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Dendrimer enriched single-use aptasensor for impedimetric detection of activated protein C

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ABSTRACT

A novel impedimetric aptasensor for detection of human activated protein C (APC) was introduced for the first time in the present study. An enhanced sensor response was obtained using poly(amidoamine) (PAMAM) dendrimer having 16 succinamic acid surface groups (generation 2, G2-PS), that was modified onto the surface of screen printed graphite electrode (G2-PS/SPE). An amino modified DNA aptamer was then immobilized onto the surface of G2-PS modified SPE. The selective interaction of APT with its cognate protein, APC was investigated using different electrochemical techniques; differential pulse voltammetry (DPV), cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The microscopic characterization was consecutively performed before/after each modification/interaction step using scanning electron microscopy (SEM) and atomic force microscopy (AFM). The selectivity of aptasensor was tested in the presence of numerous proteins; protein C, thrombin, bovine serum albumin, factor Va and chromogenic substrate in different buffer mediums. The APC detection in the artificial serum; fetal bovine serum (FBS) was also performed impedimetrically. This dendrimer modified aptasensor technology brings several advantages: being single-use, fast screening with low-cost per measurement and resulting in sensitive detection of APC with the detection limits of 0.74 μg/mL (0.46 pmol in 35 μL sample) in buffer medium, and 2.03 μg/mL (1.27 pmol in 35 μL sample) in serum.

1. Introduction

The inhibition of overcoagulation, which causes thrombus formation, is controlled by several anticoagulant mechanisms; the one of these processes is protein C pathway. The protein C (PC) named as "zymogen protein" is a vitamin K-dependent plasma protein, and its genetic mild, or severe deficiencies are related to the risk for venous thrombosis or neonatal purpura fulminans, respectively [1]. Activated protein C (APC) which is the key enzyme of the protein C pathway, is a serine protease generated from zymogen protein C [1–4]. APC generation is accomplished by two steps; PC binds to the endothelial cell PC receptor (EPCR), then thrombin/thrombomodulin complex is formed by proteolytic activation of PC [2]. APC has cytoprotective, anti-inflammatory and anti-apoptotic properties which are related to protection of endothelial barrier function [1]. Additionally, it has been known that APC are directed by inter-connected relation with various plasmatic and cellular proteins [1,3]. Inherited deficiencies of PC, or other dysfunctions of the PC pathway; such as, APC resistance cause life-long effected diseases. Acquired PC deficiency contributes to the development of microvascular thrombosis in septicemia [3]. Moreover, the recombinant APC has been used as prospective therapeutic intervention for treatment of sepsis [5,6]. Thus, there is an urgent need for development of sensitive and selective detection platforms for recognition and monitoring of APC. Several reports for detection of APC have been introduced in the literature by using a conventional method, ELISA [7–9].

Aptamers termed as analogous to antibodies are nucleic acid-based molecules that could selectively bind to any molecule of choice [10–19]. Their synthesis and selection are done by using SELEX (Systematic Evolution of Ligands by EXponential enrichment) process. Improved stability properties including resistant to denaturation and degradation, easy modification, target versatility and easy-to-stock make them more advantageous beside to antibodies and ideal candidates as protein recognition elements in a wide range of bioassays and for diagnostic applications [13–25].

There has been recently a growing interest for detection of APC by using aptamer-based assays [15,16,25]. Müller et al. [15] introduced an exosite specific single stranded DNA (ssDNA) aptamer by using recombinant APC for the first time in the literature. In their study, an oligonucleotide-based enzyme capture assay was also introduced for the application of APC detection using this

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well-characterized ssDNA aptamer [16]. They asserted that this assay allows also the quantification of APC under routine clinical conditions.

After the discovery of electroactivity in nucleic acids [26], several studies related to the development of electrochemical nucleic acid biosensors have been performed related to the pharmaceutical, clinical, environmental, and forensic applications [12,18–32]. It was shown that they can provide rapid, simple, and low-cost point-of-care detection of analytes; nucleic acid sequences, or their interactions with proteins, toxins, pathogens or drugs.

Since there has been an increasing need for miniaturizing of the sensing platforms, different strategies have been progressed for development of DNA-chip technology based on nanomaterials. In the last decade, several nanomaterials have been introduced into biosensing area; such as nanoparticles [27–29], nanofibers [30], carbon nanotubes [31–42] and dendrimers [43–54].

Dendrimers are a relatively new class of nanosized polymers with unique architectural, structural and functional features. The structures of dendrimers comprise well defined cavities [50] and terminal groups [51] that effect physical properties of dendrimers; such as solubility [52], reactivity [53] and exoreceptor characteristics of the molecule [54]. Dendrimers can also be used to modify electrode surface by providing some advantages; their good biocompatibility and adequate functional groups to generate chemical bonds [53]. There have been various dendrimer applications for nano-biomedical applications such as bioimaging, drug and gene delivery, cancer diagnosis and (bio)sensors [43–54].

An impedimetric dendrimer-based DNA biosensor was developed by using second generation poly(amidoamine) dendrimer (G2-PAMAM) with multi-walled carbon nanotube (MWNT) at glassy carbon electrode surface for electrochemical monitoring of DNA hybridization by Zhu et al. [44]. The charge transfer resistance (R_{ct}) was consecutively measured by using EIS before and after single stranded DNA probe (ssDNA) immobilization and hybridization step between ssDNA probe and its complementary target, or non-complementary sequence.

Amino-terminated G4 PAMAM dendrimer (PDR) modified disposable pencil graphite electrodes (PDR-PGE) and PDR modified multiwalled carbon nanotube functionalized screen-printed graphite electrodes (PDR-MWCNT-SPE) were introduced with good sensitivity in the literature for the first time by Erdem et al. [43]. After the microscopic and electrochemical characterization of these SPE surfaces were explored, the electrochemical analysis of an anticancer drug, daunorubicin (DNR) was studied voltammetrically by using these disposable PDR-SPE sensor platforms. The impedimetric analysis of DNR was also performed successfully using PDR-MWCNT-SPE in the study of Erdem et al. [43].

To our best knowledge no report has been introduced yet in the literature for impedimetric recognition between DNA aptamer and its cognate protein, human activated protein C (APC) based on screen printed sensor platform (SPE) modified with carboxylated poly(amidoamine) PAMAM dendrimer (generation 2, G2-PS). The single-use screen printed graphite electrodes (SPE) was firstly modified by using carboxylated G2-PS in order to enhance sensor response. Then, an amino-linked DNA aptamer (DNA-APT) was immobilized onto the surface of G2-PS modified SPE via covalent coupling between the carboxyl groups of dendrimer, G2-PS and the amino groups of DNA aptamer. The experimental conditions, such as G2-PS, APT, APC concentration and APC–APT interaction time were accordingly optimized. The selectivity of aptasensor was also tested in the presence of several proteins; protein C (PC), thrombin (THR), bovine serum albumin (BSA), factor Va (FVa) and chromogenic substrate (KS) in two different buffer media. The APC detection in the artificial serum, fetal bovine serum (FBS) was finally explored by using impedimetric aptasensor under the optimized experimental conditions.

2. Experimental

2.1. Apparatus

All experimental measurements were carried out using AUTOLAB–PGSTAT 302 electrochemical analysis system supplied with a FRA 2.0 module for impedance measurements, and GPES 4.9 software package (Eco Chemie, The Netherlands). For electrochemical measurements, differential pulse voltammetry (DPV), cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were used. All measurements were performed in the Faraday cage (Eco Chemie, The Netherlands).

The planar screen-printed electrode 3.4 cm × 1.0 cm × 0.05 cm (length × width × height) consists of three main parts; a 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. These disposable screen printed electrodes were commercially purchased from DropSens (Oviedo-Asturias, Spain).

A specific DropSens connector (ref. DSC) allows the connection of the SPEs to the potentiostat. All measurements on SPEs were performed by placing a 35 μL drop of the corresponding solution to the working area.

2.2. Chemicals

The amino-linked single stranded APC specific DNA aptamer (DNA-APT), random DNA APT and oligodeoxynucleotide (random DNA) were purchased from TIBMOLBIOL (Berlin, Germany). The ssDNA-APT was chosen according to related information given in the literature [15,16]. Activated protein C (APC) was purchased from Haemtech. The second generation poly(amidoamine) (PAMAM) dendrimer with 1,4-diaminobutane core (G2-PS) which has 16 succinamic acid surface groups, fetal bovine serum (FBS) as the artificial serum, protein C (PC), factor Va (FVa), chromogenic substrate (KS), bovine serum albumin (BSA), thrombin (THR) were purchased from Sigma.

DNA APT

5′-NH_2-C_6-GCC TCC TAA CTG AGC TGT ACT CGA CTT ATC CCG GAT GGG GCT CTT AGG AGG C-3′.

Random DNA APT

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′.

Random DNA

5′-NH_2-C_6-CAAA GAA GTG GCA GGA AGA GTC GAA GGT CTT GTT GTC ATT GCT GCA CAC CTT-3′.

2.2.1. The preparation of G2-PS

The frozen stock solution of G2-PS (10^6 μg/mL) was diluted in phosphate buffer solution containing 20 mM NaCl (PBS, pH 7.4).

2.2.2. The preparation of DNA-APT, random DNA APT and random DNA

The DNA aptamers and random DNA stock solutions (500 μg/mL) were prepared in ultrapure water and kept frozen. More diluted solutions of DNA APT were prepared in PBS (pH 7.4), or Tris-buffer solution (TBS, 10 mM Tris–HCl, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4) containing 1 mg/mL BSA according to the related information given in the literature [15]. The diluted solutions of random APT and random DNA were prepared in TBS (pH 7.4) containing 1 mg/mL BSA.