Volume 253, number 1,2, 235–238 FEB 07478 August 1989

Pulse radiolysis study on the reactivity of Trolox C phenoxyl radical with superoxide anion

Enrique Cadenas, Gabor Merényi* and Johan Lind+

*Department of Pathology II, University of Linköping, S-581 85 Linköping, *Department of Physical Chemistry, Royal Institute of Technology, S-100 44 Stockholm* and *+Department of Nuclear Chemistry, Royal Institute of Technology, S-100 44 Stockholm, Sweden*

Received 16 June 1989

The reaction between the phenoxyl radical of Trolox C, a water-soluble vitamin E analogue, and superoxide anion radical was examined by using the pulse radiolysis technique. The results indicate that the Trolox C phenoxyl radical may undergo a rapid one-electron transfer from superoxide radical [$k = (4.5 \pm 0.5) \times 10^8$ $M^{-1} \cdot s^{-1}$] to its reduced form. This finding indicates that superoxide radical might play a role in the repair of vitamin E phenoxyl radical.

Superoxide radical; Trolox C; Vitamin E; Phenoxyl radical; Pulse radiolysis

1. INTRODUCTION

The antioxidant properties of α-tocopherol, the most important component of vitamin E, and its synthetic analogues, are related to the termination of lipid chain oxidation reactions by intercepting lipid peroxyl radicals. The reactivity of α-tocopherol towards free radicals is extended to other species such as perhydroxyl radicals ($HO_2^{\cdot}$) [1,2], metal complexes [3], hydroxyl radicals ($HO^{\cdot}$) [4], as well as organic peroxyl radicals and other electrophilic radicals [5–9]. Thus, it was suggested that the antioxidant properties of α-tocopherol are not restricted to its function as a chain-breaking antioxidant, but include the interception of species that can be considered as initiators of chain reactions. The overall reaction of α-tocopherol with different electrophilic free radical species can be formulated as in reaction 1, formally regarded as an H-atom transfer [9]; however, another possibility regards electron transfer, particularly in nonpolar media such as lipids and membranes, followed by deprotonation of the antioxidant radical cation in a reaction with H_2O immersed in the lipid phase [10].

$$\alpha\text{-T-OH} + R^{\cdot} \longrightarrow \alpha\text{-T-O}^{\cdot} + RH \qquad (1)$$

The chromanoxyl radical (α-T-O$^{\cdot}$) resulting from α-tocopherol/free radical interactions (as in reaction 1) is known to be long-lived and it decays by pathways involving disproportionation or repair by electron- or hydrogen atom transfer. The former pathway (reaction 2) proceeds at modest rates in either micelles or organic systems ($2k = 3.5 \times 10^2$ $M^{-1} \cdot s^{-1}$) [11] to give a set of molecular products, but is considerably increased for the case of the water-soluble analogue of α-tocopherol, Trolox, an effect probably determined by the replacement of the C_{16}-chain in α-tocopherol by a -COOH group in Trolox [3].

$$2\alpha\text{-T-O}^{\cdot} \longrightarrow \text{products} \qquad (2)$$

The latter reaction, repair of the chromanoxyl radical of α-tocopherol, has been shown to occur with ascorbic acid [4,5], serotonin, and other

Correspondence address: E. Cadenas, Department of Pathology II, University of Linköping, S-581 85 Linköping, Sweden

Abbreviations: T-OH, Trolox C (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid); α-T-OH, α-tocopherol

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

hydroxyindole derivatives (present in the nerve cells in high concentrations) [12], and thiol-containing compounds [4].

$$\alpha\text{-T-O}^{\cdot} + \text{XH} \longrightarrow \alpha\text{-T-OH} + \text{X}^{\cdot} \quad (3)$$

In the present paper we describe in a pulse radiolysis study the reaction of the phenoxyl radical of Trolox C, a synthetic, water-soluble analogue of α-tocopherol, with $O_2^{\cdot-}$, a reaction of potential biological interest.

2. MATERIALS AND METHODS

Trolox C was obtained from Aldrich Chemical Co. (Steinheim, FRG). The pulse radiolysis experiments were conducted using the Royal Institute of Technology (Stockholm) microtron accelerator facility as previously described [13]. The optical detection system was equipped with a xenon light source. Micro-cells of 1-cm optical-path length (volume = 1 ml) were used throughout. Dosimetry was performed with aerated aqueous solutions of 10×10^{-2} M KSCN, taking $G_\epsilon(SCN)_2^{\cdot-} = 2.2 \times 10^{-4}$ m^2/J for $(SCN)_2^{\cdot-}$ at $\lambda_{500\,nm}$ [14]. The reducing radical, $CO_2^{\cdot-}$, was produced by giving short pulses (100 ns) of radiation to a water solution of Trolox containing 2×10^{-2} M $NaHCO_2$ and 10^{-3} M potassium phosphate buffer.

3. RESULTS AND DISCUSSION

Water, when radiolyzed, yields the species shown in reaction 4.

$$H_2O \longrightarrow e_{aq}^- + HO^{\cdot} + H^{\cdot} \quad (4)$$

In N_2O-saturated solutions, e_{aq}^- is converted to $HO^{\cdot}$ (reaction 5) and the addition of KBr to the N_2O-saturated solution yields the oxidizing radical $Br_2^{\cdot-}$ as indicated in reactions 6 and 7.

$$e_{aq}^- + N_2O \longrightarrow N_2 + HO^- + HO^{\cdot} \quad (5)$$

$$HO^{\cdot} + Br^- \longrightarrow HO^- + Br^{\cdot} \quad (6)$$

$$Br^{\cdot} + Br^- \longrightarrow Br_2^{\cdot-} \quad (7)$$

Oxidizing species such as $Br_2^{\cdot-}$ (with a reduction potential of +1.69 V [15]) react with Trolox (reactions 8,9) via a direct electron transfer process, at variance with $HO^{\cdot}$, which is known to form adducts with compounds such as Trolox [3]. The product, the transient phenoxyl radical, is formed upon loss of the H atom in the -OH group in Trolox.

$$Br_2^{\cdot-} + \text{T-OH} \longrightarrow 2\,Br^- + \text{T-OH}^{\cdot+} \quad (8)$$

$$\text{T-OH}^{\cdot+} \longleftrightarrow \text{T-O}^{\cdot} + H^+ \quad (9)$$

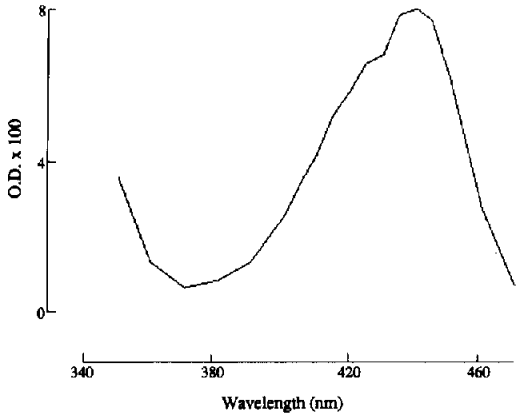


Fig.1. Absorption spectrum of Trolox C phenoxyl radical. Spectrum was obtained in a N_2O-saturated aqueous solution containing 4.5×10^{-4} M Trolox C and 0.05 M Br^- at pH 7. Dose/pulse = 34 Gy.

Fig.1 shows the transient absorption spectrum of Trolox phenoxyl radical following the pulse irradiation of a N_2O-saturated Trolox solution. The spectrum exhibits an absorption maximum at $\lambda_{440\,nm}$ ($\epsilon = 4.4 \times 10^3$ M$^{-1}\cdot$cm^{-1}) and a shoulder at about $\lambda_{424\,nm}$. The UV and visible spectra of Trolox radical, under similar conditions, have been described in detail [3,16]; the disappearance of the semiquinone form of Trolox occurs via a disproportionation reaction (as indicated in reaction 2), the rate of which varies with pH, ranging from 1.6×10^7 M$^{-1}\cdot$s^{-1} at pH 4.5 to 3.6×10^5 M$^{-1}\cdot$s^{-1} at pH 5.6 [3].

Once the absorption spectrum of the Trolox radical was established (fig.1), the reactivity of the radical with $O_2^{\cdot-}$ was investigated. On pulse radiolysis of a N_2O/O_2-saturated solution containing excess of HCO_2^-, a transient absorption is observed immediately after the pulse with λ_{max} = 260 nm, characteristic of the $O_2^{\cdot-}$ formed by reactions 10 and 11.

$$HO^{\cdot} + HCO_2^- \longrightarrow H_2O + CO_2^{\cdot-} \quad (10)$$

$$CO_2^{\cdot-} + O_2 \longrightarrow CO_2 + O_2^{\cdot-} \quad (11)$$

$O_2^{\cdot-}$ does not react with Trolox C [2,4] as does its protonated form, $HO_2^{\cdot}$, which reacts with vitamin E with a second order rate constant of 2×10^5 M$^{-1}\cdot$s^{-1} [17]. On pulse radiolysis performed

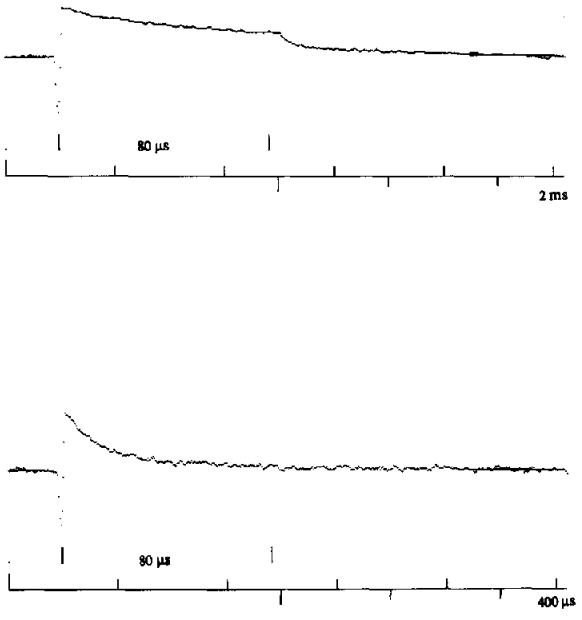


Fig.2. Time course of decay of Trolox C phenoxyl radicals. Traces recorded at λ_{440nm} after delivery of a 5×10^{-7} s electron pulse with a dose of 180 Gy to a solution containing 10^{-3} M Trolox, 0.05 M Br^-, and 0.5 M HCO_2^-, pH 7. Upper trace, N_2O saturated; lower trace, O_2 saturated.

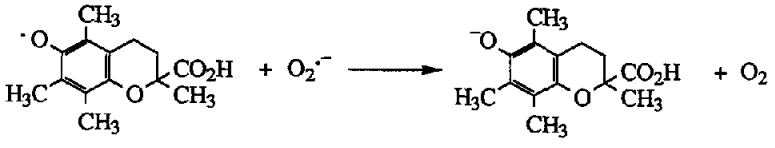
on O_2-saturated solutions of Trolox (10^{-3} M), e_{aq}^- and H· react with O_2 to yield $O_2^{\cdot-}$ according to equations 12 and 13.

$$e_{aq}^- + O_2 \longrightarrow O_2^{\cdot-} \quad (12)$$

$$H^\cdot + O_2 \longrightarrow O_2^{\cdot-} + H^+ \quad (13)$$

By adding varying amounts of Br^- and HCO_2^- to the sample, the HO· radical was scavenged through reaction 6 (followed by reaction 7) in competition with reaction 10. As can be seen, reaction 7 is followed by reactions 8 and 9 to yield T-O· while reaction 10 is ensued by reaction 11 to produce $O_2^{\cdot-}$. The amount of Br^- and HCO_2^- were adjusted so as to result in a 5–10-fold excess of $O_2^{\cdot-}$ over T-O·. The value [T-O·]/[$O_2^{\cdot-}$] initially formed in a particular experiment was determined by comparing the size of the absorbance of the T-O· radical at λ_{440nm} in O_2-saturated solutions with its corresponding size measured in N_2O-saturated Br^- solutions. (Evidently, in the latter case all primary radicals are ultimately scavenged by Trolox to form T-O·.)

Fig.2 demonstrates that the decay of the λ_{440nm} absorbance is accelerated in the presence of O_2. This decay is independent of the O_2 concentration but is dependent on the dose. In O_2-purged solutions, the decay of the absorbance at λ_{440nm} was exponential with rates proportionally increasing with the applied dose. From the measured pseudo-first order rates, the second order rate constant for the reaction of Trolox radical with $O_2^{\cdot-}$, i.e. k_{14}, was calculated to be $(4.5 \pm 0.5) \times 10^8$ $M^{-1} \cdot s^{-1}$.



(14)

Next, an air saturated aqueous solution containing 10^{-4} M of Trolox and 5×10^{-3} M Br^- was irradiated in a γ-source (dose rate 0.4 Gy·s^{-1}) at pH 7. Under such circumstances, the radical mixture produced consists of 55% $O_2^{\cdot-}$ and 45% T-O·. As both these radicals are relatively stable with respect to self recombination they should disappear predominantly through reaction 14. We found that more than 60% of the $O_2^{\cdot-}$ reverted back to O_2. This supports reaction 14 being an electron transfer. We also noted that, in pulse radiolysis, after the completion of reaction 14 the solution reverts to its original colour with no absorption above ca. λ_{300nm}. In contrast, a coloured product forms upon the radical dismutation reaction of T-O·. This finding mitigates against reaction 14 being an autoxidation of T-O· by $O_2^{\cdot-}$. The less than quantitative generation of O_2 indicates the partial formation of a hydroperoxy adduct in a minor process occurring parallel to reaction 14.

From the thermodynamic point of view the electron transfer in reaction 14 makes sense. Combining + 192 mV, the one-electron reduction potential of T-O· [18] with − 155 mV (the E° value of the $O_2/O_2^{\cdot-}$ couple at 1 M concentration [19]), we calculate a $\Delta G^\circ = -33.5$ kJ·mol^{-1} for reaction 14. Thus, the reaction is exothermic but not excessively so. High as it is, the measured rate cons-

tant k_{14} is still significantly below the diffusion-controlled limit. This is consistent with the suggestion that the rate constant of self-exchange of the $O_2/O_2^{\cdot -}$ couple is very low [20].

4. CONCLUDING REMARKS

The results presented here indicate that the phenoxyl radical of Trolox can be repaired by $O_2^{\cdot -}$, presumably by an electron-transfer mechanism. The rate value for reaction 14 is higher than the value reported for the reaction rate of ascorbate [$(8.3 \pm 0.2) \times 10^6\ M^{-1} \cdot s^{-1}$] with the Trolox radical [4]. Under cellular conditions, the repair of vitamin E chromanoxyl radical will not only be dependent on the absolute rate of electron transfer from the electron donors cited, but also on the actual concentrations of the reactants. The steady-state concentration of $O_2^{\cdot -}$ in hepatocytes has been estimated to be about 10^{-11} M [21], a value decidedly lower than the intracellular concentration of ascorbate (~ 3–4×10^{-4} M) [22]. However, the biological relevance of the repair of phenoxyl radicals by $O_2^{\cdot -}$ reported here cannot be assessed on the questionable assumption that the distribution of $O_2^{\cdot -}$ – or any other free radical species – is homogeneous within the cell. Reaction 14 can be viewed also as an efficient means to scavenge $O_2^{\cdot -}$ and, at variance with the disproportionation of $O_2^{\cdot -}$ – either spontaneous or enzyme-catalyzed – the reaction does not yield H_2O_2 as a molecular product.

Acknowledgements: This work was supported by grant 2703-B90-01XA from the Swedish Cancer Foundation, grant 7679 and 4481 from the Swedish Medical Research Council, and a grant from the Swedish Natural Science Research Council.

REFERENCES

[1] Bielski, B.H.J., Arudi, R.L. and Sutherland, M.W. (1983) J. Biol. Chem. 258, 4759–4761.
[2] Arudi, R.L., Sutherland, M.W. and Bielski, B.H.J. (1983) in: Oxygen Radicals and Their Scavenger Systems (Cohen, G. and Greenwald, R.A. eds) vol.I, pp.26–31, Elsevier, New York.
[3] Cabelli, D.E. and Bielski, B.H.J. (1986) J. Free Radicals Biol. Med. 2, 71–75.
[4] Davies, M.J., Forni, L.G. and Willson, R.L. (1988) Biochem. J. 255, 513–522.
[5] Packer, J.E., Slater, T.F. and Willson, R.L. (1979) Nature 278, 737–738.
[6] Simic, M.G. (1981) in: Oxygen and Oxy-Radicals in Chemistry and Biology (Rodgers, M.A.J. and Powers, E.L. eds) pp.109–118, Academic Press, New York.
[7] Hunter, E.P.L. and Simic, M.G. (1983) in: Oxy Radicals and their Scavenger Systems (Cohen, G. and Greenwald, R.A. eds) vol.I, pp.32–37, Elsevier, New York.
[8] Jore, D. and Ferradini, C. (1985) FEBS Lett. 183, 299–303.
[9] Willson, R.L., Dunster, C.A., Forni, L.G., Gee, C.A. and Kittridge, K.J. (1985) Phil. Trans. R. Soc. Lond. B 311, 545–563.
[10] Simic, M.G. and Hunter, E.P.L. (1985) in: Chemical Changes in Food during Processing, pp.107–119, AVI Publishing Co.
[11] Simic, M.G. (1980) in: Autoxidation in Food and Biological Systems (Simic, M.G. and Carel, M. eds) pp.17–26, Plenum Press, New York.
[12] Jovanovic, S.V. and Simic, M.G. (1985) Life Chem. Rep. 3, 124–130.
[13] Eriksen, T.E., Lind, J. and Reitberger, T. (1976) Chem. Scr. 10, 5–6.
[14] Fielden, E.M. (1982) in: The Study of Fast Processes and Transient Species by Electron Pulse Radiolysis (Baxendale, J.H. and Busi, F. eds) pp.49–62, Reidel, Dordrecht.
[15] Woodruf, W.H. and Margerum, D.W. (1973) Inorg. Chem. 12, 962–964.
[16] Bisby, R.H., Ahmed, S. and Cundall, R.B. (1984) Biochem. Biophys. Res. Commun. 119, 245–251.
[17] Fukuzawa, K. and Gebicki, J.M. (1983) Arch. Biochem. Biophys. 226, 242–251.
[18] Stenken, S. and Neta, P. (1982) J. Phys. Chem. 86, 3661–3667.
[19] Ilan, Y.A., Czapski, G. and Meisel, D. (1976) Biochim. Biophys. Acta 430, 209–224.
[20] Zahir, K., Espenson, J.H. and Bakac, A. (1988) J. Am. Chem. Soc. 110, 5059–5063.
[21] Chance, B., Sies, H. and Boveris, A. (1979) Physiol. Rev. 59, 527–605.
[22] Hornig, D. (1975) Ann. NY Acad. Sci. 258, 103–118.