Antioxidant activity of green tea polyphenols against lipid peroxidation initiated by lipid-soluble radicals in micelles

Zhi-Hua Chen, Bo Zhou, Li Yang, Long-Min Wu and Zhong-Li Liu*

National Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou, Gansu 730000, China. E-mail: liuzl@lzu.edu.cn; Fax: +86 931 8625657

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Antioxidant effects of the principal polyphenolic components extracted from green tea leaves, *i.e.*, (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epigallocatechin gallate (EGCG), against peroxidation of linoleic acid have been studied in sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide (CTAB) micelles. The peroxidation was initiated thermally by a lipid-soluble azo initiator di-*tert*-butyl hyponitrite (DBHN) and the reaction kinetics were followed by formation of linoleic acid hydroperoxides and consumption of the antioxidant. Kinetic analysis of the antioxidation process demonstrates that these green tea polyphenols are effective antioxidants in micelles used either alone or in combination with α-tocopherol (vitamin E), but their antioxidative behaviour and relative effectiveness are substantially different from those in the water-soluble initiator AMPAD-initiated peroxidations reported previously. The antioxidative action of the green tea polyphenols (GOHs) in DBHN-initiated peroxidation involves trapping the initiating *tert*-butoxyl radical and the propagating linoleic acid peroxyl radicals. However, the GOHs could not participate in the α-tocopherol recycling reaction as in the case of AMPAD-initiated reactions due to their very fast reaction with the initiating *tert*-butoxyl radical.

Introduction

A major development over the past two decades has been the realization that free radical induced lipid peroxidation and DNA damage are associated with a variety of chronic health problems, such as cancer, ageing and atherosclerosis.[1,2] Plant and food derived antioxidants, such as α-tocopherol (vitamin E), L-ascorbic acid (vitamin C), β-carotene and flavonoids, are increasingly found beneficial in protecting against these diseases,[3,4] hence antioxidant therapy[5] has become an attractive therapeutic strategy.

Green tea has been the most popular beverage in China for thousands of years. Polyphenolic compounds extracted from green tea leaves have been reported to be good antioxidants against lipid peroxidation in phospholipid bilayers,[6,7] in low density lipoprotein,[8] in epidermal microsomes,[9] in synaptosomes,[10] and in animal model systems.[11] (−)-Epigallocatechin gallate (EGCG), the most abundant constituent of green tea polyphenols, was reported to be active in preventing cancer[12] and could accelerate the apoptosis (programmed death) of cancer cells, whilst the healthy cells are left unharmed.[13]

We have undertaken kinetic and mechanistic studies on bio-antioxidants with an emphasis on structure–activity and activity–microenvironment relationships.[14–18] We found recently that green tea polyphenols exhibited a synergistic antioxidant effect with α-tocopherol in homogeneous solutions,[19] in micelles[20,21] and in human low density lipoprotein[22] when the peroxidation was initiated by a water-soluble azo initiator 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AMPAD). However, no such synergistic effect was observed in AMPAD-initiated oxidative hemolysis of human red blood cells.[23] It has been recognized that the antioxidant activity depends not only upon the chemical activity of the antioxidant molecule, but also upon the microenvironment of the reaction media and the lipophilicity of the initiator.[18,24–26] Niki[26] has reported that although vitamin C is a very effective antioxidant for lipid peroxidation in liposomes when the peroxidation was initiated in the bulk water phase, it is totally ineffective when the peroxidation was initiated in the lipid phase of the liposome. Therefore, we were motivated to see if the water-soluble green tea polyphenols are still active for lipid peroxidation initiated in the lipid phase and if they can still react synergistically with α-tocopherol. Presented herein is a kinetic and mechanistic study on the antioxidative effect of the principal polyphenolic components extracted from green tea leaves, *i.e.*, (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epigallocatechin gallate (EGCG), against peroxidation of linoleic acid initiated by a lipid-soluble azo initiator di-*tert*-butyl hyponitrite (DBHN) in sodium dodecyl sulfate (SDS) and in cetyltrimethylammonium bromide (CTAB) micelles. The interaction of these green tea polyphenols with α-tocopherol (TOH, vitamin E) was also investigated.

Results and discussion

Inhibition of formation of linoleic acid peroxides by green tea polyphenols

The primary peroxidation products of linoleic acid are hydroperoxides formed by oxygen addition at the C-9 or C-13 position with either *trans*,*cis*- or *trans*,*trans*-diene stereochemistry.[27] They showed a characteristic ultra-violet absorption at 235 nm[28] which was used to monitor the formation of the total hydroperoxides during the peroxidation after separation of the reaction mixture by high performance liquid chromatography (HPLC). A set of representative kinetic curves of the total hydroperoxides formation in SDS and CTAB micelles are shown in Figs. 1 and 2 respectively. It is seen that the concentration of the hydroperoxides increased linearly with time in the absence of antioxidants upon DBHN-initiation, demonstrating fast peroxidation of linoleic acid (line a in Figs. 1 and 2). The slope of this line corresponds to the rate of chain propagation, R_p. The peroxide formation was inhibited remarkably by the addition of α-tocopherol in the so-called

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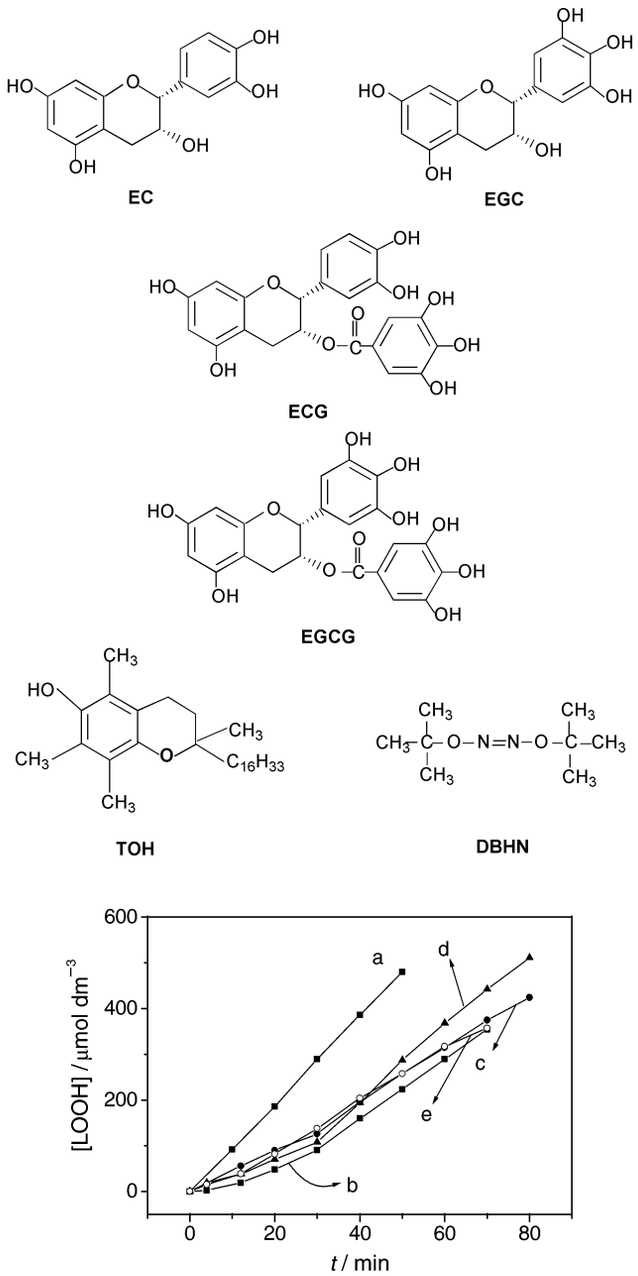


Fig. 1 Formation of total hydroperoxides (LOOH) during linoleic acid (LH) peroxidation in 0.1 mol dm^{-3} SDS micelles at pH 7.4 and 30 °C, initiated with DBHN and inhibited with green tea polyphenols (GOHs). [LH] = 15 mmol dm^{-3}; $[DBHN]_0$ = 10 mmol dm^{-3}; $[GOH]_0$ = 3 μmol dm^{-3}. (a) Uninhibited peroxidation; (b) inhibited with EGCG; (c) inhibited with ECG; (d) inhibited with EGC; (e) inhibited with EC.

'inhibition period', t_{inh}, the rate increased after the antioxidant was exhausted (line b in Figs. 3 and 4). Addition of the green tea polyphenols (GOHs) appreciably inhibited the formation of hydroperoxides in both SDS and CTAB micelles, but the kinetic behaviour is appreciably different from that of the water-soluble initiator AMPAD-initiated peroxidation reported previously.[19,20] In the AMPAD-initiated peroxidation all of these GOHs decreased the rate of propagation, but no inhibition period could be observed in homogeneous solutions (Fig. 1 in our previous paper[19]), while in micelles all of the GOHs produced a clear inhibition period and different GOHs exhibited significantly different activity (Figs. 1 and 2 in our previous paper[20]). In the present case, however, the inhibition period was not distinct and all of the GOHs showed similar activity. In addition, ECG and EC in SDS micelles and ECG in CTAB micelles produced no observable inhibition period, but only decreased the rate of propagation.

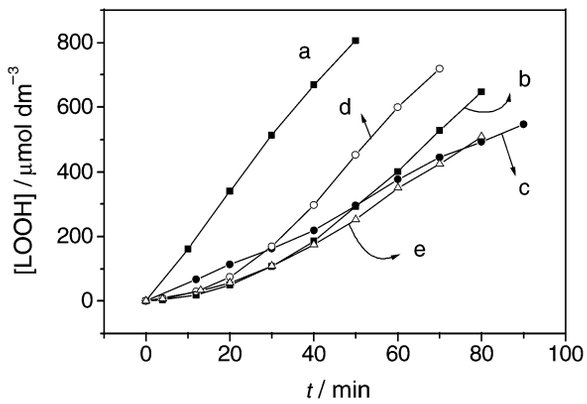


Fig. 2 Formation of total hydroperoxides (LOOH) during linoleic acid (LH) peroxidation in 0.015 mol dm^{-3} CTAB micelles at pH 7.4 and 30 °C, initiated with DBHN and inhibited with green tea polyphenols (GOHs). [LH] = 15 mmol dm^{-3}; $[DBHN]_0$ = 10 mmol dm^{-3}; $[GOH]_0$ = 1 μmol dm^{-3}. (a) Uninhibited peroxidation; (b) inhibited with EGCG; (c) inhibited with ECG; (d) inhibited with EGC; (e) inhibited with EC.

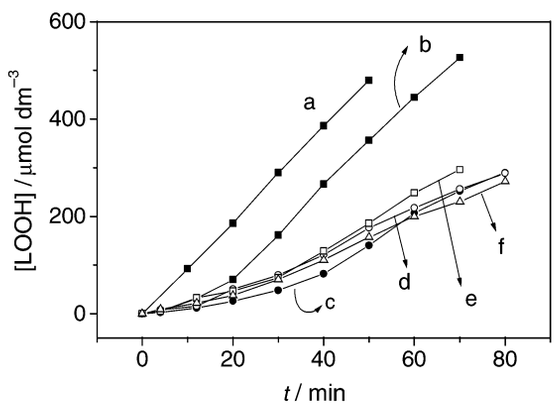


Fig. 3 Formation of total hydroperoxides (LOOH) during linoleic acid (LH) peroxidation in 0.1 mol dm^{-3} SDS micelles at pH 7.4 and 30 °C, initiated with DBHN and inhibited with green tea polyphenols (GOHs) and α-tocopherol (TOH). [LH] = 15 mmol dm^{-3}; $[DBHN]_0$ = 10 mmol dm^{-3}; $[GOH]_0$ = 3 μmol dm^{-3}; $[TOH]_0$ = 3 μmol dm^{-3}. (a) Uninhibited peroxidation; (b) inhibited with TOH; (c) inhibited with TOH + EGCG; (d) inhibited with TOH + ECG; (e) inhibited with TOH + EGC; (f) inhibited with TOH + EC.

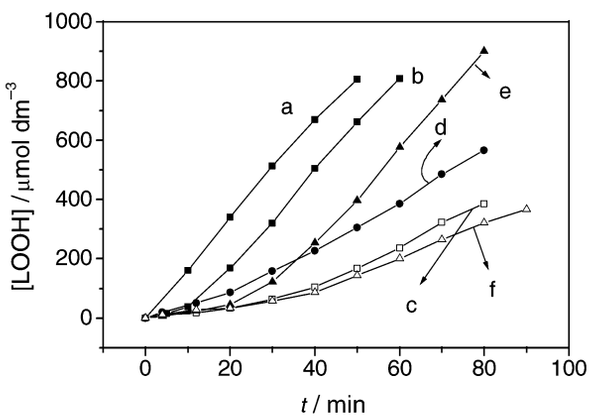


Fig. 4 Formation of total hydroperoxides (LOOH) during linoleic acid (LH) peroxidation in 0.015 mol dm^{-3} CTAB micelles at pH 7.4 and 30 °C, initiated with DBHN and inhibited with green tea polyphenols (GOHs) and α-tocopherol (TOH). [LH] = 15 mmol dm^{-3}; $[DBHN]_0$ = 10 mmol dm^{-3}; $[GOH]_0$ = 1 μmol dm^{-3}; $[TOH]_0$ = 1 μmol dm^{-3} (a) Uninhibited peroxidation; (b) inhibited with TOH; (c) inhibited with TOH + EGCG; (d) inhibited with TOH + ECG; (e) inhibited with TOH + EGC; (f) inhibited with TOH + EC.

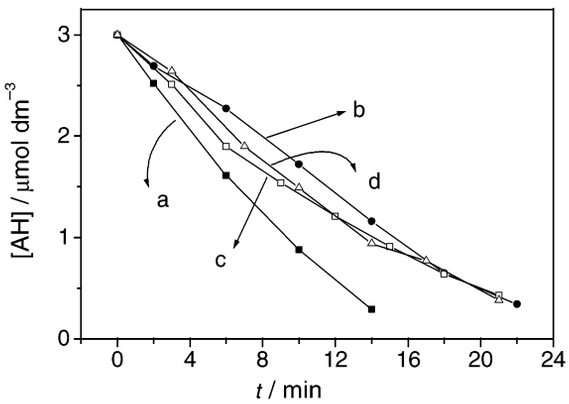


Fig. 5 Consumption of EGCG and TOH during DBHN-initiated linoleic acid peroxidation in SDS micelles. The reaction conditions were the same as described in the legend of Fig. 3. (a) Decay of TOH in the absence of EGCG; (b) decay of TOH in the presence of EGCG; (c) decay of EGCG in the absence of TOH; (d) decay of EGCG in the presence of TOH.

Antioxidant effect of green tea polyphenols in the presence of α-tocopherol

α-Tocopherol is the principal lipid-soluble chain-breaking antioxidant in plasma and erythocytes.[29] It showed typical antioxidative behaviour upon linoleic acid peroxidation in both SDS and CTAB micelles (line b in Figs. 3 and 4). It is seen that the inhibition period of α-tocopherol is substantially longer in CTAB than in SDS micelles, which is in contrast to the behaviour when initiated by AMPAD reported in our previous paper.[20] Addition of the green tea polyphenol together with α-tocopherol prolonged the inhibition period of the latter, but the effectiveness depends significantly on the reaction medium and the polyphenol. EGCG and EGC, which produced an inhibition period when used individually, only showed an additive effect with α-tocopherol, *i.e.*, the inhibition period when the two antioxidants were used in combination was the sum of those when they are used individually, while others which produced no inhibition time when they were used alone showed a remarkable synergistic antioxidant effect, *i.e.*, the inhibition period when the two antioxidants were used in combination was much longer than the sum of when they were used individually (Figs. 3 and 4). This is also different from the reaction initiated by AMPAD.[20]

Consumption of α-tocopherol and EGCG

In order to rationalize the mechanism of the antioxidant synergism of α-tocopherol and EGCG their decay kinetics were studied by HPLC separation of the reaction mixture followed by electrochemical determination of the antioxidants. It was found that α-tocopherol decayed approximately linearly with time in SDS micelles whenever used individually or in the presence of EGCG (lines a and b in Fig. 5), in accordance with the kinetic demand for antioxidation reactions (eqn. (4), *vide infra*). The decay rate of α-tocopherol used individually was 3.0 nmol dm^{-3} s^{-1}, and the rate appreciably decreased to 2.0 nmol dm^{-3} s^{-1} in the presence of EGCG. The decay of EGCG was not changed in the presence of α-tocopherol (lines c and d in Fig. 5).

Kinetics and mechanism

It has been proved that the reaction kinetics of the peroxidation in micelles and biomembranes follow the same rate law as those in homogenous solutions.[25,30] The kinetics of linoleic acid (LH) peroxidation initiated by azo-compounds and its inhibition by a chain-breaking antioxidant (AH) have been discussed in detail in our previous paper.[20] The rate of propagation (R_p) and the rate of peroxide formation in the inhibition period (R_{inh}) are

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

$$R_{inh} = k_p R_i[LH]/(nk_{inh}[AH]) \quad (2)$$

given by eqns. (1) and (2) respectively. Where k_p, k_t and k_{inh} are rate constants for the chain propagation, chain termination and chain inhibition by antioxidants, respectively, and R_i is the apparent rate of chain initiation which can be obtained by measuring the inhibition period or decay of the antioxidant (AH), (eqns. (3) and (4)).[20] Where n is the stoichiometric factor

$$R_i = n[AH]_0/t_{inh} \quad (3)$$

$$R_i = -n d[AH]/dt \quad (4)$$

that designates the number of peroxyl radicals trapped by each antioxidant molecule. Since the n value of α-tocopherol is generally assumed to be 2,[25,30] the R_i value can be determined from the inhibition period or the decay rate of α-tocopherol.

The kinetic chain length (KCL) defines the number of chain propagations initiated by each initiating radical and is given by eqns. (5) and (6) for uninhibited and inhibited peroxidation

$$KCL_p = R_p/R_i \quad (5)$$

$$KCL_{inh} = R_{inh}/R_i \quad (6)$$

respectively. The kinetic parameters deduced from Figs. 1–4 are listed in Tables 1 and 2.

It is seen from Figs. 1 and 2 and Table 1 that these GOHs are good antioxidants which appreciably reduce the rate of propagation and the kinetic chain length, and in the case of EGCG, EGC and/or EC produce an observable inhibition period, demonstrating that GOHs could trap the initiating *tert*-butoxyl radical and/or the propagating linoleic acid peroxyl radical in micelles as discussed previously.[20] The activity sequence for trapping the initiating radical (based on R_p and KCL_p) is EC > ECG > EGCG > EGC in SDS micelles, and ECG > EC > EGCG > EGC in CTAB micelles. The activity sequence for trapping the propagating peroxyl radical (based on R_{inh}, k_{inh} or KCL_{inh}) is EGCG > EGC in SDS micelles and EGCG > EC > EGC in CTAB micelles. Comparison of these data with those reported in our previous papers for the water-soluble initiator AMPAD-initiated reactions[19,20] demonstrates clearly that the character and the lipophilicity of the initiator exert significant effects on the rate of initiation and the antioxidant activity of GOHs.

The decomposition rate constant, k_d, of DBHN was reported to be 3.2×10^{-6} s^{-1},[30] but the apparent initiation rate, R_i, is substantially smaller due to the cage effect which diminishes the effective initiation. The R_i values determined from the inhibition time [eqn. (3)] are 6.7 and 3.3 nmol dm^{-3} s^{-1} for 10 mmol dm^{-3} of DBHN-initiation in SDS and CTAB micelles respectively, which are in good agreement with the values of 6.0 and 3.6 nmol dm^{-3} s^{-1} respectively determined from the decay of α-tocopherol [eqn. (4)] and that reported by Barclay and Ingold.[30] In the present case the R_i value in CTAB micelles is substantially smaller than that in SDS micelles, which is in contrast to the relative sequence for the AMPAD-initiation (3.1 and 8.3 nmol dm^{-3} s^{-1} in SDS and CTAB micelles respectively[20]). This is understood because DBHN is lipid-soluble and bearing no charge, hence its initiation efficiency depends predominantly on the microviscosity of the micelle. The microviscosity in the interior of CTAB micelle is 2.6 times larger than that of SDS micelle,[31] this makes the cage effect larger in CTAB micelles, hence the rate of initiation is smaller in CTAB than in SDS micelles. On the other hand, AMPAD is water-soluble and positively charged, hence it is prone to adsorb on the surface of SDS micelles which in turn, would reduce the effective initiation due to the cage effect.

Table 1 Inhibition of DBHN-initiated peroxidation of linoleic acid by green tea polyphenols (GOH) in micelles[a,b]

Micelle	GOH	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}/ 10^4 dm^3 mol^{-1} s^{-1}	k_{tr}/ 10^8 dm^3 mol^{-1} s^{-1}	n	KCL_p	KCL_{inh}
SDS	None	16.1						24.2	
SDS	EGCG	10.9	4.1	1.4	1.5	2.8	3.2	16.4	6.1
SDS	ECG	9.1				3.9		13.6	
SDS	EGC	13.2	5.6	1.5	1.0	1.6	3.3	19.7	8.3
SDS	EC	8.9				4.0		13.4	
CTAB	None	27.3						81.9	
CTAB	EGCG	18.9	4.1	1.9	1.0	8.8	6.2	56.7	12.3
CTAB	ECG	10.5				16.6		31.5	
CTAB	EGC	23.6	6.1	1.4	1.1	3.7	4.6	78.6	18.2
CTAB	EC	14.0	4.7	1.6	1.1	13.2	5.4	42.0	14.2

[a] The reaction conditions and the initial concentration 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 determinations which were reproducible with deviation less than 10%. [b] Taking R_i as 6.7 and 3.3 nmol dm^{-3} s^{-1} in SDS and CTAB micelles respectively, see text.

Table 2 Inhibition of DBHN-initiated peroxidation of linoleic acid by green tea polyphenols (GOH) and α-tocopherol (TOH) in micelles[a,b]

Micelle	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}/10^4 dm^3 mol^{-1} s^{-1}	n'[c]	KCL_p	KCL_{inh}	SE (%)
SDS	TOH	15.1	4.5	0.9	2.0	2.0	22.6	6.7	
SDS	EGCG + TOH	8.8	2.7	2.0	1.4	2.3	13.2	4.0	~0
SDS	ECG + TOH	7.1	4.0	1.4	1.5	1.6	10.7	6.0	60
SDS	EGC + TOH	9.5	4.2	1.9	1.0	2.1	14.2	6.3	~0
SDS	EC + TOH	6.7	3.1	1.7	1.6	1.9	10.0	4.6	80
CTAB	TOH	27.0	6.3	0.6	2.1	2.0	81.1	18.9	
CTAB	EGCG + TOH	12.3	3.3	2.4	0.9	4.0	37.1	10.0	~0
CTAB	ECG + TOH	14.3	7.1	1.8	0.6	3.0	42.9	21.2	200
CTAB	EGC + TOH	27.2	3.8	1.9	1.1	3.2	81.7	11.4	~0
CTAB	EC + TOH	9.5	3.0	2.3	1.1	3.9	28.4	8.9	~0

[a] The reaction conditions and the initial concentration of the substrates are the same as described in the legends of Figs. 3 and 4 for reactions conducted in SDS and CTAB micelles respectively. Data are the average of three determinations which were reproducible with deviations less than 10%. [b] Taking R_i as 6.7 and 3.3 nmol dm^{-3} s^{-1} in SDS and CTAB micelles respectively, see text. [c] $n' = R_i t_{inh}/([GOH]_0 + [TOH]_0)$.

It is reasonable to assume that the direct trapping of the initiating *tert*-butoxyl radicals by GOHs (eqn. (8)) decreases the rate of initiation (eqn. (7)), which in turn, decreases the rate of propagation. Therefore, the relative activity for the reaction of *tert*-butyoxyl radical towards GOH and linoleic acid (LH) can be calculated from eqn. (9) where ΔR_p is the decrease of R_p in the presence of GOHs. Taking k_i as 1.8×10^5 dm^3 l^{-1} s^{-1},[32] k_{tr} can be estimated and the data are also listed in Table 1.

$$t\text{-BuO}^{\bullet} + \text{LH} \xrightarrow{k_i} t\text{-BuOH} + \text{L}^{\bullet} \quad (7)$$

$$t\text{-BuO}^{\bullet} + \text{GOH} \xrightarrow{k_{tr}} t\text{-BuOH} + \text{GO}^{\bullet} \quad (8)$$

$$k_{tr}/k_i = \Delta R_p[\text{LH}]/\{R_p[\text{GOH}]\} \quad (9)$$

It is seen from Table 1 that k_{tr} ranges from 10^8 to 10^9 dm^3 mol^{-1} s^{-1} which is over 10^4 higher than the rate of chain-breaking reaction, k_{inh}, of GOHs. Therefore, the chain-breaking reaction could not effectively compete with the *t*-BO$^{\bullet}$-trapping reaction, making the inhibition period hardly observable, especially for the most active ECG and/or EC. This result is significantly different from the behaviour of GOHs in the AMPAD-initiated reaction in micelles where all of the GOHs showed clear inhibition period (Figs. 1 and 2 in our previous paper[20]). This comes to the fact that in the AMPAD-initiated reaction the initiating radical is an alkylperoxyl radical (ROO$^{\bullet}$) which possesses much lower activity (*ca.* 10^6 lower[33]) than *t*-BuO$^{\bullet}$, hence GOHs could effectively participate in the chain-breaking reaction, *i.e.*, the reaction with linoleic acid peroxyl radical (LOO$^{\bullet}$).

It is of interest to compare the antioxidant synergism of GOHs with α-tocopherol (TOH) in AMPAD- and DBHN-initiated reactions. It has previously been proved[19–21] that in AMPAD-initiated peroxidations GOHs could reduce α-tocopheroloxyl radicals (TO$^{\bullet}$) to regenerate TOH (eqn. (10)), hence

$$\text{GOH} + \text{TO}^{\bullet} \rightarrow \text{GO}^{\bullet} + \text{TOH} \quad (10)$$

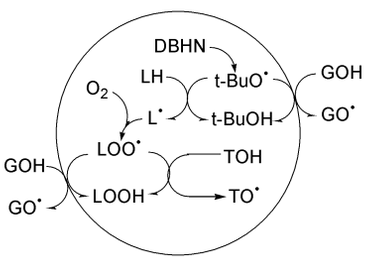
making the inhibition period when using GOH and TOH together longer than the sum of the two inhibition periods when using them individually, in addition the decay of TOH was remarkably inhibited in the presence of GOH and became faster after the GOH was consumed (Fig. 5 in our previous papers[19,20]). The antioxidant synergistic efficiency, SE%, is expressed by eqn. (11). The rate constants for reaction (10) have

$$\text{SE}\% = \{t_{inh}(\text{TOH} + \text{GOH}) - [t_{inh}(\text{TOH}) + t_{inh}(\text{GOH})]\}/[t_{inh}(\text{TOH}) + t_{inh}(\text{GOH})] \times 100 \quad (11)$$

been directly determined in this laboratory by electron paramagnetic resonance spectroscopy (EPR) to be $0.55–1.02 \times 10^3$ dm^3 mol^{-1} s^{-1} in CTAB micelles with EGCG being the most active one.[21] In the present case, however, EGCG did not show any synergistic effect with TOH. It is also seen from Fig. 5 that although the decay of TOH was diminished in the presence of EGCG, it did not become faster after the consumption of EGCG. This result implies that EGCG and TOH might react separately with LOO$^{\bullet}$ and reaction (10) did not take place in the DBHN-initiated reaction. Both α-tocopheroxyl radical and *tert*-butoxyl radical can reside in the interior of the micelles and react with EGCG and other green tea polyphenols at the micelle/water interface, and the steady state concentration of the former is higher than the latter.[21] It seems that α-tocopheroxyl radical would have more chance to react with GOHs. However, the present experimental results demonstrate

that this reaction (eqn. (10), $k \sim 10^3$ dm^3 l^{-1} s^{-1} [21]) might not be able to compete with the extremely fast reaction of GOH with *tert*-butoxyl radicals (eqn. (8), $k \sim 10^8$ dm^3 l^{-1} s^{-1}). The very high SE% values of ECG and/or EC are probably not due to reaction (10), but due to their very fast reaction with the initiating *tert*-butoxyl radical (eqn. (8)) that suppresses the effective initiation and hence prolongs the inhibition time of TOH.

In conclusion, this work demonstrates that the principal components of green tea polyphenols (GOH), *i.e.*, EGCG, EGC, ECG and EC, are effective antioxidants against the lipid-soluble DBHN-initiated linoleic acid peroxidation in SDS and CTAB micelles. However, the antioxidative behaviour is substantially different from that in water-soluble AMPAD-initiated reactions. The principal factor that makes the difference is the remarkably higher activity of the initiating *t*-BuO$^\bullet$ than ROO$^\bullet$ radicals. The antioxidative action of GOHs in DBHN-initiated peroxidation involves trapping the initiating *tert*-butoxyl radical and the propagating linoleic acid peroxyl radicals as shown



Scheme 1

in Scheme 1. It is worth noting that in the present case no α-tocopherol recycling reaction (eqn. (10)) takes place as in the case of AMPAD-initiated reactions.

Experimental

Materials

(−)-Epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epigallocatechin gallate (EGCG) 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 procedures reported previously.[34,35] Their structures and purity were confirmed by ^{1}H and ^{13}C NMR spectra and HPLC, respectively, as reported previously.[36] Linoleic acid (Sigma, Chromatographic pure) and *dl*-α-tocopherol (Merck, Biochemical reagent, >99.9%) were used as received and kept under nitrogen in a refrigerator before use. Di-*tert*-butyl hyponitrite (DBHN) was synthesised according to the available method.[37] The surfactants SDS and CTAB were recrystallized from ethyl alcohol and acetone–water (9 : 1 v/v) respectively.

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 chomatography (HPLC) analysis using a Gilson liquid chromatograph with a ZORBAX ODS reversed phase column (6 × 250 mm, Du Pont Instruments) and eluted with methanol–propan-2-ol (3 : 1 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.[28] Every experiment was repeated at least three times to ensure the experimental deviation within ±10%.

Determination of α-tocopherol and EGCG

The procedure was the same as described above for the analysis of linoleic acid hydroperoxides, except that a Gilson model 142 electrochemical detector set at +700 mV *vs.* SCE was used for simultaneous monitoring of both TOH and EGCG. The column was eluted with methanol–propan-2-ol-formic acid (80 : 20 : 1, v/v/v) containing 50 mmol dm^{-3} of sodium perchlorate as a supporting electrolyte.

Acknowledgements

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