Antioxidant synergism of tea polyphenols and α-tocopherol against free radical induced peroxidation of linoleic acid in solution

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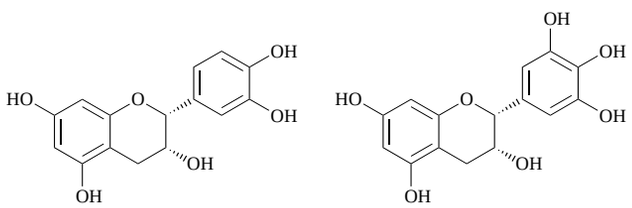
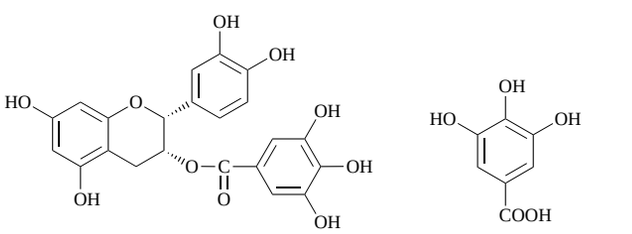
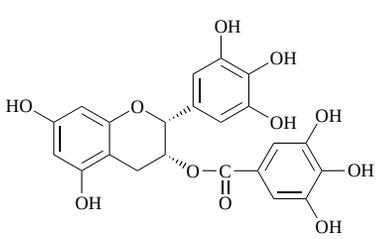
Peroxidation of linoleic acid was initiated by a water soluble azo initiator 2,2′-azo(2-amidinopropane)-dihydrochloride (AAPH) in *tert*-butyl alcohol–water solution and inhibited by α-tocopherol and polyphenols extracted from green tea, *i.e.* (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin gallate (EGCG) and gallic acid (GA), either alone or in combination. The reaction kinetics were followed by oxygen uptake, formation of linoleic acid hydroperoxides and consumption of the antioxidants. It was found that the tea polyphenols could slow the rate of peroxidation with an activity sequence of EGCG > EGC ~ ECG > EC ~ GA, but no definite inhibition period was observed. On the other hand, the tea polyphenols could significantly increase the inhibition period of α-tocopherol and protect the latter from depletion with an activity sequence of EGCG ⩾ ECG ~ EGC > GA > EC when they were used in combination. A synergistic antioxidation mechanism involving the recycling of α-tocopherol by the tea polyphenol is proposed.

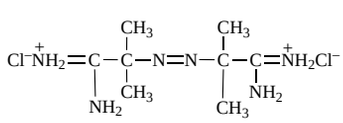
Introduction

The recent development of free radical biology and medicine has provided a large body of evidence that free radical mediated peroxidation of membrane lipids is associated with a wide variety of chronic health problems, such as cancer, atherosclerosis and ageing, and that antioxidants, such as α-tocopherol (vitamin E), L-ascorbic acid (vitamin C) and β-carotene may have beneficial effects in protecting against these diseases.[1–4] Several recent studies have shown that some polyphenols extracted from green tea leaves exhibit significant inhibition effects against lipid peroxidation in phospholipid bilayers and in animal model systems[5–8] and against tumourigenesis.[9] However, the specific mode of inhibition of lipid peroxidation by the polyphenols is still not clear.[5] It was reported that a mixture of vitamin E, vitamin C, β-carotene and glutathione exhibited remarkably enhanced anticarcinogenic activity compared to the individual components in experimental animals.[10] This fact, coupled with the well known antioxidant synergism of vitamin E and vitamin C,[11–12] and our recent finding of antioxidant synergism and mutual protective effects of vitamin E and β-carotene,[13–14] motivated us to study the chemical antioxidation mechanism of tea polyphenols and their interaction with vitamin E. In this paper, we report kinetic studies on the antioxidative effect of the major components of green tea polyphenols, *i.e.* (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin gallate (EGCG) and gallic acid (GA), and their interaction with α-tocopherol.









Results and discussion

Oxygen absorption kinetics

Linoleic acid (LH) was dissolved in *tert*-butyl alcohol–water (3 : 2 v/v) mixed solvent and kept at constant temperature under atmospheric oxygen, then water-soluble azo initiator 2,2′-azo(2-amidinopropane)dihydrochloride (AAPH) was added to initiate the peroxidation which led to rapid oxygen absorption as shown in Fig. 1(*a*). The oxygen absorption was inhibited by addition of α-tocopherol (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. 2(*b*)]. Addition of EC, EGC, ECG, EGCG or GA decreased the oxygen absorption rate, but no inhibition period was observed [Fig. 1(*b*)–(*f*)]. The decrease of the oxygen absorption rate followed the sequence of EGCG > EGC ~ ECG ⩾ EC ~ GA.

On the other hand, the inhibition period of α-tocopherol increased significantly when the tea polyphenol was added together with α-tocopherol, demonstrating a synergistic anti-

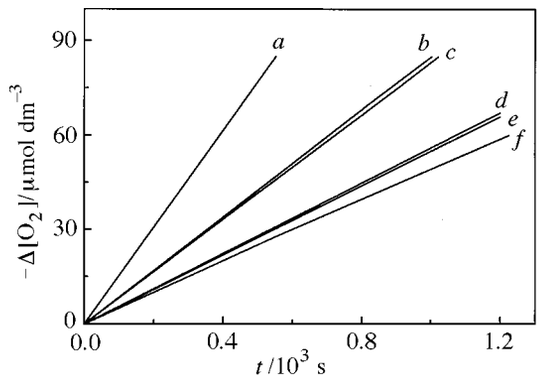


Fig. 1 Oxygen uptake recorded during the peroxidation of LH in *tert*-butyl alcohol–water (3:2 v/v) at 37 °C, initiated with AAPH and inhibited with ArOH. $[LH]_0 = 0.1$ mol dm^{-3}; $[AAPH]_0 = 10$ mmol dm^{-3}; $[ArOH]_0 = 15$ μmol dm^{-3}. (*a*) Uninhibited reaction; (*b*) inhibited with GA; (*c*) inhibited with EC; (*d*) inhibited with ECG; (*e*) inhibited with EGC; (*f*) inhibited with EGCG.

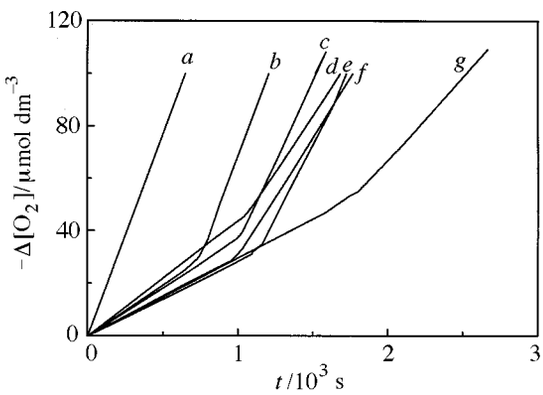


Fig. 2 Oxygen uptake recorded during the peroxidation of LH in *tert*-butyl alcohol–water (3:2 v/v) at 37 °C, initiated with AAPH and inhibited with TocH and ArOH. $[LH]_0 = 0.1$ mol dm^{-3}; $[AAPH]_0 = 10$ mmol dm^{-3}; $[TocH]_0 = 6$ μmol dm^{-3}; $[ArOH]_0 = 15$ μmol dm^{-3}. (*a*) Uninhibited reaction; (*b*) inhibited with TocH; (*c*) inhibited with TocH and EC; (*d*) inhibited with TocH and ECG; (*e*) inhibited with TocH and EGC; (*f*) inhibited with TocH and GA; (*g*) inhibited with TocH and EGCG.

oxidation effect (Fig. 2). The increase of the inhibition period followed the sequence of EGCG ⩾ ECG ~ EGC > GA > EC.

Formation of linoleic acid peroxides

The primary peroxidation products of linoleic acid are hydroperoxides formed by oxygen addition at C-9 or C-13 positions with either *trans*,*cis*- or *trans*,*trans*-diene stereochemistry.[15] They showed characteristic ultraviolet absorption at 235 nm[16] which was used to monitor the formation of the total hydroperoxides during the peroxidation after separation of the reaction mixture by HPLC. A set of representative kinetic curves of the total hydroperoxides formation is shown in Fig. 3 which exhibits the similar kinetic behaviour as that of oxygen absorption shown in Figs. 1 and 2. That is, the concentration of hydroperoxides increased linearly with time in the absence of antioxidants and in the presence of EGCG; the rate of the hydroperoxide formation decreased in the presence of EGCG; the hydroperoxide formation was inhibited in the presence of α-tocopherol; and the inhibition period of α-tocopherol was remarkably increased by addition of EGCG. These results are all in accordance with the kinetic results of oxygen absorption given above and with the kinetic demand of lipid peroxidation (*vide infra*).

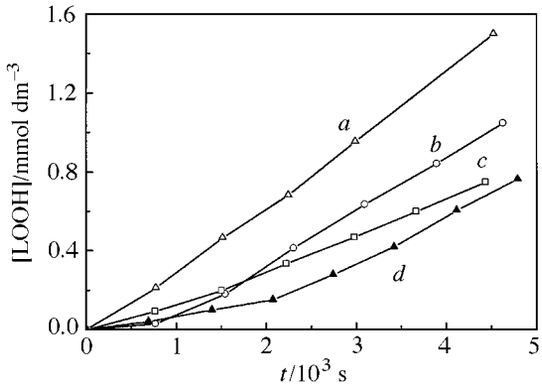


Fig. 3 Formation of total hydroperoxides (LOOH) during the peroxidation of LH in *tert*-butyl alcohol–water (3:2 v/v) at 37 °C, initiated with AAPH and inhibited with TocH and EGCG. $[LH]_0 = 0.1$ mol dm^{-3}; $[AAPH]_0 = 10$ mmol dm^{-3}; $[TocH]_0 = 6$ μmol dm^{-3}; $[EGCG]_0 = 15$ μmol dm^{-3}. (*a*) Uninhibited reaction; (*b*) inhibited with TocH; (*c*) inhibited with EGCG; (*d*) inhibited with TocH and EGCG.

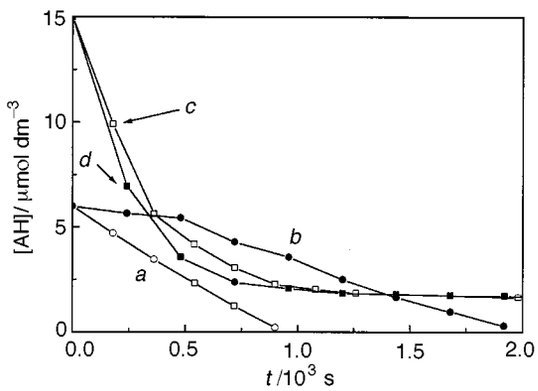


Fig. 4 Consumption of antioxidants (AH) during the antioxidation of LH in *tert*-butyl alcohol–water (3:2 v/v) at 37 °C, initiated with AAPH and inhibited with TocH and EGCG. $[LH]_0 = 0.1$ mol dm^{-3}; $[AAPH]_0 = 10$ mmol dm^{-3}; $[TocH]_0 = 6$ μmol dm^{-3}; $[EGCG]_0 = 15$ μmol dm^{-3}. (*a*) Decay of TocH in the absence of EGCG; (*b*) decay of TocH in the presence of EGCG; (*c*) decay of EGCG in the absence of TocH; (*d*) decay of EGCG in the presence of TocH.

Decay of the antioxidants

In order to rationalize the mechanism of the observed antioxidant synergism the decay kinetics of α-tocopherol and EGCG were studied by HPLC separation of the reaction mixture followed by electrochemical determination of the antioxidants. It was found that α-tocopherol decayed linearly with time, in accordance with the kinetic demand for antioxidation reactions (*vide infra*). The decay rate was 6.4×10^{-9} mol dm^3 s^{-1}. Interestingly, the decay of α-tocopherol was inhibited in the presence of EGCG. In the initial stage the decay was almost completely inhibited with a very small rate (1.1×10^{-9} mol dm^3 s^{-1}). After most EGCG had been consumed the rate increased moderately (5.5×10^{-9} mol dm^3 s^{-1}), but it was still less than the rate in the absence of EGCG. On the other hand, the decay of EGCG was exponential and was not significantly affected by the co-existent α-tocopherol (Fig. 4).

Kinetics and mechanism

The peroxidation of linoleic acid (LH) thermally initiated by azo-compounds (R–N=N–R) can be represented by the following simplified scheme [eqns. (1)–(3)] where k_d, k_p and k_t are rate constants for decomposition of the initiator, for chain propagation and for chain termination, respectively; *e* designates the fraction of the initiator which is effective in initiating the peroxidation due to the cage effect. Based on the steady-state

Initiation:

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

$$R^{\bullet} + LH \longrightarrow RH + L^{\bullet} \quad (2)$$

Propagation:

$$L^{\bullet} + O_2 \longrightarrow LOO^{\bullet} \quad (3)$$

$$LOO^{\bullet} + LH \xrightarrow{k_p} L^{\bullet} + LOOH \quad (4)$$

Termination:

$$LOO^{\bullet} + LOO^{\bullet} \xrightarrow{2k_t} \text{molecular products} \quad (5)$$

kinetic treatment, the rate of oxygen uptake can be expressed as eqn. (6), where $k_p/(2\ k_t)^{\frac{1}{2}}$ is referred to as the oxidizability of the

$$-d[O_2]/dt = R_p = [k_p/(2k_t)^{\frac{1}{2}}]R_i^{\frac{1}{2}}[LH] \quad (6)$$

substrate, representing the susceptibility of the lipid to undergo peroxidation, and the apparent rate of the chain initiation is given by eqn. (7).

$$R_i = 2k_d e[R{-}N{=}N{-}R] \quad (7)$$

In the presence of an antioxidant molecule (AH) the peroxyl radical can be trapped and a new antioxidant radical ($A^{\bullet}$) produced [eqn. (8)]. If the $A^{\bullet}$ is a stabilized radical (*e.g.* α-tocopheroloxyl radical or ascorbate radical anion) which can promote the rate-limiting hydrogen abstraction reaction [eqn. (8)] and undergo a fast termination reaction [eqn. (9)], the peroxidation would be inhibited.

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

$$LOO^{\bullet} + A^{\bullet} \xrightarrow{\text{fast}} LOOA \quad (9)$$

Table 1 Inhibition of AAPH initiated peroxidation of linoleic acid by tea polyphenols (ArOH)[a]

ArOH	$R_p/10^{-8}$ mol dm^{-3} s^{-1}	$R_{inh}/10^{-8}$ mol dm^{-3} s^{-1}	$k_{cl,p}$	$k_{cl,inh}$	$[k_p/(2k_t)^{\frac{1}{2}}]/10^{-2}$ dm$^{\frac{3}{2}}$ (mol s)$^{-\frac{1}{2}}$
None	14.3		8.7		1.1
EGCG		5.0		3.0	0.39[b]
EGC		5.5		3.3	0.45[b]
ECG		5.6		3.4	0.43[b]
EC		8.3		5.0	0.64[b]
GA		8.5		5.1	0.66[b]

[a] In *tert*-butyl alcohol–water (3 : 2 v/v) mixed solvent at 37 °C initiated with 10 mmol dm^{-3} AAPH. The initial concentration of linoleic acid and tea polyphenols was 0.1 mol dm^{-3} and 15 μmol dm^{-3} respectively. Data are the average of three measurements with deviation within ±10%. [b] Apparent oxidizability, see text.

During the inhibition period the rate of peroxyl formation by initiation equals the rate of peroxyls trapped, therefore eqns. (10) and (11) hold, where n is the stoichiometric factor which

$$R_{inh} = k_{inh}n[AH][LOO^{\bullet}] \quad (10)$$

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

designates the number of peroxyl radicals trapped by each antioxidant molecule and is given by eqn. (12).

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

From eqns. (8)–(11) we have eqn. (13).

$$-d[AH]/dt = R_i/n \quad (13)$$

The n value of α-tocopherol is generally assumed to be 2;[17–18] thus R_i 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 eqn. (14).

$$-d[O_2]/dt = R_{inh} = k_p R_i[LH]/(nk_{inh}[AH]) \quad (14)$$

The kinetic chain length defines the number of chain propagation initiated by each initiating radical and is given by eqns. (15) and (16) for inhibited and uninhibited peroxidation

$$k_{cl,inh} = R_{inh}/R_i \quad (15)$$

$$k_{cl,p} = R_p/R_i \quad (16)$$

respectively. The kinetic data obtained from Figs. 1 and 2 are listed in Tables 1 and 2, respectively.

It can be seen from Fig. 2 and Table 2 that α-tocopherol is a well-behaved antioxidant which shows a clear inhibition period during which the rate of chain propagation and the kinetic chain length are significantly decreased. The k_{inh} value (3.0×10^5 dm^3 mol^{-1} s^{-1}) was calculated from eqn. (14) by taking $k_p = 100$ dm^3 mol^{-1} s^{-1} at 37 °C.[19] This value is somewhat smaller than that in *tert*-butyl alcohol solvent reported previously (5.1×10^5 dm^3 mol^{-1} s^{-1}).[19] This reduction of k_{inh} is to be expected since the stronger hydrogen-bonding ability of water with peroxyl radicals would make the antioxidation reaction [eqn. (8)] slower (compare also $k_{inh} = 2 \times 10^5$ dm^3 mol^{-1} s^{-1} in 85% ethanol[20]). The oxidizability of linoleic acid [0.011 (mol s)$^{-\frac{1}{2}}$ dm$^{\frac{3}{2}}$] in the present solvent is also smaller than in other solvents, *e.g.* 0.029 (mol s)$^{-\frac{1}{2}}$ dm$^{\frac{3}{2}}$ in *tert*-butyl alcohol,[18] probably also due to the stronger hydrogen-bonding ability of the present solvent.

Addition of the tea polyphenol (ArOH) appreciably decreased the oxygen absorption rate (R_{inh}) and increased the kinetic chain length ($k_{cl,inh}$). This is understood because the tea

Table 2 Inhibition of AAPH initiated peroxidation of linoleic acid by α-tocopherol (TocH) and tea polyphenols[a,b]

Antioxidant	$R_p/10^{-8}$ mol dm^{-3} s^{-1}	$R_{inh}/10^{-8}$ mol dm^{-3} s^{-1}	$t_{inh}/10^2$ s	$k_{inh}/10^5$ dm^3 mol^{-1} s^{-1}	$k_{cl,p}$	$k_{cl,inh}$	$[k_p/(2k_t)^{\frac{1}{2}}]/10^{-2}$ dm$^{\frac{3}{2}}$ (mol s)$^{-\frac{1}{2}}$	SE (%)
TocH	14.3	4.7	7.3	3.0	8.6	2.8	1.1	
TocH + EGCG	6.3	3.0	17.4	2.0	3.8	1.8	0.49	138
TocH + EGC	11.0	2.9	10.9	3.1	6.6	1.8	0.85	49.5
TocH + ECG	7.7	4.0	11.0	2.4	4.6	2.4	0.60	50.4
TocH + EC	10.0	6.0	9.9	2.8	6.0	2.2	0.77	34.3
TocH + GA	8.7	3.0	10.4	3.2	5.2	1.8	0.67	42.2

[a] The reaction conditions were the same as described in the footnote [a] of Table 1. The initial concentration of α-tocopherol and the tea polyphenol were 6 μmol dm^{-3} and 15 μmol dm^{-3} respectively. [b] $R_i = 1.64 \times 10^{-8}$ mol dm^{-3} s^{-1} was obtained by using eqn. (12) which is close to the previously reported value of 1.3×10^{-6} [AAPH].[19]

polyphenols contain many phenolic hydroxyl groups which can be hydrogen-abstracted by either the initiating radical R$^{\bullet}$ and/or the propagating radical LOO$^{\bullet}$ as shown in eqns. (17) and (18), decreasing the peroxidation rate of linoleic acid. However,

$$\mathrm{ArOH + R^{\bullet} \longrightarrow ArO^{\bullet} + RH} \quad (17)$$

$$\mathrm{ArOH + LOO^{\bullet} \longrightarrow ArO^{\bullet} + LOOH} \quad (18)$$

since the intermediate tea polyphenol radical (ArO$^{\bullet}$) is not as stable as α-tocopheroloxyl radical, it is also possible to propagate the peroxidation chain by eqn. (19) that makes the tea

$$\mathrm{ArO^{\bullet} + LH \longrightarrow ArOH + L^{\bullet}} \quad (19)$$

polyphenol less efficient than α-tocopherol as an antioxidant. On the other hand, since the oxidation products of tea polyphenols, *e.g.* theaflavins, also possess antioxidant property,[21–22] the decay of tea polyphenols became slower in the later stage of the reaction [Fig. 4(*b*), *vide infra*] and no inhibition period could be observed.

If we use the apparent oxidizability, *i.e.* calculating oxidizability by using the decreased propagation rate in the presence of antioxidants, to represent the susceptibility of lipids in the presence of antioxidants, it is seen from Table 1 that the polyphenols could decrease the susceptibility of linoleic acid due to the competition of reaction (18) with reaction (4). Comparison of the oxygen absorption rate, kinetic chain length and apparent oxidizability of linoleic acid in the presence of the tea polyphenols gives their relative antioxidant efficacy decreasing in the order EGCG > ECG ~ EGC > EC ~ GA. This activity sequence is in accordance with the recent pulse radiolysis and laser photolysis results for reactions of tea polyphenols with azidyl radicals.[23]

Antioxidant synergism of tea polyphenols with α-tocopherol

It can be seen from Fig. 2 and Table 2 that addition of tea polyphenols together with α-tocopherol significantly increases the inhibition period of the latter, decreases the kinetic chain length both in the inhibition period and after the inhibition period, and decreases the oxidizability of linoleic acid. Formation of linoleic acid hydroperoxides (Fig. 3) exhibited similar kinetic behaviour with a similar inhibition period as observed in oxygen absorption experiments. These results indicate a remarkable synergistic effect in the antioxidation process. The synergistic efficiency SE% is given by eqn. (20).[24]

$$\mathrm{SE\%} = [t_{\mathrm{inh}}(\mathrm{TocH + ArOH}) - t_{\mathrm{inh}}(\mathrm{TocH})]/t_{\mathrm{inh}}(\mathrm{TocH}) \times 100\% \quad (20)$$

Accordingly, the synergistic effectiveness of the tea polyphenols can be arranged in the order EGCG ⩾ ECG ~ EGC > GA > EC. This activity sequence is somewhat different from that obtained in the absence of α-tocopherol (*vide supra*) because the former sequence deals with the reaction with alkyl and/or peroxyl radicals [eqns. (17) and (18)], while this sequence deals with the reaction with α-tocopheroloxyl radicals [eqn. (21), *vide infra*].

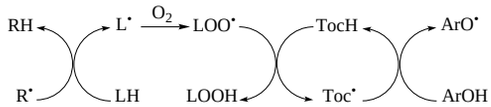
In order to rationalize the mechanism of this antioxidant synergism the decay kinetics of α-tocopherol and EGCG during the antioxidation reactions were studied. As shown in Fig. 4 α-tocopherol decayed linearly with time in the absence of EGCG, with a typical antioxidant decay as defined by eqn. (13). On the other hand, the decay of EGCG was exponential, implying a bimolecular reaction. Interestingly, although the decay rate of EGCG was similar either in the absence or in the presence of α-tocopherol, the decay of α-tocopherol was almost totally suppressed by the coexistent EGCG until most of the EGCG was consumed. These results are similar to the kinetic behaviour observed in the regeneration of α-tocopherol by L-ascorbic acid,[19] and hence strongly suggest that EGCG can reduce α-tocopheroloxyl radical to regenerate the parent molecule [eqn. (21)].

$$\mathrm{Toc^{\bullet} + ArOH \longrightarrow TocH + ArO^{\bullet}} \quad (21)$$

This reaction should be, of course, reversible. However, due to the lower oxidation potential of EGCG (0.43 V *vs.* NHE[23]) than that of α-tocopherol (0.48 V *vs.* NHE[25]), this α-tocopherol-reparative reaction should be a preferable reaction. An alternative mechanism is the electron transfer mechanism proposed recently by Jovanovic *et al*. [eqn. (22)].[23]

$$\mathrm{Toc^{\bullet} + ArOH \underset{k_{-et}}{\overset{k_{et}}{\rightleftharpoons}} Toc^{\bullet -} + ArOH^{\bullet +}} \quad (22)$$

The rate constants k_{et} and k_{-et} determined by radiolysis for the electron transfer reaction between trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a water soluble analogue of α-tocopherol) radical and EGCG are 3.3×10^4 and 3×10^3 dm^3 mol^{-1} s^{-1} respectively,[23] indicating clearly that the forward reaction is much more favourable than the reverse one. Since radical cations are much more acidic than their parent molecules,[26] this electron transfer reaction should be followed by fast deprotonation from the radical cation of EGCG and the overall reaction is the same as shown in eqn. (21). A similar electron transfer–proton tunnelling mechanism has been proposed for the antioxidation and prooxidation reaction of α-tocopherol.[27–28] Because the rate of termination reaction by two α-tocopherol radicals (2×10^3 dm^3 mol^{-1} s^{-1})[29] is over one order of magnitude smaller than the k_{et} in reaction (22) and the concentration of EGCG is much larger than that of α-tocopheroloxyl radical, the α-tocopherol repairing reaction [eqn. (21)] must be the predominant reaction in the presence of EGCG and other tea polyphenols. Therefore, the antioxidant synergistic interaction of α-tocopherol and tea polyphenols may be illustrated by Scheme 1.



Scheme 1

Experimental

Materials

Linoleic acid (Fluka, chromatographic pure) and (±)-α-tocopherol (Merck, biochemical reagent) were used as received and kept under nitrogen in a refrigerator before use. 2,2′-Azo-(2-amidinopropane)dihydrochloride (AAPH) was synthesized according to the available method.[30] (−)-Epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin gallate (EGCG) and gallic acid (GA) were isolated from green tea leaves by extraction with methanol, water and ethyl acetate consecutively and chromatographic separation on a Sephadex LH-20 column, with reference to the procedures reported previously.[31–32] Their structures and purity were checked by ^{1}H and ^{13}C NMR spectra and HPLC, respectively. Other reagents were treated by conventional methods.

Oxygen uptake measurements

The rate of oxygen uptake was measured under air in a closed glass vessel of *ca*. 2 ml in volume, thermostatted at 37 °C and provided with a magnetic stirrer, using an SP-2 oxygen electrode (Shanghai Institute of Phytobiology) which was able to record oxygen concentrations as low as 10^{-8} mol dm^{-3}. Linoleic acid was dissolved in *tert*-butyl alcohol–water (3:2 v/v) mixed

solvent and AAPH in water solution was injected to initiate the peroxidation. In the case of antioxidation experiments the antioxidant was added together with linoleic acid. The initial concentration of AAPH was 10 mmol dm^{-3}. Every experiment was repeated three times and the results were reproducible within 10% experimental error.

Determination of linoleic acid hydroperoxides

Aliquots of the reaction mixture in an open vessel were taken out at appropriate time intervals and subjected to high performance liquid chromatography (HPLC) analysis using a Gilson liquid chromatograph and a Sychropak KPP-100 reversed-phase column (4 × 250 mm) and eluted with methanol–water (95 : 5 v/v). The flow rate was set at 1.0 ml min^{-1}. A Gilson Model 116 UV detector was used to monitor the total linoleic acid hydroperoxides at 235 nm.[16] The concentration of the hydroperoxides was determined by integration of the peak area which was calibrated by iodometric determination of the hydroperoxides using molar extinction coefficient of 2.19×10^4 at 350 nm.[33]

Determination of α-tocopherol and EGCG

The procedure was the same as mentioned above for analysis of hydroperoxides, but eluted with methanol–formic acid (99 : 1 v/v) containing 50 mmol dm^{-3} of sodium perchlorate as supporting electrolyte. A Gilson Model 142 electrochemical detector was used to monitor both α-tocopherol and EGCG simultaneously by setting the oxidation potential at +700 mV.

Conclusion

The principal components of green tea polyphenols, *i.e.* EGCG, EGC, ECG, EC and GA, are effective antioxidants against linoleic acid peroxidation in homogeneous solutions, especially when they are used in combination with α-tocopherol. The synergistic antioxidative effect may be explained by recycling α-tocopherol by the tea polyphenols as illustrated in Scheme 1.

Acknowledgements

We thank the National Natural Science Foundation of China and the State Education Commission of China for financial support.

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Paper 7/06691K
Received 15*th September* 1997
Accepted 28*th November* 1997