Antioxidant effect of coumarin derivatives on free radical initiated and photosensitized peroxidation of linoleic acid in micelles

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The antioxidant activity of 4-methylcoumarin (MC), 6-hydroxy-4-methylcoumarin (HMC) and 7,8-dihydroxy-4-methylcoumarin (DHMC), respectively, in the inhibition of peroxidation of linoleic acid in micellar systems has been studied. The peroxidation was initiated either thermally by water-soluble initiator 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AMPAD) or photochemically by a triplet sensitizer benzophenone (BP) in the anionic micelle sodium dodecyl sulfate (SDS) or in the cationic micelle cetyl trimethylammonium bromide (CTAB). It was found that DHMC is a good antioxidant for both AMPAD-initiated and BP-sensitized peroxidation in both SDS and CTAB micelles, while HMC is only active for BP-sensitized peroxidation in SDS micelles. MC is ineffective in any case. The antioxidative action of the coumarin derivatives may include trapping the initiating radicals, trapping the propagating lipid peroxyl radicals, recycling α-tocopherol and/or deactivating the excited photosensitizer.

Introduction

Free radical mediated peroxidation of membrane lipids is believed to be associated with a wide variety of chronic health problems, such as cancer, atherosclerosis and aging.[1,2] Therefore, a great deal of research has been devoted to kinetic and mechanistic studies on the antioxidative effect of natural antioxidants, such as vitamin E, vitamin C, β-carotene, and polyphenols extracted from green tea.[3–11] Coumarins are a class of phenolic compounds, widely present in plants,[12] which are potential antioxidants. However, their antioxidant activity has scarcely been studied. Foti *et al.*[13] reported recently that some coumarin derivatives showed poor antioxidative activity in sodium dodecyl sulfate (SDS) micelles. It is well-known that the activity of antioxidants depends not only on their molecular structure, but also on the microenvironment of the reaction medium and the character of initiating radicals.[8–11] Therefore, it is worth investigating the structure–activity relationship of coumarins in different reaction media and under different initiating conditions. We report herein kinetic studies on the antioxidant activity of three coumarin derivatives, *i.e.* 4-methylcoumarin (MC), 6-hydroxy-4-methylcoumarin (HMC) and 7,8-dihydroxy-4-methylcoumarin (DHMC), against the peroxidation of linoleic acid in sodium dodecyl sulfate (SDS) and cetyl trimethylammonium bromide (CTAB) micelles. A water-soluble azo compound 2,2′-azobis(2-methylpropionamidine) (AMPAD) was used to thermally initiate the peroxidation and a triplet sensitizer benzophenone (BP) was used to photochemically induce the peroxidation. The interaction of these coumarins with α-tocopherol (TocH) was also studied.

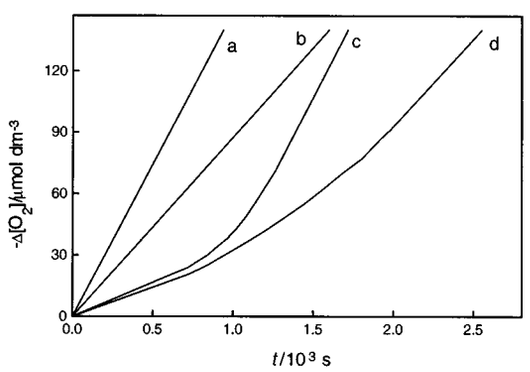
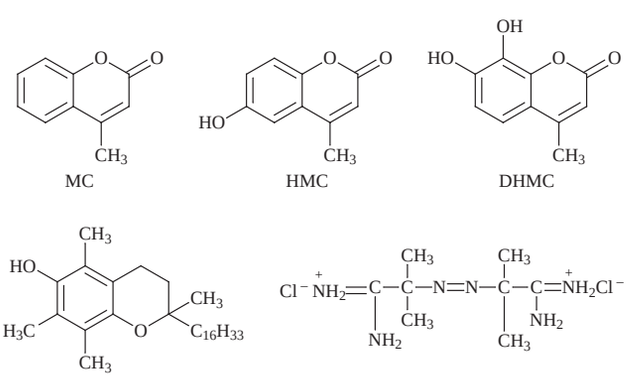


Fig. 1 Oxygen uptake recorded during the AMPAD-initiated peroxidation of LH in 0.5 mol dm^{-3} SDS micelle at 37 °C under atmospheric oxygen. $[LH]_0 = 15.8$ mmol dm^{-3}; $[AMPAD]_0 = 16.2$ mmol dm^{-3}; $[DHMC]_0 = 4.1$ μmol dm^{-3}; $[TocH]_0 = 3.0$ μmol dm^{-3}. (a) Uninhibited reaction; (b) inhibited with DHMC; (c) inhibited with TocH; (d) inhibited with DHMC and TocH.



Results and discussion

AMPAD-induced peroxidation

Linoleic acid (LH) was suspended in SDS micelles and kept at a constant temperature under atmospheric oxygen, then the water-soluble initiator AMPAD was added to initiate the peroxidation which led to rapid oxygen absorption as shown in Fig. 1(a). Addition of MC or HMC showed no appreciable effect on the oxygen absorption (data not shown), while addition of DHMC significantly decreased the rate of oxygen absorption [Fig. 1(b)]. The oxygen absorption was inhibited by addition of TocH for a so-called 'inhibition period' (t_{inh}) or 'induction period', and then recovered to the original rate when the antioxidant was exhausted [Fig. 1(c)]. Although DHMC

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Table 1 Inhibition of AMPAD-initiated peroxidation of linoleic acid by TocH and DHMC[a,b]

Medium	Antioxidant	$R_p/10^{-8}$ mol dm^{-3} s^{-1}	$R_{inh}/10^{-8}$ mol dm^{-3} s^{-1}	$t_{inh}/10^3$ s	$k_{inh}{}^c/10^4$ dm^3 mol^{-1} s^{-1}	kcl_p	kcl_{inh}	$[k_p/(2k_t)^{\frac{1}{2}}]^d$/ dm$^{\frac{1}{2}}$ (mol s)$^{-\frac{1}{2}}$
SDS	None	15.7				26.6		0.046
	TocH	15.7	1.55	1.02	3.70	26.6	2.6	0.046
	DHMC	7.38				12.5		0.022
	TocH + DHMC	6.64	1.36	1.48	2.90	11.2	2.3	0.019
CTAB	None	33.3				23.8		0.017
	TocH	19.0	1.52	2.18	1.37	13.6	1.1	0.010
	DHMC	26.8	3.41	0.72	1.85	19.1	2.4	0.014
	TocH + DHMC	18.5	1.13	2.85	1.41	13.2	0.8	0.009

[a] The reaction conditions and the initial concentrations of the substrates are the same as described in the legends of Figs. 1 and 2 for reactions conducted in SDS and CTAB micelles respectively. Data are the average of three measurements with deviation within ±10%. [b] $R_i = 5.90 \times 10^{-9}$ and 1.40×10^{-8} mol dm^{-3} s^{-1} for the initiation conducted in SDS and CTAB micelles, respectively, which is obtained by the inhibition period method [eqn. (13)]. [c]Calculated by taking $k_p = 37$ dm^3 mol^{-1} s^{-1} in micelles.[16] [d] Oxidizability in the micellar phase.

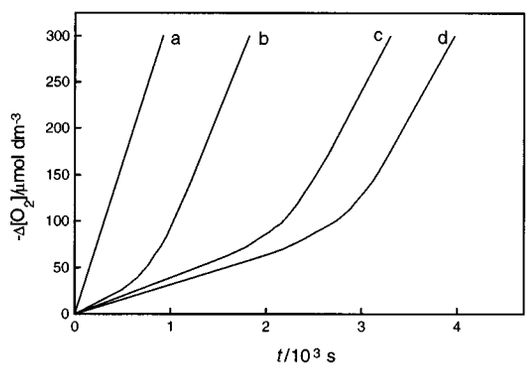


Fig. 2 Oxygen uptake recorded during the AMPAD-initiated peroxidation of LH in 0.015 mol dm^{-3} CTAB micelle at 37 °C under atmospheric oxygen. $[LH]_0 = 12.3$ mmol dm^{-3}; $[AMPAD]_0 = 11.4$ mmol dm^{-3}; $[DHMC]_0 = 3.9$ μmol dm^{-3}; $[TocH]_0 = 14.6$ μmol dm^{-3}. (a) Uninhibited reaction; (b) inhibited with DHMC; (c) inhibited with TocH; (d) inhibited with TocH and DHMC.

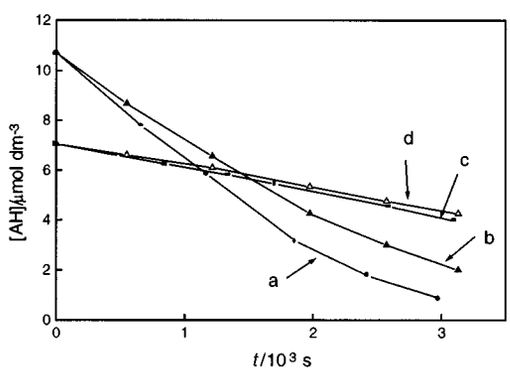


Fig. 3 Decay of antioxidants during the AMPAD-initiated peroxidation of LH in 0.5 mol dm^{-3} SDS micelle at 37 °C under atmospheric oxygen. $[LH]_0 = 15.8$ mmol dm^{-3}; $[AMPAD]_0 = 16.2$ mmol dm^{-3}; $[DHMC]_0 = 7.1$ μmol dm^{-3}; $[TocH]_0 = 10.7$ μmol dm^{-3}. (a) Decay of TocH in the absence of DHMC; (b) decay of TocH in the presence of DHMC; (c) decay of DHMC in the absence of TocH; (d) decay of DHMC in the presence of TocH.

alone did not produce any inhibition period, the inhibition period of TocH increased significantly when DHMC was added together with TocH [Fig. 1(d)]. Similar results were obtained when the reaction was conducted in CTAB micelles, but the rate of oxygen absorption was much faster than that in SDS micelles, and DHMC alone also produced a clear inhibition period. The inhibition period of (TocH + DHMC) is simply the sum of those of TocH and DHMC (Fig. 2 and Table 1).

The decay kinetics of TocH and DHMC were studied by HPLC separation of the reaction mixture followed by electrochemical determination of the antioxidants. It was found that TocH decayed approximately linearly with time within the two half-life times [Fig. 3(a) and 4(a)], in accordance with the kinetic demand for the antioxidation reaction [eqn. (14), *vide infra*]. Addition of DHMC decreased the decay rate slightly in SDS micelles, but remarkably in CTAB micelles [Fig. 3(b) and 4(b)]. The decay of DHMC is also linear and not influenced by the co-existing TocH in SDS micelles [Fig. 3(c) and (d)], but it deviates from linearity in CTAB micelles and the rate decreased slightly in the presence of TocH [Fig. 4(c) and (d)].

It has been proved that the reaction kinetics of the peroxidation in micelles follow the same rate law as that in homogenous solutions.[14] Therefore, the peroxidation of linoleic acid (LH) initiated by azo-compounds (R–N=N–R) can be described by eqns. (1)–(6), where e is the efficiency of the initiator which

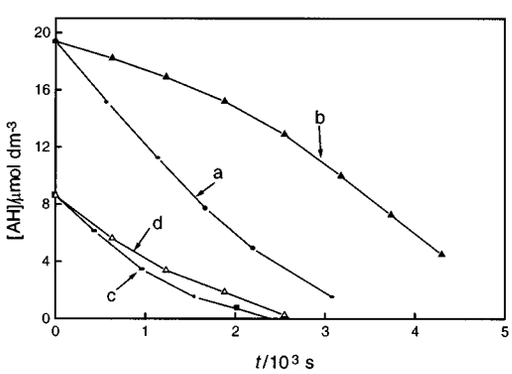


Fig. 4 Decay of antioxidants during the AMPAD-initiated peroxidation of LH in 0.015 mol dm^{-3} CTAB micelle at 37 °C under atmospheric oxygen. $[LH]_0 = 12.3$ mmol dm^{-3}; $[AMPAD]_0 = 11.4$ mmol dm^{-3}; $[DHMC]_0 = 8.6$ μmol dm^{-3}; $[TocH]_0 = 19.4$ μmol dm^{-3}. (a) Decay of TocH in the absence of DHMC; (b) decay of TocH in the presence of DHMC; (c) decay of DHMC in the absence of TocH; (d) decay of DHMC in the presence of TocH.

Initiation:

$$R\text{–}N{=}N\text{–}R \xrightarrow{k_d} 2eR^{\bullet} + N_2 + (1 - e)\,R\text{–}R \quad (1)$$

Propagation:

$$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet} \quad (2)$$

$$ROO^{\bullet} + LH \xrightarrow{k_p} L^{\bullet} + LOOH \quad (3)$$

$$L^{\bullet} + O_2 \longrightarrow LOO^{\bullet} \quad (4)$$

$$LOO^{\bullet} + LH \xrightarrow{k_p} L^{\bullet} + LOOH \quad (5)$$

Termination:

$$2\,LOO^{\bullet} \xrightarrow{2k_t} \text{Molecular products} \quad (6)$$

designates the fraction of the initiator effective in initiating the

peroxidation due to the cage effect, and where k_d, k_p and k_t are the rate constants for the decomposition of the initiator, for the chain propagation and termination, respectively. Based on the steady state kinetic treatment, the oxygen uptake rate can be expressed as eqn. (7), where $k_p/(2k_t)^{1/2}$ is referred to as the

$$-\mathrm{d}[O_2]/\mathrm{d}t = R_p = \{k_p/(2k_t)^{1/2}\} R_i^{1/2}[LH] \quad (7)$$

oxidizability of the substrate, representing the susceptibility of the lipid to undergo peroxidation; and the apparent rate of the chain initiation is given by eqn. (8).

$$R_i = 2k_d e\,[R{-}N{=}N{-}R] \quad (8)$$

In the presence of a chain-breaking antioxidant molecule (AH) the peroxyl radical can be trapped and a new antioxidant radical ($A^{\bullet}$) produced [eqn. (9)]. If the $A^{\bullet}$ is a stabilized radical

$$LOO^{\bullet} + AH \xrightarrow{k_{inh}} LOOH + A^{\bullet} \quad (9)$$

(*e.g.* α-tocopheroloxyl radical or ascorbate radical anion) which can promote the rate-limiting hydrogen abstraction reaction [eqn. (9)] and undergo a fast termination reaction [eqn. (10)], the peroxidation would be inhibited.

$$LOO^{\bullet} + A^{\bullet} \xrightarrow{\text{fast}} LOOA \quad (10)$$

During the inhibition period the rate of peroxyl radicals formed by initiation equals the rate of peroxyl radicals that are trapped, therefore eqns. (11) and (12) hold, where n is the

$$R_{inh} = k_{inh}\, n\, [AH][LOO^{\bullet}] \quad (11)$$

$$[LOO^{\bullet}] = R_{inh}/k_{inh}\, n\, [AH] \quad (12)$$

stoichiometric factor that designates the number of peroxyl radicals trapped by each antioxidant molecule and is given by eqn. (13). The k_{inh} in eqn. (11) represents the activity of the

$$n = R_i\, t_{inh}\, [AH]_0 \quad (13)$$

antioxidants. From eqns. (9)–(12) we have eqn. (14). The n value

$$-\mathrm{d}[AH]/\mathrm{d}t = R_i/n \quad (14)$$

of α-tocopherol is generally assumed to be 2,[14,15] thus the R_i value can be determined from the inhibition period or from the decay rate of α-tocopherol. The rate of oxygen uptake during the inhibition period can be derived from the steady state treatment of the above equations as:

$$-\mathrm{d}[O_2]/\mathrm{d}t = R_{inh} = k_p R_i[LH]/(nk_{inh}[AH]) \quad (15)$$

The kinetic chain length (kcl) defines the number of chain propagation steps initiated by each initiating radical and is given by eqns. (16) and (17) for inhibited and uninhibited

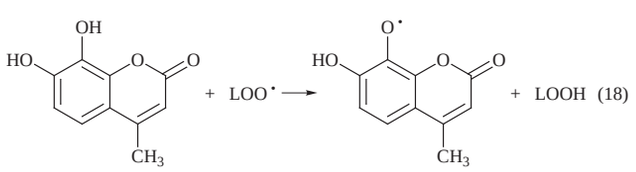
$$kcl_{inh} = R_{inh}/R_i \quad (16)$$

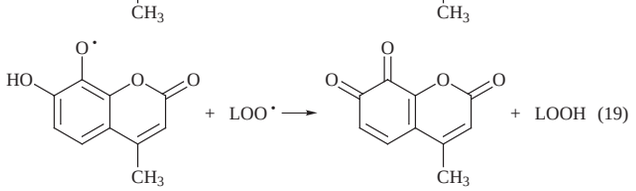
$$kcl_p = R_p/R_i \quad (17)$$

peroxidation respectively. The kinetic parameters deduced from Figs. 1–4 are listed in Table 1.

It can be seen from Figs. 1–4 and Table 1 that the reaction medium exerts a significant effect on the oxidizability of linoleic acid, on the rate of initiation, and on the antioxidant activity of TocH and DHMC. The R_i value of AMPAD in SDS micelles [5.9×10^{-9} mol dm^{-3} s^{-1} calculated by using eqn. (13)] is remarkably smaller than that in CTAB micelles (13.4×10^{-9} mol dm^{-3} s^{-1}). This is due to the fact that AMPAD is positively charged, hence it is prone to adsorb on the surface of SDS which, in turn, would reduce the effective initiation due to the cage effect [eqn. (1)]. However, the oxidizability of linoleic acid and the inhibition rate constant, k_{inh}, are higher in SDS than in CTAB micelles. This is because lipid peroxyl radicals are polar (dipole moment of *ca*. 2.6 Debye) and electrophilic,[14] hence they should move to the surface of micelles more quickly in SDS than in CTAB micelles to react with the antioxidant, according to the floating peroxyl hypothesis proposed by Barclay.[15] α-Tocopherol is an efficient antioxidant in both micelles and is more active in SDS as has been discussed previously.[14–17]

DHMC does not show an inhibition period but reduces significantly the rate of propagation, R_p, in SDS micelles (Fig. 1), whereas it also produces a clear inhibition period in CTAB micelles (Fig. 2). DHMC with a pK_a of *ca*. 9.4[18] should partially deprotonate to form phenoxide anion in the neutral medium, and the polar surface of micelles must facilitate the hydrogen-bonding interaction and the deprotonation process. Indeed, the ultra-violet absorption bands of DHMC appreciably red-shifted in CTAB micelles, demonstrating formation of the corresponding phenoxide anion. The phenoxide anion of DHMC must reside predominantly in the interior of SDS micelles, but around the surface of CTAB micelles, due to the electrostatic interaction with the charged micelle head groups. Therefore, in SDS micelles DHMC can only trap the initiating radicals in the interior of the micelle to suppress the rate of initiation, hence reducing the apparent rate of propagation [eqn. (7), Table 1]. This suppression of the initiation would, in turn, increase the inhibition period of TocH [eqn. (13)] when DHMC is used in combination with the former as observed in Fig. 1. The decay kinetics of TocH and DHMC in SDS (Fig. 3) is in accordance with this mechanism, *i.e.*, DHMC reduces the decay rate of TocH, but TocH has no effect on the decay of DHMC. In CTAB micelles, however, DHMC can trap not only the initiating radicals but also the propagating lipid peroxyl radicals effectively around the surface of the micelle, acting as a chain-breaking antioxidant to produce an inhibition period (Fig. 2). The inhibition period of TocH + DHMC is 2.85×10^3 s which is approximately the sum of the inhibition period of TocH and DHMC when they were used separately (Table 1), suggesting that the two antioxidants may act independently (see below, however). The antioxidant activity of DHMC is obviously due to its *ortho*-dihydroxy structure that makes the oxidation intermediate *o*-semiquinone fairly stable and easily oxidized to form the final product *o*-quinone [eqns. (18 and 19)].





As a matter of fact, DHMC showed a reversible cyclic voltammogram with the oxidation peak potential at 0.250 and 0.238 V (*vs*. SCE) in SDS and CTAB micelles respectively, which are close to the oxidation potential of α-tocopherol (0.160 V in both SDS and CTAB micelles).

It is worth noting that the decay kinetics of the two antioxidants in CTAB micelles (Fig. 4) is quite different from that in SDS micelles (Fig. 3). In SDS micelles DHMC decays linearly, consistently with the kinetic demand for a chain-breaking

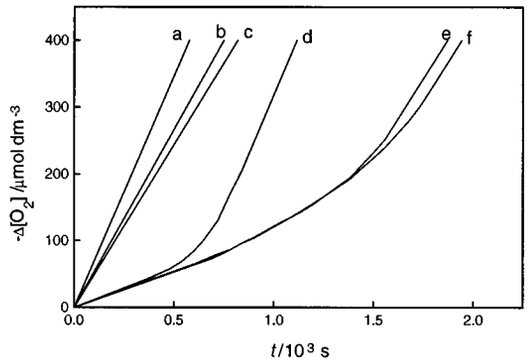


Fig. 5 Oxygen uptake recorded during the BP-photosensitized peroxidation of LH in 0.5 mol dm^{-3} SDS micelle at 30 °C under pure oxygen. $[LH]_0 = 32.2$ mmol dm^{-3}; $[BP] = 1.4$ mmol dm^{-3}; $[DHMC]_0 = 3.8$ μmol dm^{-3}; $[HMC]_0 = 41.2$ μmol dm^{-3}; $[TocH]_0 = 8.4$ μmol dm^{-3}. (a) Uninhibited reaction; (b) inhibited with DHMC; (c) inhibited with HMC; (d) inhibited with TocH; (e) inhibited with DHMC and TocH; (f) inhibited with HMC and TocH.

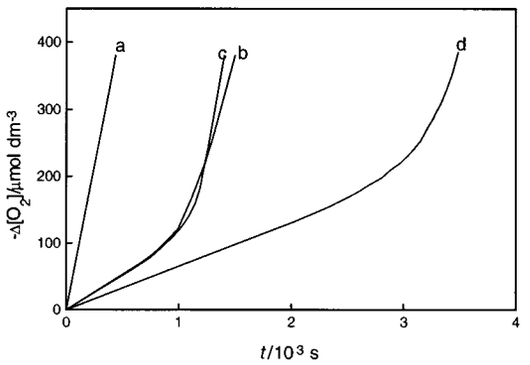
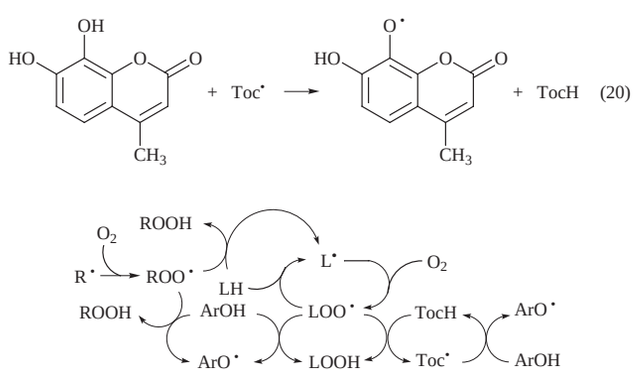


Fig. 6 Oxygen uptake recorded during the BP-photosensitized peroxidation of LH in 0.015 mol dm^{-3} CTAB micelle at 30 °C under pure oxygen. $[LH]_0 = 12.3$ mmol dm^{-3}; $[BP] = 0.7$ mmol dm^{-3}; $[DHMC]_0 = 15.6$ μmol dm^{-3}; $[TocH]_0 = 28.0$ μmol dm^{-3}. (a) Uninhibited reaction; (b) inhibited with DHMC; (c) inhibited with TocH; (d) inhibited with DHMC and TocH.

antioxidant [eqn. (14)]. However, in CTAB micelles the decay of DHMC deviates significantly from linearity and becomes exponential, implying a bimolecular reaction. In addition, the decay rate of TocH decreased remarkably in the presence of DHMC and recovered to its intrinsic value after depletion of the latter (Fig. 4). This strongly suggests a synergistic antioxidant effect, *i.e.*, TocH is recycled by DHMC according to eqn. (20). Similar antioxidant synergism and suppressed decay of TocH have been reported previously in the cooperative antioxidation of TocH with ascorbic acid,[19] and TocH with tea polyphenols.[11] Therefore, it is clear that in CTAB micelles DHMC cannot only trap the initiating radicals as it does in SDS micelles, but also acts as both a chain-breaking antioxidant [eqns. (18) and (19)] and an α-tocopherol recycling co-antioxidant [eqn. (20)] as illustrated in Scheme 1.



Scheme 1 Antioxidant mechanism of DHMC in AMPAD-initiated peroxidation in CTAB micelles, where ArOH designates DHMC.

BP-photoinduced peroxidation

Irradiation of a mixture of linoleic acid (LH) and benzophenone (BP) suspended in SDS micelle with a high pressure mercury lamp through a Pyrex filter induced rapid oxygen absorption [Fig. 5(a)], demonstrating a photo-induced peroxidation. MC showed no effect on the oxygen absorption (result not shown), while HMC decreased the rate of oxygen absorption when it was used in high concentration [Fig. 5(b)], and increased the inhibition period of TocH when it was used in combination with the latter [Fig. 5(e)]. This is significantly different from the ineffectiveness of HMC in AMPAD-induced peroxidation (*vide supra*). DHMC and TocH showed similar effects to those in the AMPAD-induced reaction, *i.e.*, in SDS micelles DHMC decreases the rate of peroxidation, TocH produces a clear inhibition period, and the inhibition period of TocH was remarkably prolonged in the presence of DHMC

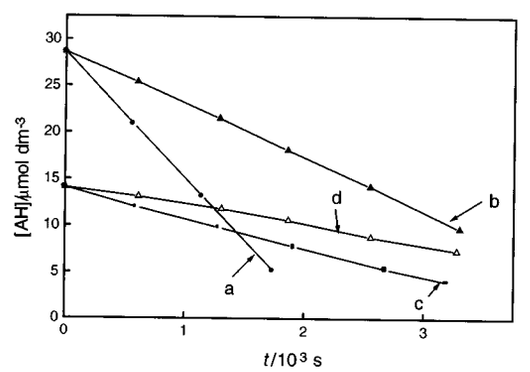


Fig. 7 Decay of antioxidants during the BP-photosensitized peroxidation of LH in 0.5 mol dm^{-3} SDS micelle at 30 °C under pure oxygen. $[LH]_0 = 32.3$ mmol dm^{-3}; $[BP]_0 = 1.41$ mmol dm^{-3}; $[DHMC]_0 = 14.1$ μmol dm^{-3}; $[TocH]_0 = 28.6$ μmol dm^{-3}. (a) Decay of TocH in the absence of DHMC; (b) decay of TocH in the presence of DHMC; (c) decay of DHMC in the absence of TocH; (d) decay of DHMC in the presence of TocH.

[Fig. 5(c, d and f)]. DHMC alone also produced a clear inhibition period in CTAB micelles and the inhibition period of (DHMC + TocH) is significantly longer than the sum of those of DHMC and TocH, demonstrating a synergistic antioxidant effect (Fig. 6). However, HMC showed no effect in CTAB micelles even when at very high concentration.

The decays of TocH and DHMC during the BP-induced peroxidation are shown in Figs. 7 and 8. In SDS micelles TocH decayed completely linearly with time and the decay rate decreased remarkably in the presence of DHMC [Fig. 7(a) and (b)]. DHMC also decayed linearly and the decay rate decreased in the presence of TocH [Fig. 7(c) and (d)]. The decay kinetics in CTAB micelles (Fig. 8) are similar to those of the AMPAD-induced reaction (Fig. 4).

The photosensitized reaction of oxygen with an organic substrate may proceed *via* hydrogen abstraction from the substrate by the sensitizer triplet followed by free radical autoxidation (Type I mechanism) or *via* energy transfer from the sensitizer to form singlet oxygen which can react with the hydrogen donor in a variety of ways (Type II mechanism).[20,21] Barclay and co-workers[22,23] have proved that benzophenone-sensitized photooxidation of linoleic acid in SDS micelles proceeded *via* a Type I (free radical) mechanism, since allylic hydrogens of linoleic acid are known to be effective quenchers of benzoquinone triplets by hydrogen abstraction[24] and peroxyl radicals have lifetimes orders of magnitude longer than singlet oxygen in SDS micelles.[25] Our results are consistent with this conclusion. Therefore, the kinetic treatment used in the AMPAD-initiated peroxidation mentioned in the previous section can also be used

Table 2 Inhibition of BP-photosensitized peroxidation of linoleic acid by TocH, HMC and DHMC[a,b]

Medium	Antioxidant	$R_p/10^{-8}$ mol dm^{-3} s^{-1}	$R_{inh}/10^{-8}$ mol dm^{-3} s^{-1}	$t_{inh}/10^3$ s	k_{inh}[c]/10^4 dm^3 mol^{-1} s^{-1}	kcl_p	kcl_{inh}	$[k_p/(2k_t)^{\frac{1}{2}}]$[d]/ dm$^{\frac{3}{2}}$ (mol s)$^{-\frac{1}{2}}$	SE%
SDS	None	69.4				24.8		0.046	
	TocH	65.5	11.8	0.59	1.71	23.4	4.2	0.043	
	DHMC	53.3				19.0		0.035	
	HMC	48.8				17.4		0.032	
	TocH + DHMC	53.3	11.5	1.40	0.74	19.0	4.1	0.035	
	TocH + HMC	46.0	10.4	1.51	0.76	16.4	3.7	0.030	
CTAB	None	83.3				18.0		0.023	
	TocH	83.3	10.2	1.22	0.37	18.0	2.2	0.023	
	DHMC	69.4	11.1	0.96	0.43	15.0	2.4	0.019	
	TocH + DHMC	52.0	6.58	3.28	0.21	11.3	1.4	0.014	50.4

[a] The reaction conditions and the initial concentrations of the substrates are the same as described in the legends of Figs. 6 and 7 for reactions conducted in SDS and CTAB micelles respectively. Data are the average of three measurements with deviation within ±10%. [b] $R_i = 2.80 \times 10^{-8}$ and 4.62×10^{-8} mol dm^{-3} s^{-1} for the initiation conducted in SDS and CTAB micelles, respectively, which is obtained by the inhibition period method [eqn. (13)]. [c]Calculated by taking $k_p = 37$ dm^3 mol^{-1} s^{-1} in micelles.[16] [d] Oxidizability in the micellar phase.

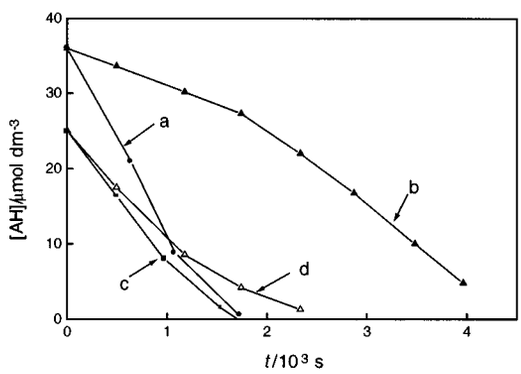
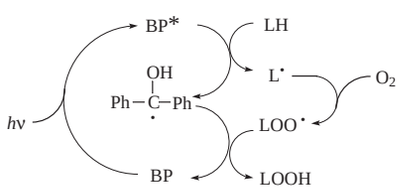


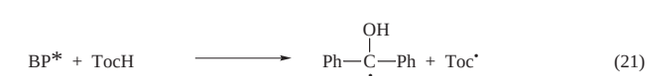
Fig. 8 Decay of antioxidants during the BP-photosensitized peroxidation of LH in 0.015 mol dm^{-3} CTAB micelle at 30 °C under pure oxygen. $[LH]_0 = 12.3$ mmol dm^{-3}; $[BP]_0 = 0.7$ mmol dm^{-3}; $[DHMC]_0 = 25.0$ μmol dm^{-3}; $[TocH]_0 = 36$ μmol dm^{-3}. (a) Decay of TocH in the absence of DHMC; (b) decay of TocH in the presence of DHMC; (c) decay of DHMC in the absence of TocH; (d) decay of DHMC in the presence of TocH.

for the BP-sensitized reaction and the results are listed in Table 2. The only difference is that benzophenone can be recycled *via* hydrogen abstraction from the intermediate ketyl radical by the peroxyl radical (Scheme 2).



Scheme 2 Mechanism of BP-photosensitized peroxidation.

It can be seen from Tables 1 and 2 that the k_{inh}values of α-tocopherol and DHMC in the photo-initiated reactions are smaller than those in the AMPAD-initiated ones, possibly due to the consumption of α-tocopherol and DHMC through their direct reaction with the triplet benzophenone [*e.g.*, eqn. (21)].



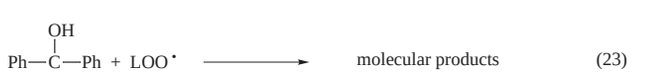
(21)

The antioxidant effect of HMC observed in SDS micelles might be due to its ability to deactivate the benzophenone triplet rather than trapping the peroxyl radicals, since it exhibits no activity in AMPAD-initiated peroxidation (*vide supra*). It was reported that the triplet energy of coumarin (62 kcal mol^{-1}) is significantly lower than that of benzophenone (69 kcal mol^{-1}).[26] Therefore, triplet-energy transfer from the benzophenone triplet to HMC should readily take place which would deactivate the benzophenone triplet. Since the triplet energy of coumarin is too low to initiate the peroxidation, the initiation efficiency is reduced. DHMC may also be able to quench the benzophenone triplets, hence the decay rate of TocH is remarkably decreased in the presence of DHMC (Fig. 7). The ineffectiveness of HMC in CTAB micelles is understandable because HMC should predominantly locate around the surface of the CTAB micelles, hence is too far away to carry out energy transfer with benzophenone triplets which reside in the interior of the micelle.

The antioxidant synergism of TocH and DHMC in CTAB micelles in the BP-sensitized peroxidation is similar to that in the AMPAD-initiated reaction, but the inhibition period of TocH and DHMC when they were used in combination is significantly longer than the sum of their inhibition periods when they were used separately, with the synergistic efficiency SE% of 50.4 (Table 2). The synergistic efficiency is defined by eqn. (22).[27]

$$SE\% = [t_{inh}(TocH + DHMC) - t_{inh}(TocH) - t_{inh}(DHMC)]/[t_{inh}(TocH) + t_{inh}(DHMC)] \times 100\% \quad (22)$$

This is probably due to the fact that the propagating lipid peroxyl radicals can also be terminated by the intermediate ketyl radical of BP [eqn. (23)], hence more TocH and DHMC could be spared.



(23)

Conclusion

This work demonstrates that the antioxidant activity of coumarin derivatives in micelles depends not only on their structure but also on the microenvironment of the reaction medium and the initiation conditions. DHMC is a good antioxidant for both AMPAD-initiated and BP-sensitized peroxidation in both SDS and CTAB micelles, while HMC is only active for BP-sensitized peroxidation in SDS micelles. MC is ineffective in any case. The antioxidative action of the coumarin derivatives may include trapping the initiating radicals, trapping the propagating lipid peroxyl radicals, recycling α-tocopherol and/or deactivating the excited benzophenone triplets.

Experimental

Materials

4-Methylcoumarin (MC) was prepared by the condensation

of phenol and ethyl acetoacetate using anhydrous aluminum chloride as the catalyst in nitrobenzene.[28] 6-Hydroxy-4-methylcoumarin (HMC) and 7,8-dihydroxy-4-methylcoumarin (DHMC) were synthesized by the condensation of resorcinol and pyrogallol, respectively, with ethyl acetoacetate using sulfuric acid as the catalyst.[28] 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AMPAD) and benzophenone (BP) were purchased from Aldrich and used as received. Linoleic acid (Fluka, chromatographic pure), (±)-α-tocopherol (Merck, biochemical reagent) were used as received and kept under nitrogen in a refrigerator before use. The surfactants SDS and CTAB were recrystallized from ethanol and acetone–water (9 : 1 v/v) respectively.

Oxygen uptake measurements

The rate of oxygen uptake was measured with an SP-2 oxygen uptake apparatus equipped with an oxygen electrode able to record oxygen concentrations as low as 10^{-8} mol dm^{-3}. Every experiment was repeated at least three times to ensure the experimental deviation within 10%. The reaction was conducted under atmospheric oxygen at 37 ± 0.1 °C for AMPAD-initiated peroxidation, while conducted under pure oxygen at 30 ± 0.1 °C for the BP-photosensitized peroxidation and irradiated with a 200 W high pressure mercury lamp through a Pyrex filter. Experimental details have been described previously.[17]

Determination of α-tocopherol and DHMC

Aliquots of the reaction mixture were taken out at appropriate time intervals and subjected to high performance liquid chromatography (HPLC) analysis using a Gilson chromatograph and a Sychropak KPP-100 reversed-phase column (4 × 250 mm) and eluted with methanol–propan-2-ol–formic acid (9 : 1 : 0.01 v/v/v) containing 50 mmol dm^{-3} of sodium perchlorate as supporting electrolyte. A Gilson Model 142 electrochemical detector was used to monitor α-tocopherol and DHMC simultaneously by setting the oxidation potential at +700 mV.

Determination of oxidation potential

The oxidation potentials of MC, HMC and DHMC were determined with a PAR model 173 potentiostat using a glassy carbon electrode as described previously.[11] The potential was recorded relative to a saturated calomel electrode (SCE) reference electrode.

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