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Reduction of lapachones and their reaction with L-cysteine and mercaptoethanol on glassy carbon electrodes

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Abstract

The electrochemical reduction of β-lapachone and its 3-sulphonic salt was studied by cyclic, square wave and differential pulse voltammetry in aqueous media using a glassy carbon electrode. These compounds have a wide range of biological activities, including antibacterial, cytotoxic, antifungal, trypanocidal and anticancer action. The reduction of β-lapachone in the presence of L-cysteine and 2-mercaptoethanol was studied and the results, together with others already published, suggest that the anticancer mechanism of β-lapachones can be explained via interaction with topoisomerase. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: β-Lapachone; Cytotoxic action; Electrochemical reduction; Topoisomerase

1. Introduction

β-Lapachone is a naturally occurring quinone, easily synthesized from lapachol or lomatiol [1]. It has been shown to have a wide range of biological activities, including anticancer activity [2,3]. Its mechanism of action as antibacterial, cytotoxic and trypanocidal is related to its redox cycling nature, generating reactive oxygen species, which can damage DNA [4]. However, the clinical efficacy of this drug remains to be explored, and such studies await elucidation of its mechanism of action. The inhibitory effect of β-lapachone on human DNA topoisomerase II was investigated [5]. It is suspected that β-lapachone binds directly to the enzyme to prevent DNA unwinding by topoisomerase I. It was also suggested that the cytotoxic actions of naphthoquinones derive, in part, from alkylation of exposed thiol residues on topoisomerase II-DNA complexes [6]. The majority of the above-mentioned biological activities of β-lapachone are dependent on bioreduction. The present work aims to obtain electrochemical data to help in the elucidation of the mechanism of the anticancer action of β-lapachones.

2. Experimental

Calf thymus DNA (sodium salt, Type I) was obtained from Sigma and L-cysteine and 2-mercaptoethanol from Aldrich. β-Lapachone (**1**), 3-sulphonic acid (**2**) and α-lapachone (**3**) used as a model, were synthesized according to the literature [1]. In an aqueous ethanolic phosphate buffer pH 7.0, **1** and **3** were prepared, while in aqueous buffer solutions, **2** was prepared. The experimental conditions were described elsewhere [7]. The electrochemical DNA-biosensor was prepared as described [8].

3. Results and discussions

Cyclic voltammograms of β-lapachones at a glassy carbon electrode show a reversible process involving the same number of electrons and protons (Fig. 1), and the reduction potential of β-lapachone is pH-dependent (slope 59 mV per pH unit) [7].

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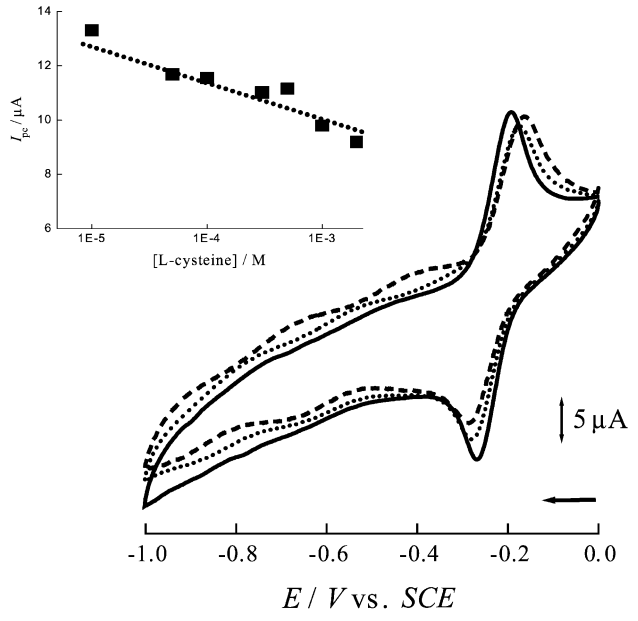


Fig. 1. Cyclic voltammograms of 0.1 mM β-lapachone: (—) absence and presence of (– – –) 1 mM and (•••) 0.1 mM L-cysteine. GCE, pH 7.0 0.2 M phosphate buffer, v=100 mV s^{-1}. Insert: plot of I_{pc} vs. concentration of L-cysteine.

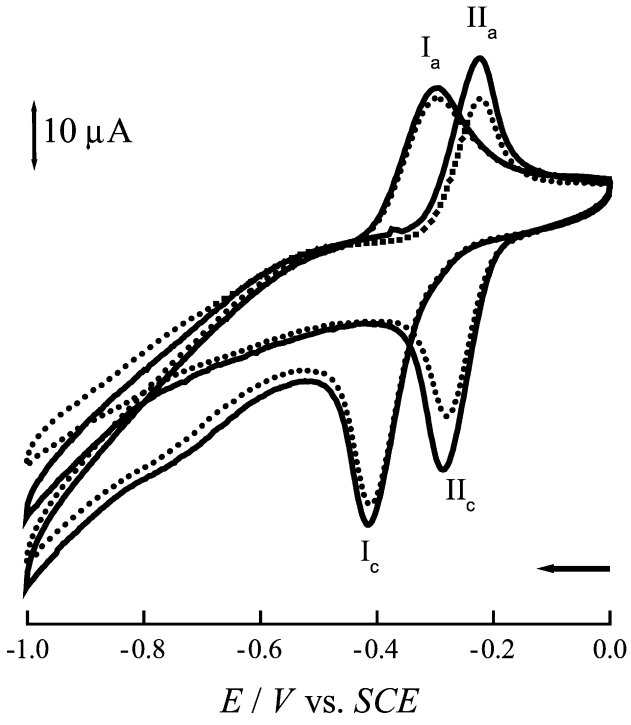
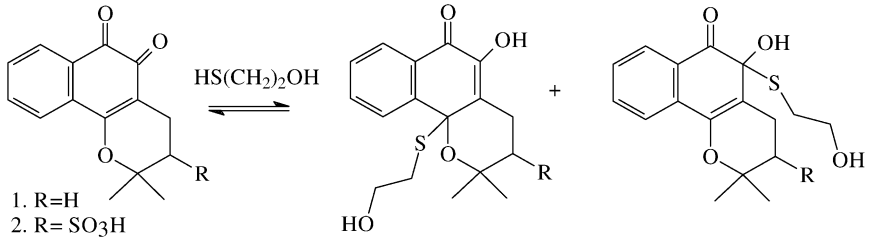


Fig. 2. Cyclic voltammograms of 0.1 mM of (I) α-lapachone and (II) β-lapachone: (—) absence and (•••) presence of 1 mM mercaptoethanol. GCE, pH 7.0 0.2 M phosphate buffer, v=100 mV s^{-1}.

Electrochemical experiments, with both β-lapachone and dsDNA in solution, did not show any evidence of interaction after 48 h. Using the DNA-biosensor in a solution containing ssDNA, the peaks corresponding to the oxidation of the bases guanine and adenine were not affected by the presence of β-lapachones. The electrochemical DNA-biosensor was prepared by covering a GCE with dsDNA and enables the evaluation and prediction of DNA damage by detection of the electrochemical oxidation of the DNA purine bases, guanine and adenine [8]. These results suggest that β-lapachones do not damage DNA directly, as already evidenced by other methods. DNA topoisomerases are certainly poisoned by β-lapachone, despite the uncertainty of the exact mechanism of interaction [5,6]. Topoisomerase II has 15 essential cysteine residues, any of which, if favourably located, may be susceptible to oxidation or any other reaction with quinones. Some thiol reagents, like mercaptoethanol [6], cysteine or *N*-acetylcysteine mimic the reactivity of thiol-containing enzymes, such as topoisomerase. Fig. 1 shows cyclic voltammograms of β-lapachone in the absence and in the presence of L-cysteine. When L-cysteine was added to the medium, the cathodic peak decreased significantly in size. Also the anodic peak was affected and new cathodic peaks were observed. This change is concentration-dependent and at high concentrations, the colour of the reaction solution changes immediately from bright yellow to pale yellow. These effects can be attributed to the reaction of β-lapachone and L-cysteine. A similar behaviour to that obtained with L-cysteine, but more intense, was observed using 2-mercaptoethanol, (Fig. 2). In the case of quinones, 1,2- and 1,4-Michael-type adducts are formed by the addition of the thiol group to the quinone ring, as suggested in the reaction scheme of β-lapachone with 2-mercaptoethanol [6] (see below).

A small change in the voltammogram is observed for α-lapachone. In the case of β-lapachone, the adducts formed are not stable and retroconversion to the starting products is observed.



4. Conclusions

β-Lapachones do not interact directly with dsDNA or ssDNA. The electrochemical observation of the reaction of β-lapachone with L-cysteine and 2-mercaptoethanol corroborates results already obtained and constitute additional evidence for the anticancer mechanism of β-lapachones based on the interaction with topoisomerase and/or DNA-bound topoisomerase.

Acknowledgements

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