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Aptasensor platform based on carbon nanofibers enriched screen printed electrodes for impedimetric detection of thrombin

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ABSTRACT

Herein, impedimetric detection of THR could be achieved using an aptasensing platform based on carbon nanofibers enriched screen-printed electrodes (CNF-SPE). The resistance to charge transfer (R_{ct}) was recorded using electrochemical impedance spectroscopy (EIS) technique before/after the immobilization of amino-modified DNA aptamer (APT) selective to thrombin (THR) onto the surface of CNF-SPEs and the specific interaction between APT and THR. The selectivity of the aptasensor was also tested in the presence of a random DNA oligonucleotide and a DNA aptamer that were different from THR specific APT. The impedimetric aptasensing of target protein was also explored in the fetal bovine serum (FBS) medium at different concentration levels of THR. Additionally, the selectivity of the aptasensor was tested against bovine serum albumin (BSA) and protein C (PC) in FBS medium. This CNF-SPE based aptasensor platform allows a reliable, sensitive and selective impedimetric monitoring of THR.

1. Introduction

Aptamers are known as a new class of nucleic acids which are used to design sensitive, selective and robust biodetection platforms. The electrochemical biosensing technology can be combined with aptamers for recognition of nucleic acids, proteins, drugs, and toxins. Thus, there have been many reports in the literature about development of electrochemical aptamer based sensors (aptasensors) [1–22]. Elshafey et al. [12] reported the selection of a high affinity DNA aptamer and development of an impedimetric aptasensor by using gold electrode for sensitive and selective detection of Anatoxin-a (ATX) that is cyanobacterial toxin known as the smallest potent neurotoxin. The selectivity of the impedimetric aptasensor was also tested against cylindrospermopsin, Microcystin-LR and their mixture. In the study of Gao et al. [14], an electrochemical aptasensor for detection of hemin which was a well-known natural porphyrin to iron complex and applied in pharmacy, environmental and food industry was developed. Glassy carbon electrode (GCE) was modified with the nanocomposite comprised of hemin binding aptamer and carboxylated graphene, then, the interaction of hemin and its aptamer was monitored.

Thrombin (THR) is the last serine protease involved in the coagulation cascade and its concentration in blood is of relevance in many pathological situations [23–26]. Fibrinogen is converted into insoluble fibrin in the presence of THR. Moreover, THR is involved in thrombosis and platelet activation. Whereas THR is nearly absent in blood under normal conditions, its presence or absence under pathological conditions can be indicative of coagulation abnormalities [25]. Therefore, sensitive detection of THR has a vital importance not only for diagnosis of cardiovascular disease, but also of different types of cancer.

The thrombin-binding aptamer (HD1) was the first aptamer selected *in vitro*, specific for a protein without nucleic acid-binding properties [26]. The interaction between THR and the HD1 has been taken as a model system by many authors. G-quartet structure of HD1 and the binding site of THR with HD1 has been characterized thoroughly [27,28]. Due to the importance of THR, many sensors for thrombin detection, using different techniques and approaches have been developed [1–6]. Label-free electrochemical detection of human α-THR in human blood serum was explored by Kwon et al. [1] by using thiolated aptamers immobilized gold electrodes. It was reported that positively charged ferrocene-coated gold nanoparticles were electrostatically bound to the negatively charged aptamers and accordingly, the electrochemical response was recorded by cyclic voltammetry and differential pulse voltammetry (DPV). Li et al. [4] developed a novel electrochemical aptasensor for the voltammetric detection of THR based on multi-walled carbon nanotubes modified GCE surface.

Evtugyn and co-workers [20] introduced an aptasensor platform for impedimetric detection of thrombin by using neutral red attached and thiacalix[4]arene modified GCE.

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Carbon nanofibers (CNFs) are hollow cylinders, which have different diameters varying from 50 to 500 nm and a few tens of microns-size lengths. Due to the fact that they have high aspect ratios (length/diameter > 100), they can align homogeneously at the surface, on which they were immobilized [29,30]. In addition, it was found that they can provide a stable surface for robust biosensor applications [31–38].

Detection of dopamine and serotonin in the presence of excess ascorbic acid, a biosensor based on CNF modified GCE in combination with DPV technique was described by Rand et al. [32]. The detection limits (DLs) were found to be as low as 50 nM and 250 nM respectively for dopamine and serotonin. Periyakaruppan and co-workers developed a selective and sensitive assay based on CNF modified nanoelectrode in combination with EIS to analyze a toxic protein, ricin [33]. An amperometric lactate biosensor was also developed by using screen printed carbon electrodes modified with platinum nanoparticles supported on graphitized carbon nanofibers (PtNps/GCNF) [37].

In our study, the impedimetric detection of THR using an aptasensor platform was comprehensively investigated. To the best of our knowledge, no report describing an impedimetric detection of aptamer–THR interaction at the surface of carbon nanofibers enriched screen printed electrodes (CNF-SPEs) is available as of yet. A sensitive and selective THR detection was performed herein at the surface of CNF-SPEs using EIS technique. The surface morphologies of CNF-SPE were explored using scanning electron microscopy (SEM) technique. The effect of different experimental conditions upon the sensor response was examined; e.g. the concentration of DNA aptamer and THR. The selectivity of the aptasensor was also investigated using random DNA sequence, or random DNA aptamer. The impedimetric detection of interaction between DNA aptamer and its cognate protein was also investigated even in the presence of a complex medium, fetal bovine serum (FBS). Moreover, the selectivity of the aptasensor was tested against bovine serum albumin (BSA) and protein C (PC) in FBS medium.

2. Experimental

2.1. Apparatus

Electrochemical measurements were performed by using AUTOLAB-PGSTAT 302 electrochemical system supplied with a FRA 2.0 module for impedance measurements and NOVA software package (Eco Chemie, The Netherlands). Electrochemical impedance spectroscopy (EIS) measurements were performed in the Faraday cage (Eco Chemie, The Netherlands).

2.2. Chemicals

The amino-linked DNA aptamers (APT) and random DNA (DNA ODN) oligonucleotides were synthesized from Ella Biotech (Germany). The DNA APTs were designed according to the information given in the literature [39].

Anti-thrombin DNA aptamer-1 (APT-1):
5′-NH_2-C_6-GGT TGG TGT GGT TGG – 3′
Anti-thrombin DNA aptamer-2 (APT-2):
5′-NH_2-C_6-GGT TGG TGT GGT TGG AAA AAA AAA AAA AAA AGT CCG TGG TAG GGC AGG TTG GGG TGA CT-3′
Non-binding point mutant of DNA aptamer (APT-MM):
5′-NH_2-C_6-GGT AGG TGT GGT TGG-3′
Random DNA oligonucleotide (DNA ODN):
5′-NH_2-TCA-AAT-CAG-GTT-GCT-TA-3′

The stock solutions of the DNA APTs and random DNA ODN were prepared using fresh ultrapure triple distilled water and kept frozen. The diluted solutions of the APTs and DNA ODN were prepared with 5 mM Tris–HCl buffer containing 20 mM NaCl (TBS, pH 7.00).

Thrombin (THR), bovine serum albumin (BSA) and protein C (PC) were purchased as lyophilisates from Sigma-Aldrich (Germany). The stock solutions of proteins were prepared by dissolving the lyophilisates in fresh ultrapure triple-solutions. Diluted solutions of proteins were prepared in 50 mM phosphate buffer solution containing 20 mM NaCl (PBS, pH 7.40). Fetal bovine serum (FBS) was purchased from Sigma-Aldrich (Germany). Other chemicals were supplied from Sigma (USA) and Merck (Germany) in analytical reagent grade.

2.3. Carbon nanofibers enriched screen printed electrodes (CNF-SPEs)

Graphitized carbon nanofibers enriched screen printed electrodes (CNF-SPEs) were purchased from DropSens (Oviedo-Asturias, Spain). CNF-SPEs were designed for the development of (bio)sensors with an enhanced electrochemical active area. The planar screen-printed electrode 3.3 × 1.0 × 0.05 cm (length × width × height) consists of three main parts, which are graphitized carbon nanofiber modified graphite working electrode, a graphite counter electrode and a silver pseudo-reference electrode. The graphite working screen printed electrode surface is 4 mm in diameter.

A specific DropSens connector (ref. DSC) allows the connection of CNF-SPE to the potentiostat. The electrodes were pretreated by applying + 0.9 V for 60 s with 40 μL droplet of acetate buffer solution (ABS, pH 4.8).

2.4. Microscopic characterization of bare (unmodified) SPE and CNF-SPE by scanning electron microscopy (SEM)

The microscopic characterizations of bare SPE and CNF-SPE were obtained by Quanta 400 FEI, field emission scanning electron microscope (FE-SEM) (Tokyo, Japan) with 10.0 kV acceleration voltage with the resolution in various magnitudes; 500 nm, 1 μm, 2 μm and 4 μm.

2.5. DNA APT/DNA ODN immobilization onto the surface of CNF-SPE, THR-APT interaction at CNF-SPE surface and impedance measurements

EIS measurements were performed in the redox probe solution containing 2.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) prepared in 0.1 M KCl. The impedance was measured in the frequency range from 100 mHz to 100 KHz in a potential of + 0.23 V versus silver reference with a sinusoidal signal of 10 mV. The frequency interval was divided into 98 logarithmically equidistant measure points. The respective semicircle diameter corresponds to the charge-transfer resistance, R_{ct}, the values of which are calculated using the fitting program AUTOLAB 302 (FRA, version 4.9 Eco Chemie, The Netherlands).

EIS measurements were performed by placing a 40 μL drop of the corresponding solution onto the working area at the surface of pretreated CNF-SPE. Before and after the immobilization of various types of aptamers, or control oligonucleotides onto the surface of CNF-SPE, EIS measurements were performed by dropping 40 μL of redox probe onto the electrode surface.

For DNA APT/DNA ODN immobilization onto the electrode, the surface of CNF-SPE was covered by 40 μL of the indicated amount of aptamer, or control oligonucleotides and it was kept for 30 min. Thereby the formation of peptide bounds between carboxylic groups of the nanofibers and amine groups of aptamer, or DNA ODN was occurred at the electrode surface without using any chemicals (EDC, NHS) used for covalent attachment. Similar immobilization process for amino linked DNA APT specific for lysozyme was applied for its immobilization onto the surface of carboxylated multiwalled carbon nanotube modified SPE in earlier works [40,41]. Each electrode was then rinsed with TBS (pH 7.0) for 5 s to remove unbound aptamer/DNA ODN from the surface. Then, EIS measurements were performed as described above.

40 μL of the indicated concentration of THR or BSA was added on the surface of aptamer or oligonucleotide-modified electrode and incubated for 30 min. Subsequently, each electrode was rinsed with PBS (pH 7.4)