, nano-needles and nanotubes for fabricating electrochemical DNA-based biosensors [24]. The properties of CNTs, such as high electronic conductivity and high mechanical resistance, have also led to their use in the preparation of electrochemical sensors [25]. DNA is an important biological polymer, which is classified as a natural, negatively charged polyelectrolyte due to its phosphate groups. It can be immobilized onto CNTs and TiO_2 via covalent and noncovalent interactions. However, the results are unsatisfactory because negatively charged CNTs and TiO_2 repulse the negatively charged DNA. To overcome this problem, nanoparticles are usually dispersed on such positively charged polyelectrolytes as poly dialyldimethyl ammonium chloride (PDDA) or chitosan [26].

The study of the interactions between drugs and DNA is a relatively little known area which forms an attractive field, which is essential for gaining a deeper understanding of the mechanisms of interaction and developing efficient methods for detecting these mechanisms. The knowledge thus gained will be used for designing new, efficient drugs and for their in vitro screening [27]. Some studies have demonstrated that DNA damage, which may occur by induction of DNA hypomethylation and generation of reactive oxygen species [28,29]. Induction of oxidative damage (oxidative stress) is a noticeable event to explain metal-induced mutagenicity and genotoxicity. Various carcinogenic metals such as cobalt, chromium, lead, mercury, and copper cause redox reactions in living systems [29]. These metals can produce the reactive oxygen species and reactive nitrogen species in vivo and vitro systems in experimental and clinical medicine [30–32].

In this study, to improve the immobilization of ds-DNA on the surface, a mixture of nanomaterials and positively charged polyelectrolytes was immobilized at the surface of a pencil graphite electrode (PGE). At first, two positively charged polyelectrolytes, namely poly dialyldimethylammonium chloride (PDDA) and chitosan, were compared for DNA immobilization at the surface of MWCNTs and titanium dioxide nanoparticles (TiO_2NPs). In another study, a comparison was made between MWCNTs and TiO_2NPs in order to recognize the effect of nanoparticle on DNA immobilization at the electrode surface. In order to investigate the efficiency of the prepared PGE in the determination ds-DNA and small molecules interactions, methylene blue was chosen as a model. The interaction between methylene blue and guanine bases of ds-DNA is well known in the literature [33–35]. The interaction between methylene blue and ds-DNA has generally been investigated by means of the oxidation peak current value of methylene blue [36,37]. However, we investigated the interaction between methylene blue and ds-DNA by means of the changes in the oxidation peaks current of guanine and adenine bases (of the ds-DNA) on PGE. These studies were carried out using differential pulse voltammetry technique.

2. Experimental

2.1. Chemicals

All solutions were prepared using reagent grade chemicals and doubly distilled water was used through the work. Double-stranded salmon sperm DNA (ds-DNA, catalog No. D8899) was purchased from Sigma (St. Louis, USA). Reagent grade Tris–HCl, CH_3COOH, CH_3COONa, H_3PO_4, EDTA, NaCl and NaOH were purchased from Aldrich Chemicals (Milwaukee, USA). Chitosan was obtained from Acros Chemical. Their solution was prepared by dissolving a certain amount of chitosan in 10 mL CH_3COOH (120.1 mg mL^{-1}) containing 29.2 mg mL^{-1} NaCl by aid of sonication for 30 min. PDDA (low molecular weight) were purchased from Sigma. Aqueous solutions of PDDA were initially prepared with 29.2 mg mL^{-1} NaCl. Multiwall carbon nanotubes (MWCNTs) powder (diameter 70–100 nm, length 5–9 μm) was obtained from Fluka. Titanium dioxide nanoparticles (TiO_2NPs) powder (30 nm) was obtained from Fluka. Their suspensions were prepared in chitosan and PDDA solutions after sonication for 3 h to obtain a homogeneous suspension.

2.2. Apparatus

Electrochemical measurements were performed using an Autolab PGSTAT 12, potentiostat/galvanostat connected to a three-electrode cell, Metrohm, Model 663 VA stand, with a GPES 4.9 software package (Eco Chemie, The Netherlands). The raw data was treated using the Savitzky and Golay filter (level 2) of the GPES software, followed by the GPES software moving average baseline correction with a "peak width" of 0.01. The reference electrode was Ag/AgCl and the counter electrode was a platinum wire. A standard one-compartment three-electrode cell of 20 mL capacity was used in all experiments. PGE was the working electrode. A Noki pencil was used as a holder for Pentel graphite leads. Electrical contact with the lead was obtained by soldering a metallic wire to the metallic part. The pencil was hold vertically with 12 mm of the lead extruded outside (9 mm of which was immersed in the solution). The pencil leads were used as received. All the electroanalytical measurements were performed at room temperature.

2.3. Functionalization and purification of MWCNTs

MWCNTs were purified and functionalized as described elsewhere [38]. A mass of 120 mg of MWCNTs was stirred in 10 mL of a 190.0 mg mL^{-1} nitric acid solution for 20 h. The solid product was collected on a filter paper and washed several times with distilled water until the filtrate solution became neutral (pH 7.0). The functionalized MWCNTs thus obtained were then dried in an oven at 80 °C for 24 h. Nitric acid usually causes a significant destruction of carbon nanotubes and introduces –COOH groups at the ends or at the sidewall defects of the nanotube structure.