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REPAIR OF AMINO ACID RADICALS BY A VITAMIN E ANALOGUE

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Summary: Free radicals derived from one-electron oxidation of the amino acids tryptophan, tyrosine, methionine and histidine have been found to be rapidly ($k=10^7$ -10^9 dm^3 mol^{-1} s^{-1}) and efficiently repaired by Trolox C, a vitamin E analogue. The reactions form a relatively stable phenoxyl radical of Trolox C (λ_{max} = 440 nm; $\varepsilon=5.4 \times 10^3$ mol dm^{-3} cm^{-1}). The radical cation of tryptophan is more rapidly repaired than the neutral tryptophan radical. Repair of tryptophanyl radicals in the enzyme lysozyme has also been observed. The results suggest that a function of α-tocopherol in membranes may be the repair of radicals of integral membrane proteins.

Deficiency of tocopherol (Vitamin E) in vivo leads to a wide range of physiological disturbances (1). At the molecular level, it is known that α-tocopherol has a strong inhibitory effect (2-5) on the free radical autoxidation of lipids (6) in model and cellular systems. Recent pulse radiolysis investigations have shown that α-tocopherol is capable of repairing alkyl peroxy radicals with rate constants of the order of 10^7 dm^3 mol^{-1} s^{-1} (7,8). The resulting tocopheryl radical is itself repaired by ascorbate (9). Hence the role of α-tocopherol has been thought to be as an intermediary in the overall repair of lipid radicals by reductants such as ascorbate. α-Tocopherol has also recently shown to react with $HO_2^{\cdot}$ at a rate ($k= 2 \times 10^5$ dm^3 mol^{-1} s^{-1}) which is approximately two orders of magnitude greater than the rate of reaction with unsaturated fatty acids ($k= (1.2 - 3.0) \times 10^3$ dm^3 mol^{-1} s^{-1}) (10,11). Consequently it has been suggested that an alternative role for α-tocopherol is the scavenging of radicals involved in the initiation of lipid peroxidation (10-12).

Trolox C (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) is a water soluble analogue of α-tocopherol which also possesses antioxidative properties (13,14). The solubility of Trolox C in aqueous solutions has enabled the rates

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of repair of amino acid radicals by this compound to be measured, using the technique of pulse radiolysis (15).

MATERIALS AND METHODS

Trolox C was a gift from Hoffman LaRoche (Nutley, New Jersey). Solutions were prepared in baked glassware using triply distilled water as diluent. Chemicals were AnalaR grade when available. Prior to radiolysis, solutions were saturated with N_2O in glass syringes as described previously (16). Pulse radiolysis was performed with a Febetron 705 producing single 50 ns pulses of 1.5 MeV electrons. Transient absorptions were recorded with a Datalab 902 transient recorder and transferred to an Apple II+ microcomputer for kinetic analysis. Doses of between 150 and 300 rads per pulse were used for kinetic measurements. Dosimetry was performed with air saturated KSCN (10^{-2} mol dm^{-3}), taking G $(SCN)_2^{\cdot-}$ = 2.8 and ε(480 nm) = 7,600 dm^3 mol^{-1} cm^{-1}. Intensities of transient spectra are shown as the product of radical G-value (molecules formed heV^{-1} of energy absorbed) and extinction coefficient (ε, units dm^3 mol^{-1} cm^{-1}).

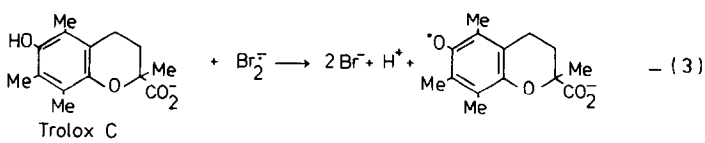
RESULTS

Reactions of amino acid radicals with Trolox C were investigated in solutions saturated with N_2O. In such solutions radiolysis leads to the formation of $^{\cdot}OH$ and $^{\cdot}H$ as the only primary free radicals with G-values of 6.0 and 0.55 respectively

$$H_2O \rightsquigarrow {}^{\cdot}OH,\ {}^{\cdot}H,\ e^-_{aq},\ H_2O,\ H_2 \qquad - (1)$$

$$N_2O + e^-_{aq} \rightarrow N_2 + {}^{\cdot}OH + OH^- \qquad - (2)$$

The second order rate constant for reaction of $^{\cdot}OH$ with Trolox C was determined by the competition method (17,18) using either I^- or SCN^- as reference solutes. The results gave a value for k($^{\cdot}OH$ + Trolox C) = (2.5 ± 0.5) x 10^{10} dm^3 mol^{-1} s^{-1} at pH 7.0. Either $Br_2^{\cdot-}$ or $N_3^{\cdot}$, formed by scavenging of $^{\cdot}OH$ by Br^- or N_3^- respectively, were employed as selective radicals (19,20) to generate the amino acid radicals. Pulse radiolysis of solutions containing Br^- (0.1 mol dm^{-3}) and Trolox C (0.2 mmol dm^{-3}) at pH 7.15 gave rise to the spectra shown in Figure 1. The initial spectrum shown at 2 μs after the radiation pulse with λ_{max} = 360 nm is due to $Br_2^{\cdot-}$. This absorption decays exponentially and is simultaneously replaced (see inset to Figure 1) by the Trolox radical spectrum (λ_{max} = 440 nm; ε(440 nm) = 5.4 x 10^3 dm^3 mol^{-1} cm^{-1})



– (3)

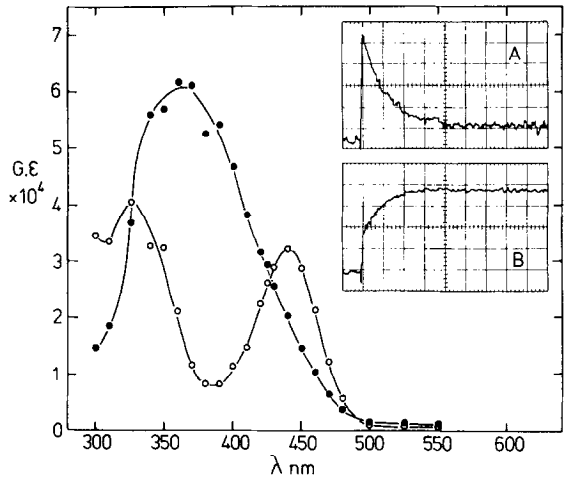


Figure 1 Transient spectra obtained on pulse radiolysis of N_2O-saturated solutions containing KBr (0.1 mol dm^{-3}) and Trolox C (0.2 mmol dm^{-3}) at pH 7.15. Measured ● 1μs and O 100μs after the pulse. Inset: A-decay of $Br_2^{\cdot-}$ at 380nm; B-formation of Trolox radical at 460nm; abscissae-time 10μs/division; ordinate-absorption 0.91%/division (A) and 0.0455%/division (B).

which appears to be typical of a phenoxyl radical (20,21) and is similar in intensity and spectral shape to the phenoxyl radical of α-tocopherol (7-9). The second order rate constant for reaction of $Br_2^{\cdot-}$ with Trolox C at pH 7.0, determined by varying the Trolox C concentration, was found to be 3.8×10^8 dm^3 mol^{-1} s^{-1}. Figure 2 shows the results obtained on pulse radiolysis of a

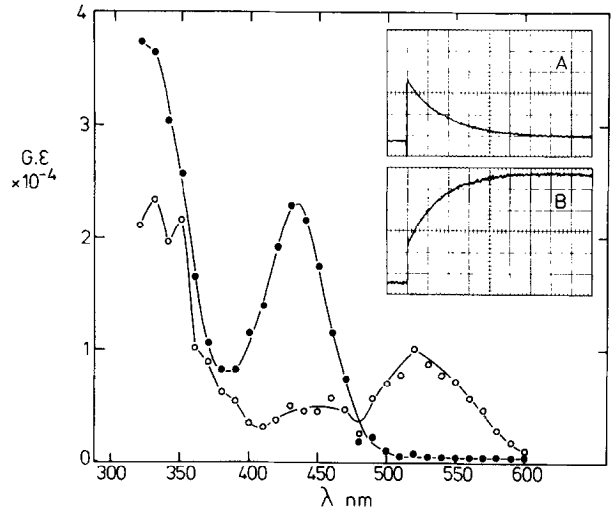
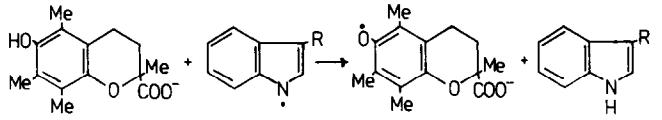


Figure 2 Transient spectra obtained on pulse radiolysis of N_2O saturated solutions containing NaN_3 (0.1 mol dm^{-3}), tryptophan (4 mmol dm^{-3}) and Trolox C (0.4 mmol dm^{-3}) at pH 7.1. Measured O 2μs and ● 300μs after the pulse. Inset: transients measured in N_2O-saturated solutions containing NaN_3 (0.1 mol dm^{-3}), tryptophan (2 mmol dm^{-3}) and Trolox C (0.2 mmol dm^{-3}) at pH 7.0. A-decay of tryptophan radical at 520nm; B-formation of Trolox radical at 440nm. Abissae-time 50μs/division, ordinate-absorption 0.0455%/division.

solution containing N_3^- (0.1 mol dm^{-3}), tryptophan (4 mmol dm^{-3}) and Trolox C (0.4 mmol dm^{-3}) at pH 7.1. The $N_3^\cdot$ radicals (which are transparant in the wavelength region examined here) react rapidly with tryptophan ($k = 4.1 \times 10^9$ dm^3 mol^{-1} s^{-1} (19)) to form the neutral tryptophan radical with λ_{max} = 520 nm. At the concentrations used in the experiment, the tryptophan radical is present 2 μs after the radiation pulse and decays exponentially to be simultaneously replaced by the Trolox radical :



- (4)

The rate of repair of the tryptophan radical by Trolox C (equation (4)) was determined by calculation of both the rate of decay of the tryptophan radical at 520 nm, and the rate of formation of the Trolox radical at 440 nm. Figure 3 shows the results obtained at pH 6.3 and demonstrates that similar rates are obtained by both methods (see Table 1). The observed efficiency of repair of tryptophan radicals by Trolox C depends on both Trolox C and tryptophan radical concentration due to competition between reaction (4) and the second order of decay of tryptophan radicals ($Trp^\cdot$) :-

$$Trp^\cdot + Trp^\cdot \rightarrow \text{products} \qquad - (5)$$

The fraction of tryptophan radicals repaired by Trolox C can be estimated from the intensity of the Trolox radical absorption at 440 nm ($G\varepsilon_{obs}$) and related to

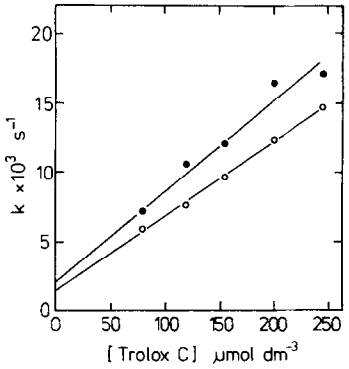


Figure 3 First order rate versus concentration of Trolox C for repair of tryptophan radicals at pH 6.3. Measured ● from formation of Trolox radical at 440nm; O from decay of tryptophan radical at 520nm.

Table 1. Efficiences and rate constants for repair of amino acid radicals by Trolox C

Compound	pH	Rate constant for repair* (amino acid radical decay)	Rate constant for repair* (Trolox radical formation)	Estimated maximum efficiency of repair (%)
Tryptophan	7.0	3.9×10^7	5.2×10^7	94
Tryptophan	1.65	1.72×10^9	1.86×10^9	82
Histidine	7.2	-	8.05×10^8	100
Tyrosine	7.0	-	3.83×10^8	95
Methionine	7.0	7.06×10^8	-	-
Lysozyme	7.0	2.1×10^7	5.3×10^7	-

* units of $dm^3\ mol^{-1}\ s^{-1}$, error ± 5%

the respective solute concentrations and maximum yield of the Trolox radical absorption ($G\varepsilon_{max}$) :

$$\frac{1}{G\varepsilon_{obs}} = \frac{1}{G\varepsilon_{max}} + \frac{k_5}{k_4} \cdot \frac{[Trp^{\cdot}]}{[Trolox\ C]} \cdot \frac{1}{G\varepsilon_{max}} \qquad - (6)$$

A plot of equation (6) is shown in Figure 4. The value of $G\varepsilon_{max}$ obtained indicates that 94% of tryptophan radicals are repairable by Trolox C, assuming G (tryptophan radicals) = 6.0. Below pH5 the rate constant for repair of tryptophan radicals by Trolox C increased corresponding to an estimated pK of 4.2 ± 0.1. This is in good agreement with the previously determined pK of 4.3 for the protonation of the tryptophan radical (22), and shows that the tryptophan radical cation is more rapidly repaired by Trolox C than the neutral tryptophan

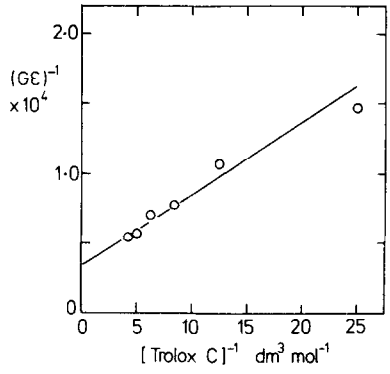


Figure 4 Plat of $(G\varepsilon)^{-1}$ versus $[Trolox\ C]^{-1}$ according to equation (6) for radical transfer from tryptophan to Trolox C at pH 7.0.

radical. The rate constant for repair of the tryptophan radical cation by Trolox C at pH 1.65 is included in the Table.

Similar experiments were conducted with other amino acids (histidine, tyrosine and methionine) which are readily oxidised by $N_3^{\cdot}$ or $Br_2^{\cdot-}$. (19,20). The results shown in the Table indicate that radicals formed by one-electron oxidation of these amino acids are also rapidly and efficiently repaired by Trolox C. Also included in the Table are the results from an experiment in which the radical(s) formed by one-electron oxidation of lysozyme is repaired by Trolox C. The rate constant for repair of the lysozyme radical(s) is similar to that found with tryptophan, and is consistent with previous reports that tryptophan is the predominant residue in lysozyme which is oxidised by selective radical anions (20,23).

DISCUSSION

The free radical transfer reactions described here are similar to intramolecular reactions observed between tryrosine or methionine and tryptophan radicals in proteins (24,25). However, the intermolecular radical transfer from tryptophan to tyrosine does not readily occur, indicating the more favourable redox potential of Trolox C for this process. The high efficiencies and rapid rates for repair of amino acid radicals by Trolox C indicates that α-tocopherol may have an important function in repairing free radical sites on membrane bound proteins. The amino acids studied here are those which are most likely to be oxidised by cellular free radical processes. By analogy with cholesterol (26), α-tocopherol would be expected to be orientated in a membrane bilayer with the hydroxyl group at the membrane-aqueous interface and the alkyl chain aligned with the fatty acyl chains of the lipid molecules towards the hydrophobic bilayer interior. This orientation might be expected to be unfavourable for the direct transfer of a one-electron reducing equivalent from the -OH group of α-tocopherol to a radical site on a fatty acyl chain. However, this orientation would favour both scavenging of free radicals attacking the membrane from the aqueous phase, and repair of protein radicals generated by such attack at the regions of membrane proteins close to the membrane surface. The importance of repair of

membrane proteins may be judged from reports indicating free radical mediated damage to membrane proteins, having transport and enzymatic functions, on exposure to radiation (27, 28) or high oxygen pressures (29, 30).

ACKNOWLEDGEMENTS

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