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Reusable potentiometric screen-printed sensor and label-free aptasensor with pseudo-reference electrode for determination of tryptophan in the presence of tyrosine

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ABSTRACT

Analysis of L-tryptophan (Trp) in biological samples has great importance for biomedical studies. Amino acid tyrosine (Tyr) that usually coexist with Trp in biological fluids can significantly interfere with reliable determination of Trp. In the current study, we demonstrate the development of two ultra-sensitive electrochemical sensor and label-free aptasensor for selective analysis of Trp in biological samples (i.e., cow's milk and human plasma, saliva and urine samples). In addition, without using AgCl/KCl, an Ag pseudo-reference screen printed electrode (Ag-PR-SPE) was exploited as a reference electrode. To prepare the engineered Trp sensor/aptasensor, a gold SPE was first modified with multiwall carbon nanotube (MWCNT-AuSPE) and then armed with Trp aptamer molecules (Apt-MWCNT-AuSPE). The prepared sensors were characterized using constant current-potentiometric stripping analysis (CC-PSA) and electrochemical impedance spectroscopy (EIS). The MWCNT-AuSPE and Apt-MWCNT-AuSPE were compared with respect to the linear detection range, limit of detection (LOD), accuracy, precision, repeatability. MWCNT-AuSPE and Apt-MWCNT-AuSPE demonstrate fast near-Nernstian response for PSA of Trp over the concentration ranging from 1.0×10^{-9} to 2.0×10^{-4} mol L^{-1} and 1.0×10^{-11} to 1.0×10^{-4} mol L^{-1} with detection limits of 3.6×10^{-10} mol L^{-1} and 4.9×10^{-12} mol L^{-1}, respectively. Common interfering species present in the biological fluids (i.e., tyrosine, uric acid, ascorbic acid) showed no effects on the determination of Trp using CC-PSA. MWCNT-AuSPE and Apt-MWCNT-AuSPE represented well reproducibility and great precision with relative standard deviation (RSD) of 2.9% and 5.3% respectively. In comparison with the MWCNT-AuSPE, Apt-MWCNT-AuSPE provided higher sensitivity, selectivity and accuracy of Trp detection in real samples. Based on these findings, we propose the developed Apt-MWCNT-AuSPE as a simple detection method for analysis of Trp in biological samples.

1. Introduction

L-tryptophan (Trp) is one of the 22 standard amino acids that are essential to human life. This amino acid plays critical actions in healthy and diseased conditions; hence, detection of this important amino acid in biological fluids (blood, urine, saliva, extracellar matrix, and celeberospinal fluids, etc.) has great diagnostic importance in clinical studies and also great significance in basic medical studies particularly in neuroscience and oncology [1]. So far a variety of analytical methods such as high performance liquid chromatography (HPLC), fluorometric analysis, capillary electrophoresis (CE) and chemiluminescence (CL) approaches as well as electrochemical assays have been developed for determination of Trp [2–14] content of biological fluids; however, a miniturized electrochemical biosensor for ultraselective, ultrasensitive and label-free determination of Trp in such fluids has not been developed yet. In addition, such biosensors demonstrate potential capability to be integrated with lab-on-a-chip (LOC) systems, microfluidics, and Micro Total Analysis Systems (μTAS) for point of care testing (POCT) applications. Selection of proper miniturized platform for three electrode system (i.g., screen printed electrode), reliable miniatured reference electrode (i.g., pseudo reference electrodes) and bio-recognition element (i.g., aptamer, antibody, ect.) for modification of miniturized working electrode in selective determination of Trp are main issuses that need to be addressed. Pseudo reference electrodes (PRE) are practical aletrnative to conventinal reference electrodes in developing miniturized electrochemical systems. However, in many cases normally simple metal wires of platinum or gold or silver

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serve as pseudo- or quasi-reference electrodes [15–17]. These electrodes offer advantages such as simplicity of use, minimum ohmic resistance and contamination with test solutions without liquid junction potential [18].

Technically, conventional three electrode-driven development of miniature, inexpensive, and disposable biosensors capable of analyzing pretreatment-free biological samples has been possible by using screen printed electrodes (SPE) in recent years. In general, screen-printing technology is used to scale down the Ag/AgCl reference electrode, taking the advantage of commercial or homemade printing pastes containing silver and/or silver chloride to obtain layers of these materials [19–22]. For potentiometric applications the key issue is to achieve supply of electrolyte (KCl) to stabilize potential over the electrode life; however, for electrochemical techniques based on recording current usually the presence of Ag or Ag/AgCl layers is sufficient for ensuring significant potential stability [23]. Howeve, so far a potentiometric study of pseudo- or quasi-reference electrodes on screen printed electrodes has not been undertaken.

Recently aptamers, synthetic DNA or RNA sequences that are selected through SELEX (systematic evolution of ligands by exponential enrichment) processes from a random sequence bank [24], have attracted much more attention as bio-recognition elements because of their higher affinity, stability and specific binding to the ligand. Especially aptamers can be successfully selected to detect small molecules [25], whereas other recognition elements hardly recognize small molecules with adequate affinity [26–31].

Among advanced nanomaterials used for the modification of electrode surface, carbon nanotubes (CNTs) especially MWCNTs, discovered in 1991 [32], consisted of multiple rolled layers of grapheme, possess individual characteristics due to their porous and large surface area, excellent electrical conductivity, good chemical stability, interfacial adsorption properties, enhanced electrocatalytic activity and excellent mechanical properties, significantly reducing the modification steps of biosensor preparation [33]. In addition, electrodes modified with MWCNTs exhibits antifouling effects and large surface area to increase the number of aptamer mounted on the electrode surface [34].

The PSA operation obviates the need for oxygen removal, offers low background contributions, and minimizes interferences [35]. There are a few reports on the use of CC-PSA coupled to an integrated screen-printed three-electrode system. Kadara et al. carried out their PSA experiments with gold (Au) SPE in which potassiem chloride (KCl) was used for assemly of reference electrode [36]. Chloride mediums were selected because of the chloride concentration dependency of the silver/silver chloride (Ag/AgCl) reference electrode on the three-electrode integrated strip. Novel methods of immobilizing the bio-recognition elements of the biosensors have been emphasized and it is clear that researchers are designing simpler methods of manufacture.It is clear that researchers are designing simpler methods of manufacture.

In the present paper, we domonstrate the development of a novel aptamer-modified SPE that concurrently benefit from the advantages of exploiting multiwall carbon nanotubuse (MWCNs) and constant current potentiometry stripping analysis (CC-PSA) by the Ag pseudo-reference screen printed electrode (Ag-PR-SPE) rather than using conventional Hg_2Cl_2/Hg, 3 mol L^{-1} KCl (SCE) electrode in Trp determination. From electro-analytical point of view, broader linear dynamic range of analysis is obtained with PSA, which is a modified form of potentiometry. This means analyzing over a broad range concentration without the need for deluting orginal biological samples. In this work for first time, we aimed to develope pseudo-reference electrode-based screen printed sensing platforms and to apply them for ultra-sensitive, ultra-selective, label free and unexpensive determination of Trp in complex biological samples, which can offer several advantages over the conventional electrodes such as simplicity of mass production, size tunibility.

2. Experimental

2.1. Chemicals

The sequence of RNA aptamer for Trp has previously been reported by Majerfeld et al. [37]. It was synthesized by MWG Co (Ebersberg, Germany) and its sequence is that as follows:

5′GGGAUCCUAAGCGACGAAGUUGAGGACCGGUACGGCCGCCACUCAGUAUCUACGCAUCGGA3′. The randomly selected sequence, non-functional control RNA which is similar to the original aptamer only in nucleotide content, is as follows:

5′CGACGCGCAACAACAUCUCGGCGUCCCGCGGUUCGUUGAACCGACUACAACGUUACCCCGA3′.

Trp, $K_4[Fe(CN)_6]$, $K_3[Fe(CN)_6]$, K_2HPO_4 and KH_2PO_4 were procured from Merck Co., (Darmstadt, Germany). Different amino acids, carboxylated multiwall carbon nanotubes (MWCNTs) (purity > 80%, average diameter 9.5 nm, length ~ 1.5 μm, carboxylate (COOH) content > 8.00 wt%) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Aminoven™ 10%, which contains the mixture of essential and non-essential amino acids as a commercially available pharmaceutical formulation for parenteral nutrition of parients) was prepared from local drug store (Tofigi) of Sina hospital in Tabriz, Iran. 1000 ml of Aminoven 10% contain: Isoleucine 5.00 g, Leucine 7.40 g, Lysine acetate 9.31 g = Lysine 6.60 g, Methionine 4.30 g, Phenylalanine 5.10 g, Threonine 4.40 g, Tryptophan 2.00 g, Valine 6.20 g, Arginine 12.00 g, Histidine 3.00 g, Alanine 14.00 g, Glycine 11.00 g, Proline 11.20 g, Serine 6.50 g, Tyrosine 0.40 g, Taurine 1.00 g. Diethylpyrocarbonate (DEPC) for preparation of aptamer solution was prepared from Sinagen Co. (Tehran, Iran). All solutions were prepared using bidistilled water obtained from Merck Millipore (Darmstadt, Germany). All chemicals not listed were of the analytical grade and used as received without any further purification.

2.2. Apparatus

All electrochemical measurments other than electrochemical impedance spectroscopy (EIS) analyses were performed using Autolab PGSTAT30 Potentiostat/Galvanostat equipped with GPES 4.7 software (Eco-Chemie, Utrecht, Netherland). The EIS measurements were also carried out using AutoLab PGSTAT302N (Metrohm Co., Schiedam, Netherlands) equipped with frequency response analyzer (FRA) module and Nova 1.8 software. Commercial screen printed electrodes (Metrohm Co., Schiedam, Netherland, code: 6.1208.210) comprising a working electrode (gold, diameter of 4 mm) and carbon-based counter electrode and silver pseudo-reference electrode. The connection of SPEs to the autolab is provided by a specific homemade connector. All laboratory experiments were conducted at ambient condition (25 °C degree of celcius) and measurements on SPEs were performed by placing a 50 μl drop of the corresponding solution to the working area. Some measurements which were carried using Au electrode (φ = 3 mm) together with platinum wire counter and Hg_2Cl_2/Hg (3 mol L^{-1} KCl) reference electrodes (SCE) (Metrohm Co., Schiedam, Netherland) were performed in Metrohm's 10 ml electrochemical cell. Open circuit potential (OCP) measurements were carried using conventional AgAgcl (3 mol L^{-1} KCl) or Ag-PR-SPE as working electrode together with (SCE) as a reference electrodes.