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Kinetic study of the degradation of a potential local anesthetic drug in serum using the DNA-based electrochemical biosensor

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Abstract

DNA-drug association interaction at the DNA modified screen-printed electrode for 1-methyl-2-piperidinoethylester of 2-hexoxyphenylcarbamic acid was found leading to an accumulation of the drug within the DNA layer. A procedure for the determination of drug in blood serum matrix using the protein precipitation and voltammetric measurement of the electroactive drug with the DNA biosensor was obtained and an effort was done to apply it for an assay of the drug enzymatic degradation in human and rabbit sera at 37 °C.

Keywords: Local anesthetics; Serum; Drug degradation; DNA electrochemical biosensor; DP voltammetry

1. Introduction

The derivatives of alkoxyphenylcarbamic acids exhibit local anesthetic activity [1] and in vitro interact significantly with the surface-attached DNA by an electrostatic association with the negatively charged dsDNA backbone [2]. This association does not lead to DNA damage and the drug molecules can be removed at the dissociation of the drug-DNA adducts in blank buffer solution. The HPLC technique is typically used for the determination of such drugs in biological matrices and the drug degradation by various esterase enzymes [3–5].

These local anesthetics are electroactive at both bare and chemically modified voltammetric electrodes. Recently, we reported a procedure for the differential pulse voltammetric (DPV) determination of such type of the drugs at a screen-printed carbon electrode modified by the thin DNA layer (DNA/SPE) [6]. The aim of this work was an effort for the first time to use this new procedure for an assay of the drug degradation in blood serum. For this purpose, two different spiked sera have been chosen, namely lyophilized human serum and original rabbit serum stored in a frozen state. A change of both the drug concentration and in the association ability towards the surface attached DNA was of interest.

2. Experimental

A computerized voltammetric analyzer ECA pol, model 110 (Istran, Bratislava, Slovakia) fitted with a screen-printed three-electrode assembly (FACH, Prešov, Slovakia) was used for voltammetric measurements. The working electrode was preconditioned electrochemically in 0.005 M phosphate buffer pH 7.0 at 1.7 V for 60 s, covered with 2 μl of the DNA stock solution and leaving to dry overnight. The measurements were carried out at room temperature (25 °C).

Calf thymus dsDNA was obtained from Merck (1.24013.0100) and used as received. Its stock solution (5 mg/ml) was prepared in 0.010 M Tris–HCl buffer of pH 8.0 with 1×10^{-3} M EDTA solution and stored at −4 °C. The drug hydrochloride of 1-methyl-2-piperidinoethylester

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of 2-hexoxyphenylcarbamic acid (pK_a=7.32) was synthesized as described previously [1]. Its 1×10^{-2} M stock standard solution in water was stored at 4 °C. Lyophilized human serum Exapat was obtained from Imuna (Šarišské Michal'any, Slovakia) and its solution was prepared by the addition of 5.0 ml deionized water to the commercial tube. Rabbit blood serum was obtained from the Faculty of Pharmacy, Comenius University. All other chemicals were of analytical reagent grade purity. Deionized, double distilled water was used throughout.

For the drug determination, the analytical procedure published in Ref. [2] was modified in order to enhance the sensitivity of the determination as follows: the drug was accumulated in the DNA layer from its solution in 0.005 M phosphate buffer pH 7.0 using −0.20 V polarization potential of the working electrode for 60 s under stirring. The DPV record was obtained immediately within the potential range of 0.40–0.85 V at the pulse amplitude 200 mV, pulse duration 40 ms and scan rate of 10 mV s^{-1} and the DPV peak current was evaluated after the baseline correction.

Two spiked serum samples were prepared containing 0.02 mg/ml drug in human blood serum and 0.008 mg/ml drug in rabbit blood serum. The samples were kept at 37 °C in a thermostat for a given time period. Then 250 μl of spiked blood serum were pipetted to a test tube and 500 μl of acetonitrile was added. The precipitate was separated by centrifugation for 2 min. Then, 550 μl of the solution phase were transferred to another test tube and evaporated to dryness under air purge at room temperature. The residue was dissolved in 300 μl of double distilled water and 20 or 50 μl of the resulting solution were pipetted into voltammetric cell containing 4.0 ml of 0.005 M phosphate buffer pH 7.0. The DPV record was obtained. Two standard additions of 1×10^{-4} M drug solution served for the drug quantification.

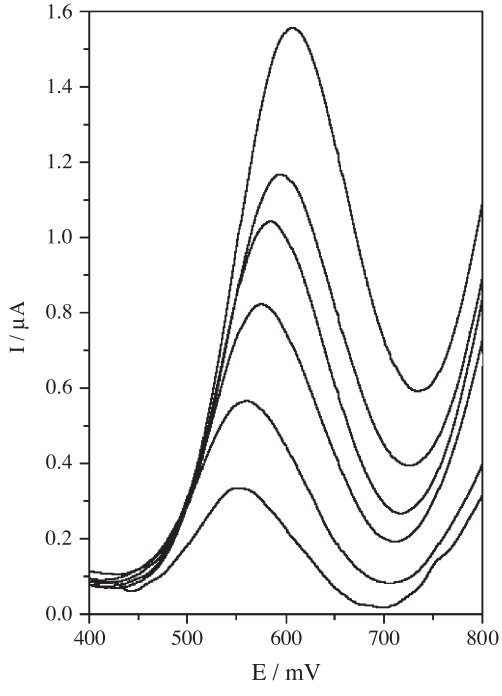

Fig. 1. Differential pulse voltammograms of the drug in test solutions with 0.05, 0.15, 0.3, 0.5, 0.7, and 1.0 μM drug. Conditions: 1 h incubation of spiked rabbit blood serum at 37 °C followed by the protein precipitation and drug extraction, then dilution with 0.005 M phosphate buffer solution pH 7.0 and the drug accumulation on DNA/SPE at −0.20 V for 60 s under stirring, DPV measurement with pulse amplitude 200 mV, pulse duration 40 ms, scan rate of 10 mV s^{-1}.

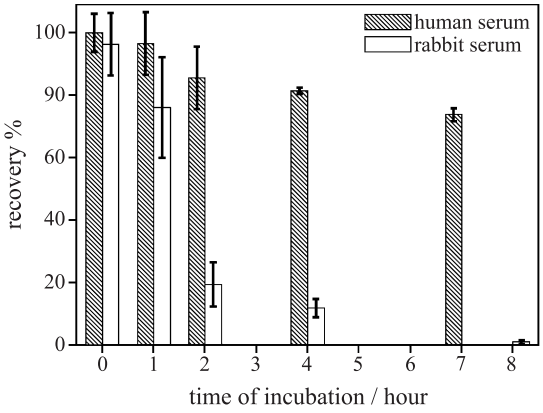

Fig. 2. Drug degradation profile during the incubation of serum at 37 °C expressed as the dependence of the original drug recovery on time. Conditions: the initial drug spike concentration 20 μg/ml in human serum and 8 μg/ml in rabbit serum. Error bars represent the standard deviation from the average value (n=3).

3. Results and discussion

The drug molecule undergoes an electrooxidation at the SPE and DNA/SPE in the region of 0.6 V vs. Ag/AgCl-SPE in 0.005 M phosphate buffer pH 7.0 [2]. A non-specific adsorption of the drug at a bare SPE and a specific association of the drug with the surface confined DNA have been observed with the saturation of the electrode surface within 60 s. Due to the accumulation of the drug traces within the DNA layer, about three times higher analytical signal was obtained at the DNA/SPE comparing to bare SPE.

The serum matrix has a strong influence on the voltammetric drug signal leading to a suppression of the peak current and the shift of the peak potential. For the determination of low drug concentration in blood serum, proteins have to be eliminated. For this purpose, the protein precipitation by acetonitrile, as described in Ref. [7], has been found to be effective. After that the drug signal is concentration dependent (Fig. 1) with the linearity from 0.05 to 1.00 μM for rabbit blood serum extract and 0.013–0.200 μM for human blood serum extract. The detection limits were found to be 0.06 μM for rabbit blood serum and 0.016 μM for human blood serum [8].

Chemical stability of the original drug has been studied in both pure aqueous medium of the drug stock solution as

well as in spiked human blood and rabbit blood sera. In pure aqueous medium of the buffer solution, no drug degradation was detected after a week of storage at room temperature. Due to a dramatically lower sensitivity of the voltammetric signal of the drug added as a spike to the blood serum matrix at room temperature, the procedure of protein precipitation followed by the drug accumulation on the DNA biosensor and DPV measurement was utilized as described in Experimental. Using a previous incubation of the spiked blood sera at 37 °C, the drug signal at the DNA/SPE decreases with time of incubation. Evidently, a deep decomposition of the original drug molecule takes place to products which are not electrochemically active within the potential region used for the voltammetric determination of the original drug molecule or do not associate with the DNA electrode modifier. Fig. 2 shows the drug degradation in serum at 37 °C expressed as a recovery of the original drug amount. Observed rate constants of the degradation process can be calculated using the linear dependence: $\ln(c/c_0)=f(t)$ (first order) and $1/c=f(t)$ (second order). For human blood serum, the first-order and the second-order rate constants observed are $(1.14\pm0.18)\times10^{-5}$ s^{-1} (correlation coefficient of $r=0.964$) and (0.27 ± 0.04) M^{-1} s^{-1} ($r=0.973$), respectively, while for the rabbit blood serum the values are $(1.53\pm0.14)\times10^{-4}$ s^{-1} ($r=0.989$) and $(1.44\pm0.36)\times10^{2}$ M^{-1} s^{-1} ($r=0.919$), respectively. According to the values of the correlation coefficients it is difficult to select a true order of the rate constants. At this point an agreement is found with the degradation study of this drug in another sample of the rabbit serum using HPLC as the detection technique [4]. The reason for it is evidently a rather complicated drug decomposition process with the participation of esterase enzyme which cannot be described by simple kinetic model. Moreover, the results could be influenced also by the amount of drug spike, the presence of other (drug) substances in serum as well as by serum storage conditions and storage time which all together may influence the enzyme activity [5].

4. Conclusion

In this paper new type of biosensing was tested as a simple and inexpensive method for the drug determination in serum matrix. It was found that the sensitive electrochemical drug determination at the DNA-based biosensor can be used for an evaluation of the drug degradation. The new procedure represents an alternative to conventional methods such as HPLC. The association of the original drug molecule with the surface attached DNA as well as the drug degradation in serum are of great interest regarding an understanding of the drug activity and interaction with the DNA biopolymer.

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