Available online at www.sciencedirect.com
SCIENCE DIRECT®

Bioelectrochemistry 59 (2003) 85–87

Bioelectrochemistry

www.elsevier.com/locate/bioelechem

Electrochemical study of oxygen interaction with lapachol and its radical anions

Marília O.F. Goulart[a,*], Piotr Falkowski[b], Tadeusz Ossowski[b], Adam Liwo[b]

[a] Departamento de Química-CCEN Campus, Universidade Federal de Alagoas, Tabuleiro dos Martins, Maceio, Alagoas 57072-970, Brazil
[b] Faculty of Chemistry, University of Gdansk, 80-952, Poland

Received 11 October 2002; received in revised form 27 December 2002; accepted 28 December 2002

Abstract

Cyclovoltammetric studies were performed with lapachol [2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone], in the absence and presence of oxygen, which aimed to investigate possible oxygen interaction with lapachol and its radical anion. The obtained results clearly indicate the consumption of the semiquinone anion-radicals by oxygen in an EC type reaction, generating the deprotonated form of lapachol and $HOO^{\bullet}$. The observed generation of reactive oxygen species (ROS) after electron transfer can be related to the mechanism of biological action of lapachol.

Keywords: Lapachol; Oxygen interactions; Biological mechanism of action; Oxidative stress

1. Introduction

Lapachol, 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone (**1**), is known to possess antitumor, antibiotic, antimalarial, antiinflammatory and antiulceric activities [1]. Recent results revealed significant molluscicidal [2,3], cercaricidal [4] and trypanocidal activities [2,5] of lapachol and its potassium salt, and also strong interceptive effect in rats with 100% fetal/embryo mortality, without affecting the mothers [6]. The mechanism where hydroxy- and hydroxyalkyl-1,4-naphthoquinones exert their toxic effects is of particular interest. Two major mechanisms of quinone cytotoxicity have been proposed [7–9]: oxidative stress and alkylation of cellular nucleophiles. Quinones are highly redox active molecules, which can redox cycle with their semiquinone radicals, leading to the formation of reactive oxygen species (ROS) that include the superoxide anion radical, the peroxyl radical, hydrogen peroxide and, ultimately, the hydroxyl radical. ROS production can cause severe oxidative stress within cells through the formation of oxidized cellular macromolecules, including lipids, proteins and DNA [9]. A recent paper described the participation of P450 reductase in the bioactivation of lapachol, which led to the generation of superoxide anion radical [10] that promotes DNA scission.

For peroxidation in the presence of oxygen to occur, the presence of hydroxyl groups in the quinone moiety has been demonstrated to be essential [11].

The present work reports cyclovoltammetric studies performed with **1**, in the absence and presence of oxygen. Cyclic-voltammetry investigations of quinones in the presence of oxygen in aprotic media have been considered a useful tool for studying interaction of oxygen and the superoxide anion radical with quinones and their radical anions [11]. Non-aqueous aprotic solvents should be better models of membrane environment in which peroxidation processes take place, because both the superoxide anion radical and its conjugated acid, the hydroperoxyl radical, are virtually unstable in water and other protic solvents, owing to fast disproportionation [11].

2. Experimental

Electrochemical studies were carried out in dimethylsulfoxide (DMSO) containing 0.1 mol l^{-1} tetraethylammonium perchlorate (TEAP), with and without oxygen, using a glassy carbon electrode (0.0491 cm^2) as a working elec-

---

* Corresponding author. Tel./fax: +55-82-2141389.
E-mail addresses: mofg@qui.ufal.br, mariliagoulart@aol.com.br (M.O.F. Goulart).

1567-5394/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S1567-5394(03)00005-7

trode, the platinum-wire electrode as a counter electrode, and the standard saturated calomel electrode (SSCE; Hg, Hg_2Cl_2, NaCl sat.) as the reference electrode [11]. The ferrocene–ferricinium redox system was used to standardize the potential. Oxygen concentration was estimated as described previously [11].

3. Results and discussion

Electrochemical studies of the reduction of lapachol in DMF/TBAP 0.1 mol l^{-1} on Hg and glassy carbon electrode have been published recently [12]. The voltammograms are represented by two main pairs of peaks, the first cathodic one, designated Ic, with a related anodic peak (Ia), and the second pair, named IIIc (cathodic) and IIIa (anodic), the processes having irreversible and quasi-reversible nature, respectively. Intermediate shoulders (IIc) are also present. They showed strong dependence on scan rate [12]. Self-protonation mechanism and hydrogen-bonded intermediates explain the nature of the first peaks and shoulders [12]. The first pair of peaks is related to the semiquinone ($NQOH^{\cdot}$) and the last pair to the quasi-reversible electronic reduction of the respective anion of the lapachol or more generally to the conjugated base of 2-hydroxynaphtoquinones (NQO^{-}) [12]. The behaviour in DMSO/TEAP follows the same pattern (Fig. 1), with less evident shoulders IIc (Fig. 1).

Addition of O_2 to the system causes remarkable changes in the position of the first reduction peak potential as well as in the shape of the curves (Fig. 1). The presence of even small amounts of oxygen causes the increase of the height and anodic shift of the first cathodic wave Ic, related to the generation of the semiquinone (Fig. 1).

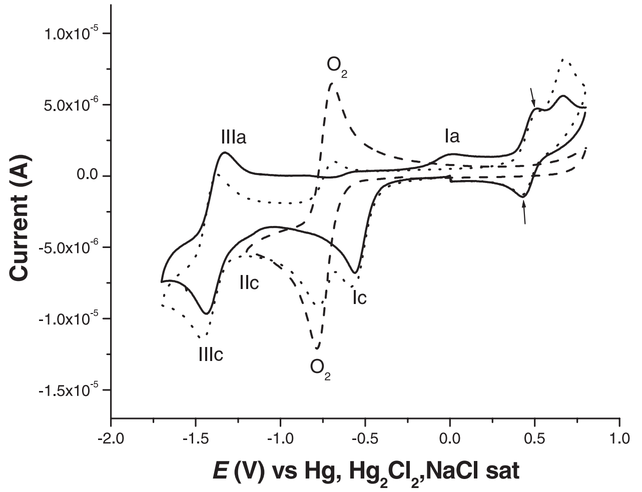

Fig. 1. Cyclic voltammograms in DMSO/TEAP 0.1 mol L^{-1}, on a glassy carbon electrode vs. SSCE (Hg, Hg_2Cl_2, NaCl sat.), of lapachol at concentration 0.73 mmol L^{-1}, in the absence of oxygen (solid line), oxygen at concentration 0.29 mmol L^{-1} (dashed line), and lapachol at concentration 0.73 mmol L^{-1} in the presence of oxygen at concentration 0.23 mmol L^{-1} (dotted line). v=0.100 V s^{-1}. Arrows indicate the ferrocene-ferricinium internal redox standard system.

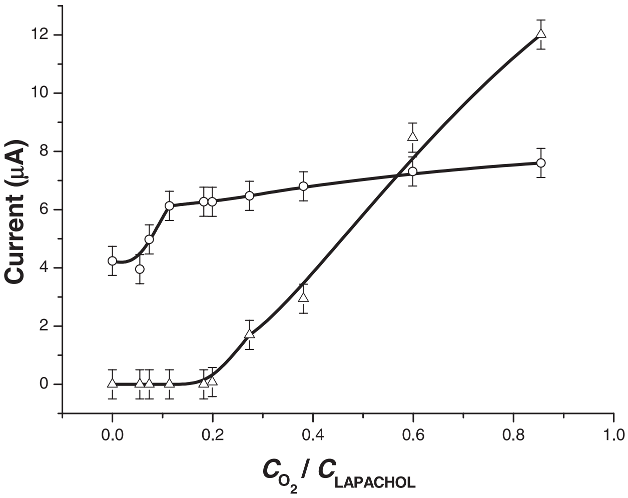

Fig. 2. Change of the current of the first reduction wave of lapachol (○) at concentration C_1=0.73 mmol L^{-1} and of the current of the first reduction wave of oxygen (△), as a function of oxygen concentration. DMSO/TEAP 0.1 mol L^{-1}, on a glassy carbon electrode vs. SSCE (Hg, Hg_2Cl_2, NaCl sat.). v=0.100 V s^{-1}.

A detailed study of the influence of oxygen concentration on E_{pIc} and I_{pIc} was performed, as described previously [11]. The graphs in Fig. 2 show that the peak of oxygen reduction ($E_{p\mathrm{O}_2} = -0.779$ V) starts only at oxygen/lapachol concentration ratio close to 0.2, while the reduction current related to lapachol (Ic) increases sharply (46%) at low O_2 concentrations. The position of lapachol reduction peak also changes, with shifts toward more positive potentials, with oxygen concentration (Fig. 3). The larger shift (ca. 0.048 V) occurs at oxygen/lapachol concentration ratio close to 0.12. The occurrence of an irreversible chemical reaction following one electron reduction of lapachol is also manifested by the disappearance of the anodic reoxidation peak (Ia) almost immediately after O_2 intake.

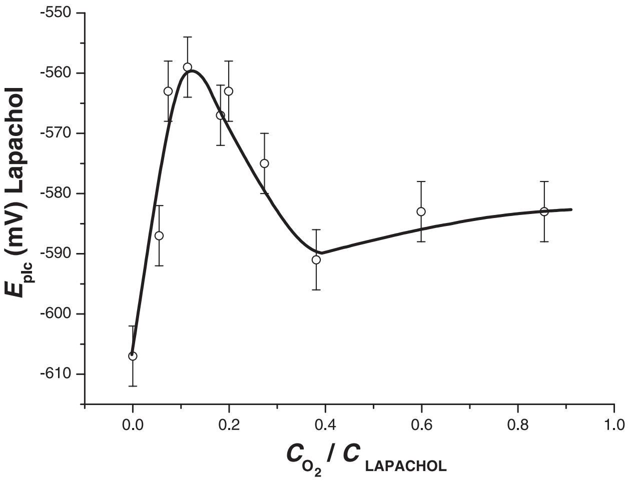

Fig. 3. Plot of potential of the first reduction wave of lapachol at concentration C_1=0.73 mmol L^{-1} ($E\mathrm{p_{Ic}}$) vs. oxygen concentration. DMSO/TEAP 0.1 mol L^{-1}, on a glassy carbon electrode vs. SSCE (Hg, Hg_2Cl_2, NaCl sat.). v=0.100 V s^{-1}.

All of these phenomena can be explained by assuming the EC mechanism, i.e., the consumption of the semiquinone anion-radicals by oxygen, in a EC type reaction, generating the deprotonated forms of **1** and $HOO^{\bullet}$ (Eqs. (1) and (2)).

The deprotonated lapachol reduces reversibly at potentials close to IIIc ($E_{pc} = -1.465$ V, $E_{pa} = -1.385$ V). The assignment of this wave to the conjugated base of lapachol was already reported [12].

$$\mathrm{NQOH} + e^{-} \rightleftharpoons \mathrm{NQOH}^{\bullet -} \tag{1}$$

$$\mathrm{NQOH}^{\bullet -} + O_2 \rightleftharpoons \mathrm{NQO}^{-} + \mathrm{HOO}^{\bullet} \tag{2}$$

4. Conclusions

In this study, using electrochemical methods, we have demonstrated that the lapachol anion-radical interacts with O_2, according to an EC mechanism, giving the deprotonated lapachol and the peroxyl radicals. These facts support the possible intermediacy of ROS in the mechanism of action of lapachol, corroborating the results of earlier studies in the presence of NADPH–cytochrome P450 reductase [10].

Acknowledgements

We thank CAPES, CNPq and FAPEAL for grants and financial support.

References

[1] S. Subramanian, M.M.C. Ferreira, M. Trsic, A structure-activity relationship study of lapachol and some derivatives of 1,4-naphthoquinones against carcinosarcoma Walker 256, Struct. Chem. 9 (1998) 47–57.

[2] A.F. dos Santos, P.A.L. Ferraz, F.C. de Abreu, E. Chiari, M.O.F. Goulart, A.E.G. Sant'Ana, Molluscicidal and trypanocidal activities of lapachol derivatives, Planta Med. 67 (2001) 92–93.

[3] A.F. dos Santos, P.A.L. Ferraz, A.V. Pinto, M.C.R.F. Pinto, M.O.F. Goulart, A.E.G. Sant'Ana, Molluscicidal activity of 2-hydroxy-3-alkyl-1,4-naphthoquinones and derivatives, Int. J. Parasitol. 30 (2000) 1199–1202.

[4] N.M.F. Lima, A.F. dos Santos, Z. Porfírio, M.O.F. Goulart, A.E.G. Sant'ana, Toxicity of lapachol and isolapachol and their potassium salts against *Biomphalaria glabrata*, *Schistosoma mansoni cercariae*, *Artemia salina* and *Tilapia nilotica*, Acta Trop. 83 (2002) 43–47.

[5] M.O.F. Goulart, C.L. Zani, J. Tonholo, F.C. De Abreu, D.S. Raslan, S. Starling, A.B. Oliveira, E. Chiari, Trypanocidal activity and redox potentials of heterocyclic and 2-hydroxy-naphthoquinones, Bioorg. Med. Chem. Lett. 7 (1997) 2043–2048.

[6] M.O. Guerra, A.S.B. Mazoni, M.A.F. Brandão, V.M. Peters, Interceptive effect of lapachol in rats, Contraception 60 (1999) 305–307.

[7] P.J. O'Brien, Molecular mechanisms of quinone cytotoxicity, Chem.–Biol. Interact. 80 (1991) 1–41.

[8] T.J. Monks, P. Hanzlik, G.M. Cohen, D. Ross, D.G. Graham, Quinone chemistry and toxicity, Toxicol. Appl. Pharmacol. 112 (1992) 2–16.

[9] J.L. Bolton, M.A. Trush, T.M. Penning, G. Dryhurst, T.J. Monks, Role of quinones in toxicology, Chem. Res. Toxicol. 13 (2000) 135–160.

[10] Y. Kumagai, Y. Tsurutani, M. Shinyashiki, S.H. Takeda, Y. Nakai, T. Yoskikawa, N. Shimojo, Bioactivation of lapachol responsible for DNA scission by NADPH-cytochrome P450 reductase, Environ. Toxicol. Pharmacol. 3 (1997) 245–250.

[11] T. Ossowski, P. Pipka, A. Liwo, D. Jeziorek, Electrochemical and UV-spectrophotometric study of oxygen and superoxide anion radical interaction with anthraquinone derivatives and their radical anions, Electrochim. Acta 45 (2000) 3581–3587.

[12] P.A.L. Ferraz, F.C. De Abreu, J. Tonholo, E. Chiari, A.V. Pinto, V. Glezer, M.O.F. Goulart, Electrochemical aspects of the reduction of biologically active 2-hydroxy-3-alkyl-1,4-naphthoquinones, J. Electroanal. Chem. 507 (2001) 275–286.