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ELECTROCHEMICAL OXIDATION AND INTERACTION OF NEWLY SYNTHESIZED ACRIDINE DERIVATIVES WITH DNA

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ABSTRACT

Oxidation of newly synthesized acridine derivatives was studied using cyclic voltammetry at glassy carbon electrode. Oxidation occurs as irreversible, diffusion-controlled process at pH 4.6 for compounds 1-3 and as adsorption controlled process for compound 4. The interaction between newly synthesized acridine compounds (compounds 1-4) and dsDNA was studied using a multilayer dsDNA biosensor applying square wave voltammetry. Peak current corresponding to deoxyadenosine decreased after 30 minutes of interaction suggesting interaction with dsDNA.

INTRODUCTION

It has been proven that acridine derivatives may be used as anti-inflammatory, anticancer and antimicrobial agents [1]. Activity of acridine derivatives can be attributed to their semiplanar structure and to their redox properties as they can intercalate between double stranded DNA (dsDNA) base pairs [2]. Synthesis and activity testing for acridine derivatives has been the subject of many research papers [3] as well as investigation of their redox activity [4]. DNA-drug interaction can be investigated using DNA biosensor where the adsorbed DNA at glassy carbon electrode (GCE) surface undergoes charge-transfer reactions, producing signals which correspond to deoxyguanosine (dG) and deoxyadenosine (dA) oxidation.

The aim of this work was to investigate the oxidation of some newly synthesized acridine dervatives [3] and their interaction with dsDNA.

METHODS

Voltammetric measurements were performed using an μAutolab analyzer (EcoChemie, Utrecht, The Nederlands) controlled by the GPES 4.9 software. A conventional three-electrode system was used with Ag/AgCl as a reference (3.00 M KCl) and Pt as an auxiliary electrode. A GCE (d = 3 mm, CH Instruments, Inc., Austin, TX, USA) was used as the working electrode. Before the measurements, the GCE was polished on a smooth polishing pad with an aqueous slurry of Al_2O_3 powder (particle size 0.05 μm). After polishing, the GCE was sonicated in bidistilled water for 2 minutes and then in absolute ethanol for 2 minutes. Calf thymus DNA (dsDNA, $MW_r = 10 – 15 \times 10^6$) was obtained from Sigma−Aldrich, and acridine derivatives: (S)-methyl 2-(acridin-9-ylamino)-3-phenylpropanoate (compound 1), (S)-methyl 2-(acridin-9-ylamino)-3-(1H-imidazol-4-yl)propanoate (compound 2), (S)-methyl 2-(acridin-9-ylamino)-3-(1-methyl-1H-indol-3-yl)propanoate (compound 3) and (S)-2-(acridin-9-ylamino)-3-(1-methyl-1H-indol-3-yl)propanoic acid (compound 4) were synthesized in our laboratory [3]. Final concentrations of the compounds 1-4 (2.4×10^{-4} M for cyclic voltammetry (CV) and 1×10^{-4} M for square wave voltammetry (SWV)) were obtained by diluting the stock solution with appropriate volume of supporting electrolyte (acetate buffer, pH 4.6). Solutions were

de-aerated for 10 minutes with high purity nitrogen. The experiments were performed at room temperature (25 °C).

The multilayer DNA biosensor was prepared by covering the surface of glassy carbon electrode succesively with three drops of DNA (5 μL, 73.95 μg mL^{-1}) [4]. After placing each drop on the top of the electrode surface, the electrode was dried under nitrogen atmosphere, and rinsed with water to remove the unabsorbed DNA. The DNA biosensor was immersed in acridine derivative solutions and allowed to incubate for 30 minutes. After the incubation time, the biosensor was removed from the solution, washed with bidistilled water in order to remove the unbounded molecules and placed in the electrochemical cell containing only the supporting electrolyte.

The transduction for biosensor was performed using SW voltammetry. The experimental parameters for SWV were: frequency 25 Hz, potential increment 0.001 V, effective scan rate of 0.0025 V/s and pulse amplitude of 0.005 V. Oxidation of acridine derivatives was examined using CV. CV was performed between 0 V and + 1.6 V. Scan rate was ranging from 10 mV/s up to 100 mV/s with step potential of 0.005 V.

RESULTS AND DISCUSSION

The oxidation of synthesized acridine derivatives (compounds 1-4) of 2.4×10^{-4} M was studied by CV at pH 4.6. The cyclic voltammograms were recorded in three successive scans at a scan rate 10 – 100 mV/s (Fig. 1). Anodic peak which appeared at $E_p \sim 0.9 – 1.0$ V was of interest for our research since the oxidation of the synthesized compounds was monitored during the DNA interaction study. The corresponding cathodic peak was absent, meaning that the oxidation process of compounds was irreversible. The scan rate effect on the compounds oxidation peak was monitored. The peak current increased linearly with the square root of ν for compounds 1-3 indicating that the oxidation process was diffusion controlled [5]. The corresponding slopes of log I_p *vs.* log ν dependences, which were close to theoretical value of 0.5 for diffusion controlled process, proved the diffusion nature of the oxidation of the compounds 1-3.

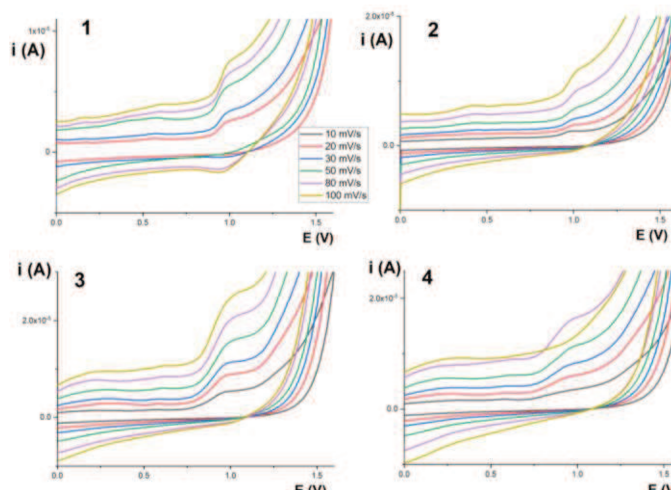

Figure 1. Cyclic voltammograms of compounds 1-4 at different scan rates

Table 2. Equations I_p *vs.* $\nu^{1/2}$ or I_p *vs.* ν and log I_p *vs.* log ν for compounds 1- 4

Comp	I_p *vs.* $\nu^{1/2}$ or I_p *vs.* ν	log I_p *vs.* log ν
1	I_p (A) $=3.34 \times 10^{-6} \nu^{1/2}$ (V/s)$^{1/2}$ -7.11×10^{-8}, $R^2 = 0.9904$	log I_p (A) = 0.55 log ν (V/s) −5.44
2	I_p (A) $= 1.84 \times 10^{-6}\nu^{1/2}$ (V/s)$^{1/2}$ $+ 1.91 \times 10^{-9}$, $R^2 = 0.8960$	log I_p (A) = 0.45 log ν (V/s) −5.80
3	I_p (A) $=1.34 \times 10^{-5} \nu^{1/2}$ (V/s)$^{1/2}$ -1.18×10^{-7}, $R^2 = 0.9788$	log I_p (A) = 0.47 log ν (V/s) −4.03
4	I_p (A) $= 2.72 \times 10^{-5} \nu$ (V/s) -6.13×10^{-8}, $R^2 = 0.9989$	log I_p (A) = 0.93 log ν (V/s) −4.71

On the other hand, compound 4 showed linear dependence of I_p *vs.* ν, and the log I_p *vs.* log ν curve slope close to the unity value, indicating its adsorption nature. (Table 1).

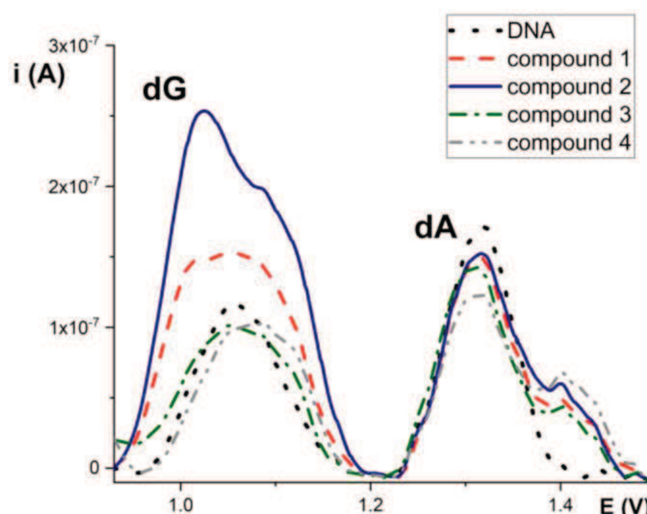

Figure 2. SW voltammograms of acridine derivatives interaction with DNA

The interaction of compounds with dsDNA was examined using SWV at pH 4.6. The changes in the purine base (deoxyguanosine, dG and deoxyadenosine, dA) oxidation peak currents were monitored after the incubation of DNA biosensor with 1×10^{-4} M solutions of synthesized compounds during 30 minutes [6].

As the oxidation peak of compounds and dG of DNA overlapped, only the change in dA peak was informative.

After the interaction, the height of the dA peak decreased (comparing to control dsDNA biosensor) indicating an interaction. According to the results presented in Figure 2, compound 4 indicated most intensive interaction with DNA, probably as a consequence of its adsorption at GCE.

CONCLUSION

The oxidation of compounds was examined applying cyclic voltammetry using a glassy carbon electrode and it occurred as irreversible, diffusion controlled process for compounds 1-3 and adsorption controlled process for compound 4. The glassy carbon electrode covered with three drops of dsDNA solution was used as electrochemical biosensor with aim to examine the possible

interaction of compounds with DNA. The results of our study indicate potential chemotherapeutical role for used compounds, where compound 4 has the most intensive interaction considering the dA peak current decrease.

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