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Ultrasensitive and reusable electrochemical aptasensor for detection of tryptophan using of $[Fe(bpy)_3](p\text{-}CH_3C_6H_4SO_2)_2$ as an electroactive indicator

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ABSTRACT

In this paper, we report the application of a reusable electrochemical aptasensor for detection of tryptophan by using $[Fe(bpy)_3](p\text{-}CH_3C_6H_4SO_2)_2$ as an electroactive indicator and based on the target-compelled aptamer displacement. The aptasensor fabricated by self-assembling the thiolated probe on the surface of graphite screen-printed electrode modified with gold nanoparticles/multiwalled carbon nanotubes and chitosan nanocomposite (AuNPs/MWCNTs-Chit/SPE). Afterward, Trp aptamer (Apt) immobilized on the modified electrode surface through hybridization. In the absence of Trp, a sharp peak of $[Fe(bpy)_3](p\text{-}CH_3C_6H_4\ SO_2)_2$ can be observed in differential pulse voltammetry (DPV) study. The introduction of Trp led to the formation of aptamer-Trp complex and dissociation of the aptamer from the DNA-Apt duplex on the electrode surface into the solution and decreases the peak current intensity of electroactive indicator. This is because, $[Fe(bpy)_3](p\text{-}CH_3C_6H_4SO_2)_2$ tends to bind to the two strands DNA. Therefore, the peak current of $[Fe(bpy)_3](p\text{-}CH_3C_6H_4\ SO_2)_2$ linearly decreased with increasing the concentration of Trp over a range of 3.0 nM- 100 μM. The detection limit (3 σ) was 1.0 nM. In addition, we examined the selectivity of the constructed biosensor for tyrosine, histidine, arginine, lysine, valine and methionine that belonged to the amino acid family.

The obtained results showed that the fabricated sensor had a good selectivity for Trp against the other examined amino acids. Also, the potential applicability of the aptasensor was investigated by detecting the Trp in a complex media such as human blood plasma spiked with Trp.

1. Introduction

Diagnosis and quantitative determination of amino acids play an essential role in biomedical studies, pharmaceutical industries and clinical applications [1,2]. Tryptophan (Trp) is a required amino acid in humans and animals for brain functions and a precursor for some hormones and vitamins) serotonin, melatonin and niacin) [3]. The concentration of TRP in human blood is very important, because it is a probable cause of many diseases [4]. Therefore, development of a highly sensitive and selective technique for recognition and determination of TRP is so important.

In recent years, affinity biosensors have attracted much consideration and there has been great interest in the development of biosensors that use aptamers as the biorecognition element (aptasensors) [5]. Aptamers are artificial single-stranded oligonucleotides, which are selected from random RNA or DNA libraries for the first time in 1990 by SELEX[1] [6,7]. Since their discovery, aptamers have attracted considerable attention in biosensor development [8–11].

Numerous aptasensor have been developed using different analytical methods, including optical [12], electrochemical techniques [13,14], fluorimetry [15], quartz crystal microbalance (QCM) [16] and colorimetry [17]. Among various aptamer-based biosensors, electrochemical aptasensors have received much attention due to distinct benefits such as good excellent sensitivity and selectivity for detection of various targets, portability, low production cost and fast response [5].

A considerably improved performance for aptasensors can be obtained by modifying the electrode surface with different nano

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[1] systematic evolution of ligands by exponential enrichment

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particles [18–20]. Among the various nanomaterials, a carbon nanotube (CNT) is highly regarded due to exclusive properties such as high mechanical resistance, conducting, good biocompatibility and high specific surface area [21,22]. Furthermore, we used chitosan, a biocompatible and nontoxic polymer, for the preparation of stable nanocomposite. As a result, through noncovalent binding, multiwalled CNTs (MWCNTs) can be uniformly dispersed in a chitosan film and leads to a homogeneous black solution [23]. Then, gold nanoparticles (AuNPs) were electrodeposited on the MWCNTs- Chit modified screen printed electrode (SPE) for attachment of the thiolated probe. Also, in this work, $[Fe(bpy)_3](p\text{-}CH_3C_6H_4SO_2)_2$ was used as an electrochemical indicator and a reporter for the detection events. Transition metal complexes and pyridine derivatives have important biological activities as the part of some vitamins, enzymes, proteins and also have been widely applied in cancer therapy [24]. Commonly transition metal complexes can interact with nucleic acid by intercalation or groove-binding.

In the present study, we report a simple, label-free and reusable electrochemical aptasensor for the determination of Trp based on the target-compelled strand displacement mechanism from DNA/apt duplex to apt/target complex. The fabrication of reusable Trp aptasensor was based on the self-assembly of thiolated DNA (part of the complementary strand of aptamer) onto the AuNps/MWCNTs-Chit/SPE and then, immobilization of the aptamer on the surface via its hybridization with the thiolated DNA. Upon Trp binding, aptamer was taken off from the electrode due to the formation of a complex between the aptamer and Trp, and part complementary strands remains at the modified electrode surface. Thus with this technique, a reusable sensing system will be provided [25]. As mentioned above, transition metal complexes tend to interact with nucleic acid by intercalation, so with dissociation of the Trp/aptamer complex from the electrode surface, the peak current of $[Fe(bpy)_3](p\text{-}CH_3C_6H_4SO_2)_2$ decreased.

The main advantages and novelties of this work are: a) making a reusable sensor for detection and determination of Trp; b) construction of AuNps/MWCNTs -Chit/SPE with large specific surface area and also high conductivity; c) using $[Fe(bpy)_3](p\text{-}CH_3C_6H_4SO_2)_2$ as an electroactive indicator for determination of Trp and d) $[Fe(bpy)_3](p\text{-}CH_3C_6H_4SO_2)_2$/DNA-Apt/AuNps/MWCNTs-Chit/SPE was used for the first time to detect Trp, which shows a high sensitivity and specificity.

2. Materials and methods

2.1. Reagents and instruments

Trp aptamer and partly complementary DNA strand with 5'-thiol modification was purified with HPLC and obtained from Bioneer Co. (South Korea). The sequence of the selected aptamer for Trp and partly complementary strand DNA was as follows [26]:

Aptamer sequence: 5≠AGCACGTTGGTTAGGTCAGGTTT-GGGTTTCGTGC-3′.

DNA strand: 5′-SH-$(CH_2)_6$-GCACGAAACCCAAACCTG-3′.

All chemicals were of analytical grade and supplied from Aldrich or Merck Company. A 0.1 mM stock solution of aptamer was prepared by 0.05 M Tris-HCl, 1.0 mM EDTA (Tris-EDTA buffer, pH 8.0) and afterwards the prepared solution was kept at −20 °C. All solutions were prepared using ultrapure water.

Graphite screen-printed electrodes (GSPEs), consisted of the carbon working electrode, carbon counter electrode and silver pseudo reference electrode were bought from Florence, Italy.

Autolab PGstut 30 electrochemical analysis system controlled by GPES 4.9 and FRA software (Eco Chemie Netherlands) was used for all voltammetry and electrochemical impedance spectrometry (EIS) measurements. Also in each step of electrode modification, scanning electron microscopy images were obtained by Mira 3-XMU Field Emission SEM (FESEM).

2.2. Fabrication of the electrochemical Trp aptasensor

For the preparation of MWCNT-Chit nano composite, Chit (5.0 mg) was dissolved in 1.0 mL 1% acetic acid. Then, MWNTs (2.0 mg) was dissolved in prepared Chit solution and sonicated until a homogeneous mixture was obtained [27]. After the preparation of nanocomposite, the surface of the working electrode was modified with 7.0 μL of nano composite and allowed to dry at room temperature. Then, the synthesis of AuNPs was done by chronoamperometry at -0.23 V for 300 s in a solution containing 0.5 mM $HAuCl_4$ for the formation of gold nanoparticles on the surface of MWCNT-Chit/SPE. Subsequently, 8.0 μL of 2.0 μM thiolated DNA solution was placed on the AuNps/MWCNT-Chit/SPE for 6.0 h at room temperature. In the next step, 8.0 μL of 2.0 μM 34-bases Trp aptamer was immobilized on the surface of the prepared electrode through DNA hybridization reaction at room temperature for construction of DNA-Apt/AuNps/MWCNTs-Chit/SPE. After 100 min, 8.0 μL of (5 μM) $[Fe(bpy)_3](p\text{-}CH_3C_6H_4SO_2)_2$ was added onto the modified electrode surface for 20 min. After these processes, for determination of Trp, 8.0 μL of Trp solutions with different concentrations was dropped onto the modified electrode surface for 45 min. After each step, the surface of modified electrode was rinsed with double distilled water. At last, the peak current of $[Fe(bpy)_3](p\text{-}CH_3C_6H_4\ SO_2)_2$ was used to evaluate the Trp concentration. Scheme 1 illustrates the step by step fabrication procedure and the sensing mechanism of aptasensor.

3. Results

3.1. Optimization of effective parameters on aptasensor response

We optimized some of the experimental parameters such as hybridization and incubation time of the aptasensor for the analysis of the Trp. The effect of the hybridization time of DNA on the response of the aptasensor was optimized by EIS method. Fig. S1A shows the dependence of the R_{ct} to the hybridization time of the DNA. As seen, R_{ct} of the aptasensor obviously increased with increasing the hybridization time from 10 to 100 min and then starts to level off. Therefore, the optimum hybridization time between DNA and aptamer was found to be 100 min.

Furthermore, the effect of Trp incubation time on the response of the fabricated aptasensor was studied from 0 to 60 min (Fig. S1B). The interaction time includes both the interaction time of the Trp with the aptamer and the departure time of the aptamer-Trp complex from the electrode surface. As shown in Fig. S1B, with increasing the interaction time from 5 min to 45 min, R_{ct} decreased and reaches a minimum at 45 min but no significant change in R_{ct} was observed over 45 min. Thus, according to the obtained results, 45 min was chosen as the optimal incubation time. After 45 min hybridization, the sensing interface was approximately regenerated and can be reused.

3.2. Morphology study of the modified electrode

The morphology of modified SPE was investigated by FE-SEM images at each step. Fig. 1 (A–C) represents the FE-SEM images of the bare SPE, MWCNT-Chit/SPE and AuNPs/ MWCNT-Chit/SPE. As can be seen, Fig. 1B shows the forming of homogeneous and spaghetti-like porous MWCNT- Chit nanocomposites on the surface of the SPE. The obtained nanocomposite has good practicality, because of the high specific surface area and porous structure of the nanocomposite. After electrodeposition, the gold nanoparticles with sizes ranging from 21 nm to 28 nm were well distributed and