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Cyclic voltammetry of echinomycin and its interaction with double-stranded and single-stranded DNA adsorbed at the electrode

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Abstract

Interactions of echinomycin (Echi) with DNA was studied by cyclic voltammetry (CV) with hanging mercury drop electrode (HMDE). Echinomycin was electrochemically active, yielding several signals. Interaction of Echi with dsDNA attached to a hanging mercury drop electrode resulted in high Echi signals, suggesting a strong binding of Echi to dsDNA by bis-intercalation at the electrode surface. Under the same conditions, interaction of Echi with ssDNA produced almost no Echi signal. This behavior is in agreement with a strong binding of Echi to dsDNA and a very weak binding of Echi to ssDNA observed earlier in solution. Echi, thus, appears to be a good candidate for redox indicator in electrochemical DNA hybridization sensors. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Electrochemistry of DNA; Interaction of DNA with echinomycin; Hanging mercury drop electrode; DNA hybridization sensors; DNA bis-intercalator

1. Introduction

Antimicrobial and antitumor activity of quinoxaline antibiotic has been known for several decades [1,2]. Echinomycin (Echi) represents one of the quinoxaline antibiotics. Echi interaction with dsDNA was described many years ago [3], and later, the mechanism of binding was named as bis-intercalation [4]. Echi binds to the minor groove of DNA duplex showing strong preference for the CpG sequence [5–8]. The two aromatic rings are oriented in an optimum configuration for bis-intercalation, separated by 10–11 Å, and can accommodate two base-pairs between the rings as required for binding by the neighbor exclusion principle. Significantly higher DNA-binding constants and slower dissociation rates from DNA are characteristics for bis-intercalators relative to monointercalators [9]. The results in this field were reviewed, e.g. Refs. [10–13]. On the other hand, less is known about electrochemical behavior of quinoxaline antibiotics, including Echi. Here, we present a preliminary report on interaction of Echi with ss- and dsDNA using adsorptive transfer stripping cyclic voltammetry (AdTSCV) at hanging mercury drop electrode (HMDE) [14,15].

2. Materials and methods

Calf thymus DNA was isolated as described previously [16]. DNA was denatured by heating at 100 °C for 6 min with subsequent cooling in an ice-bath. Echinomycin was purchased from Sigma-Aldrich (Germany). All reagents were of analytical grade: solutions were prepared from triple distilled water. Voltammetric measurements with a hanging mercury drop electrode HMDE were performed with an AUTOLAB analyzer (EcoChemie, The Netherlands) with VA-Stand 663 Metrohm (Zurich, Switzerland). The HMDE mode with an area of 0.4 mm^2 and a three-electrode system were used (Pt wire served as an auxiliary electrode and an Ag/AgCl/3M KCl served as reference electrode). DNA was adsorbed from 8-μl sample drop on the HMDE surface. After washing, the electrode was transferred into the solution of Echi, washed and transferred into the background electrolyte where usual voltammetric measurements were done. CV measurements were carried out in deaerated solutions at room temperature.

3. Results and discussion

We have found that Echi is an electroactive species yielding several CV signals at neutral pH. With 10 μM

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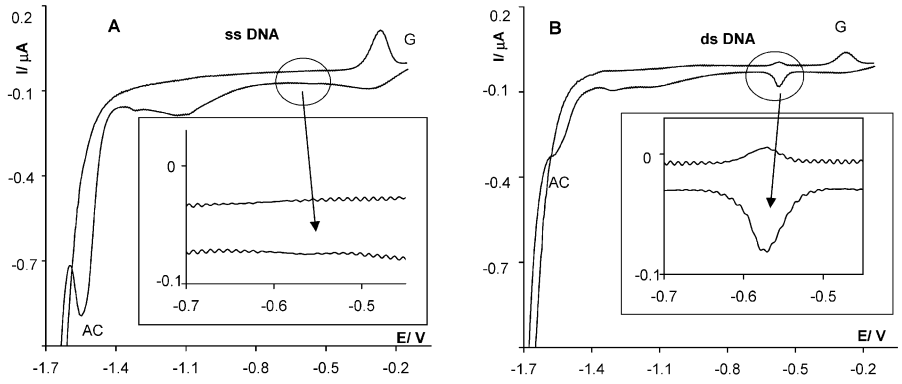

Fig. 1. Cyclic voltammograms of Echi complex with (A) ssDNA and (B) dsDNA. The curves were obtained using double-transfer step technique. DNA-modified HMDE (100 ppm of calf thymus DNA, t_A 120 s) was dipped into 10 μM Echi for 60 s, washed and transferred into 0.3 M ammonium formate with sodium phosphate, pH 6.9. Scan rate 0.5 V/s, starting potential − 0.1 V, switching potential − 1.85 V, HMDE. Only the second scans are shown.

Echi in 0.3-M ammonium formate, phosphate buffer, and pH 6.9, a redox couple peak around − 0.53 V, a low broad cathodic peak around − 1.08 V, and a sharp cathodic peak around − 1.69 V were observed (not shown). More details about the behavior of Echi will be published elsewhere. Here, we tested Echi as a potential bis-intercalating redox indicator for a DNA hybridization sensor. We measured only the most positive signals of Echi at − 0.53 V. DNA was adsorbed at the open current circuit from 0.2 M NaCl for the accumulation time (t_A) 120 s. After accumulation, the electrode with immobilized DNA was transferred into solution of 10 μM Echi in 0.2 M NaCl and incubated for 60 s. Then, the second electrode transfer into the blank background electrolyte (0.3 M ammonium formate with phosphate buffer, pH 6.9) followed, and after electrolyte deaerating, the CV scan was applied. In case of thermally denatured ssDNA, we could observe on voltammograms only signals of DNA, i.e. cathodic peak CA of cytosine and adenine or anodic peak G due to the oxidation of the reduction product of guanine as described earlier [17,18] (Fig. 1A). Contrary to ssDNA, with dsDNA, new redox couple appeared at about − 0.5 V (Fig. 1B) corresponding to peaks of free Echi. These results show a striking difference in voltammetric responses with ds- and ssDNA; dsDNA–Echi complex produced specific DNA and Echi signals in agreement with the strong binding of Echi to dsDNA by bis-intercalation [7]. If multiple CV scan was used, the difference between the voltammograms obtained with ds- and ssDNA was observed at all measured scans. On the other hand, some changes at the voltammograms of dsDNA displaced some changes on the successive scans. Fig. 1 shows only the second scans; more details will be published elsewhere. Under the same conditions, interaction of Echi with ssDNA resulted in high DNA signals and almost no Echi signals, suggesting only very weak binding of Echi to ssDNA at the electrode surface. Echi, thus, appears to be a good candidate for a redox indicator in electrochemical DNA hybridization sensors [19].

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