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Fabrication of a highly sensitive adenosine aptasensor based on covalent attachment of aptamer onto chitosan-carbon nanotubes-ionic liquid nanocomposite

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ABSTRACT

The present study describes the fabrication of a novel electrochemical aptasensor for the label-free determination of adenosine. The immobilization surface is prepared by the modification of a glassy carbon (GC) electrode with a robust nanocomposite containing multiwalled carbon nanotubes, ionic liquid and chitosan(MWCNTs-IL-CHIT). Amine-terminated 12-mer capture probe(ssDNA1) is covalently attached onto the nanocomposite using glutaraldehyde (GA) as the linking agent, a 32-mer adenosine-specific aptamer (ssDNA2) immobilized onto the electrode surface through hybridization with the **s**sDNA1 and methylene blue (MB) used as the redox probe.The peak current of MB decreased linearly with increasing adenosine concentration due to the formation of aptamer-adenosine complex and displacement of the aptamer from the modified electrode surface.The aptasensor showed alow detection limit of 150 pM and high sensitivity of 0.67 μAnM^{-1} at a concentration range of up to 0.4 μM.Through the control experiments performed by using some other nucleosides such as guanosine, cytidine and uridine, the excellent specificity of this sensor toward adenosine detection is demonstrated. The potential applicability of the aptasensor is successfully applied for measuring adenosine concentration in blood serum and drug formulation samples.The herein described methodology may hold great promise for fabrication of other aptasensors and immunosensors.

1. Introduction

Adenosine is an endogenous nucleoside which plays an important role in the regulation of the physiological activity of various tissues and organic function(Latini and Pedata, 2001).It also acts as a regulator of cerebral blood flow in the central nervous system (Phillis, 1989) and modulates neurotransmission (Brundege and Dunwiddie, 1997).The determination of urinary adenosine has been applied to display the progress of diseases (Yang et al., 2002), and direct monitoring of adenosine variations under physiological conditions can be used in investigations related to heart and brain physiology (Giglioni et al., 2008). A variety of detection methods such as HPLC and GC/LC-MS have been used for adenosine determination (Huang et al., 2004). However, these methods have some disadvantages such as time consumption, sample pretreatment, complicated procedure and expensive instrumentation. Thus, a simple, rapid, sensitive and selective method for the determination of adenosine is of great interest.

Aptamers are specific single-stranded DNA or RNA molecules that have been selected in vitro from large randomized oligonucleotide libraries bySELEX (systematic evolution of ligands by exponential enrichment) (Tuerk and Gold, 1990). Very similar to antigens and antibodies, aptamers can bind specifically to abroad range of targets such as small molecules, proteins, amino acids, drugs and even viruses and whole cells(Zou et al., 2007).Owing to their numerous advantages such as easy labeling, good stability, reproducibility for synthesis, highly specific binding abilities, wide target range and wide applicability to extreme conditions, aptamers are considered to be ideal candidates as molecular recognition elements in biosensors (Luzi et al., 2003). A variety of aptasensors have been developed using different analytical methods including optical transduction (Yang et al., 2005), circular dichroism (Nagatoishi et al., 2007), electrochemical techniques (Feng et al., 2008), fluorimetry (Chen et al., 2010), colorimetry (Wei et al., 2007), atomic force microscopy (AFM) (Basnar et al., 2006), surface plasmon resonance (SPR)(Li etal., 2006)and quartz crystal microbalance(QCM) (Fang et al., 2008). Among various

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aptamer-based biosensors, electrochemical aptasensors are proved to be powerful analytical tools in the detection of proteins and small molecules due to high sensitivity and selectivity as well as simple instrumentation.

Two transduction strategies including target-induced conformational change of the surface-bound strand (Wang et al., 2009) and the use of target-induced aptamer displacement mechanism (Wu et al., 2007) have been used for fabrication of electrochemical aptasensors. For conformational change-based aptasensors, the interaction of the target with the aptamer induces an alteration in the conformation of aptamer. This causes some changes in the electron-transfer communication of the redox center with the surface of the electrode, a process that can be electrochemically monitored. In the target-induced aptamer displacement mechanism, either the short complementary strand or the longer aptamer strand releases from the electrode surface due to the formation of a target-aptamer complex. Despite the various aptamer-based biosensors explored so far, many of them need various steps of the aptamer or target labeling with a probe molecule. These labeling procedures make experiments more complex and thus, limit the wide-use application of such aptasensors. Therefore, the development of label-free aptasensors for determination of adenosine would be of great significance.

A significantly improved performance for aptamer-based biosensors has been recently reported through combination of aptamers with novel nanomaterials. Among different nanomaterials, carbon nanotubes (CNTs) have attracted great interestbecause of their attractive electronic, chemical and mechanical properties. The excellent compatibility and electrochemical applications of CNTs to immobilize a variety of species have been reported (Chen et al., 2001; Salimi et al., 2006; Salimi et al., 2008). However, lack of stability and homogeneity of CNTs directly immobilized on the surface of electrode can be a problem. CNTs also tend to aggregate through strong π–π stacking and van der Waals attraction among tubes (Li et al., 2008).The use of ionic liquids (ILs) can prevent the aggregation of CNTs (Zhou et al., 2009; Salimi et al., 2010; Teymourian, 2012). Furthermore, the stability of CNTs in aqueous chitosan(CHIT) solution has greatly improved the sensitivity of DNA sensors (Li et al., 2005).

In the present study, the MWCNTs-IL-CHIT nanocomposite was used for the fabrication of a label-free aptasensor for the determination of adenosine using methylene blue (MB) as the redox label. The fabrication of the aptasensor was based on the covalent attachment of amine-terminated capture aptamer onto the nanocomposite via GA as the linking agent and then, immobilization of the adenosine-specific aptamer probe to the surface of MWCNTs-IL-CHIT through its hybridization with the capture probe. In the presence of adenosine, the MB peak current decreased due to the aptamer release from the capture probe and the formation of a complex between the aptamer and adenosine.The applicability of the aptasensor was also evaluated forthe analysis of adenosine in blood serum sample.

2. Experimental

2.1. Chemicals and Reagents

1-Butyl-1-methyl pyrolydiniumbis(trifluoro-methyl-sulfonyl) imide [$C_{11}H_{20}F_6N_2O_4S_2$] (IL), cytidine, uridine, guanosine, adenosine,glutaraldehyde (GA), chitosan (CHIT), phosphate buffer solution (PBS) and methylene blue (MB) werepurchased from Sigma. Oligonucletides, designed according to the literature (Zou et al., 2007), were custom-synthesized by Bioneer Co. (South Korea). The sequence of 3′-amine-terminated capture probe containing 12 baseswas (3′-NH_2-$(CH_2)_6$-TCTCTTGGACCC-5′) and sequence of aptamer probe containing 32 bases was (3′-TGGAAGGAGGCGTTATGAGGGGGTCCAAGAGA-5′).This sequence was designed to hybridize with the 12-base capture probe and specifically recognize adenosine.MWCNTs with a purity of 95%, surface specific area of 480 m^2g^{-1}, diameter of 20–30 nm and 1 μm length were obtained from Nanolab (Brighton, MA).All other chemicals were of analytical reagent grade and used without further purification.

All sample solutions (cytidine, uridine, guanosine and adenosine) at various concentrations were prepared by dissolving analytes in PBS(pH 7.4) and DNA solutions were prepared by dissolving in distilled water. A mixture of MB (20 μM): Tris–HCl (10 mM) at a ratio of 1:1 was applied as the redox indicator.A solution containing 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) and 0.5 M KCl was used in the electrochemical impedance spectroscopy (EIS) experiments. The human serum sample was obtained from a local clinical laboratory (Verdi Laboratory, Sanandaj-Iran) and subjected to ultrafiltration by loading into a centrifugal filtration tube at 5000 rpm for 30 min.Then, the ultrafiltrate was diluted 50 times with PBS (0.1 M) and DPVs were conducted in order to determine the adenosine concentration.

2.2. Apparatus and measurements

All electrochemical measurements were carried out with a conventional three-electrode system comprising a modified glassy carbon electrode(GC) as the working electrode, an Ag/AgCl (3 M KCl) and platinum wire as the reference and counter electrode, respectively. Cyclic voltammetry (CV), differential pulse voltammetry (DPV) and EIS was performed with aμ-AUTOLAB type III computer controlled Potentiostat/Galvanostat (ECO-Chemie, The Switzerland). For EIS measurements, a frequency response analysis (FRA) system software under an oscillation potential of 5 mV over a frequency range of 10 kHz to 0.1 Hz was used. The electrochemical signals were obtained in PBS by using DPV with amplitude of 25 mV and pulse width of 0.05 s.

2.3. Preparation of the GC/MWCNTs-IL-chit electrode

Prior to coating, the GC electrode was carefully polished with 0.03 μm alumina powder on polishing cloth, ultrasonicated in ethanol and doubly distilled water in order to remove adsorbed particles, and finally allowed to dry at room temperature. The MWCNTs-IL-CHITnanocomposite prepared according to our previously reported procedure (Kavosi, 2013; Khezrian, 2013). Briefly, 3 mg chitosan was dispersed into 10 μL IL on a glass slide to make a uniform paste. Then, 2 mg MWCNTs were mixed in the mixture of CHIT/IL film to obtain a homogeneous paste. The MWCNTs-IL-CHIT modified GC electrode was fabricated by rubbing some of this nanocomposite on the electrode surface until the forming a uniform layer.

2.4. Fabrication of the sensing interface

A10 μLGA aqueous solution (0.25% v/w) was dropped onto the surface ofMWCNTs-IL-CHIT modified GC electrode. Then,10 μL of 20 μM 3′-amine capture probe (ssDNA1) was droppedon the surface of the modified electrode and kept for 2 h at 4 °C. In this way, the capture probe was covalently attached to the amine group of CHIT through amide coupling.After rinsing with water, 10 μL of 20 μM 32-base adenosine aptamer(ssDNA2) was added and incubated on the prepared electrode for 4 h at 4 C°to hybridize with the ssDNA1.Afterwards, MB was accumulated onto the aptamer-modified electrode surface by dropping 5 μL of MB (20 μM)in Tris–HCl (10 mM) solution at a ratio of 1:1for 30 min.Finally, this electrode was thoroughly rinsed with high ionic strength rinsing buffer (10 mMTris-HCl, pH 7.4, containing 10 mMKCl, 10 mM