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Fabrication of an ultrasensitive impedimetric buprenorphine hydrochloride biosensor from computational and experimental angles

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ABSTRACT

For the first time, an ultrasensitive impedimetric buprenorphine hydrochloride (BN) biosensor based on immobilization of bovine serum albumin (BSA) onto multi-walled carbon nanotubes (MWCNTs)/glassy carbon electrode (BSA/MWCNTs/GCE) has been developed using initial characterization by computational methods and complementing them by experimental observations. Computational results showed that the BSA hydrophobically binds to MWCNTs which is energetically favorable and leads to spontaneous formation of the stable BSA/MWCNTs nanobiocomposite (bioconjugate). Computational results also showed that the interaction of BN with BSA is mainly driven by hydrophobic interactions. The interactions of BSA with MWCNTs and BN with BSA were also monitored by fluorescence and UV–vis spectroscopic techniques, and their results were consistent with the computational results. Morphology and electrochemical properties of the fabricated composite electrodes were examined by scanning electron microscopy (SEM), cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS). Besides complementing the computational studies, experimental results showed that the addition of MWCNTs to the surface of the GCE greatly facilitated the electron transfer reactions, and also showed that the presence of BSA inhibits the interfacial electron transfer in some extent due to the non-conductive properties of BSA. On the other hand, the presence of BN may form an electroactive complex with BSA which accelerates the interfacial electron transfer and leads to obvious Faradaic impedance changes. The Faradaic impedance responses were linearly related to BN concentration between 5.0 nM and 72.0 nM and a limit of detection (LOD, $3S_b/b$) of 1.5 nM was achieved. Finally, the proposed biosensor was successfully applied to determination of BN in urine samples of both healthy and addict volunteers. The results were satisfactory and comparable to those obtained by applying the reference method based on high performance liquid chromatography-ultraviolet detection (HPLC-UV). It is expected that the distinctive features of BSA/MWCNTs nanobiocomposite would make it potentially advantageous for a broad range of biosensing, and clinical applications.

1. Introduction

Buprenorphine (BN, Fig. 1A) is a strong semi-synthetic opiate painkiller with a sovereignty of 20–40 times higher than that of morphine [1]. It is commonly used at higher doses for treating opioid addiction and at lower doses for controlling both moderate to acute pains in non-opioid-tolerant individuals and moderate chronic pains [1]. BN causes three major effects of diminished respiration, gladness, and reduced pain. In fact, at high doses and under certain circumstances, BN not only blocks the effects of full opioid agonists but also precipitates withdrawal symptoms [2]. As an analgesic, it has been successfully used via intramuscular,

Abbreviations: BSA, bovine serum albumin; HSA, human serum albumin; BN, buprenorphine hydrochloride; MWCNTs, multi-walled carbon nanotubes; GCE, glassy carbon electrode; SEM, scanning electron microscopy; CV, cyclic voltammetry; EIS, electrochemical impedance spectroscopy; LOD, limit of detection; UPW, ultrapure water; SCE, saturated calomel electrode; MVD, molegro virtual docker; PBS, phosphate buffered solution; RSD, relative standard deviation; HPLC-UV, high performance liquid chromatography-ultraviolet; DMF, dimethylformamide

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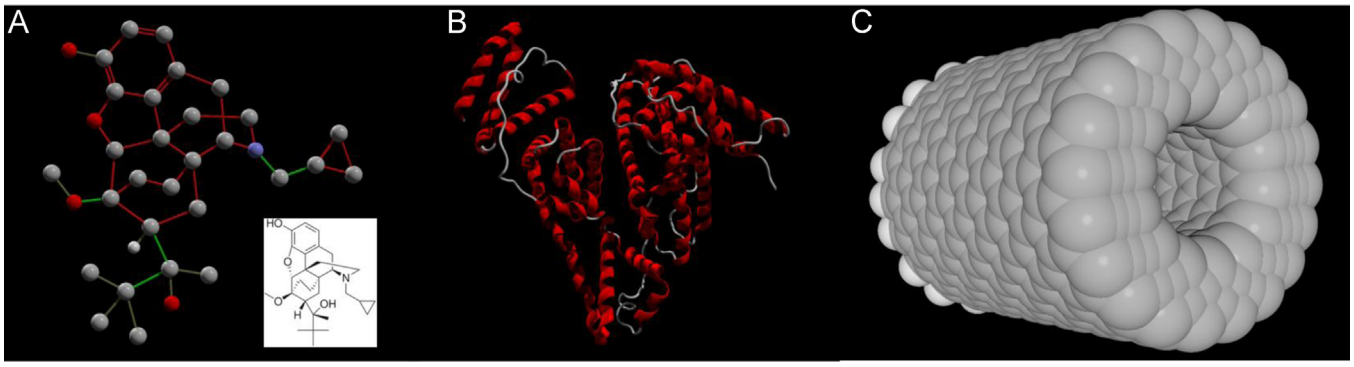

Fig. 1. (A) Molecular structure of BN represented by the ball and stick model, (B) three-dimensional structure of BSA (chain A) represented by solid ribbons, and (C) structure of the MWCNTs designed by Nanotube Modeler software and represented by the CPK model.

intravenous, or sublingual paths for appeasing moderate to severe and chronic pains [3]. Like other opiates, it has been reportedly abused [4] as in the doping of racehorses [5]. Therefore, the matrices in which BN could be defined are very different, especially in biological samples.

Different methods have been reported for the determination of BN, including adsorptive stripping voltammetry [6], gas chromatography–mass spectrometry [7–10], radioimmunoassay [5], and high performance liquid chromatography (HPLC) [11–17]. As a powerful technique for the determination of pharmaceutical compounds in biological fluids, electrochemical techniques can be considered as a suitable and sensitive alternative to other instrumental methods [18–22]. Among the electrochemical methods, electrochemical impedance spectroscopy (EIS) technique becomes increasingly popular because it offers several advantages such as simplicity, high sensitivity and serving as an elegant way to interface biorecognition events and signal transduction.

Among biomacromolecules, serum albumin is a soluble protein, which is a major constituent of the circulatory system, and it commonly serves as a depository and a transport molecule for many exogenous compounds. Bovine serum albumin (BSA, Fig. 1B) is one of the most extensively studied of this group of proteins, particularly because of its structural homology with human serum albumin (HSA). The direct electron transfer between proteins and the electrode surface has received considerable attention, because it can be used in fabricating new generation of biosensor devices. Since redox centers are deeply buried within the protein molecules, the electron transfer between proteins and the electrode is difficult. Therefore, many efforts have been devoted to immobilizing proteins on the electrode surface modified with various films. With good conductance, electrocatalytic properties and chemical stability, carbon nanotubes (CNTs) have been investigated extensively [23–25].

The objective of this study is to develop a novel, ultrasensitive, selective and simple impedimetric BN biosensor with good stability, reproducibility and repeatability for determination of BN using unique properties of BSA/MWCNTs nanobiocomposite (bioconjugate) which can be directly applied in urine samples without expensive and time-consuming pretreatments. To demonstrate the general design of the BSA/MWCNTs nanocomposite based biosensing platform, we first used computational studies for a proof of concept and then completed them by experimental observations. This valuable study describes the results obtained by a combination of computational and experimental techniques to develop an ultrasensitive BSA/MWCNTs/GCE biosensor for direct determination of BN in urine samples of both healthy and addict volunteers. To the best of our knowledge, there are only two works in the literature related to the determination of BN using electrochemical methods [6,26], and this work is the first report on the impedimetric BN biosensor.

2. Experimental

2.1. Chemicals and solutions

The BSA (M=66487, free of fatty acids with an electrophoresis grade), BN, and MWCNTs (purity > 95%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents employed were of analytical grade and received from Merck. A concentration of 0.067 M phosphate buffer solution (PBS, prepared from NaH_2PO_4 and Na_2HPO_4) was used to control the pH at 7.4. $[Fe(CN)_6]^{3-/4-}$ solution (redox probe, 5.0 mM) was prepared in PBS (0.067 M, pH 7.4) and used for the measurements. A stock standard solution of BN was prepared in methanol with a concentration level of 0.01 M, and was stored in a freezer at −20 °C. Working solutions were prepared by appropriate dilution of the stock standard solution. A blank urine sample (drug-free) was collected from a healthy volunteer and an actual urine sample was obtained from a Medical Diagnostic Laboratory in Kermanshah, Iran, and stored at −20 °C prior to use. All solutions were prepared by ultrapure water (UPW).

2.2. Instruments and softwares

Electrochemical experiments were performed using a μ-AutolabIII/FRA2 controlled by the Nova software (Version 1.8). A conventional three-electrode cell was used with a saturated calomel electrode (SCE) as a reference electrode, a Pt wire as a counter-electrode and a bare or modified GCE as a working electrode. The EIS measurements were performed in the redox probe solution and plotted in the form of complex plane diagrams (Nyquist plots). The SEM experiments were performed by a KYKY-EM 3200 scanning electron microscope. All fluorescence spectra were measured using a Cary Eclipse fluorescence spectrophotometer equipped with a water bath and a 1.0 cm quartz cell. The UV–vis spectra were measured using an Agilent 8453 UV–vis Diode-Array spectrophotometer controlled by the Agilent UV–vis ChemStation software. A JENWAY-3345 pH-meter equipped with a combined glass electrode was used for pH measurements. High performance liquid chromatography-ultraviolet detection (HPLC-UV) analyses reported in this study were carried out in a Medical Diagnostic Laboratory in Kermanshah, Iran whose instrument was an 1100 Series HPLC from Agilent Technologies (Wilmington, DE, USA) equipped with a binary pump, degasser, auto-sampler, solvent tray and multiple wavelength detector. The statistical analysis of the sequence of BSA (chain A) was performed using CLC Main Workbench software (Version 6.0). The three-dimensional structure