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Determination of lidocaine based on electrocatalysis of a chemically modified electrode

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The potential application of biochemically modified electrodes was investigated for construction of a drug biosensor. The electrocatalytic response of lidocaine was examined through modified electrodes using the electron transfer ability of hemoglobin (Hb) immobilized on gold film modified with a self-assembled monolayer (SAM) of 3-mercaptopropionic acid (MPA). According to the results of voltammetric studies, Au/MPA/Hb electrodes exhibited high sensitivity and good electrocatalytic activity for the reduction of lidocaine, motivating us to construct drug biosensors. With this study, a new electroanalytical procedure is proposed for the determination of lidocaine level in medical applications or therapeutic formulations containing lidocaine as the active ingredient.

Key Words: Lidocaine, hemoglobin, chemically modified electrodes, drug biosensor, electrochemistry

Introduction

Local anesthetics work in general by binding to sodium channel receptors inside the nerve, and thereby preventing depolarization of nerve cells by binding to cell membrane voltage-dependent sodium channels and inhibiting the passage of sodium ions, thus blocking the action potential and neural conduction.[1] Local anesthetic molecules are composed of 3 parts: an aromatic group, an intermediate ester or amide chain, and a secondary or tertiary amino terminus.[2] The aromatic portion of the molecule is responsible for the lipophilic or more accurately hydrophobic properties of the molecule, whereas the amine end confers water solubility. Lipid solubility is essential for the penetration of the nerve. The greater lipid solubility enhances diffusion through

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nerve sheaths, as well as the neural membranes of axons.[3] The intermediate chain provides the separation between the hydrophilic and hydrophobic ends of the molecule. Activity of local anesthetics depends on their lipid solubility, diffusibility, affinity for protein binding, percent ionization at physiological pH approximately, and vasodilating properties. Local anesthetics reversibly block nerve conduction when applied to nerve tissue at appropriate concentrations.[4] However, the toxicity of these drugs seems to constitute the main obstacle to their medical uses. Many side effects, particularly cardiovascular and neurological, may occur in the case of increasing toxicity. Lidocaine is one of the most commonly used local anesthetics and peripheral analgesics in surgery,[5] gynecology,[6] and dentistry.[7] It may have some serious side effects on cardiovascular systems, like ventricular arrhythmia in the case of overdosed intake. In adequate concentrations of 0.5%-2%, lidocaine produces a rapid onset of intense motor and sensory nerve blockade.[8] Higher concentrations were used for spinal anesthesia, until reports of transient radicular irritation suggested that these concentrations may be also neurotoxic.[9] This is because the quantitative determination of local anesthetics in blood and other biological materials is obviously around the considerable point in assessment of toxicity, metabolism, and distribution of these drugs following various routes of administration.[10,11]

Various approaches, such as flow-injection analysis,[12] high-performance liquid chromatography (HPLC),[13,14] and gas chromatography (GC) combined with mass spectrophotometric[15] and ultraviolet (UV) spectroscopic detectors,[16] have been developed for the determination of anesthetic drugs and their metabolites in pharmaceutical preparations as well as in biological samples. Although these methods are specific and highly sensitive, they require complex controlling systems for temperature and vacuum that makes the methods expensive. Separation processes and sample preparation are also difficult in these systems, so that electrochemical methods have been found to be cheaper/faster alternative techniques.[17] So far, a few applications of lidocaine determination in electrochemistry have been reported. Kulapina and Barinova developed ion selective electrodes sensitive towards a group of nitrogen-containing drugs, including lidocaine.[18,19] They used tetraphenyl borate (TPB) as the counterion in electrode-active components of ion selective electrodes for the potentiometric determination of drugs. Halbert and Baldwin used glassy carbon electrodes for the electrochemical detection of lidocaine and its metabolites in blood serum after separation by liquid chromatography.[20] Sun and colleagues developed a method for simultaneous determination of lidocaine, proline, and lomefloxacin in urine samples by capillary electrophoresis-electrochemiluminescence detection with $Ru(bpy)_3^{2+}$.[21] Under optimized conditions, satisfactory results such as high sensitivity, good linearity, and reproducibility were obtained.[21] Solid-state electrochemiluminescence detectors that employ tris(2,2′-bipyridyl)ruthenium(II) ($Ru(bpy)_3^{2+}$)-Zirconia–Nafion composite modified glassy carbon disk electrodes and microchips have been used to detect pharmaceuticals such as tramadol, lidocaine, and ofloxacin.[22] The detection limit for lidocaine was found to be 5.0×10^{-6} mol L^{-1}. As all these methods are complex, it is of great importance to develop a new rapid, simple, and sensitive method for the quantitative determination of lidocaine. Thus, modifying the electrode surfaces has been considered a very effective approach to achieve these specifications. In this sense, Oliveira et al. recently developed a new electroanalytical method, based on cyclic voltammetry and square wave voltammetry techniques with boron-doped diamond electrodes as working electrodes, for lidocaine determination in pharmaceutical preparation.[23] Another group immobilized cytochrome P450 2B6 (CYP2B6) on zirconium dioxide nanoparticles (ZrO_2) that incorporated colloidal platinum and poly-lysine on glassy carbon electrodes. The film has shown good electrocatalytic behavior on reduction of lidocaine.[24]

We modified gold electrodes with a self-assembled monolayer (SAM) and immobilized the redox protein hemoglobin (Hb) on these surfaces in order to perform lidocaine analysis by depending on the electroactive character of the protein. It is well established that direct electron transfer of redox proteins on electrode surfaces serve as a model to understand electron transfer mechanism in biological systems and fabricate bioelectronic devices and electrochemical biosensors.[25] Among the electroactive proteins, Hb is an ideal molecule recently used in electrocatalysis and biosensor development.[26] However, it is usually difficult for the redox proteins to transfer electrons directly at naked solid electrodes because redox centers are deeply buried inside hemoglobin and adsorption of the protein at solid surfaces leads to conformational variations and loss of activity.[27,28] In addition, the absorption of Hb on the electrode surface such as platinum and glassy carbon may result in the denaturation and thus loss of its electrochemical activities and bioactivities.[29–32] Gold could be an alternative substrate for stable immobilization of biomolecules without affecting their bioactivity.[33] Moreover, electron transfer between redox proteins and electron surfaces is facilitated. This is induced by many factors, such as high surface-to-volume ratio, high surface energy, and decreased proteins-metal particles distance, as well as the acting as electron conducting pathways between prosthetic groups and the electrode surface from the gold.[34] In order to obtain the direct electrochemistry of the heme protein on the basis of biosensors, using electrodes modified with functional nanomaterials,[27] self-assembled monolayers,[35] polymer films,[36] or surfactants,[37] has attracted great efforts. These surfaces have been widely used in the electrocatalysis of hydrogen peroxide,[26] nitric oxide,[38] trichloroacetic acid,[36] and some drugs[39] due to the enzymatic activity of Hb. In this work, the electrochemical behavior of Au/MPA/Hb electrodes was studied by adsorbing Hb on gold electrodes modified with 3-mercaptopropionic acid (MPA). The electrocatalytic response to the local anesthetic drug lidocaine was analyzed via Au/MPA/Hb electrodes.

Materials and methods

Reagents

Bovine hemoglobin, MPA (99%) and lidocaine (98%) were purchased from Sigma. All the other chemicals were of analytical grade. A 100 mM phosphate buffer solution (PBS, pH 7.0) was prepared from $NaH_2PO_4 \cdot 2H_2O$ (Aldrich) and $Na_2HPO_4 \cdot 2H_2O$ (Aldrich). A 10 mM MPA solution was prepared with absolute ethanol and 20 mg/mL Hb solutions were prepared freshly at PBS pH 7.0 and stored at 4 °C. Stock solution of lidocaine was prepared by dissolving lidocaine in ethanol (0.23 g/mL) and then diluting 100 μL of this stock solution in 10 mL of PBS to get a final concentration of 10 mM, pH 7.0. Stock solution preparation can also be found in the literature.[40]

Electrode preparation

Au/MPA electrode preparation

The gold electrode was polished with 0.1 and 0.05 μM alumina powder and cleaned in a mixture of absolute ethanol and distilled water. Then the electrode was electrochemically pretreated by cycling the potential from +0.2 V to +1.6 V vs. the saturated calomel reference electrode (SCE) in 1 M H_2SO_4, until reproducible voltammograms were obtained. The electrode was rinsed and immersed in 10 mM MPA solution for 30 min.

The resulting electrode was then washed with absolute ethanol followed by a wash step in triple distilled water to remove physically adsorbed thiol molecules. This electrode was named as the Au/MPA electrode.

Au/MPA/Hb electrode preparation

Five microliters of Hb solution (20 mg/mL) was cast on the surface of the Au/MPA electrode and allowed to dry at 4 °C overnight. Finally, the electrodes were washed with PBS and denoted as Au/MPA/Hb electrodes.

Electrochemical measurements

All electrochemical measurements were performed with a CHI 660C (USA) model electrochemical workstation. In this study, a conventional 3-electrode system was used with a film modified or bare gold disk electrode with an area of 0.0314 cm^2, SCE, and a platinum wire as counter electrode. Voltammetry on modified electrodes was performed in buffer solutions purged with nitrogen gas prior to the experiments.

Results and discussion

A quantitative understanding of the redox profile of the drugs is important to obtain knowledge about their toxicological mechanisms and for design of new analytical assays.[40] In this manner, the electrochemical behavior of lidocaine at physiological pH on biochemically modified electrodes is important for the advance of knowledge of molecular toxicological mechanism of this drug and the development of new analytical assays for the quantitative determination in biological systems and pharmaceutical formulations. Figure 1 shows the chemical structure of lidocaine. As can be seen, it is an amide-derivate of diethylamino acetic acid.

As mentioned, it is aimed to get Au/MPA/Hb surfaces. Gold surfaces have been used as suitable candidates for modifying electrodes utilizing Au-S bonds due to alkanethiol covalent binding to the surface.[41] The gold surface also allows the enhancement of direct electron transfer between redox biomolecules and electrode surfaces, providing faster and more effective processes based on charge transfer. Direct electron transfer of immobilized Hb on the SAM modified gold electrode was facilitated due to the suitable interfacial property of the alkanethiol molecule. Electrostatic attraction between the negatively charged –COOH terminal group of MPA and the positively charged iron in the heme group of protein was considered to provide the necessary scaffold background for the protein to be adsorbed at optimal distance and in correct orientation.[42,43] The electrochemical behavior of the fabricated electrodes was examined in order to control the direct electron transfer of Hb. Cyclic voltammetry was performed vs. SCE in PBS pH 7.0 between –0.4 V and 0.0 V.

As depicted in Figure 2a, the Au/MPA/Hb electrode showed well defined redox peaks at –0.15 V (Ep_a) and –0.33 V (Ep_c) in a solution of PBS. This result denotes the characteristic Fe(III)/Fe(II) redox couple of heme protein indicating direct electron transfer between Hb and electrode. In similar test conditions, the cyclic voltammogram obtained with only the Au/MPA electrode (Figure 2b) shows no redox peaks in the potential range. These results exhibited the electrochemical behavior of Hb on Au/MPA electrodes. Hence, Au/MPA/Hb was considered to be usable as a working electrode for investigation of the electrochemical behavior of lidocaine and determination of the drug by using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) in aqueous solutions of lidocaine.

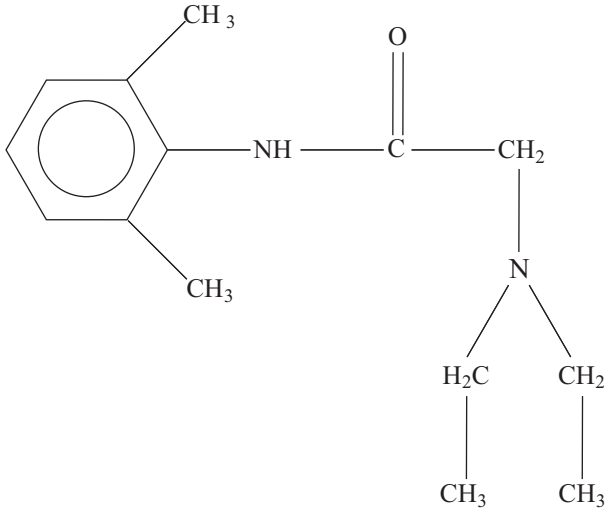

Figure 1. Chemical structure of lidocaine.

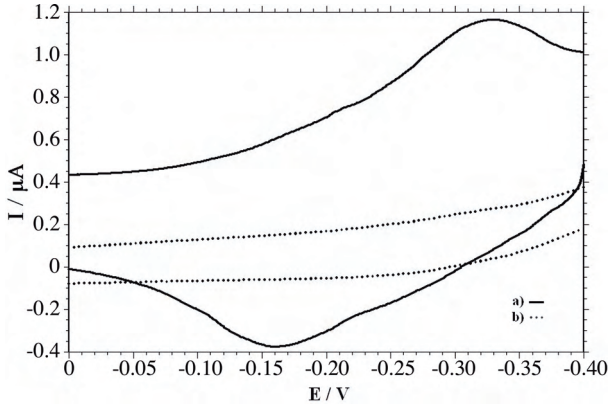

Figure 2. Cyclic voltammograms in PBS pH 7.0 at 0.1 V/s of (a) Au/MPA/Hb electrode and (b) Au/MPA electrode.

Figure 3 illustrates the cyclic voltammograms obtained in the presence and absence of lidocaine in PBS, respectively. Voltammetric behavior shown in Figure 3a indicates an irreversible peak at +1.53 V, due to the oxidation of lidocaine on the Au/MPA/Hb electrode at pH 7.0 PBS. This is in agreement with results reported in the literature.[23]

DPV has been applied as a sensitive analytical method for determination of sub-micromolar levels of organic compounds.[44] Figure 4 shows typical DPVs of 10 mM lidocaine in PBS pH 7.0 at different electrodes. At bare Au (Figure 4a) and Au/MPA (Figure 4b) electrodes, oxidation peak currents of lidocaine were relatively weak, with peak potential of +1.6 V and +1.5 V, respectively. In contrast, the Au/MPA/Hb electrode showed enhanced signals at +1.53 V leading to better electroactivity on the response of lidocaine. This indicated that Hb exhibits an electrocatalytic effect on the oxidation of the drug.[45]

DPVs for solutions containing various concentrations of lidocaine with the Au/MPA/Hb electrode are shown in Figure 5. The obtained voltammograms illustrate that the peak current increased with lidocaine concentration in a dynamic range from 5×10^{-7} M to 2.9×10^{-6} M, with a regression equation of $i_p = 2.531C + 5.775$ (i_p in μA and C in μM) (Figure 6). In our studies, the detection limit was obtained at 2.9×10^{-7} M, which is lower than the 5.0×10^{-6} mol L^{-1} detected on a tris(2,2′-bipyridyl)ruthenium(II) ($Ru(bpy)_3^{2+}$)–zirconia–nafion composite modified glassy carbon electrode[22] and comparable with the detection limit obtained with a boron-doped diamond electrode.[23] The sensitive and fast catalytic response of the Hb electrode may be attributed to the good direct electrochemistry of Hb immobilized on Au/MPA. The stability of the film was examined by storing the electrodes in PBS at 4 °C for 7 days, and it was found that the electrode almost retained its initial activity during this time.

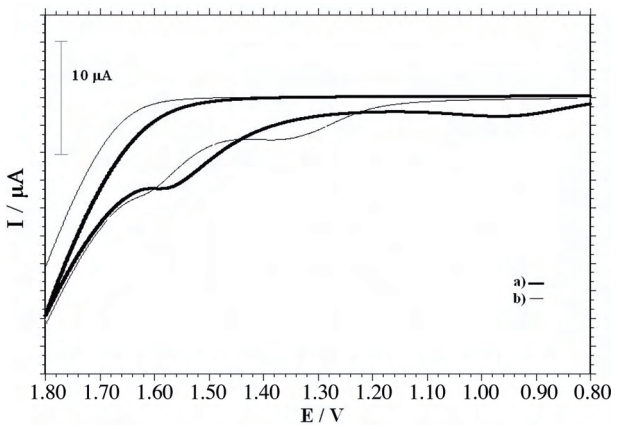

Figure 3. Cyclic voltammograms of Au/MPA/Hb electrode in (a) lidocaine 10 mM in PBS, pH 7.0, (b) PBS, pH 7.0. Scan rate 0.1 V/s.

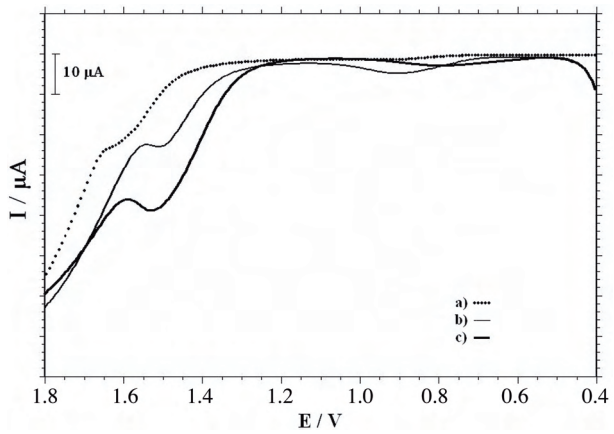

Figure 4. Differential pulse voltammograms of 10 mM lidocaine in PBS pH 7.0 at different electrodes: (a) Au, (b) Au/MPA, and (c) Au/MPA/Hb electrode. Pulse amplitude 50 mV, pulse width 50 ms.

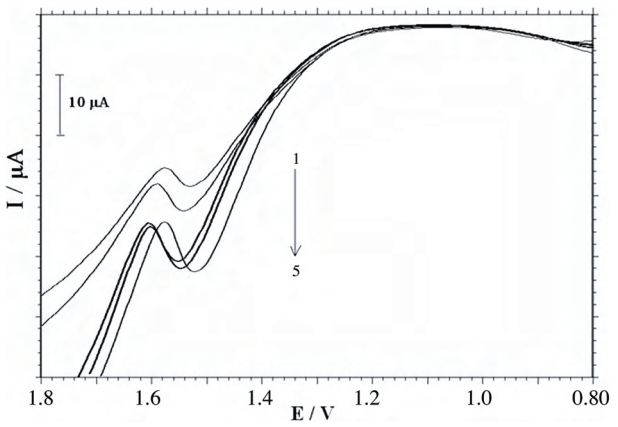

Figure 5. Differential pulse voltammograms for increasing concentrations of lidocaine at pH 7.0 PBS on Au/MPA/Hb electrode: pulse amplitude, 50 mV, pulse width, 50 ms. Lidocaine concentration (1) 4.99×10^{-7} M, (2) 9.90×10^{-7} M, (3) 1.96×10^{-6} M, (4) 2.91×10^{-6} M, and (5) 1.33×10^{-5} M.

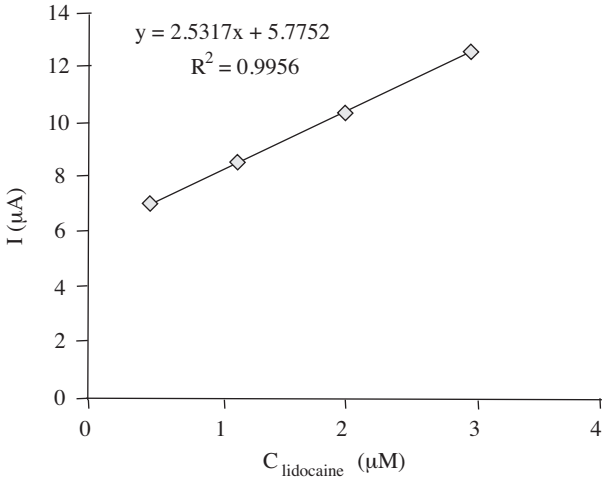

Figure 6. Calibration curve for lidocaine concentrations.

Conclusion

Modern electrochemical methods are now sensitive, selective, rapid and easy. These techniques are applicable to analysis in pharmaceutical fields and most areas of analytical chemistry. They are probably the most versatile of all types of trace pharmaceutically active compound analysis. Voltammetric techniques have also been

extremely useful in measuring blood levels, metabolites, and urinary excretion of drugs following low doses, especially when coupled with chromatographic methods.[46] In recent years, biomolecule-based electroanalysis methods have often been used for monitoring concentrations of analytes. Biomolecule-based analytic systems can meet the needs for real-time monitoring and replace the time consuming analytical techniques used in industrial and clinical chemistry and medicine.

In the present study, heme protein-based biosensor mechanism is studied by using chemically modified electrodes by means of coupled hemoglobin to Au/MPA composite. After the electrochemical studies, it is concluded that lidocaine, one of the most widely used local anesthetics, has an oxidative peak when cyclic and differential pulse voltammetry experiments were conducted using Au/MPA/Hb electrodes. It was clearly demonstrated that the Au/MPA/Hb acted as an efficient promoter to enhance electrochemical response of lidocaine. The electrochemical performance of the modified electrode on lidocaine is comparable with the other techniques used in the literature but it is cheaper and easier to perform, providing a new promising electrochemical sensing platform to determine trace amounts of lidocaine successfully in tissue and blood samples as well as pharmaceutical formulations.

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