Reactions of Excited Triplet Duroquinone with α-Tocopherol and Ascorbate: A Nanosecond Laser Flash Photolysis and Time-Resolved Resonance Raman Investigation§

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Abstract: Nanosecond laser flash photolysis and time-resolved resonance Raman spectroscopy (TR^3) have been used to study reactions between the antioxidants α-tocopherol and ascorbate and the triplet excited states of duroquinone (DQ) and ubiquinone (UQ). In nonaqueous solvents ^3DQ is reduced by both antioxidants at near diffusion-limited ($>10^9$ dm^3 mol^{-1} s^{-1}) rates. Rate constants obtained by direct measurement of the rate of decay of ^3DQ as a function of antioxidant concentration were in good agreement with those determined indirectly from reduction in singlet oxygen luminescence yield, showing that the antioxidants are capable of reacting quantitatively to suppress the sensitized formation of singlet oxygen from triplet states. In SDS micellar solution, the rate of reaction of ^3DQ with ascorbate is governed by the rapid exit of ^3DQ from the micelle. Time-resolved resonance Raman (TR^3) and absorption spectra were used to show that ^3DQ reacts with both 6-palmitoyl-L-ascorbate and α-tocopherol in SDS micelles at neutral pH by hydrogen atom transfer. The spectra also allowed observation of deprotonation of the resulting neutral durosemiquinone radical after diffusion from the micelle into the aqueous phase. Implications of these results to the biological role of antioxidants are discussed.

Introduction

Triplet excited state carbonyl compounds are well-known to react with ground state oxygen to give the highly reactive singlet excited ($^1\Delta_g$) molecular oxygen ("singlet oxygen", $^1O_2$).[1,2] Singlet oxygen reacts with protein, nucleic acid, and lipid components of cells and is therefore highly damaging in biological systems.[3,4] Triplet state aliphatic carbonyls are formed in biochemical systems by a number of enzymic and non-enzymic mechanisms such as in lipid peroxidation and through the actions of peroxidases[5,6] and may transfer their energy to a suitable aromatic carbonyl present. Duroquinone (DQ) is a representative aromatic quinone whose triplet state reactivity has been extensively investigated.[7,8] The reduction potential of the $^3DQ/DQ^{-\bullet}$ couple is estimated[8] at +2.17 V, showing ^3DQ to be a relatively powerful one-electron oxidant. Other studies[9] have shown that triplet ubiquinone (^3UQ) is also a powerful oxidant, capable of one-electron oxidation of water. The π,π^* nature of triplet duroqinone predisposes it to react via hydrogen atom transfer, whereas n,π^* triplet carbonyls tend to react by electron transfer.[7,8,10] Triplet carbonyl states may therefore produce damage in biological systems either directly by reaction of the oxidizing triplet state with a suitable cellular target or indirectly by acting as sensitizers of singlet oxygen production. Some quinones are recognized as potentially damaging photodynamic sensitisers in cell and tissue systems.[11,12]

Cellular protection against singlet oxygen damage may be provided by a number of compounds capable of acting as quenchers.[13−16] The most important physiological quenchers are carotenoids and phenols, notably the tocopherols (vitamin E). The reactivity of phenols with singlet oxygen increases with increasing oxidizability, although the quenching is >90% via the physical mechanism.[17] Phenols, such as α-tocopherol (α-T-OH), are good one-electron reductants (for α-tocopherol $E^{\ominus\prime}$-[α-T-O$^\bullet$,H$^+$/α-T-OH] = 0.48 V)[18] and are rapidly oxidized by lipid peroxyl radicals,[19] accounting for their widely recognized function as chain breaking antioxidants in cell membranes.[20] Ascorbate (vitamin C) has also been recognized as a weak singlet oxygen quencher.[21,22] It is a better one-electron reductant

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than α-tocopherol[18] with $E^{\ominus\prime}[Asc^{-\bullet},H^+/AscH^-] = 0.23$ V and is thought to recycle the α-tocopheroxyl radical in vivo.[23-25] The lipophilic derivative 6-palmitoyl-L-ascorbic acid ($PASCH_2$) is a good antioxidant in model systems[26] and is also effective in cellular systems.[27] The one-electron reduction potentials show that both α-tocopherol and ascorbate should be readily oxidized by ^3DQ. In this report we show that ^3DQ reacts with a rate constant near the diffusion-controlled limit with antioxidants such as α-tocopherol and 6-palmitoyl-L-ascorbate, offering protection by deactivation of the oxidizing triplet state and by preventing singlet oxygen formation. Time-resolved absorption and resonance Raman spectroscopy provide direct evidence that both reactions occur by hydrogen atom transfer rather than by electron transfer.

Materials and Methods

The antioxidants used were DL-α-tocopherol (Fluka, >98%) and 6-palmitoyl-L-ascorbic acid (Merck). Other reagents were the highest purity grade available commercially. Micellar solutions were prepared by rapid injection of solutes into the vortexing solution.[25]

Laser flash photolysis employed an excimer laser (308 nm, 10 ns, 15 mJ at sample). Time-resolved resonance Raman (TR^3) spectra were measured as described previously.[9] Pump and probe laser energies were 0.2−0.5 mJ in a 10 ns pulse. Spectra were typically accumulated over about 10 min at a 10 Hz repetition rate. The deoxygenated sample flowed through a silica capillary tube (2 mm i.d.) to waste. Raman spectra were recorded using either a Princeton Instruments CCD detector (CSMA LN/CCD-1024/TKB/I) or IR-700 UV enhanced gated intensified diode array coupled to a Spex Triplemate spectrograph. Raman spectra were calibrated from solvent spectra measured under identical conditions; wavenumbers are expected to be ±2 cm^{-1}.

Time-resolved singlet oxygen luminescence decays from air-saturated samples at 25 C were recorded using a North Coast EO-817P germanium photodiode at 77 K, detected through a 1270 nm interference filter at right angles to the laser (355 nm, 10 ns) excitation. The laser pulse was passed through a liquid light guide to provide uniform sample illumination. A photodiode sampling the laser beam was used to correct for variations in laser energy (*ca.* 200 ± 20 μJ/pulse).

Results

1. Reactions of ^3DQ with Antioxidants Studied by Laser Flash Photolysis and Transient Absorption Spectroscopy. (a) Rate of Reaction of ^3DQ with Antioxidants Determined by Flash Photolysis. Rates of reaction of ^3DQ with antioxidants were measured from the decay of the 480 nm absorption of ^3DQ produced on laser flash photolysis of DQ solutions at 308 nm. In deaerated ethanol and acetonitrile solutions the triplet lifetimes were found to be about 3 and 5 μs, respectively. Second order rate constants were obtained from plots of first order decay rate versus antioxidant concentration, as shown in the inset to Figure 1A for α-tocopherol and $PASCH_2$ in ethanol. The intercept of these plots represents mixed bimolecular decay and reaction with solvent.[2,7] In ethanol, the second order rate constants for reaction of ^3DQ with $PASCH_2$ and α-tocopherol were (1.75 ± 0.05) × 10^9 dm^3 mol^{-1} s^{-1} and (2.77 ± 0.18) × 10^9 dm^3 mol^{-1} s^{-1}, respectively. The second order rate constant for reaction of ^3DQ with $PASCH_2$ in acetonitrile was (3.46 ± 0.28) × 10^9 dm^3 mol^{-1} s^{-1}. The rates of these reactions are close to the diffusion-controlled values, as expected from the highly exergonic nature of these reactions due to the large differences between the one-electron reduction potentials of ^3DQ and the antioxidant radicals.[7,18]

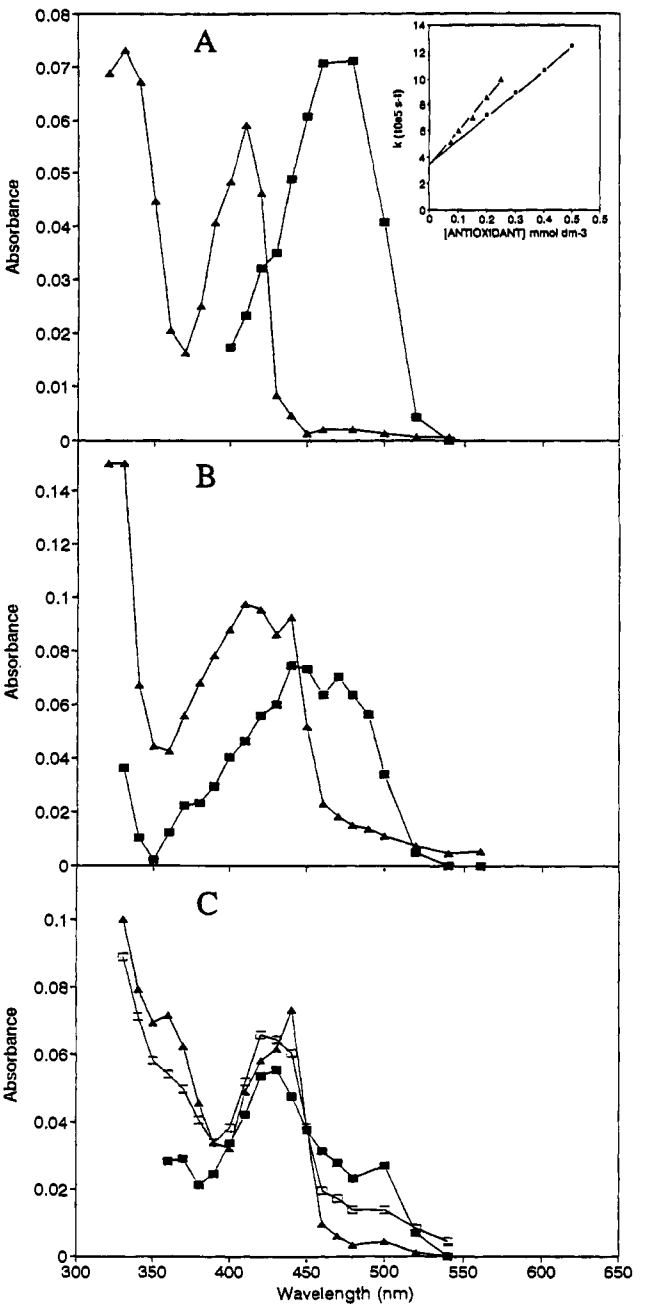

Figure 1. Transient absorption spectra from reaction of ^3DQ with antioxidants following flash photolysis at 308 nm in argon bubbled solutions. A: in acetonitrile solution of DQ (2 mmol dm^{-3}) and $PASCH_2$ (400 μmol dm^{-3}) measured 100 ns (■) and 500 ns (▲) after the flash, [INSET- effect of α-tocopherol (▲) and $PASCH_2$ (■) concentrations on the first order rate constant for decay of ^3DQ in ethanol solutions]; B: in acetonitrile solution of DQ (2 mmol dm^{-3}) and nitrite (10 mmol dm^{-3}) containing 1% water measured 100 ns (■) and 500 ns (▲) after the flash; and C: in aqueous SDS micellar solution containing DQ (2 mmol dm^{-3}), $PASCH_2$ (1 mmol dm^{-3}), SDS (50 mmol dm^{-3}), phosphate buffer (50 mmol dm^{-3}, pH 7.3) and EDTA (50 μmol dm^{-3}), measured 100 ns (■), 400 ns (□) and 2.5 μs (▲) after the flash.

In deaerated micellar solutions of sodium dodecyl sulfate (SDS, 50 mmol dm^{-3}) at pH 7.3 the lifetime of ^3DQ was 3 μs. In SDS solutions at pH 7 containing up to 4 mmol dm^{-3} sodium ascorbate the rate of decay of ^3DQ was first order in ascorbate concentration, giving a second order rate constant for reaction of ^3DQ with ascorbate of (2.0 ± 0.1) × 10^9 dm^3 mol^{-1} s^{-1}. In view of our previous results[25] which demonstrated a strong

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suppression of the reaction between the negatively charged ascorbate anion and α-tocopheroxyl radical in SDS micelles, this must show that ^{3}DQ reacts with ascorbate in the aqueous phase after leaving the SDS micelle with an exit rate of $\geq 10^7$ s^{-1}. This is consistent with exit rates from SDS micelles measured for small aromatic hydrocarbons[28] of up to 4×10^6 s^{-1}. With both DQ and $PASCH_2$ solubilized within SDS micelles, the rate constant for reaction of ^{3}DQ cannot be quantified so readily since the micelles are expected to contain varying numbers of $PASCH_2$ molecules according to a Poisson distribution.[29] In solutions of SDS (50 mmol dm^{-3}) containing $PASCH_2$ (1 mmol dm^{-3}) the lifetime of ^{3}DQ was found to be about 430 ns, comparable with that in solutions containing the same concentration of sodium ascorbate.

(b) Competition between Reaction of Triplet Duroquinone with Oxygen and Antioxidants. In solutions containing oxygen, the reaction of ^{3}DQ with antioxidant (AH) occurs in competition with the natural decay of ^{3}DQ and its reaction with oxygen to yield singlet oxygen (reactions 1−3). The antioxidants may also act as quenchers of singlet oxygen by physical (reaction 4a) or chemical (reaction 4b) mechanisms.

$$^3DQ \rightarrow DQ \text{ (ground state)} \quad (1)$$

$$^3DQ + AH \rightarrow DQH^\bullet + A^\bullet \quad (2)$$

$$^3DQ + {}^3O_2 \rightarrow DQ + {}^1O_2 \quad (3)$$

$$^1O_2 + AH \rightarrow {}^3O_2 + AH \quad (4a)$$

$$^1O_2 + AH \rightarrow O_2^{-\bullet} + A^\bullet + H^+ \quad (4b)$$

According to this scheme, the quantum yields for production of singlet oxygen, ϕ_0 and ϕ, in the absence and presence of AH respectively are related by

$$\frac{\phi_0}{\phi} = 1 + \frac{k_2[AH]}{k_1 + k_3[O_2]} \quad (5)$$

The relative yield of singlet oxygen in ethanol solution was determined by extrapolation to zero time of the 1270 nm time-resolved luminescence decay of singlet oxygen obtained by excitation of DQ with a 10 ns laser pulse at 355 nm. Plots of ϕ_0/ϕ versus [AH] were linear and gave values for k_2 in ethanol of $(3.7 \pm 0.1) \times 10^9$ dm^3 mol^{-1} s^{-1} for α-tocopherol and $(1.88 \pm 0.03) \times 10^9$ dm^3 mol^{-1} s^{-1} for $PASCH_2$, using values of k_1, k_3, and oxygen concentration in aerated ethanol given by Darmanyan and Foote.[2] These values of k_2 are in reasonable agreement with values measured directly from the rate of the ^{3}DQ absorbance decay (see above).

The same time-resolved singlet oxygen luminescence data also provided second order rate constants for singlet oxygen quenching (k_4) from linear plots of the first order rate constant for singlet oxygen decay versus antioxidant concentration. The value of k_4 for α-tocopherol was found to be $(2.0 \pm 0.1) \times 10^8$ dm^3 mol^{-1} s^{-1}, whereas $PASCH_2$ was more than two orders of magnitude less efficient with $k_4 = (9.9 \pm 2.5) \times 10^5$ dm^3 mol^{-1} s^{-1}. The value of k_4 for α-tocopherol is reported to be strongly solvent dependent, and the value reported here is consistent with the literature.[14,30] Literature values[21,22] for the second order rate constant, both determined indirectly, for quenching of singlet oxygen by ascorbic acid (in pyridine) and ascorbate (in aqueous solution) are both 8×10^6 dm^3 mol^{-1} s^{-1}, about an order of magnitude greater than the present value for $PASCH_2$ in ethanol.

(c) Transient Absorption Spectroscopy. The transient absorption spectra in Figure 1A show the decay of the ^{3}DQ absorption at 460−480 nm in the presence of $PASCH_2$ in acetonitrile solution. Concurrent with decay of the ^{3}DQ absorption is the formation of a peak at 410 nm attributed to the neutral durosemiquinone radical ($DQH^\bullet$).[31] The reaction is therefore a simple hydrogen atom transfer or concerted electron/proton transfer:

$$^3DQ + PASCH_2 \rightarrow DQH^\bullet + PASCH^\bullet \quad (6)$$

There was no indication of product formation at 360 nm indicative[32] of the ascorbate radical anion ($PASC^{-\bullet}$). That the durosemiquinone radical anion ($DQ^{-\bullet}$) is not formed in this reaction is demonstrated by comparison with the transient spectra observed on electron transfer from nitrite to ^{3}DQ in acetonitrile solution containing 1% water

$$^3DQ + NO_2^- \rightarrow DQ^{-\bullet} + NO_2^\bullet \quad (7)$$

which are shown in Figure 1B. Here the characteristic peak at 440 nm ($\epsilon = 7.6 \times 10^4$ dm^3 mol^{-1} cm^{-1})[31] of the $DQ^{-\bullet}$ radical anion is observed, slightly distorted by the much weaker underlying absorption due to the $NO_2^\bullet$ radical ($\lambda_{max} = 400$ nm, $\epsilon = 200$ dm^3 mol^{-1} cm^{-1}).[33] In SDS micellar solutions at pH 7.3, the transient spectra in Figure 1C show an overlapping spectrum 400 ns after the laser pulse of the neutral semiduroquinone radical and ^{3}DQ. By 2 μs after the laser pulse there are indications of a growth in absorbance at 360 and 440 nm whilst that at 410 nm decays slightly. These spectra suggest an initial hydrogen atom transfer reaction, followed by deprotonation of $DQH^\bullet$ to $DQ^{-\bullet}$. Our previous investigation[34] of the effect of pH on the rate of reaction between α-tocopheroxyl radical and $PASCH_2$ revealed a first pK_a of 6.3 for $PASCH_2$ in SDS micelles and showed that, as expected, $PASCH^-$ was a much stronger reductant than $PASCH_2$. Therefore the initial reaction is (8), followed by (9) which occurs as the radical diffuses from the micellar to aqueous phase.

$$^3DQ + PASCH^- \rightarrow DQH^\bullet + PASC^{-\bullet} \quad (8)$$

$$DQH^\bullet \rightleftharpoons DQ^{-\bullet} + H^+ \quad (9)$$

The pK of the ascorbyl radical[35] is −0.4 whilst that for $DQH^\bullet$ is 5.0,[31] indicating that at pH 7 both species should exist as the corresponding anions. Assuming k_{-9} is of the order of 10^{11} dm^3 mol^{-1} s^{-1}, k_{+9} is estimated to be about 10^6 s^{-1}, consistentwith the formation of the 440 nm absorption over a microsecond period as observed. Whilst these results are

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suggestive of the mechanism proposed, the detailed analysis is obscured by strong overlap between the ^{3}DQ and radical absorption spectra. Further investigation using time-resolved resonance Raman spectroscopy proved to be more revealing.

2. Time-Resolved Resonance Raman (TR^3) Spectroscopy of ^{3}DQ Reactions. (a) TR^3 Spectra of Duroquinone Radicals. Transients due to ^{3}DQ, DQH$^\bullet$, and DQ$^{-\bullet}$ were observed using nanosecond time-resolved resonance Raman spectroscopy with a pump wavelength of 351 or 355 nm. The intermediates were probed at wavelengths (425 nm for durosemiquinone radicals and 500−520 nm for ^{3}DQ) where the intermediates display significant absorption.[7,8,31,39] Frequencies of the principal bands in the spectra of these intermediates in acetonitrile and ethanol are shown in Table 1. TR^3 spectra in acetonitrile solution are shown in Figure 2A. When probed at 500−520 nm, ^{3}DQ in acetonitrile produced a strong Raman band with a peak at 1533 cm^{-1}, which was curve-fitted to a major band at 1533 cm^{-1}, assigned to the C=C (ν_{8a}) stretching vibration, and a weaker band at 1585 cm^{-1}. Additional weaker bands at 966 cm^{-1}, probably due to a ring C−C breathing mode, and 1166 cm^{-1} were also observed. In ethanol and ethanol−water (50:50v/v) solutions the ν_{8a} band of ^{3}DQ shifted to 1542 and 1549 cm^{-1}, respectively. When probed at 425 nm, where ^{3}DQ absorbs less strongly, the 1533 cm^{-1} band in acetonitrile was much weaker. The durosemiquinone neutral and anion radicals were generated by the same methods as employed by McCubbin et al.[39] The radical anion (DQ$^{-\bullet}$), formed by reduction of the triplet by sodium nitrite in acetonitrile containing water (2% v/v), has two strong bands at 1475 and 1612 cm^{-1}. The radical anion was also observed in ethanol solutions containing between 5 and 50% v/v water with bands at 1463 and 1614 cm^{-1}. The neutral radical (DQH$^\bullet$), formed by protonation of the radical anion in acidified acetonitrile/H_2O (5% v/v) solutions, displays bands at 1501 and 1588 cm^{-1}. The neutral radical was formed in ethanol either by protonation of the radical anion from reaction of ^{3}DQ with nitrite in acidified solution or by direct oxidation of the ethanol by the triplet state and has bands at 1501 and 1597 cm^{-1}, unaffected by up to 5% water in the solution. Assignments of the major bands to the C=O and C=C stretching vibrations (Wilson ν_{7a} and ν_{8a}, respectively) are derived from comparison with spectra of similar species reported previously.[9,36,37] The significant shift in the high frequency band (ν_{8a}, C=C stretch) on changing solvent recalls the solvent sensitivity of the frequency of the C=C band of the α-tocopheroxyl radical.[38] In addition, the frequency of the ν_{7a} (C=O) stretching vibration of DQ$^{-\bullet}$ is sensitive to solvent, moving from 1475 cm^{-1} in acetonitrile to 1463 cm^{-1} in ethanol.

The frequencies of the ν_{7a} vibration in the TR^3 spectrum of both durosemiquinone radicals measured here accord well with those previously reported,[39] but there is a large discrepancy in the frequencies of the ν_{8a} vibration of DQ$^{-\bullet}$ and DQH$^\bullet$. Similarly there is a substantial difference between the position of the main band of ^{3}DQ in ethanol reported here (1542 cm^{-1}) and in the literature (1562 cm^{-1}).[39] The instrumentation used here has been significantly developed since the previous study,[39] and so the present results are regarded as more reliable and are confirmed by detailed calibration of the spectrometer. In addition, the spectra of durosemiquinone radicals presently determined are much more similar to the recently reported TR^3 spectra of semiquinoid radicals from ubiquinone[8] and tocopherol[38] than those previously reported for duroquinone.[39]

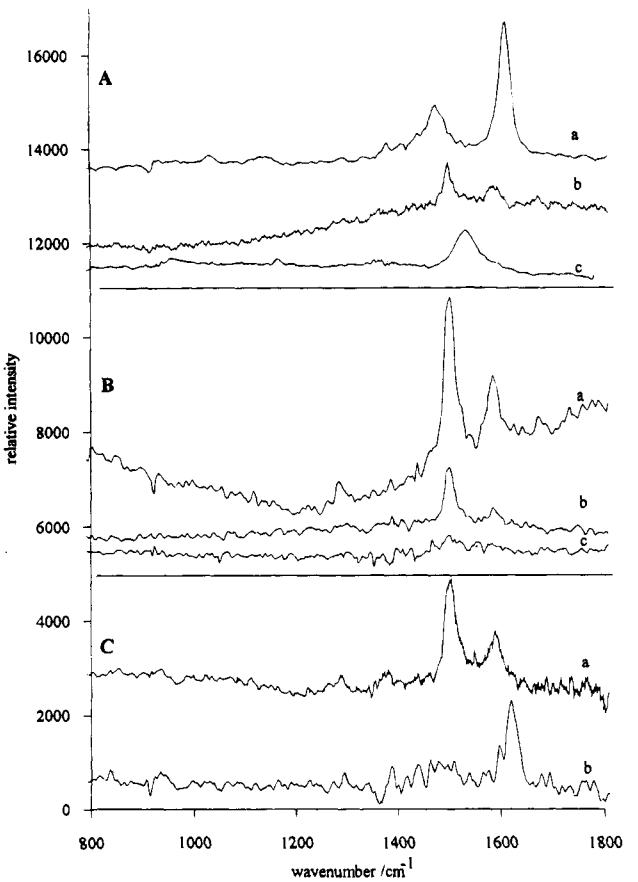

Figure 2. A: TR^3 spectra of ^{3}DQ and durosemiquinone radicals in argon-bubbled solutions. (a) Spectrum of DQ$^{-\bullet}$ in acetonitrile solution containing DQ (7 mmol dm^{-3}), 2% water and sodium nitrite (10 mmol dm^{-3}), probe pulse at 425 nm delayed 80 ns after the pump pulse at 351 nm; (b) spectrum of DQH$^\bullet$ obtained by subtraction of spectrum (a) from that obtained in a solution of DQ (5 mmol dm^{-3}) in acetonitrile containing 5% water, sodium nitrite (10 mmol dm^{-3}), and HCl (10 mmol dm^{-3}); (c) spectrum of ^{3}DQ in an acetonitrile solution of DQ (5 mmol dm^{-3}) with probe pulse (520 nm) 30 ns after the pump pulse (355 nm). B: TR^3 spectra from reaction of ^{3}DQ with α-tocopherol and $PASC_2$ in acetonitrile solution using a 351 nm pump pulse and 425 nm probe pulse (delayed by 1 μs): (a) in solution containing DQ (5 mmol dm^{-3}), α-tocopherol (4 mmol dm^{-3}); (b) in solution containing DQ (5 mmol dm^{-3}) and $PASCH_2$ (2 mmol dm^{-3}); (c) in a solution containing only α-tocopherol (4 mmol dm^{-3}). C: TR^3 spectra from reactions of ubiquinone (UQ). (a) spectra from reaction of ^{3}UQ in argon-saturated acetonitrile solution containing UQ (5 mmol dm^{-3}) and α-tocopherol (10 mmol dm^{-3}); (b) in acetonitrile plus water (5%) solution containing UQ (5 mmol dm^{-3}) and nitrite (10 mmol dm^{-3}). In both cases the 425 nm probe pulse was delayed 2 μs after the 351 nm pump pulse.

(b) TR^3 Spectroscopy of Reactions between ^{3}DQ and Antioxidants. TR^3 spectroscopy was used to study the reaction between ^{3}DQ and antioxidants in acetonitrile solution. Results are shown in Figure 2B. Following reaction between ^{3}DQ and $PASCH_2$, the observed spectrum is that of DQH$^\bullet$. The ascorbate radical has been reported not to be observed by resonance Raman spectrum presumably due to the upper state being dissociative:[40] in any case the ascorbate radical (λ_{max} = 360 nm) is not in resonance at a probe wavelength of 425 nm. There is no evidence from this spectrum for the formation of the durosemiquinone radical anion under these conditions. There-

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Table 1. Resonance Raman Frequencies of C=C (ν_{8a}) and C=O (ν_{7a}) Vibrations in Durosemiquinone and Tocopheroxyl Radicals and Triplet Duroquinone[g]

	solvent			
	acetonitrile		ethanol	
radical	ν_{7a}	ν_{8a}	ν_{7a}	ν_{8a}
$DQ^{-\bullet}$	1475[a]	1612[a]	1463[c] (1464)[e]	1614[c] (1646)[e]
DQH$^{\bullet}$	1501[b]	1588[b]	1501[d] (1510)	1597[d] (1645)
^{3}DQ		1533		1542 1549[e] (1562)
α-tocopheroxyl[f]	1502	1586	1504	1586

[a] Solutions containing 2% H_2O (v/v). [b] Solutions containing H_2O (5% v/v) and 10^{-2} mol dm^{-3} HCl. [c] Solutions containing 5% or 50% H_2O (v/v). [d] In 100% ethanol or ethanol plus H_2O (5% v/v). [e] Solutions containing 50% H_2O (v/v). [f] From Parker and Bisby, 1993 (ref 34). [g] Values in parentheses are from McCubbin et al., 1987 (ref 39).

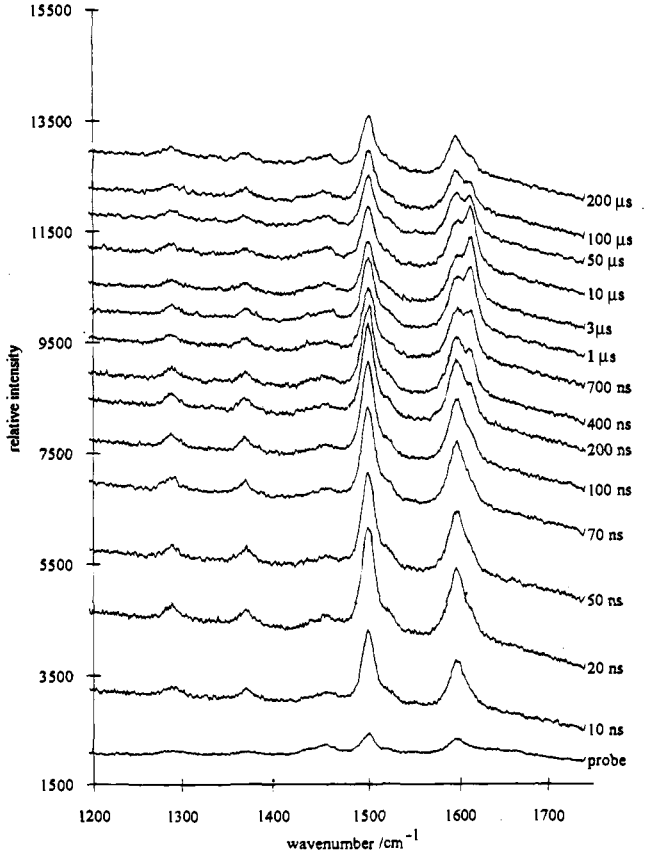


Figure 3. TR^3 spectra from aqueous solutions containing DQ (1 mmol dm^{-3}), α-tocopherol (2 mmol dm^{-3}), SDS (50 mmol dm^{-3}), EDTA (50 µmol dm^{-3}), and phosphate buffer (50 mmol dm^{-3}, pH 7.0). The pump and probe wavelengths were 355 and 425 nm, respectively, with delays as indicated on the figure. The probe-only spectrum shows weak excitation of DQ by the 425 nm probe pulse.

fore in acetonitrile solution, ^{3}DQ and $PASCH_2$ react by hydrogen atom transfer as expected. Reaction between ^{3}DQ and α-tocopherol leads to product(s) with peaks in the TR^3 spectrum at ca. 1502 and 1588 cm^{-1}. Examination of Table 1 shows that the α-tocopheroxyl and neutral durosemiqionone radical have overlapping peaks at these two frequencies which cannot be resolved. However, again there is no evidence from the spectrum of $DQ^{-\bullet}$ formation, and ^{3}DQ and α-tocopherol must also react in acetonitrile by hydrogen atom transfer. Similar results were obtained for reaction between ^{3}DQ and α-tocopherol or $PASCH_2$ in ethanol solution.

The reactions of triplet ubiquinone (^{3}UQ) with α-tocopherol

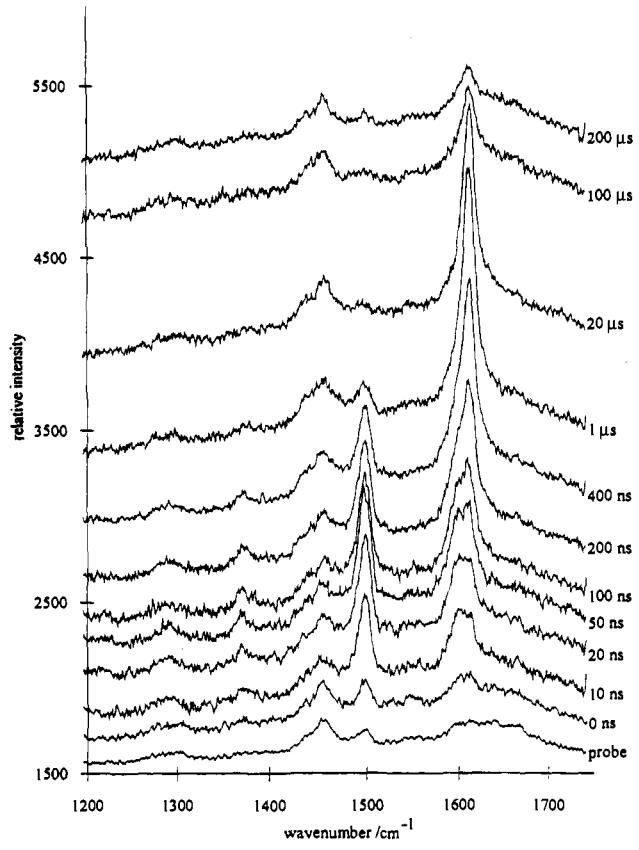


Figure 4. TR^3 spectra from aqueous solutions containing DQ (1 mmol dm^{-3}), $PASCH_2$ (2 mmol dm^{-3}), SDS (50 mmol dm^{-3}), EDTA (50 µmol dm^{-3}), and phosphate buffer (50 mmol dm^{-3}, pH 7.0). The pump and probe wavelengths were 355 and 425 nm respectively, with delays as indicated on the figure.

and nitrite in acetonitrile were also studied by TR^3 spectroscopy. Figure 2C shows that the spectrum observed following reaction between ^{3}UQ and α-tocopherol in acetonitrile. Peaks at 1503 and 1590 cm^{-1} were observed and are assigned to the α-tocopheroxyl radical. The neutral ubisemiquinone radical (UQH$^{\bullet}$) has a weak peak at 1608 cm^{-1} (in ethanol)[9] which was not resolved. However, the more intense peak at 1618 cm^{-1} due to ubisemiquinone radical anion was absent, although it could easily be observed in an acetonitrile solution of ubiquinone containing water (10% v/v) and sodium nitrite (10 mmol dm^{-3}). ^{3}UQ therefore also reacts with α-tocopherol by a hydrogen atom transfer mechanism.

(c) Reaction between ^{3}DQ and Antioxidants in SDS Micelles. In the above discussion of electron versus hydrogen atom transfer in the oxidation of α-tocopherol and ascorbate by ^{3}DQ, it may be that the nonaqueous solvents employed do not favor $DQ^{-\bullet}$ formation and hydrogen atom transfer is the predominant mechanism. Radical ions formed by electron transfer in aqueous media would be solvated and electron transfer might then become more favored. The low solubility of these compounds in water prevents their reactions being studied in aqueous solution. However, the reactants are readily solubilized in aqueous micellar systems.

Figure 3 shows nanosecond TR^3 spectra obtained during the oxidation of α-tocopherol by ^{3}DQ in SDS micelles at pH 7. The spectrum observed at the shortest interval (10 ns) between pump and probe pulses has peaks at 1502 and 1590 cm^{-1}. These increase in intensity up to an interval of about 50 ns between pump and probe pulses and represent the overlapping spectra of α-tocopheroxyl and neutral durosemiquinone radicals. At longer time delays between pump and probe pulses, up to about 3 µs, a peak at 1620 cm^{-1} grows in, representing the delayed

formation of the durosemiquinone radical anion ($DQ^{-\bullet}$). The 1620 cm^{-1} peak decays during the following 200 μs, in accord with a second order decay of $DQ^{-\bullet}$ with a rate constant[7] of about 10^8 dm^3 mol^{-1} s^{-1}. Following this, only the spectrum of the persistent α-tocopheroxyl radical remains. Despite the inability to resolve the resonance Raman spectra of α-tocopheroxyl and $DQH^\bullet$ radicals, the results are clearly indicative of an initial hydrogen atom transfer reaction followed by deprotonation of $DQH^\bullet$. A similar reaction sequence is more clearly demonstrated on oxidation of $PASCH_2$ by ^3DQ in SDS micelles, as shown in Figure 4. In this instance only the durosemiquinone radicals are observed in the TR[3] spectra. Figure 4 shows that the intensity of the 1502 cm^{-1} band (now due only to $DQH^\bullet$) increases to a maximum at about 50 ns after the pump pulse. Again, this decays and is replaced by the 1620 cm^{-1} band of $DQ^{-\bullet}$ which itself then decays over hundreds of microseconds.

The overall kinetics of the reaction between ^3DQ and $PASCH_2$ were analyzed according to the following simplified Scheme 1.

A number of approximations are made to simplify analysis: (a) that ^3DQ reacts with antioxidant within the micelle by a pseudo-first order reaction ($[AH] \gg [^3DQ]$) with rate constant k_0; (b) there is no formation of $DQ^{-\bullet}$ within the micelle and there is no diffusion of $DQH^\bullet$ back into the micelle; (c) $DQ^{-\bullet}$ decay is exponential with rate constant k_d. The scheme is further simplified by considering exit of $DQH^\bullet$ from the micelle (rate constant k_e) and its subsequent ionization (deprotonation, rate constant k_i) to be represented by a single first order process with rate constant k_t. Decay of the radicals, including that by geminate recombination, inside the micelle is indicated by k_g.

For Scheme 1 the following kinetic equations are obtained:

$$[DQH^\bullet]_t = A\{\exp(-k_0 t) - \exp(-\gamma t)\} \quad (10)$$

$$[DQ^{-\bullet}]_t = B\exp(-k_0 t) + C\exp(-\gamma t) + D\exp(-k_d t) \quad (11)$$

where

$$\gamma = k_t + k_g \quad (12)$$

$$A = \frac{k_0[^3DQ]}{\gamma - k_0} \quad (13)$$

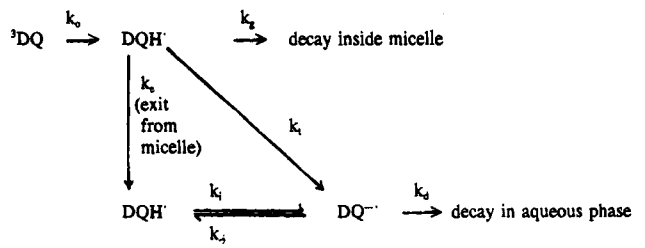
$$B = \frac{k_t}{k_d - k_0}A \quad (14)$$

$$C = -\frac{k_t}{k_d - \gamma}A \quad (15)$$

$$D = B + C \quad (16)$$

Whilst TR[3] spectroscopy may have limitations in the accurate determination of kinetic constants, nevertheless useful insight was obtained using this model. The intensities due to $DQ^{-\bullet}$ and $DQH^\bullet$ within each spectrum were determined by least squares fitting of one Gaussian peak at 1500 cm^{-1} and two Lorentzian profiles with maxima at 1600 and 1615 cm^{-1}. Equations 10 and 11, with the addition of a constant term to account for weak excitation by the probe pulse, were fitted by a non-linear least squares algorithm (Grafit, Erithacus Software Ltd.). The resulting fit to the data for formation and decay of $DQH^\bullet$ is shown in Figure 5A from which the values of k_0 and γ were found to be $(9 \pm 1) \times 10^7$ s^{-1} and $(1.73 \pm 0.11) \times 10^6$ s^{-1}, respectively. Formation and decay of $DQ^{-\bullet}$ are shown in Figure 5 (parts B and C) together with the fit according to the three exponential model of eq 11. This gave a value of $k_d = (1.7 \pm 0.2) \times 10^4$ s^{-1}. Values for B and C were unreliable, preventing individual evaluation of k_t and k_g. However, it is possible to make some observations regarding the relative contributions of k_g and k_t to γ. Evans et al.[41] have shown that for the α-tocopheroxyl-butyrophenone ketyl radical pair in SDS micelles at room temperature, k_g is about 3×10^6 s^{-1}. About 75% of these radicals undergo geminate recombination since the rate constant for escape of the butyrophenone ketyl radical from the micelle is only $\sim 10^6$ s^{-1}. If the rate constant for escape of the durosemiquinone radical is similar to that for the acetophenone radical anion (7.8×10^6 s^{-1})[42] and for ^3DQ ($\geq 10^7$ s^{-1}), then geminate recombination within the micelle is unlikely to be an important process in the present instance and can be ignored. This is consistent with the relatively high yield (>80%) of $DQ^{-\bullet}$ from ^3DQ indicated by both the transient absorption and Raman spectra. The rise in the $DQ^{-\bullet}$ intensity (Figure 5C) mirrors the decay in the $DQH^\bullet$ signal (Figure 5B). If the rate for exit of $DQH^\bullet$ from the micelle is similar to that for ^3DQ, then deprotonation would be rate-limiting in the formation of $DQ^{-\bullet}$, rather than exit from the micelle. The value of γ therefore represents the rate of this deprotonation step. With $pK_a(DQH^\bullet) = 5.0$, one calculates $k_i = 10^6$ s^{-1} if k_{-i} is diffusion controlled (10^{11} dm^3 mol^{-1} s^{-1}). The value of γ, 1.7×10^6 s^{-1}, is slightly higher than this calculated value of k_i, but this could be due to the phosphate buffer acting as a base catalyst.

Scheme 1



Conclusions

The TR[3] experiments described above provide a convincing demonstration that in micellar solution the reactions of ^3DQ with both $PASCH_2$ and α-tocopherol involve an initial hydrogen atom transfer. This is followed by durosemiquinone radical anion formation in the aqueous compartment. Njus and Keeley[43,44] have argued on theoretical grounds that both ascorbate and α-tocopherol owe many of their biological properties to their abilities to act as hydrogen atom donors rather than as electron donors. With a triplet quinone as oxidant, the experiments described here provide direct evidence in support of this mechanism. Further details of the molecular dynamics of this hydrogen atom (or concerted proton-electron) mechanism might be obtained in future by pico- or femtosecond studies of weak charge transfer complexes which we have found are formed between duroquinone and α-tocopherol and which appear similar to those reported for α-tocopherol and α-tocopheroquinone.[45]

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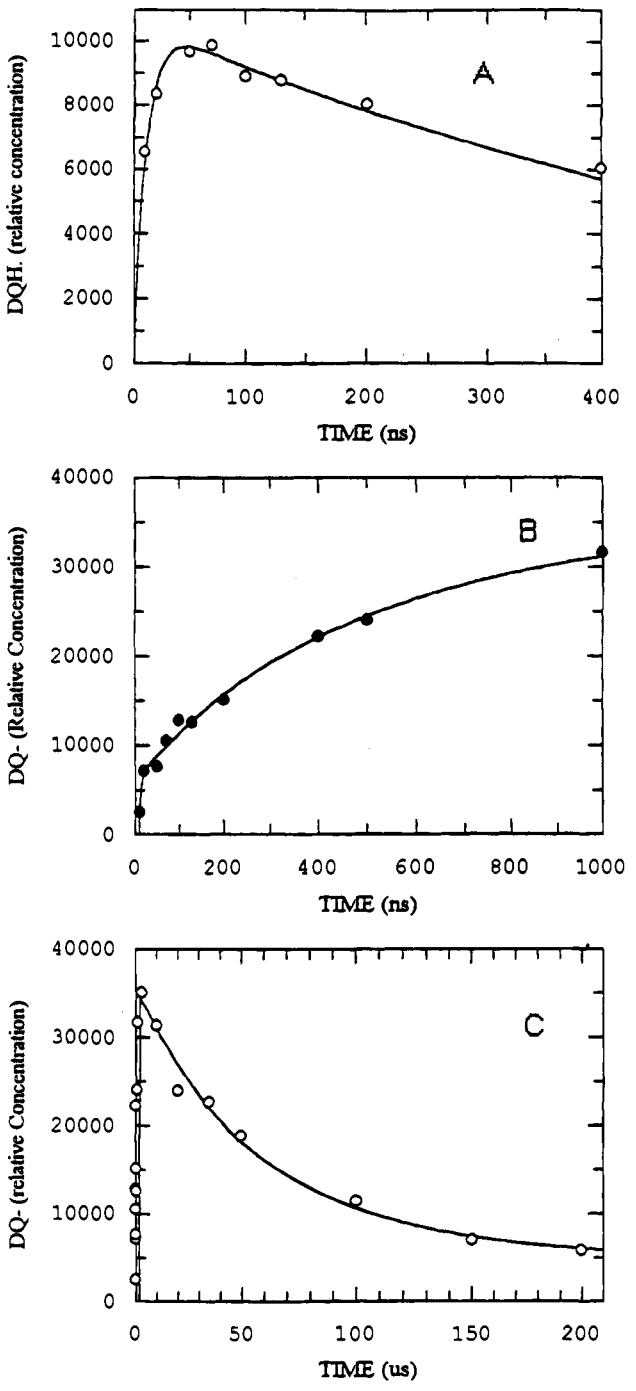

Figure 5. Kinetics of $DQH^{\bullet}$ and $DQ^{-\bullet}$ formation and decay derived from the data shown in Figure 4. The lines indicate the best fit of the data (points) to equations [10] and [11]. A: Formation and decay of $DQH^{\bullet}$; B: formation of $DQ^{-\bullet}$; C: formation and decay of $DQ^{-\bullet}$.

It is clear that α-tocopherol is a versatile protectant in biological systems. Whilst its major role is undoubtably that of a free radical chain breaking antioxidant in cellular membranes, it also possesses reactivity as a singlet oxygen quencher and in repairing free radical damage to proteins. We have demonstrated here that it is also capable of deactivating highly reactive excited states of quinones. The activity of α-tocopherol as a singlet oxygen quencher is surpassed by carotenoids,[16] so that for example in photosynthetic organelles α-tocopherol may be more important in *suppressing* singlet oxygen formation by removing excited state precursors of singlet oxygen. This is reminiscent of the reported activity of carotenoids.[46] Although good singlet oxygen quenchers, carotenoids are believed to offer protection by accepting excitation energy from chlorophyll excited states which would otherwise photosensitise formation of singlet oxygen. Aromatic ketones are also effective photoinitiators of lipid oxidation,[47] and so α-tocopherol may directly prevent the light-induced initiation of peroxidation by these compounds. Triplet quinones may also be generated nonphotochemically during redox reactions.[6,48] Therefore α-tocopherol may have a secondary role in preventing iniatition of oxidative damage, in addition to its well-known activity as a chain-breaking antioxidant.[20] The results show that the reducing properties of α-tocopherol which allow it to deactivate triplet quinones are also matched by ascorbate. However, the lipophilic nature of α-tocopherol provides it with access to lipidic sites in membranes and lipoproteins at which ascorbate cannot act. In the case of quinones bearing hydrophobic chains, as with ubiquinone, the excited state would be confined to the lipidic phase, and the lipidic nature of α-tocopherol would confer an important advantage.

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Supplementary Material Available: Materials and methods used in this study (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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