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Reactions of the α-tocopheroxyl radical in micellar solutions studied by nanosecond laser flash photolysis

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Laser flash photolysis of α-tocopherol in methanol and in aqueous micellar solutions has been shown to produce the α-tocopheroxyl radical. The reaction between the α-tocopheroxyl radical and ascorbate in positively charged hexadecyltrimethylammonium chloride (HTAC) micelles occurred with a second order rate constant of 7.2×10^7 $M^{-1} \cdot s^{-1}$, whereas in negatively charged sodium dodecyl sulphate (SDS) micelles the rats constant was only 3.8×10^4 $M^{-1} \cdot s^{-1}$. The α-tocopheroxyl radical was found to be relatively long-lived in HTAC micelles ($t_{1/2} \geq 5$ min), allowing the slow disappearance of the α-tocopheroxyl radical by reaction with glutathione to be observed.

α-Tocopherol; Vitamin E; Radical; Ascorbate; Flash photolysis; Micelle

1. INTRODUCTION

α-Tocopherol (vitamin E, α-Toc-OH) is an essential lipophilic membrane component which protects cellular membranes against lipid peroxidation by acting as a chain-breaking antioxidant [1]. Although α-tocopherol is only a minor membrane component it is effective because the α-tocopheroxyl free radical, formed in the chain-breaking reaction (1) with the lipid peroxyl radical (ROO•), is thought [2] to be rapidly repaired in reaction (2) by the much larger pool of ascorbate ($AscH^-$) in the aqueous phase.

$$ROO^\bullet + \alpha\text{-Toc-OH} \rightarrow ROOH + \alpha\text{-Toc-O}^\bullet \quad \ldots\ldots(1)$$
$$\alpha\text{-Toc-O}^\bullet + AscH^- \rightarrow \alpha\text{-Toc-OH} + Asc^{-\bullet} \quad \ldots\ldots(2)$$

This scheme is supported by several reports which show that ascorbate interacts synergistically with α-tocopherol in inhibiting lipid peroxidation [3–5]. It is reported that thiols such as glutathione may also regenerate α-tocopherol [6], although protein factors may be required in vivo [7,8]. The rate of reaction (2), k_2, has been directly measured to be 1.5×10^6 $M^{-1} \cdot s^{-1}$ in a water/2-propanol/acetone mixture by pulse radiolysis [9]. Reaction (2) has also been studied in solution by stopped flow for a number of α-tocopherol analogues [10]. An estimate of $k_2 = 2 \times 10^5$ $M^{-1} \cdot s^{-1}$ for the α-tocopheroxyl radical in phosphatidylcholine bilayer membranes has been obtained from ESR experiments [3].

Photolysis of solvent or solute molecules has been used to create radicals which react with α-tocopherol to form the α-tocopheroxyl radical [11–13]. Direct excitation or photo-ionisation of α-tocopherol to form the radical in a manner analogous to that reported for other phenoxyl radicals [14] is also possible [8]. The present paper shows that nanosecond laser flash photolysis at 308 nm may be used to directly produce the α-tocopheroxyl radical in micellar solution, and to measure the rate of the electron/hydrogen atom transfer reaction (2) across the micelle/aqueous interface.

2. MATERIALS AND METHODS

DL-α-Tocopherol (>98%) was obtained from Fluka. Sodium dodecyl sulphate (SDS) and hexadecyltrimethylammonium chloride were purchased from BDH Ltd and Kodak, respectively. The methanol used was HPLC grade (Rathburn Chemical Ltd.). α-Tocopherol was solubilised in detergent micelles by injection of a concentrated α-tocopherol solution in methanol (0.2 M) into a warm (~40°C) vortexing detergent solution.

Laser flash photolysis experiments were undertaken using a Lumonics HE460 XeCl excimer laser, producing 10 ns pulses (~120 mJ) at 308 nm. For kinetic measurements the beam was attenuated by a factor of ~10. Laser power was measured with a Gentec ED500 pyroelectric energy detector with an absolute accuracy of ±10%. The optical detection system consisted of a 150 W tungsten-halogen lamp, Monospec 0.6 m monochromatotor and a BPW34 photodiode (supplied by Farnell Electronic Components Ltd, UK) or Thorn-EMI 9698QB photomultiplier. Steady-state spectra were recorded with a Shimadzu UV-160 double beam spectrophotometer. For α-tocopherol $\varepsilon_{308nm} \simeq 385$ $M^{-1} \cdot cm^{-1}$. At the concentrations used, SDS and HTAC have negligible absorbance (less than 3% of that of α-tocopherol) at 308 nm. The sample was irradiated in a quartz cell with a laser beam 5 mm wide and 2 mm high. The analysing light was collimated (2 mm diameter) through the sample cell. The laser and analysing beams were arranged

Abbreviations: HTAC, hexadecyltrimethyl ammonium chloride; SDS, sodium dodecyl sulphate; α-Toc-OH, α-tocopherol

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in perpendicular geometry with the analysing light beam traversing very close to the inside edge of the cuvette facing the incoming laser pulse.

3. RESULTS AND DISCUSSION

3.1. *Formation and decay of the α-tocopheroxyl radical*

Laser flash photolysis of α-tocopherol in either methanol or aqueous micellar solutions produced transient absorptions, measured immediately after the flash, with maxima at 420–440 nm and at 600–700 nm. Spectra obtained in solutions of α-tocopherol in SDS are shown in Fig. 1. In deaerated solutions the long wavelength absorption was found to decay rapidly ($\tau \sim 10\ \mu s$ in the SDS solution) and was absent in N_2O-saturated solutions. The absorption at ~700 nm may therefore be assigned to the hydrated electron. The 430 nm absorption was found to be relatively persistant ($\tau \sim 1$ s in methanol), and may be assigned to the α-tocopheroxyl radical by comparison with previously reported spectra of this species [11–13,15–18]. In methanol, the intensity of the α-tocopheroxyl radical absorption was found to be directly proportional to laser power intensity (results not shown). These results indicate that the α-tocopherol is directly photoionised, producing the radical via a monophotonic mechanism. The absence of any other absorptions in the range 350–500 nm is evidence for the non-involvement of triplet states, which may have lifetimes of several microseconds in deaerated solution [14]. In the presence of oxygen, the electron produced by photoionisation will be solvated and scavenged to give superoxide radical:

$$\alpha\text{-Toc-OH} \xrightarrow{h\nu} \alpha\text{-Toc-O}^{\bullet} + e^-_{solv} + H^+ \quad \ldots\ldots (3)$$

$$e^-_{solv} + O_2 \rightarrow O_2^{-\bullet} \quad \ldots\ldots (4)$$

In these aerated solutions, the α-tocopheroxyl radical decay contained an initial rapid component, as illustrated in the inset to Fig. 1. It was found (results not shown) that in aerated methanol solutions the transient spectrum did not change significantly during this initial rapid decay. The initial decay corresponds to the reaction of superoxide with the α-tocopheroxyl radical:

$$\alpha\text{-Toc-O}^{\bullet} + O_2^{-\bullet} + H^+ \rightarrow \alpha\text{-Toc-OH} + O_2 \quad \ldots\ldots (5)$$

For the water soluble α-tocopherol analogue, Trolox C, reaction 5 has been shown by pulse radiolysis to have a rate constant of $4.5 \times 10^8\ M^{-1}\cdot s^{-1}$ in aqueous solution [18]. From the present results k_5 in methanol is estimated to be $\sim 10^9\ M^{-1}\cdot s^{-1}$, close to the diffusion-controlled limit. The decay of the α-tocopheroxyl radical absorption in O_2-saturated methanol also indicates, as previously noted [11], the lack of reaction of $O_2^{-\bullet}$ with α-tocopherol which, if it occurred, would produce additional α-tocopheroxyl radical. The inset to Fig. 1 shows that

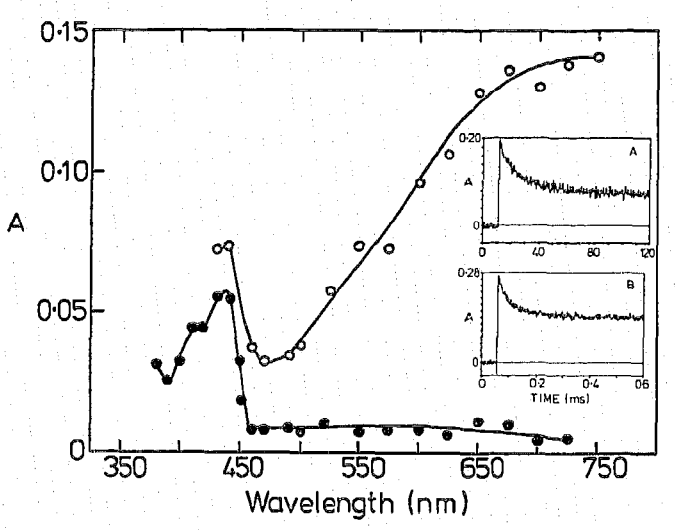

Fig. 1. Transient absorption spectra from laser flash photolysis at 308 nm of a deaerated (argon-saturated) solution of α-tocopherol (0.8 mM), SDS (40 mM) and phosphate buffer (25 mM, pH 7.2). Spectra measured immediately after the laser pulse (○) and after 20 μs (●). Inset: decays of the α-tocopheroxyl radical absorption (430 nm) in air saturated solutions of (A) SDS, pH 7.2; and (B) HTAC, pH 6.8. Note the difference in timescales.

for α-tocopherol in a micellar solution of HTAC the lifetime of the initial decay component is ~57 μs and that the rate of reaction (5) is about 400 times faster than in SDS solution, where the lifetime is ~22 ms. This difference may be attributed to the effect of positive or negative micellar charge on the reaction of the negatively charged superoxide radical with the α-tocopheroxyl radical located within the micelle.

In micellar solutions it was found that the spectrum of the α-tocopheroxyl radical could be observed for up to several minutes after flash photolysis. Fig. 2 shows the characteristic spectrum of the α-tocopheroxyl radical recorded in a double beam spectrometer approximately two minutes after photolysis. The α-tocopheroxyl radical is reported to decay slowly in organic solvents with, for example, a second order rate of 3.5×10^2

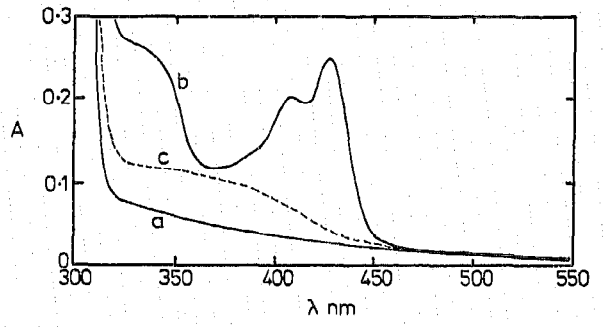

Fig. 2. Absorption spectra of air saturated aqueous solutions containing α-tocopherol (1 mM), HTAC (40 mM), phosphate buffer (25 mM, pH 6.8) and EDTA (50 μM); (a) before photolysis; (b) approximately 2 minutes after exposure to 300 laser pulses (~120 mJ/pulse, 0.2 Hz); and (c) solution from (b) after addition of ~1 mM GSH.

$M^{-1}\cdot s^{-1}$ in cyclohexane [15]. In more polar solvents its decay rate is much greater: in water the soluble analogue Trolox C disproportionates with a rate constant of 10^5–10^7 $M^{-1}\cdot s^{-1}$ depending on pH [19]. For α-tocopherol itself, a second order rate constant of ~10^8 $M^{-1}\cdot s^{-1}$ is estimated from the results of reference [9] for decay of the radical in a mixture of 2-propanol, water and acetone. The long lifetime of the α-tocopheroxyl radical in HTAC micelles may therefore be ascribed to the relatively apolar environment experienced by the radical within the micelle and perhaps also, at the radical concentrations studied, to isolation of single radicals within individual micelles leading to a lack of a radical partner for dimerisation or disproportionation reactions.

The spectra of products of photolysis in air saturated solutions of α-tocopherol in methanol were measured several minutes after flash photolysis. The spectra (Fig. 3) show consumption of α-tocopherol (λ_{max} = 295 nm, $\varepsilon = 3 \times 10^3$ $M^{-1}\cdot cm^{-1}$ and formation of products absorbing at ≥ 240 nm and 300–450 nm. These most likely represent formation of α-tocopheryl quinone by disproportionation of the radical.

3.2. *Reactions of the α-tocopheroxyl radical with ascorbate and glutathione*

Reaction (2) was studied with the α-tocopheroxyl radical in a micellar environment and ascorbate in the aqueous phase. The rate of decay of the α-tocopheroxyl radical absorption was measured after flash photolysis of α-tocopherol in solutions of SDS or HTAC containing varying amounts of ascorbate. From the results in Fig. 4, second order rate constants of (7.2 ± 0.2) × 10^7 $M^{-1}\cdot s^{-1}$ and (3.8 ± 0.2) × 10^4 $M^{-1}\cdot s^{-1}$ were obtained in micelles of HTAC and SDS respectively. These rate constants show a large effect of micellar charge on the reaction of the singly negatively charged ascorbate ion at neutral pH, and span the values previously determined or estimated for reaction (2) with α-tocopherol in solution (1.5 × 10^6 $M^{-1}\cdot s^{-1}$) [9] or in membranes (2 × 10^5 $M^{-1}\cdot s^{-1}$) [3]. These results show that access of ascorbate to the α-tocopheroxyl radical in the micelle is not particularly restricted, and that even in SDS micelles the rate of (2) at physiological concentrations of ascorbate (up to millimolar) is still very much greater than the rate of decay of the α-tocopheroxyl radical in the absence of ascorbate.

Fig. 2 demonstrates that the addition of a small amount of reduced glutathione (GSH) to a solution of the α-tocopheroxyl radical, generated by laser photolysis of α-tocopherol in HTAC micelles, caused the loss of the α-tocopheroxyl radical absorption. Fig. 5 shows the decay of the α-tocopheroxyl radical absorption in HTAC solutions containing up to 857 μM GSH. In the absence of GSH, the α-tocopheroxyl radical has a half-life of several minutes. In the presence of increasing GSH concentration the rate of decay increases. Although the decays do not strictly follow pseudo-first order kinetics, the initial slopes of semi-log plots of the data in Fig. 5 give an apparent second order rate constant of ~25 $M^{-1}\cdot s^{-1}$ for the reaction between the α-tocopheroxyl radical and GSH.

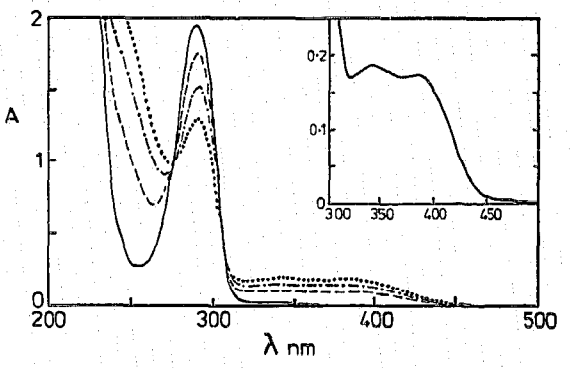

Fig. 3. Stable absorption spectra obtained after 308 nm laser photolysis of air saturated solutions of α-tocopherol (0.64 mM) in methanol. Spectra measured in a double beam spectrometer before photolysis (—) and after exposure to 100 (----), 200 (-.-.-) and 300 (.....) laser pulses (indicent power ~120 mJ/pulse, 0.2 Hz repetition rate). Inset: expanded spectrum in the region 300–500 nm after 300 laser pulses.

From a thermodynamic viewpoint, reaction (2) is quite favourable with E^0[$Asc^{-\bullet}$/$AscH^-$]≈330 mV and E^0[α-TocO•/α-TocOH]≈480 mV [20]. In contrast, the thiyl radical should be capable of oxidising α-tocopherol since E^0[$RS^\bullet$/RSH]≈900 mV [20]. This is supported by pulse radiolysis experiments [11]. Our observation that the optical absorption of the α-tocopheroxyl radical disappears on addition of glutathione, supported by other reports using ESR [6,11], indicates that the reduction of the α-tocopheroxyl radical by glutathione must be coupled to further exergonic reactions such as disulphide formation [21].

In conclusion, the technique of nanosecond laser flash photolysis has been shown to be capable of generating the α-tocopheroxyl radical instantaneously. This method enables reactions of the α-tocopheroxyl radical

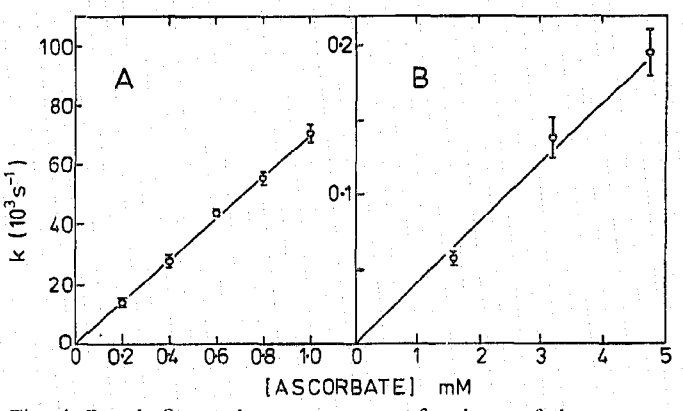

Fig. 4. Pseudo-first order rate constants for decay of the α-tocopheroxyl radical absorption at 430 nm in A-HTAC micelles at pH 6.8, and B- SDS micelles at pH 7.2 versus ascorbate concentration. Solutions were air saturated and contained α-tocopherol (1 mM), detergent (40 mM), phosphate buffer (25 mM) and EDTA (50 μM). Laser power was ~12 mJ/pulse.

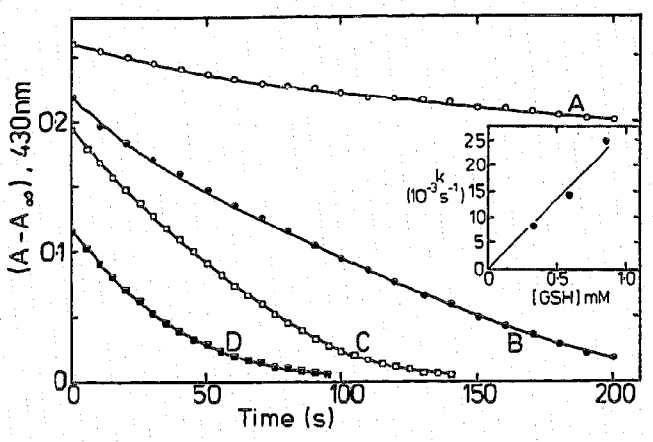

Fig. 5. Decay of the α-tocopheroxyl radical absorption at 430 nm in air saturated solutions of α-tocopherol (1 mM), HTAC (40 mM), phosphate (25 mM, pH 6.8) and EDTA (50 μM). Samples in cuvettes were exposed to laser irradiation (~120 mJ/pulse, 5 Hz for 2 min) before addition of GSH to final concentrations of 0 (a); 0.302 mM(b); 0.588 mM (c); and 0.857 mM (d). Inset: apparent first order rate constants from initial slopes of semilog plots versus GSH concentration, from the data in the main figure.

to be measured on a much faster timescale and in more complex media than is possible using other techniques such as stopped flow and pulse radiolysis. The experiments reported here show that the α-tocopheroxyl radical in a micellar environment is readily recycled to α-tocopherol by ascorbate and are to be extended to model membrane systems.

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REFERENCES

[1] Burton, G.W. and Ingold, K.U. (1986) Acc. Chem. Res. 19, 194–201.
[2] McCay, P.B. (1985) Annu. Rev. Nutr. 5, 323–340.
[3] Scarpa, M., Rigo, A., Maiorino, M., Ursini, F. and Gregolin, C. (1984) Biochim. Biophys. Acta 801, 215–219.
[4] Barclay, L.R.C., Locke, S.J. and McNeil, J.M. (1985) Can. J. Chem. 63, 366–374.
[5] Doba, T., Burton, G.U. and Ingold, K.U. (1985) Biochim. Biophys. Acta 835, 298–303.
[6] Motoyama, T., Miki, M., Mino, M., Takahashi, M. and Niki, E. (1989) Arch. Biochem. Biophys. 270, 655–661.
[7] McCay, P.B., Brueggemann, G., Lai, E.K. and Powell, S.R. (1989) Ann. NY Acad. Sci. 570, 32–45.
[8] Packer, L., Maguire, J.J., Melhorn, R.J., Serbinova, E. and Kagan, V.E. (1989) Biochem. Biophys. Res. Commun. 159, 229–235.
[9] Packer, J.E., Slater, T.F. and Willson, R.L. (1979) Nature 278, 737–738.
[10] Mukai, K., Nishimura, M., Ishizu, K. and Kitamura, Y. (1989) Biochim. Biophys. Acta 991, 276–279.
[11] Davies, M.J., Forni, L.G. and Willson, R.L. (1988) Biochem. J. 255, 513–522.
[12] Zahalka, H.A., Robillard, B., Hughes, L., Lusztyk, J., Burton, G.W., Janzen, E.G., Kotake, Y. and Ingold, K.U. (1988) J. Org. Chem. 53, 3739–3745.
[13] Evans, C., Ingold, K.U. and Scaiano, J.C. (1988) J. Phys. Chem. 92, 1257–1262.
[14] Bensasson, R.V., Land, E.J. and Truscott, T.G. (1983) Flash Photolysis and Pulse Radiolysis, Pergamon, Oxford.
[15] Simic, M.G. (1980) in: Autoxidation in Food and Biological Systems (Simic, M.G. and Karel, M., eds.) pp. 17–26, Plenum, London.
[16] Bisby, R.H., Ahmed, S. and Cundall, R.B. (1984) Biochem. Biophys. Res. Commun. 119, 245–251.
[17] Jore, D., Patterson, L.K. and Ferradini, C. (1986) Free Rad. Biol. Med. 2, 405–410.
[18] Cadenas, E., Medrenyi, G. and Lind, J. (1989) FEBS Lett. 253, 235–238.
[19] Cabelli, D.E. and Bielski, B.H.J. (1986) J. Free Rad. Biol. Med. 2, 71–75.
[20] Wardman, P. (1989) J. Phys. Chem. Ref. Data 18, 1637–1755.
[21] Wardman, P. and Wilson, I. (1987) Free Rad. Res. Commun. 2, 225–232.