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Direct-printed amiodarone electrochemical sensor. Identification of DNA based bioreceptor

Marcin Urbanowicz[1], Magdalena Urbanowicz[2], Agnieszka Paziewska-Nowak[1], Kornelia Bobrowska[1], Anna Sołdatowska[1], Marek Dawgul[1], Dorota G. Pijanowska[1]
[1] Nalecz Institute of Biocybernetics and Biomedical Engineering, Polish Academy of Sciences, Ks. Trojdena 4 St., 02-109 Warsaw, Poland,
[2] Falsified Medicines and Medical Materials Department, National Medicines Institute, Chełmska 30/34 St., 00–725 Warsaw, Poland

Summary: This study focuses on the electrochemical behaviour of amiodarone, a widely used antiarrhythmic drug, to develop a direct-printed electrochemical sensor. The oxidation of amiodarone was confirmed via voltammetric techniques. A comparative analysis of graphite electrodes fabricated using direct printing (DPE) and screen printing (SPE) technologies revealed the superior performance of DPE sensors, with a sensitivity of 1.403 µA/µM – nearly twice that of SPE-based sensors. In the second part of the study, we screened for double-stranded DNA sequences capable of forming complexes with amiodarone. High-performance liquid chromatography (HPLC) identified the 5'-AGCTATAAAT-3' sequence as a promising bioreceptor. The interaction between amiodarone and DNA was further confirmed by NMR spectroscopy, based on diffusion coefficient measurements and chemical shift changes observed in NOESY and TOCSY spectra. These findings demonstrate the potential of integrating printed sensor technologies with molecular interaction studies for drug screening and biosensing applications.

Keywords: Amiodarone, Direct printing, DNA, Interaction analysis

Introduction

Amiodarone is one of the primary drugs used in the treatment of cardiac arrhythmias. However, its use is associated with many adverse effects, including pulmonary toxicity, liver damage, thyroid dysfunction, and visual disturbances. Therefore, the ability to personalize therapeutic dosing remains a significant clinical challenge. Biosensors offer a convenient tool for monitoring drug concentrations in patients, though their effectiveness relies heavily on high sensitivity and selectivity. This is particularly important for amiodarone, which has a narrow therapeutic window ranging from 0.5 to 2.5 µg/mL depending on individual patient characteristics. Reports of its potential genotoxicity suggest that amiodarone may strongly interact with DNA [1], making DNA a potential component for molecular recognition in biosensor applications [2].

Methods

Commercially available screen-printed graphite electrodes (SPE) and directly printed electrodes (DPE), fabricated using a programmable microdispensing robot, were used for sensor construction (diameter 1.6 mm). The redox behaviour of amiodarone was studied using glassy carbon electrodes (GCE) and cyclic voltammetry (CV) at a scan rate of 50 mV/s. Calibration curves were obtained using differential pulse voltammetry (DPV) with a pulse amplitude of 100 mV, a pulse duration of 0.005 s, and a scan rate of 20 mV/s. All electrochemical measurements were conducted in PBS buffer (pH 6.0).

For DNA interaction studies, 30 double-stranded DNA sequences (10 base pairs each) were screened to identify potential binding motifs. HPLC analysis was performed using a Phenomenex Gemini 5 µm NX-C18 column (250 mm × 4.6 mm) with a gradient of mobile phases: (A) 1 mM NH_4COOH (pH 6) and (B) acetonitrile. The gradient program ranged from 5–15% B (0–8 min), 15–90% B (8–18 min), held at 90% B (to 32 min), and returned to 5% B (50–60 min), with a flow rate of 1 mL/min and UV detection from 190–900 nm. NMR studies were conducted in D_2O buffer (25 mM NaCl / 25 mM K_3PO_4) with TSPA-d_4 as an internal standard. Standard pulse sequences were used for 1D and 2D experiments. DOSY was performed with 1024 transients, diffusion time 150–220 ms, and gradient strength ranging from 6 to 50 G/cm. TOCSY and NOESY spectra were acquired with a spectral width of 5000 Hz, 1024 × 512 complex points, 64 scans, and relaxation delays of 1 s. TOCSY spin-lock time was 80 ms; NOESY mixing time was 200 ms.

Results

In the first stage, measurement conditions, such as pH, ionic strength, and voltammetric parameters were optimized. The electrochemical activity of amiodarone was then investigated. The results confirmed that amiodarone undergoes an irreversible electrochemical oxidation at a potential of 850 mV (Fig. 1).

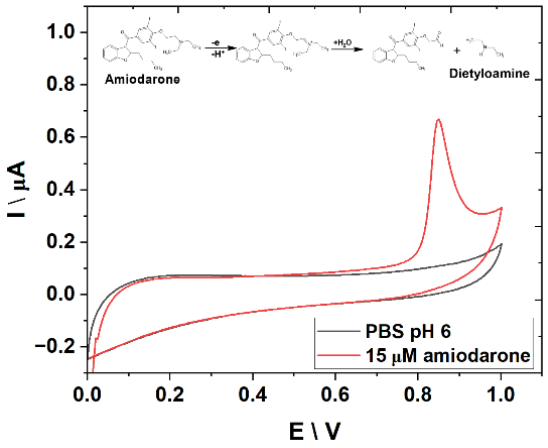

Fig. 1. GCE: redox mechanism of amiodarone.

Subsequently, calibration curves for graphite SPE and DPE electrodes were obtained using the DPV technique, as shown in Fig. 2. For electrodes of identical diameter, the DPE sensors demonstrated significantly higher sensitivity and a broader linear range compared to SPE.

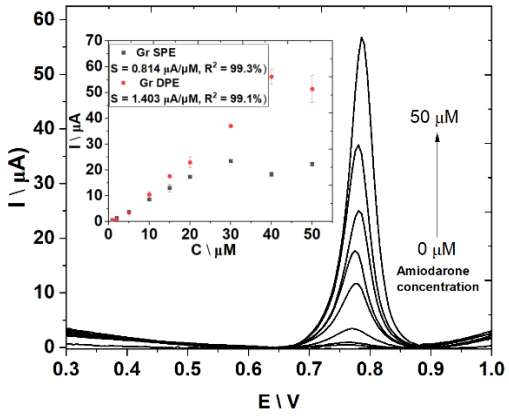

Fig. 2. Differential pulse voltammograms for Gr DPE with various concentrations of amiodarone. Inset: calibration curves for Gr-SPE (black markers) and DPE (red markers).

The second part of the study focused on identifying a DNA sequence capable of forming a complex with amiodarone. HPLC analysis (Fig. 3) of the drug's interaction with 30 different double-stranded DNA sequences revealed that the greatest decrease (by 53%) in free DNA absorbance was observed for the sequence 5'-AGCTATAAAT-3'. Additionally, a new peak at retention time (RT) = 1.909 min suggests the formation of a DNA-amiodarone complex.

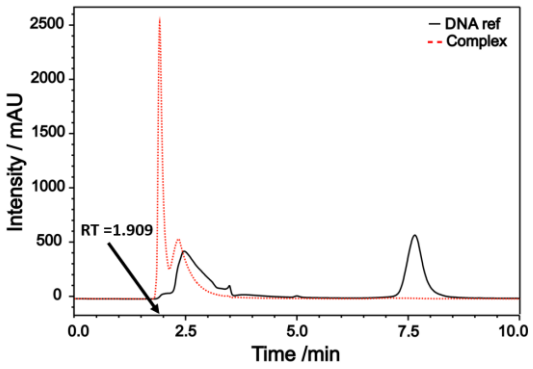

Fig. 3. HPLC chromatogram of 5'-AGCTATAAAT-3' dsDNA and complex of this DNA with amiodarone.

The interaction was further confirmed using NMR spectroscopy. Diffusion coefficient values enabled the calculation of the association constant (K_a = 2.12 M^{-1}) and dissociation constant (K_d = 0.47 M). Moreover, TOCSY (Fig. 4) showed chemical shift changes of approximately 0.06 ppm, providing strong evidence for specific binding between amiodarone and the selected DNA sequence and further confirming complex formation.

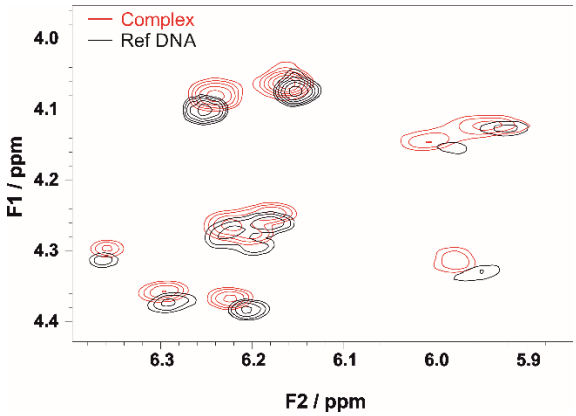

Fig. 4. TOCSY spectra of amiodarone-DNA complex after 2 h incubation.

The results provide a foundation for using the identified DNA sequence as a bioreceptor for amiodarone, enabling the development of a selective biosensor for clinical application.

References

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