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ANTIOXIDANT SYNERGISM AND MUTUAL PROTECTION OF α-TOCOPHEROL AND β-CAROTENE IN THE INHIBITION OF RADICAL-INITIATED PEROXIDATION OF LINOLEIC ACID IN SOLUTION

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Peroxidation of linoleic acid was initiated by azobis(isobutyronitrile) in *tert*-butyl alcohol and inhibited by α-tocopherol, β-carotene and retinal, either alone or in combination. Significant antioxidant synergism and a novel mutual protection of α-tocopherol and β-carotene were found and the possible involvement of retinal in the process is discussed.

INTRODUCTION

Interests in β-carotene (βC) has recently been rekindled since there is increasing epidemiological, clinical and biochemical evidence that carotenoids may play a role in prevention of cancer,[1-3] stroke,[4] and cardiovascular diseases.[5,6] One of the most interesting proposals relating β-C to the disease prevention is that it may act as an antioxidant to trap active free radicals, which would otherwise initiate harmful reactions such as lipid peroxidation and eventually induce the diseases.[1,7-9] However, despite ample accumulation of research data showing the beneficial effect of β-C on health, critical gaps remain in our knowledge of its precise antioxidant mechanism. Burton and Ingold[10] have pointed out that β-C is 'an unusual type of lipid antioxidant' because it exhibits good radical trapping behaviour at lower oxygen pressures, whereas it shows an autocatalytic, pro-oxidant effect at higher oxygen pressures in homogeneous solutions. Similar oxygen pressure-dependent antioxidant/pro-oxidant behaviour was also observed in soybean phosphatidylcholine liposomes.[11] In addition, in contrast to the well known antioxidant synergism of α-tocopherol (VE) and L-ascorbic acid (VC), namely, recycling of VE by VC,[12,13] the antioxidant synergism of–C and VE, if any, is still not well understood. It has been mentioned that VE might suppress the autoxidation of β-C, but no details were given.[11,14] Some *in vivo* experiments showed a synergistic antioxidant effect of β-C and VE,[15,16] but no such an effect was observed in other cases.[17,18] Palozza and Krinsky reported that β–C and VE exerted an additive effect in inhibiting radical-initiated lipid peroxidation in homogeneous solutions,[19] whereas in rat liver microsomes the effect was found to be synergistic[20] and influenced by several factors, including the type of the radical initiator involved and the site and rate of the radical production.[21] Obviously, further investigations are required to elucidate the mechanism of β-C and VE interactions in detail.

This paper describes oxygen uptake and ultraviolet (UV) spectroscopic studies on the azobis(isobutyronitrile) (AIBN)-initiated peroxidation of linoleic acid in *tert*-butyl alcohol under atmospheric oxygen which was inhibited by VE, β-C and retinal (RCHO), either alone or in combination. Our experiments showed that either VE or β-C inhibits the peroxidation effectively and the combination of the two antioxidants exhibits a much higher inhibition effect than an additive effect, i.e. a significant synergistic effect. Most interestingly, the depletion of the two antioxidants when they are used in combination is both slower than when they are used separately, demonstrating a novel mutual protection effect. Further, although retinal (RCHO) alone shows no observable antioxidant activity against linoleic acid peroxidation, it works very well synergistically with VE. Since retinal and other β-apo-carotenals are known to be the major oxidative degradation products of β-C,[14] we propose that retinal may play a role in the antioxidant synergism of VE and β-C.

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RESULTS AND DISCUSSION

Oxygen uptake measuremens

Linoleic acid was dissolved in *tert*-butyl alcohol and kept at constant temperature (50 °C) under atmospheric oxygen, then AIBN was added to initiate the peroxidation which led to rapid oxygen absorption as shown in Figure 1(a). The oxygen absorption was suppressed by adding VE to the system and recovered to its original rate after the antioxidant was exhausted [Figure 1(b)]. β-C showed similar antioxidant activity [Figure 1(c)] and a combination of VE and β-C gave much longer induction period (see below) than the additive effect when VE and β-C were used alone, indicating an antioxidant synergism of the two antioxidants [Figure 1(d)].

The peroxidation of linoleic acid (LH), thermally initiated by azo compounds (RN=NR), can be described by the following simplified scheme:

Initiation

$$RN{=}NR \xrightarrow{k_d} 2eR^{\cdot} + N_2 + (1-e)RR \qquad (1)$$

Propagation:

$$R^{\cdot} + O_2 \longrightarrow ROO^{\cdot} \qquad (2)$$

$$ROO^{\cdot} + LH \longrightarrow ROOH + L^{\cdot} \qquad (3)$$

$$L^{\cdot} + O_2 \longrightarrow LOO^{\cdot} \qquad (4)$$

$$LOO^{\cdot} + LH \xrightarrow{k_p} LOOH + L^{\cdot} \qquad (5)$$

Termination:

$$2LOO^{\cdot} \xrightarrow{k_t} \text{molecular products} \qquad (6)$$

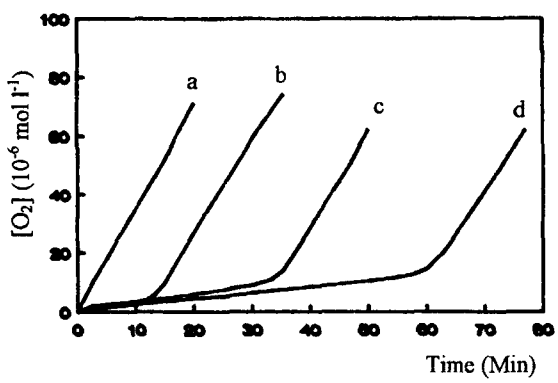

Figure 1. Oxygen uptake during the peroxidation of linoleic acid in *tert*-butyl alcohol at 50 °C initiated with AIBN. $[LH]_0 = 19{\cdot}3$ mmol l^{-1}, $[AIBN]_0 = 6{\cdot}09$ mmol l^{-1}. (a) uninhibited reaction; (b) inhibited with VE (6·96 μmol l^{-1}); (c) inhibited with β-C (11·2 μmol l^{-1}); (d) inhibited with VE (6·96 μmol l^{-1}) and β-C (11·2 μmol l^{-1})

where k_d, k_p and $2k_t$ represent the rate constants for the decomposition of the initiator, chain propagation and termination reactions, respectively, and e is the efficiency of the initiator, namely the fraction of the radicals which can diffuse out from the solvent cage to initiate the peroxidation.

Based on the steady-state kinetic treatment of reactions (1)–(6) the rate of oxygen uptake can be expressed as follows:[22]

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

where $k_p/(2k_t)^{1/2}$ is generally referred to as the oxidizability of the substrate, representing the susceptibility of the substrate to undergo peroxidation, and R_i is the apparent rate of the chain initiation which, equals $2ek_d[RN{=}NR]$.

When an antioxidant AH is added, peroxyl radicals will be trapped and the chain reaction stopped:

$$LOO^{\cdot} + AH \xrightarrow{k_{inh}} LOOH + A^{\cdot} \qquad (8)$$

$$A^{\cdot} + LOO^{\cdot} \longrightarrow \text{molecular products} \qquad (9)$$

where the rate constant k_{inh} represents the activity of the antioxidant. The rate of oxygen uptake in the presence of an antioxidant can be derived from the steady-state treatment of equations (1)–(9) as

$$\frac{-\mathrm{d}[O_2]}{\mathrm{d}t} = \frac{k_p R_i[LH]}{nk_{inh}[AH]} \qquad (10)$$

where n is the stoichiometric factor, which designates the number of peroxyl radicals trapped by each antioxidant molecule. R_i and n can be determined by induction period method;[23] the induction period, t_{inh} is the time during which the peroxidation is suppressed by the antioxidant added, i.e. the lag from the inhibition of oxygen uptake to the turning point of restoration of the oxygen uptake. The n value of VE is known to be 2,[23] hence we have

$$R_i = 2{\cdot}0[VE]_0/t_{inh} \qquad (11)$$

$$n = R_i t_{inh}/[AH]_0 \qquad (12)$$

Substituting equation (12) into equation (10) and integrating gives

$$\Delta[O_2] = -\frac{k_p}{k_{inh}}[LH]_0 \ln\left(1 - \frac{t}{t_{inh}}\right) \qquad (13)$$

where $\Delta[O_2]$ is the amount of oxygen absorbed by reaction (4) in the time interval t. The observed oxygen consumption, $\Delta[O_{12}]_{obs}$, includes the oxygen consumption by reaction (2), and therefore we have

$$\Delta[O_2] = \Delta[O_2]_{obs} - R_i t \qquad (14)$$

Plots of $\Delta[O_2]$ versus $-\ln(1 - t/t_{inh})$ give fairly good straight lines from which the ratio k_p/k_{inh} can be evaluated. Taking the estimated k_p of 230 $mol^{-1}\,s^{-1}$ at 50 °C,[24] the k_{inh} values can be calculated and are given in Table 1. Also listed are the rate of initiation, the oxidizability, the induction period, the stoichiometric factor, and the effective stoichiometric factor of the synergism, which is defined as[25]

$$n(VE) = R_i[t_{inh}(VE + \beta\text{-C}) - t_{inh}(\beta\text{-C})]/[VE]_0 \quad (15)$$

It is seen from Table 1 that both VE and β-C can inhibit the peroxidation of linoleic acid effectively. The stoichiometric factor of 4.1 for β-C is an interesting observation. It is known that β-C does not have the structural feature commonly associated with chain-breaking antioxidants, namely no abstractable hydrogens for the chain-carrying peroxyl radicals.[10,26] Burton and Ingold[10] has suggested that the resonance-stabilized carbon-centred radical formed by the addition of a peroxyl radical to the conjugated system of β-C may be responsible for its antioxidant capability, since this radical has the potential to trap another peroxyl radical to break the chain:

$$LOO^{\cdot} + \beta\text{-C} \rightarrow LOO - \beta\text{-C}^{\cdot} \quad (16)$$

$$LOO - \beta\text{-C}^{\cdot} + LOO^{\cdot} \rightarrow \text{molecular products} \quad (17)$$

According to this mechanism, one β-C$^{\cdot}$ should trap two peroxyl radicals with a stoichiometric factor of 2. Moreover, if the concurrent pro-oxidant action due to the autoxidation of β-C [equation (18)] was taken into account, the n value should be smaller than 2. It is therefore clear that something else must be contributing to the antioxidant activity of β-C:

$$LOO - \beta\text{-C}^{\cdot} + O_2 \rightarrow LOO - \beta\text{-C}{-}OO^{\cdot} \quad (18)$$

Furthermore, β-C can work synergistically with VE to prolong the induction period much longer (3600 s) than the additive effect of the induction period when the two antioxidants are used separately (2820 s, see Table 1). This result is in accord with the synergistic antioxidation of VE and β-C in rat liver liposomes observed by Palozza *et al.*[21] They suggested that VE gave protection against the autoxidation of β-C because VE was consumed faster in the presence of β-C. However, we observed that both VE and β-C were consumed more slowly when they were used in combination than when used separately (see below). Moreover, the effective stoichiometric factors are 4.4 and 5.5 for VE and β-C, respectively, when they are used in combination, i.e. much greater than their intrinsic n values. This means that each molecule of VE can 'spare' (i.e. act as the equivalent of) about 1.1 molecules of β-C and each molecule of β-C can 'spare' about 2·75 molecules of VE. This demonstrates that the synergistic interaction of VE and β-C cannot be a simple recycling mechanism such as the synergism of VE and VC[13] in which VC reduces VE radical to regenerate VE with effective n values for VC ranging from about 0·4 to 0·7.[25]

Ultraviolet spectroscopic measurements

VE and β-C exhibit characteristic UV absorption at 292 and 450 nm respectively, in *tert*-butyl alcohol, which allows the easy measurement of depletion of the antioxidants. Under similar experimental conditions to those in the oxygen uptake experiment, the UV spectra of the reaction system were measured at definite time intervals to follow the reaction. It was found that VE was depleted linearly with time either in the absence or in the presence of β-C, which is in accord with the kinetic requirements for a chain-breaking antioxidant [equation (8)]. On the other hand, the depletion of β-C was very fast and sigmoidal, demonstrating the self-catalytic autoxidation character. A representative spectrum is shown in Figure 2 and the kinetic traces are depicted in Figure 3. It can be seen from Figure 3 that the decay rate of β-C was substantially decreased (by over one order of magnitude) on adding VE to the system and the decay kinetics changed from autoxidative to linear, namely antioxidant decay. This indicates

Table 1. Inhibition of peroxidation of linoleic acid by VE and β-C[a]

$[AH]_0$ (μmol l^{-1})		R_i	R_p	$k_p/(2k_t)^{1/2}$	t_{inh}	k_{inh}	
VE	β-C	(10^{-8} mol $l^{-1}\,s^{-1}$)	(10^{-8} mol $l^{-1}\,s^{-1}$)	[$10^{-2}\,l^{1/2}$ (mol s)$^{-1/2}$]	(s)	(10^5 l $mol^{-1}\,s^{-1}$)	n
6·96		2·25[b]	5·44[c]	1·94	660	20·5	2·0
	11·2	2·25	5·66	2·05	2160	3·99	4·1
6·96	11·2	2·25	4·35	1·50	3600	3·72	4·4 (VE)[d]
							5·5 (β-C)[d]

[a] In *tert*-butyl alcohol at 50 °C with $[LH]_0 = 1{\cdot}93 \times 10^{-2}$ mol l^{-1} and $[AIBN]_0 = 6{\cdot}09 \times 10^{-3}$ mol l^{-1}.
[b] Calculated by $R_i = 2ek_d[AIBN]$ with $k_d = 2{\cdot}6 \times 10^{-6}\,s^{-1}$ and $e = 0{\cdot}70$.[33] This value is in good agreement with the value of $2{\cdot}11 \times 10^{-8}$ mol $l^{-1}\,s^{-1}$ calculated using equation (11).
[c] Rate of propagation after induction period.
[d] Effective stoichiometric factor [see equation (15)].

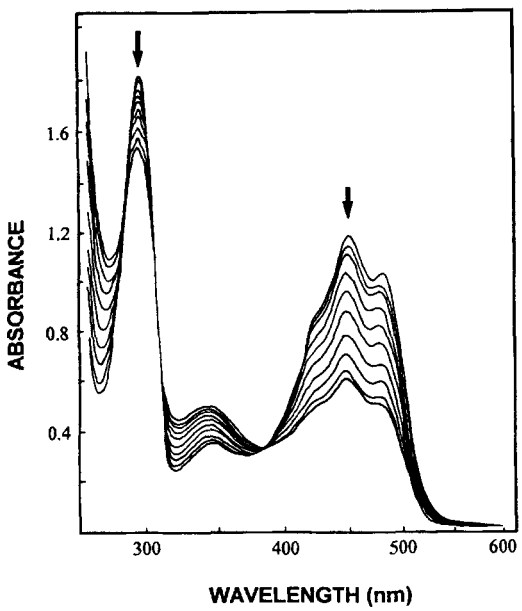

Figure 2. UV spectra recorded during the VE ($5{\cdot}13 \times 10^{-4}$ mol l^{-1}) and β-C (6·91 μmol l^{-1}) co-inhibited peroxidation of LH (11·8 mmol l^{-1}) initiated with AIBN (17·4 mmol l^{-1}) in *tert*-butyl alcohol at 50 °C. The spectra were recorded at time intervals of 25 min, but only a selection of the experimental lines is shown for clarity. Peaks that appeared and increased in intensity at *ca* 340 nm came from unidentified degradation products of β-C

that the autoxidation of β-C has been inhibited by VE, affording good experimental evidence for the previous proposals about the cooperative antioxidant/pro-oxidant behaviour of β-C and the role of VE in protecting β-C against autoxidation.[10,11,20] However, the decay of VE is also decreased by about half in the presence of β-C compared with that in the absence of the latter. Obviously, this result cannot be explained by the above mechanism, which should require an increase in the depletion of VE. Therefore, there must be some way to regenerate VE by β-C.

The direct recycling of VE radical to VE by β-C seems improbable because β-C is a poorer hydrogen or electron donor than VE.[27] However, a recent paper[14] reported that retinal and a number of β-apo-carotenals were the major fragmentation products of the autoxidation of β-C. Since retinal and other carotenals possess an abstractable aldehyde hydrogen, we answer these aldehydes may contribute to the antioxidant synergism with VE.

Role of retinal and/or other β-apo-carotenals on the antioxidation

An oxygen uptake experiment was carried out with retinal alone and with VE-retinal in combination.

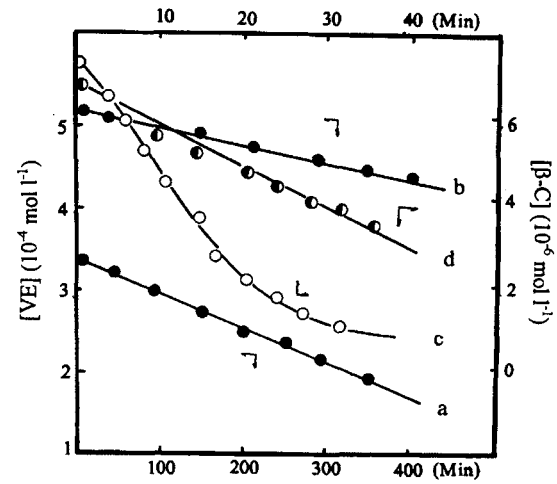

Figure 3. Kinetic decay of VE and β-C during the antioxidation reaction against the AIBN-initiated peroxidation of linoleic acid in *tert*-butyl alcohol at 50 °C. $[LH]_0 = 11{\cdot}8$ mmol l^{-1}, $[AIBN]_0 = 17{\cdot}4$ mmol l^{-1}. (a) Decay of VE ($3{\cdot}4 \times 10^{-4}$ mol l^{-1}) when it was used alone; (b) decay of VE ($5{\cdot}13 \times 10^{-4}$ mol l^{-1}) in the presence of 6·91 μmol l^{-1} of β-C; (c) Decay of β-C (7·56 μmol l^{-1}) when it was used alone; (d) decay of β-C (6·91 μmol l^{-1}) in the presence of $5{\cdot}13 \times 10^{-4}$ mol l^{-1} of VE

Retinal (RCHO) alone did not show an appreciable induction period against linoleic acid peroxidation but slowed the rate of oxygen uptake [Figure 4(b)]. On the other hand, it works synergistically with VE to suppress the oxygen absorption very well and the more RCHO used, the longer was the induction period produced

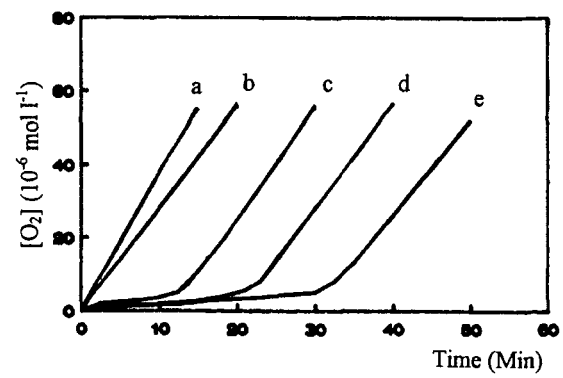

Figure 4. Oxygen uptake during the peroxidation of linoleic acid in *tert*-butyl alcohol at 50 °C initiated with AIBN. $[LH]_0 = 19{\cdot}3$ mmol l^{-1}, $[AIBN]_0 = 6{\cdot}09$ mmol l^{-1}. (a) Uninhibited reaction; (b) inhibited with RCHO (10·5 μmol l^{-1}); (c) inhibited with VE (6·96 μmmol l^{-1}); (d) inhibited with VE (6·96 μmol l^{-1}) and RCHO (10·5 μmol l^{-1}); (e) inhibited with VE (6·96 μmol l^{-1}) and RCHO (21·0 μmol l^{-1})

[Figure 4(d) and (e)]. The experimental results are given in Table 2. It is immediately seen that the effective n value of VE is dependent on the concentration of RCHO, while that one of RCHO is independent of the concentration of RCHO and much smaller than that of β-C (compare with the data in Table 1).

It is known that the bond dissociation energy (BDE) of VE is significantly lower than that of phenol owing to the steric hindrance of the *ortho*-methyls and the conjugation of the lone-pair electrons on the *para*-oxygen atom with the unpaired electron in the phenoxyl *oxygen.*[28] The reported BDE values of VE range from 76·0[29] to 80·4 kcal mol^{-1} (1 kcal = 4·184 kJ).[28] The BDE of retinal is not available in the literature. However, the C_{15} hydrogen of retinyl acetate was reported to be very active and the BDE of this C_{15}—H bond was estimated to be 65·9 kcal mol^{-1}.[30] Since the bonding of a hydrogen to a carbonyl carbon is significantly more weakened than that to an ester group,[31] it is reasonable to assume that the BDE of the C_{15}—H bond in retinal should be lower than that in retinyl acetate. In other words, the BDE of retinal should be lower than that of VE by at least *ca* 10–15 kcal mol^{-1}. This would make the regeneration of VE radical by retinal (RCHO) energetically feasible:

$$VE^{\cdot} + RCHO \rightarrow VE + R\dot{C}{=}O \qquad (19)$$

Although the retinoyl radical thus formed can abstract hydrogens from linoleic acid to continue the chain reaction that may explain the inefficiency of retinal alone against peroxidation, a diversity of termination processes may compete with the chain-carrying reaction and thus slow the rate of propagation. Indeed, the propagation rate which can be determined from the slope of the oxygen uptake line decreased in the presence of retinal [see Figure 4(b) and Table 2]. Termination reactions of retinoyl radical may involve decarbonylation, which produces a resonance-stabilized carbon-centred radical R$^{\cdot}$, which would able to trap a peroxyl radical similar to the LOO—β-C$^{\cdot}$ radical [equation (16)], and the reaction with oxygen to produce an acyl peroxyl radical, which would react with another molecule of retinal to form retinoic acid or with the R$^{\cdot}$ radical to terminate the chain as shown below:

$$R^{\cdot} + LOO^{\cdot} \rightarrow \text{molecular products} \qquad (20)$$

$$R\dot{C}{=}O + O_2 \rightarrow RCO{-}OO^{\cdot} \qquad (21)$$

$$RCO{-}OO^{\cdot} + RCHO \rightarrow RCOOH + RCOO^{\cdot} \qquad (22)$$

$$RCOO^{\cdot} + R^{\cdot} \rightarrow \text{molecular products} \qquad (23)$$

All of these termination reactions would compete with the chain-carrying reaction which, in turn, should slow the propagation step as we have observed, and in the presence of VE make the VE regeneration reaction (19) operative. The proportionality between the induction period and the concentration of retinal in the synergistic antioxidation reaction of VE and retinal indicates that this must be the case. The effective n value of 1·2 for retinal, which is significantly smaller than 2, is indicative of the competition of the VE regeneration reaction with the termination reactions. Other β-apo-carotenals may act in a similar way to take part in the synergistic antioxidant reaction with VE. Because one β-C molecule may fragment into more than two β-apo-carotenals, the effective n value of β-C is much larger than 2 in either the absence or presence of VE (see above).

The regeneration of VE by retinal [equation (19)] is also supported by the decay kinetics of VE during the inhibition of LH autoxidation, which was monitored by UV spectroscopy. As shown in Figure 5, VE exhibits a linear decay, as expected, when it was used alone [Figure 5(a)]. The decay rate was slowed in the presence of either β-C [Figure 5(b)] or retinal [Figure 5(c)], with the former being slightly more effective. It is interested that after *ca* 200 min the decay rate of VE in the presence of retinal turned to its intrinsic rate, i.e. the rate in the absence of retinal. This is similar to what we observed in the oxygen uptake experiments, in which the oxygen uptake recovered to the original rate when the antioxidant was exhausted. Obviously, retinal has regenerated VE to slow its depletion before all of the retinal was consumed.

Table 2. Inhibition of peroxidation of linoleic acid by VE and RCHO[a]

$[AH]_0$ (μmol l^{-1})							
VE	RCHO	R_i (10^{-8} mol l^{-1} s^{-1})	R_p (10^{-8} mol l^{-1} s^{-1})	$k_p/(2k_t)^{1/2}$ [10^{-2} l$^{1/2}$ (mol s)$^{-1/2}$]	t_{inh} (s)	k_{inh} (10^5 l mol^{-1} s^{-1})	n
6·96		2·25[b]	5·44[c]	1·94	660	20·5	2·0
	10·5	2·25			0		
6·96	10·5	2·25	4·66	1·45	1260	1·77	3·8 (VE)[d] 1·2 (RCHO)[d]
6·96	21·0	2·25	4·33	1·33	1860	1·14	5·6 (VE)[d] 1·2 (RCHO)[d]

[a–d] See footnotes in Table 1.

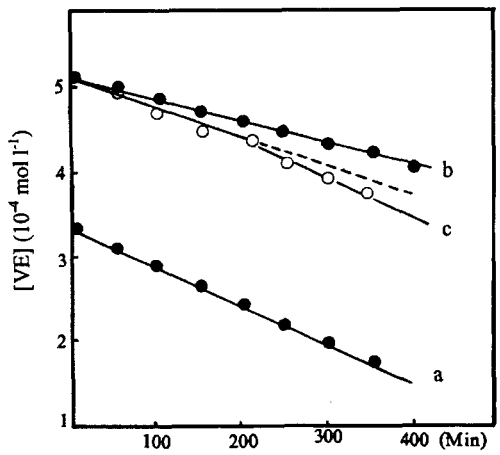

Figure 5. Kinetic decay of VE during the antioxidation reaction against the AIBN-initiated peroxidation of linoleic acid in *tert*-butyl alcohol at 50 °C. (a) Decay of VE when it was used alone ($[VE]_0 = 3{\cdot}4 \times 10^{-4}$ mol l^{-1}); (b) decay of VE in the presence of 6·9 μmol l^{-1}) of β-C ($[VE]_0 = 5{\cdot}13 \times 10^{-4}$ mol l^{-1}); (c) Decay of VE in the presence of 44·5 μmol l^{-1} of RCHO ($[VE]_0 = 5{\cdot}13 \times 10^{-4}$ mol l^{-1})

CONCLUSION

VE can work synergistically with β-C or RCHO to inhibit the peroxidation of linoleic acid initiated by thermal decomposition of AIBN in *tert*-butyl alcohol and, in the process, VE and β-C exhibit a mutual protection effect. This novel phenomenon may be rationalized by the coexistence of the following reactions:

1. antioxidation of β-C due to the formation of a resonance-stabilized carbon-centred radical LOO—β-C· [equation (16)], as proposed by Burton and Ingold;[10]
2. autoxidation of β-C [equation (18)], which would eventually fragment the molecule into retinal and other smaller β-apo-retinals;[14]
3. protection of β-C by VE against the autoxidation of the former as proposed previously;[10,11,20] this may explain the diminished consumption of β-C in the presence of VE;
4. recycling VE by retinal and other β-apo-carotenals [equation (19)]. This may explain the diminished consumption of VE in the presence of β-C.

Although further work will be required to elucidate quantitatively the relative contributions of these reactions, which may be influenced by a diversity of factors such as the partial oxygen pressure,[10,11] the type of radical initiator, the microenvironment of the reaction media and other interfering radicals or molecules, our results leave no doubt about the antioxidant synergism and the mutual protection of VE and β-C and provides a basis for further study.

EXPERIMENTAL

Linoleic acid (Fluka, chromatographically pure), *d,l*-α-tocopherol (Merck, biochemical reagent) and all-*trans*-retinal (Sigma) were used as received and kept under nitrogen in a refrigerator before use. β-*carotene* (Merck, biochemical reagent) was purified before use by column chromatography on neutral alumina with carbon tetrachloride as the eluent. Azo-bis(isobutyronitrile) (Shanghai 4th Chemicals) was recrystallized from absolute ethanol before use.

The rate of oxygen uptake was measured with an SP-2 oxygen uptake apparatus equipped with an oxygen electrode which was able to record oxygen concentrations as low as 10^{-8} mol l^{-1}. All experiments were performed at 50 ± 0·1 °C and every experiment was repeated at least three times to ensure the experimental error was within ±10%. Experimental details have been described previously.[32]

A Hitachi model 557 UV spectrophotometer was used to determine the concentrations of VE and β-C during their antioxidation reactions against the AIBN-initiated peroxidation of linoleic acid in *tert*-butyl alcohol at 50 ± 0·1 °C. The absorption maxima for VE and β-C were 292 and 450 nm with molar absorption of $3{\cdot}12 \times 10^3$ and $1{\cdot}52 \times 10^5$ l mol $^{-1}$ cm $^{-1}$, respectively. The spectra were recorded at regular time intervals to monitor the decay kinetics of the antioxidants.

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