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Bioantioxidants: From chemistry to biology*

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Abstract: Bioantioxidants, or dietary antioxidants, are dietary substances in food that significantly decrease the adverse effects of reactive oxygen species (ROS) on normal physiological functions in humans. Inhibition of ROS-induced oxidative damages by supplementation of bioantioxidants, the so-called "antioxidant therapy", has become an attractive therapeutic strategy to reduce the risk of ROS-related diseases and has led to flourishing research in the past decade. However, many questions dealing with the correlation between chemical and biological activities, the bioavailability and "non-antioxidant" effects of bioantioxidants are still under debate.

This article outlines our current kinetic and mechanistic studies on naturally occurring antioxidants, including vitamin E, green tea polyphenols (GOHs), and resveratrol, as well as their synthetic analogs in micelles, in red blood cells, in low-density lipoprotein (LDL) and in microsomes. The cytotoxicity and apoptosis-inducing activity of these antioxidants against cancer cells were also studied. It was found that there was significant correlation between the chemical and biological antioxidant activities, as well as between the antioxidant activity and the cytotoxic and apoptosis-inducing activities.

Keywords: Peroxidation; antioxidation; vitamin E; green tea polyphenols; resveratrol; apoptosis.

INTRODUCTION

The birth of free radical biology may be traced back to the mid-1950s when Gerschman [1] proposed that "oxygen poisoning and radiation injury have at least one common basis of action, possibly the formation of oxidizing free radicals". Two years later, Harman [2] articulated a "free radical theory" of ageing, speculating that endogenous generation of oxygen radicals in cells increases with increasing metabolic activity and that ageing and degenerative diseases may be associated with free radical-induced accumulative damage on cellular constituents. A decade late, Fridovich [3] provided mechanistic support for Harman's hypothesis by identification of superoxide dismutase (SOD), an enzyme capable of destroying the superoxide radical anion. In the mid-1970s, Pryor [4] pointed out that free radicals could be produced within biological systems and might play a significant role in cellular pathology. In the mid-1980s, Sies [5] described the physiological state associated with increased production of reactive oxygen species (ROS) as a state of "oxidative stress". Keith Ingold [6], Diplock [7], and Halliwell [8] were the primary investigators dealing with chemical and biological aspects of bioantioxidants. In a landmark article in 1977, Engel [9] suggested that the challenge for biomedicine was to find a new model for understanding the origin of disease. It was also proposed that oxidants and an-

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tioxidants might play a significant role in shaping our future thoughts about medical therapy [10]. These led to the suggestion of using "antioxidant therapy" as a new medicinal strategy [11]. In the last year of the last century, the Chemoprevention Working Group to the American Association for Cancer Research [12] published its report "Prevention of cancer in the next millennium", demonstrating that chemoprevention by using antioxidants has become a viable alternative means in cancer control.

It is now well recognized that free radicals play a significant role in the regulation of cell function and tissue viability [13]. Free radicals are generated as a result of normal intracellular metabolism in mitochondria and peroxisomes, as well as from a variety of cytosolic enzyme systems, such as lipoxygenase, NADPH oxidase, and cytochrome P450. In addition, a number of external agents, such as UV light irradiation, ionizing radiation, inflammatory cytokines, and environmental toxins, can trigger free radical production. Free radicals present in living organisms include hydroxyl, superoxide, peroxyl, and nitric oxide. Peroxynitrite, hydrogen peroxide, and singlet oxygen are not free radicals, but can easily convert to free radicals in living organisms. All of these species are called ROS. The nitrogen-containing ROS are also called reactive nitrogen species (RNS). It has also a sophisticated enzymatic and nonenzymatic antioxidant defense system in our body, including SOD, catalase (CAT), and glutathione peroxidase, as well as vitamin E (TOH), vitamin C (VC), and caratenoids, which can trap or cut down the free radical chain reaction to regulate the overall ROS levels to maintain physiological homeostasis. Under normal physiological conditions, ROS generation and quenching by antioxidants keep in balance. But under some pathological conditions, the balance may be destroyed.

Lowering ROS levels below the homeostatic set point may interrupt the physiological role of free radicals in cell proliferation and host defense. However, increased ROS, the so-called "oxidative stress" [5], "an imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage", may also be detrimental and lead to cell death or to an acceleration of ageing and age-related diseases, such as cardiovascular disease, cataracts, cancer, and Alzheimer's disease [16]. Originally, the impairment caused by oxidative stress was thought to result from random damage of lipids, proteins, and DNA. However, recent studies have revealed that oxidative stress may also constitute a stress signal that activates specific redox-sensitive signaling pathways, which in turn, leads to a variety of damages [15].

Therefore, "antioxidant therapy" [11] and "redox therapy" [16] were suggested a decade ago as an attractive new medical strategy. The idea is, whenever the oxidative stress takes place (i.e., more than enough free radicals are produced), people can take bioantioxidants to trap these free radicals and/or regulate the redox status in the cell to maintain good health and prevent ageing. In the beginning of the 21st century, the National Academy of Sciences defined a dietary antioxidant as "a dietary substance in food that significantly decreases the adverse effects of ROS, RNS, or both, on normal physiological functions in humans" [17]. It is obvious that studies on bioantioxidants have become a flourishing research field in the past decade. In particular, much attention has been paid to cancer prevention with antioxidative phytochemicals [12,18], antioxidant nutritional supplements for cancer patients [19], and antioxidant therapy for neurodegenerative diseases [20]. On the other hand, however, there is no doubt that free radical biology and medicine and antioxidant therapy are still in their infancy. Many mechanisms on the action of antioxidants in vitro and in vivo are still unclear, and many questions are still under debate [21].

Our program has focused on kinetic and mechanistic studies on natural antioxidants and their synthetic analogs in chemical and biological systems, with emphasis on the structure/activity relationship and the correlation between their chemical and biological activities. The recent results are outlined in this article.

ANTIOXIDANT EFFECTS OF GREEN TEA POLYPHENOLS IN MICELLES AND IN BIOMEMBRANES

Green tea has been the most popular beverage in China for thousands of years. Drinking tea, especially green tea, is believed to be associated with the lower incidence of human cancer [22]. (–)-Epigallocatechin gallate (EGCG), the most abundant constituent of green tea polyphenols (GOHs), was reported to be active in preventing cancer by inhibiting angiogenesis [23], by inhibiting urokinase activity [24], and by accelerating the apoptosis of cancer cells, whilst the healthy cells are left unharmed [25]. We found recently that GOHs could reverse malignant phenotypic characteristics and induce redifferentiation of human hepatoma cells (SMMC-7721) [26]. The desirable cancer protective or putative therapeutic properties of GOHs have also been considered to depend on their antioxidant properties [18b,27]. Therefore, we isolated from green tea leaves the principal polyphenolic components, i.e., (–)-epicatechin (EC), (–)-epicatechin gallate (ECG), (–)-epigallocatechin (EGC), EGCG, and gallic acid (GA) (Fig. 1), and studied their antioxidative effect against free radical-induced peroxidation in solution [28], in micelles [29–31], in human low-density lipoprotein (LDL) [32], in human red blood cells [33] and their ghosts [34], and in rat liver microsomes [35].

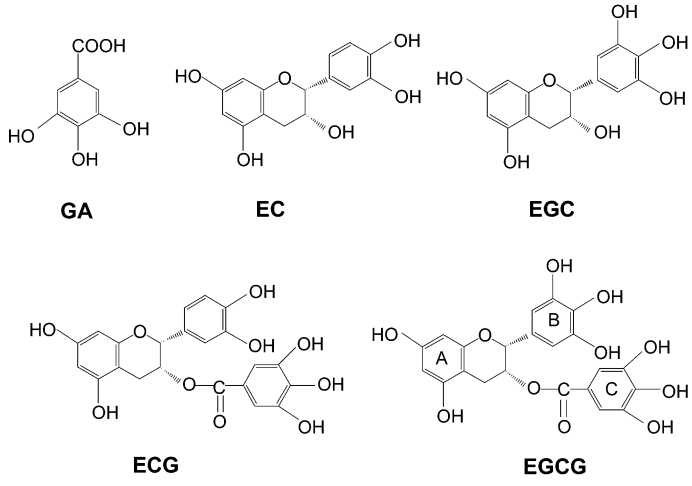


Fig. 1 Principal polyphenols from green tea.

It has been proved that the reaction kinetics of lipid peroxidation in micelles and biomembranes follow the same rate law as that in homogenous solutions [36]. The kinetics of lipid (LH) peroxidation initiated by azo-compounds and its inhibition by a chain-breaking antioxidant (AH) have been discussed in detail previously [28,36], and can be easily determined by monitoring the oxygen uptake and/or the formation of lipid peroxides. The rate of propagation (R_p) and the inhibited rate by an antioxidant in the inhibition period (R_{inh}) are given by eqs. 1 and 2, respectively.

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

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

Here, 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 rate of the antioxidant (AH), (eqs. 3 and 4, respectively).

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

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

Here, t_{inh} and n are the inhibition period and stoichiometric factor, respectively, designating the time interval of the inhibited peroxidation by the antioxidant and the number of peroxyl radicals trapped by each antioxidant molecule, respectively. The antioxidant efficacy can be assessed by comparing the inhibition rate constant, k_{inh}, and/or the inhibition period, t_{inh}.

It was found that all of these GOHs are effective antioxidants in the above-mentioned systems and their antioxidant activity depends not only on the chemical structure and the oxidative potential of the molecule, but also on the microenvironment of the reaction media and the character of the radical initiator [28–35]. For instance, these GOHs could only decrease the rate of initiation in homogeneous solutions (Fig. 2, [28]), while they could produce clear inhibition period in micelles (e.g., Fig. 3 [29]), in human LDL (Fig. 4 [32]), and in human erythrocyte ghosts (Fig. 5 [34]). These facts demonstrate that in homogeneous solution, GOHs can only trap the initiating radicals, while in micelles and biomembranes, they can also trap the propagating lipid peroxyl radicals (LOO•). The chemical activity of these GOHs in micelles correlated well with their in vitro biological activities [29–34].

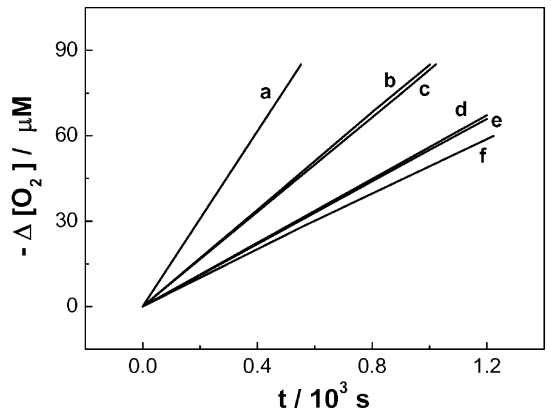


Fig. 2 Oxygen uptake recorded during the AAPH-initiated and GOH-inhibited peroxidation of LH in *tert*-butyl alcohol-water (3:2 v/v) at 37 °C. $[LH]_0$ = 0.1 mM; $[AAPH]_0$ = 10 mM; $[GOH]_0$ = 15 μM. (a) uninhibited peroxidation; inhibited with: (b) GA; (c) EC; (d) ECG; (e) EGC; (f) EGCG [28].

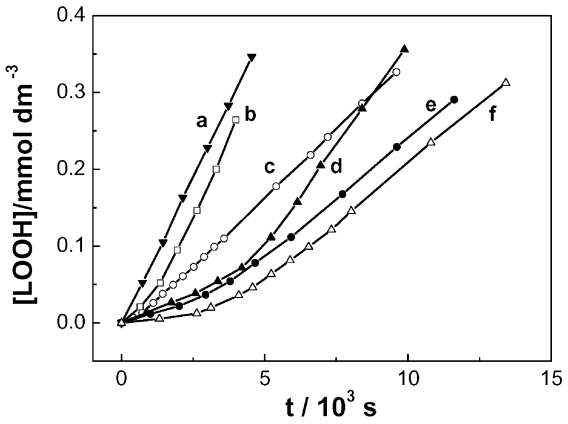


Fig. 3 Formation of LOOH during the AAPH-initiated and GOH-inhibited peroxidation of LH in 0.1 M SDS micelles at pH 7.4 and 37 °C. $[LH]_0$ = 15.2 mM, $[AAPH]_0$ = 6.3 mM, $[GOH]_0$ = 10 μM. (a) uninhibited peroxidation; inhibited with: (b) GA; (c) EC; (d) ECG; (e) EGC; (f) EGCG [29].

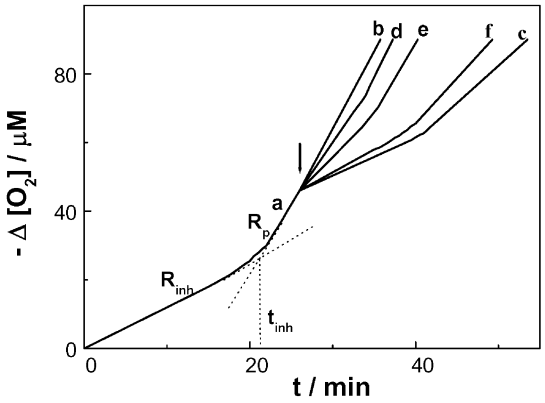


Fig. 4 Oxygen uptake recorded during the AAPH-initiated and GOH-inhibited peroxidation of human LDL in PBS (pH 7.4) at 37 °C under atmospheric oxygen. GOHs were added after the depletion of the endogenous antioxidants present in the native LDL. [LDL] = 0.3 μM; $[AAPH]_0$ = 10.0 mM; [GOH] = 2.0 μM; (a) Native LDL containing 2.3 μM of TOH; inhibited with: (b) GA; (c) EC; (d) EGC; (e) ECG; (f) EGCG [32].

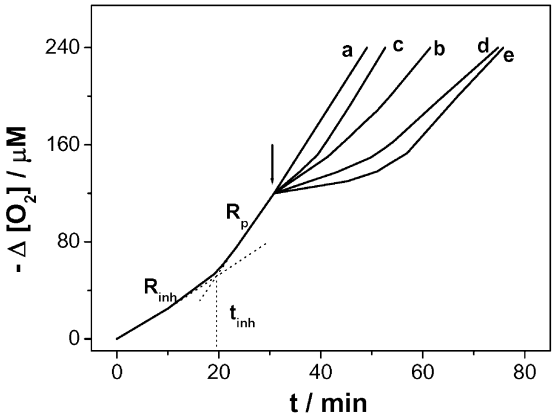


Fig. 5 Oxygen uptake recorded during the AAPH-initiated and GOH-inhibited peroxidation of human erythrocyte ghosts (2.9 mg/ml protein) in PBS (pH 7.4) at 37 °C. GOHs were added after the depletion of the endogenous antioxidants present in the native ghosts. $[AAPH]_0$ = 13.2 mM, [GOH] = 4.2 μM. (a) Native ghost; inhibited with: (b) EGCG; (c) EGC; (d) ECG; (e) EC [34].

ANTIOXIDANT SYNERGISM OF GREEN TEA POLYPHENOLS WITH VITAMIN E AND VITAMIN C

An interesting observation was that these GOHs could remarkably prolong the inhibition period of TOH (e.g., compare Figs. 3 and 6). It is seen from Fig. 6 that when EGCG was added together with TOH, the inhibition period produced was 75 % longer than the sum of the inhibition periods when the two antioxidants were added individually. The consumption of vitamin E during the antioxidation reaction was also inhibited by the coexistent EGCG (Fig. 7). This result coupled with the stopped-flow kinetic EPR study (Fig. 8) proved that this is due to the reduction of vitamin E radical (TO•) by the GOH that regenerates TOH (eq. 5) [37].

$$TO\bullet + GOH \rightarrow TOH + GO\bullet \quad (5)$$

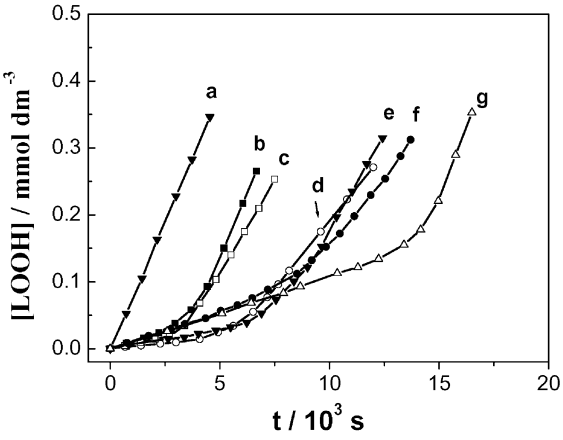


Fig. 6 Formation of LOOH during the AAPH-initiated peroxidation of LH in 0.1 M SDS micelles at pH 7.4 and 37 °C. The peroxidation was inhibited by GOHs and TOH. $[LH]_0$ = 15.2 mM, $[AAPH]_0$ = 6.3 mM, $[GOH]_0$ = 10 μM, $[TOH]_0$ = 6.3 μM. (a) uninhibited peroxidation; inhibited with: (b) TOH; (c) GA + TOH; (d) EC + TOH; (e) ECG + TOH; (f) EGC + TOH; (g) EGCG + TOH [29].

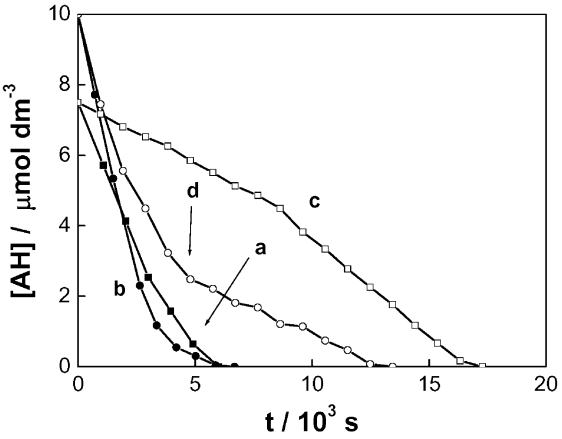


Fig. 7 Consumption of antioxidants (AH) during the peroxidation of linoleic acid in SDS micelles. The reaction conditions were the same as described in the legend of Figs. 3 and 6. (a) decay of TOH in the absence of EGCG; (b) decay of EGCG in the absence of TOH; (c) decay of TOH in the presence of EGCG; (d) decay of EGCG in the presence of TOH [29].

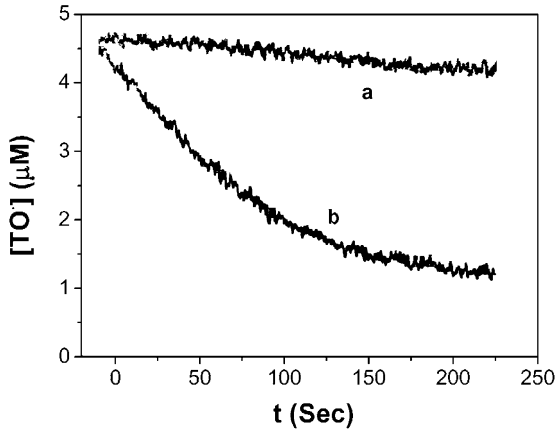


Fig. 8 Decay of TO• in 0.2 M SDS micelles at pH 7.4 and 20 °C under air determined by stopped-flow EPR. $[TOH]_0$ = 2 mM. (a) intrinsic decay; (b) in the presence of 0.1 mM of ECG [37].

Addition of vitamin C (VC) together with TOH and EGCG doubled the inhibition period compared to the inhibition periods of VC, VE, and EGCG when they were used individually (Fig. 9). The decay kinetics of these antioxidants (Fig. 10) demonstrate that the decay of VC was not affected by the coexistent VE and EGCG, while the decay of VE was remarkably suppressed by the coexistent EGCG and further suppressed by the coexistent VC, and that the decay of EGCG was also inhibited by the coexistent VC (unpublished work). Therefore, the antioxidant synergism of GOH, TOH, and VC in micelles can be rationalized by the mechanism outlined in Scheme 1. TOH, being the only lipophilic antioxidant in the system, appears to react firstly with LOO• in the interior of the micelle and form α-tocopheroloxyl radical (TO•). The latter can be reduced by GOH to regenerate TOH, hence prolonging the inhibition period. GOH can also react directly with LOO• in the surface of the micelle. VC can also reduce GO• in the bulk water phase to regenerate GOH. The oxidation potentials of TOH, EGCG, and VC were reported to be 0.26 [38], 0.23 [31], and 0.04 [38] V vs. SCE, respectively, demonstrating that these redox cycles are energetically feasible. The role of the GOHs against the water-soluble free radical initiator, 2,2'-azobis(2-methylpropionamide) dihydrochloride (AAPH), initiated lipid (LH) peroxidation in micelles and biomembranes might involve trapping the initiating radicals (R•) in the bulk water phase, trapping the propagating LOO• in the water-membrane interface and reducing TO• to regenerate vitamin E, as depicted in Scheme 1. All the rate constants in micelles [29,31,37] have been determined as shown in the scheme.

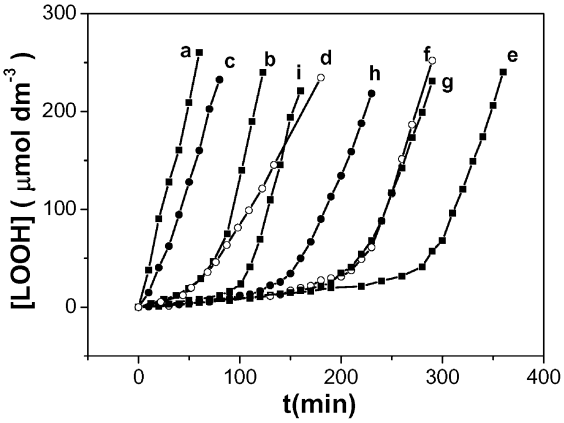


Fig. 9 Formation of LOOH during the AAPH-initiated peroxidation of LH in 0.1 M SDS micelles at pH 7.4 and 37 °C. The peroxidation was inhibited by GOHs, VC, and TOH. $[LH]_0$ = 15.2 mM, $[AAPH]_0$ = 6.3 M, $[GOH]_0$ = 10 μM, $[VC]_0$ = 10 μM, $[TOH]_0$ = 7.5 μM. (a) Uninhibited peroxidation; inhibited with: (b) TOH; (c) VC; (d) EGCG; (e) EGCG + VC + TOH; (f) ECG + VC + TOH; (g) EGC + VC + TOH; (h) EC + VC + TOH; (i) GA + VC + TOH.

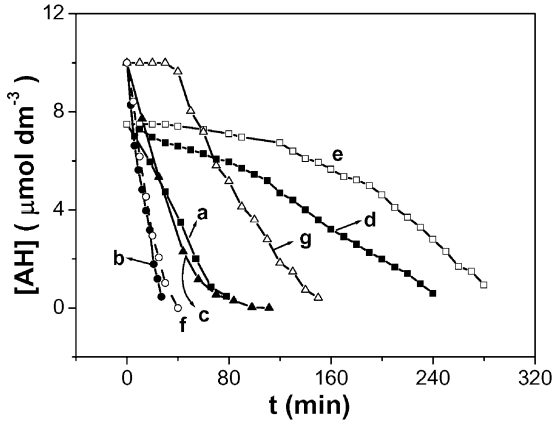
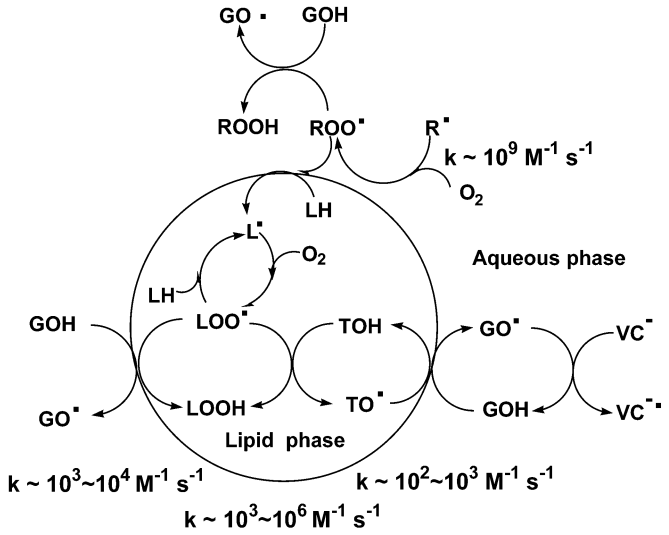


Fig. 10 Decay kinetics of antioxidants (AH) during the inhibition of linoleic acid peroxidation in SDS micelles. The reaction conditions were the same as described in the legend of Fig. 9. (a) Decay of TOH in the absence of VC and EGCG; (b) Decay of VC in the absence of TOH and EGCG; (c) Decay of EGCG in the absence of TOH and VC; (d) Decay of TOH in the presence of EGCG. (e) Decay of TOH in the presence of VC and EGCG. (f) Decay of VC in the presence of TOH and EGCG. (g) Decay of EGCG in the presence of TOH and VC.



Scheme 1 The anatioxidation mechanism of GOHs in micelles and biomembranes.

ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF RESVERATROL AND ITS ANALOGS

Resveratrol (3,5,4'-trihydroxyl-*trans*-stilbene) is a naturally occurring phytoalexin present in grapes and other plants. It has been suggested that its presence in red wine with concentrations ranging between 0.1 and 15 mg/L [39] is linked to the low incidence of heart diseases in some regions of France, the so-called "French paradox", that is, that despite a high fat intake, mortality from coronary heart disease is lower due to the regular drinking of wine [40]. In addition, resveratrol has been shown to possess cancer chemopreventive activity [41]. Therefore, the past few years have witnessed intense research devoted to the biological activity, especially the antioxidative activity, of this compound [42]. We have synthesized resveratrol and related *trans*-stilbene analogs, that is, 4-hydroxy-*trans*-stilbene (4-HS), 3,5-dihydroxy-*trans*-stilbene (3,5-DHS), 4,4'-dihydroxy-*trans*-stilbene (4,4'-DHS), 3,4-dihydroxy-*trans*-stilbene (3,4-DHS), 3,4,5-trihydroxy-*trans*-stilbene (3,4,5-THS), and 3,4,4'-trihydroxyl-*trans*-stilbene (3,4,4'-THS) (Fig. 11) and studied their antioxidant activity against free radical-induced peroxidation of linoleic acid in SDS and CTAB micelles [43], against peroxidation of rat liver microsomes

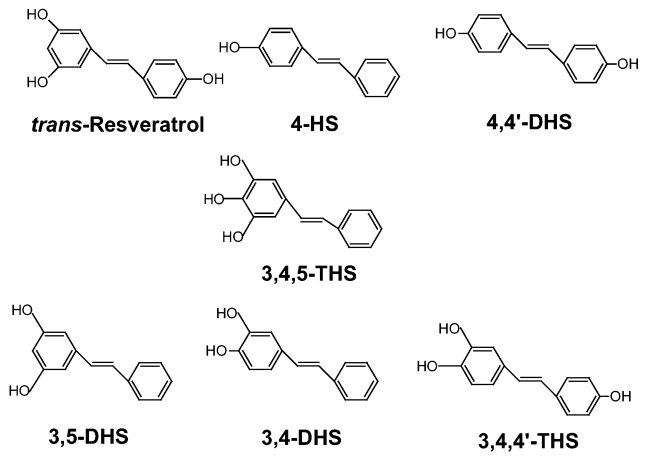


Fig. 11 Molecular structures of resveratrol and its analogs.

[44] and against hemolysis of human red blood cells [45]. The interaction of these resveratrol analogs with vitamin E was also studied.

It was found that all of these resveratrol analogs exhibit significant antioxidant activity in the above-mentioned chemical and biological systems as illustrated in Figs. 12–14 [43–45]. It is worth noting that the antioxidative activities of 3,4-DHS, 3,4,5-THS, and 3,4,4'-THS, that is, the molecules bearing *ortho*-dihydroxyl functionality, are appreciably higher than those of resveratrol and molecules bearing no such functionality, and that the antioxidant activity is correlated with the electrochemical behavior of the molecule. Molecules with lower oxidation potentials and reversible cyclic voltammograms, that is, 3,4-DHS, 4,4'-DHS, 3,4,4'-THS, and 3,4,5-THS, exhibit higher activity, while molecules with higher oxidation potentials and irreversible cyclic voltammograms, that is, 4-HS, 3,5-DHS, and resveratrol, are less active [43]. These facts suggest that electron-transfer antioxidation might take place simultaneously with a direct hydrogen-abstraction reaction, as exemplified in Scheme 2, and the formation of *ortho*-quinone intermediate might contribute to the high activity.

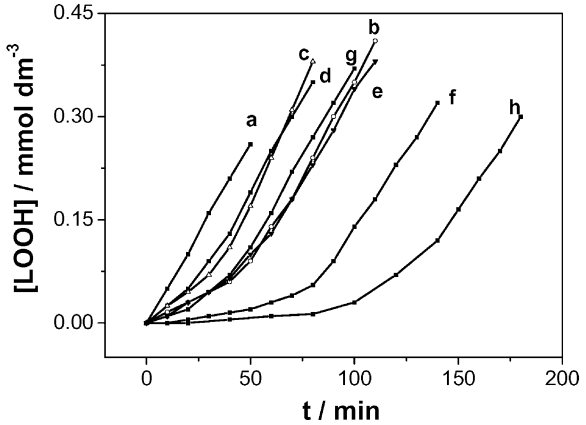


Fig. 12 Formation of LOOH during the AAPH-initiated peroxidation of LH in 0.1 M SDS micelles at pH 7.5 and 37 °C. The peroxidation was inhibited with ROHs. $[LH]_0$ = 15.2 mM, $[AAPH]_0$ = 6.3 mM, $[ROH]_0$ = 11.2 μM. (a) Uninhibited peroxidation; inhibited with: (b) resveratrol; (c) 4-HS; (d) 3,5-DHS; (e) 4,4'-DHS; (f) 3,4-DHS; (g) 3,4,5-THS; (h) 3,4,4'-THS [43].

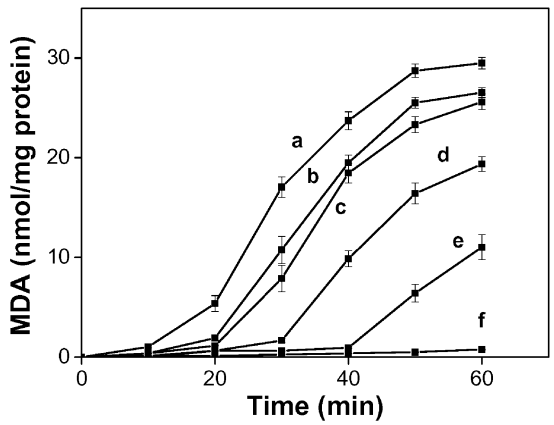


Fig. 13 Inhibition of malondialdehyde (MDA) formation during the Fe^{2+}/ascorbate-induced peroxidation of rat liver microsomes by ROHs at 37 °C. The rat liver microsomes were suspended in 0.1 M potassium phosphate buffer (pH 7.5) at the protein concentration of 0.3 mg/ml. $[FeSO_4]$ = 10 μM; [ascorbate] = 100 μM; $[ROHs]_0$ = 0.5 μM. (a) Native microsomes; inhibited with: (b) 3,5-DHS; (c) 4-HS; (d) Resveratrol; (e) 4,4'-DHS; (f) 3,4-DHS [44].

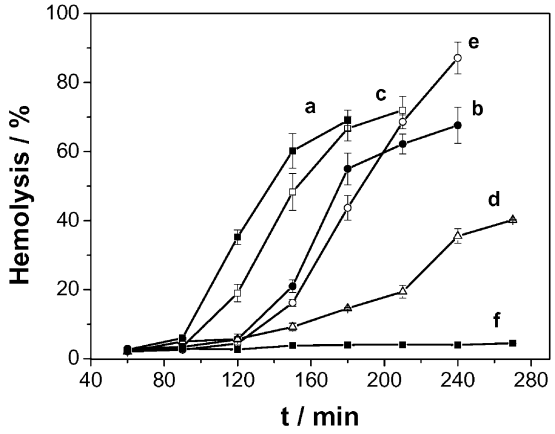
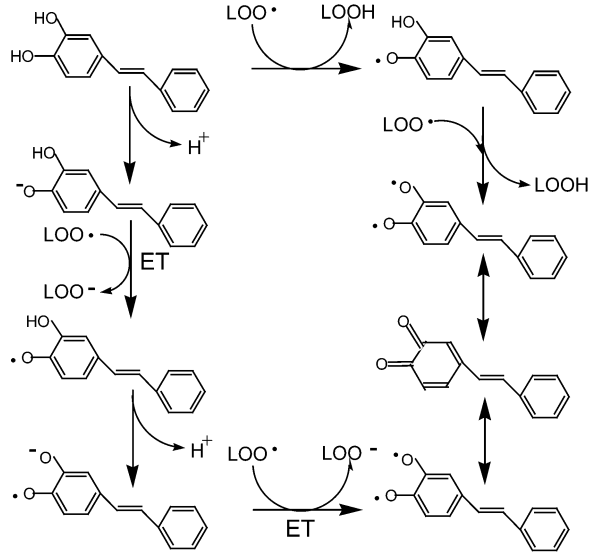


Fig. 14 Inhibition of AAPH-induced hemolysis of human erythrocytes by ROHs. $[AAPH]_0$ = 51.6 mM, $[ROH]_0$ = 15 μM. (a) native RBCs; inhibited with: (b) resveratrol; (c) 3,5-DHS (d) 3,4-DHS; (e) 4,4'-DHS; (f) control ($[AAPH]_0$ = 0) [45].



Scheme 2 The antioxidant mechanism of 3,4-DHS [43].

It was also found that the consumption of vitamin E could be significantly inhibited by resveratrol and its analogs as exemplified in Fig. 15, and the self-decay of TO• was accelerated by resveratrol and its analogs as exemplified in Fig. 16 [43]. These facts indicate that resveratrol and its analogs can reduce TO• and regenerate vitamin E, similar to the case of GOHs mentioned in the previous section.

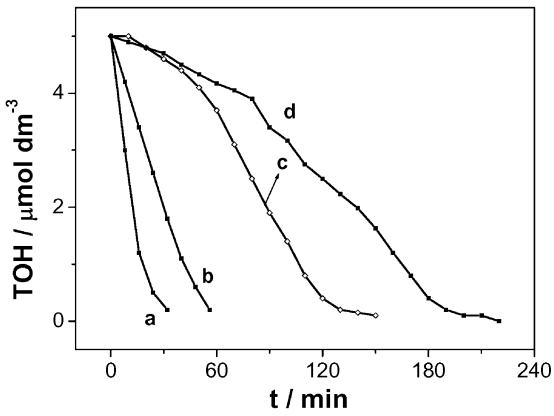


Fig. 15 Consumption of TOH during the AAPH-initiated peroxidation of linoleic acid in micelles at pH 7.5 and 37 °C. The peroxidation was inhibited by TOH and/or 3,4,4'-THS (ROH). $[LH]_0$ = 15.2 mM, $[AAPH]_0$ = 6.3 mM, $[ROH]_0$ = 11.2 μM, $[TOH]_0$ = 5 μM. (a) Decay of TOH in the absence of 3,4,4'-THS in 15 mM CTAB micelles; (b) decay of TOH in the absence of 3,4,4'-THS in 0.1 M SDS micelles; (c) decay of TOH in the presence of 3,4,4'-THS in CTAB micelles; (d) decay of TOH in the presence of 3,4,4'-THS in SDS micelles [43].

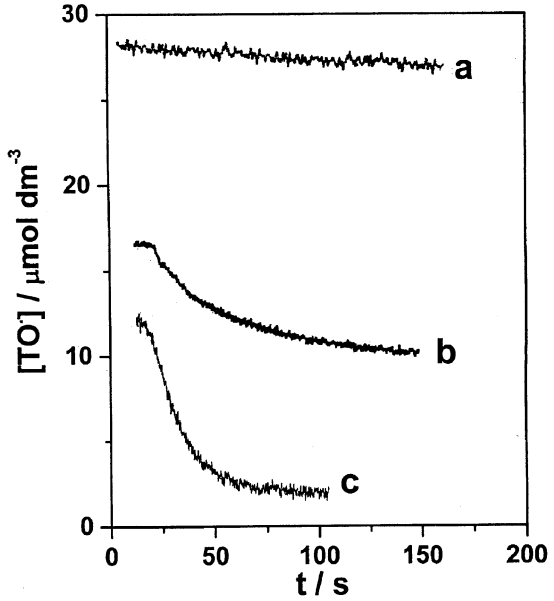


Fig. 16 The decay of TO• in 15 mM CTAB micelles at pH 7.4 and room temperature in the air. (a) intrinsic decay; (b) in the presence of 0.78 mM resveratrol; (c) in the presence of 0.13 mM 3,4-DHS [43].

These resveratrol analogs were found to be able to protect DNA from free radical-induced strand breakage as illustrated in Figs. 17 and 18 (unpublished work). Treatment of supercoiled plasmid DNA with AAPH induced the DNA strand breakage, producing the corresponding circular and linear DNA. Addition of resveratrol and its analogs significantly inhibited the strand breakage. Again, the molecules bearing *ortho*-dihydroxyl functionality exhibited remarkably higher activity than resveratrol and molecules bearing no such functionality, and than EGCG, Trolox (a water-soluble analog of vitamin E) and quercetin (a flavonol antioxidant).

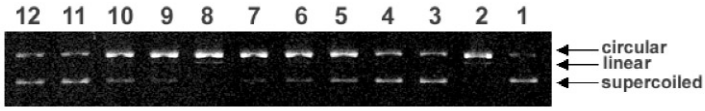


Fig. 17 Electrophoretograms of plasmid pBR 322 DNA strand breakage. The strand breakage was induced by 10 mM of AAPH and inhibited by 10 μM of resveratrol and its analogs, Trolox, EGCG, and quercetin, in tris-HCl buffer (pH 8.0) for 1 h at 37 °C. Lane 1: native pBR 322 DNA.; Lane 2: DNA damage induced by AAPH; Damage inhibited by: Lane 3: 3,4,4'-THS; Lane 4: 3,4-DHS; Lane 5: resveratrol; Lane 6: 2,4-DHS; Lane 7: 3,5-DHS; Lane 8: 3,5,4'-TMS; Lane 9: 3,4,5-THS; Lane 10: Trolox; Lane 11: EGCG; Lane 12: quercetin.

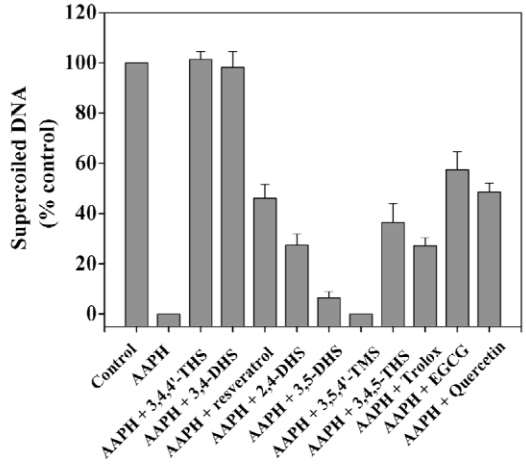


Fig. 18 Quantitative analysis of the protective effect of antioxidants on AAPH-induced pBR 322 DNA strand breakage. DNA damage is represented by the percentage of supercoiled DNA to native DNA.

The most interesting observation was that resveratrol analogs with lower oxidation potentials possess higher antioxidant activity, and particularly, also exhibit higher cytotoxicity (Fig. 19) and higher apoptosis-inducing activity (Fig. 20) on human promeolocytic leukemia (HL-60) cells [46]. The results are summarized in Table 1. Although the precise mechanism is still unclear, these results provide evidence for the correlation between the antioxidant and anticancer activities, hence they support the free radical theory of cancer and give us useful information for antioxidant and chemoprevention drug design.

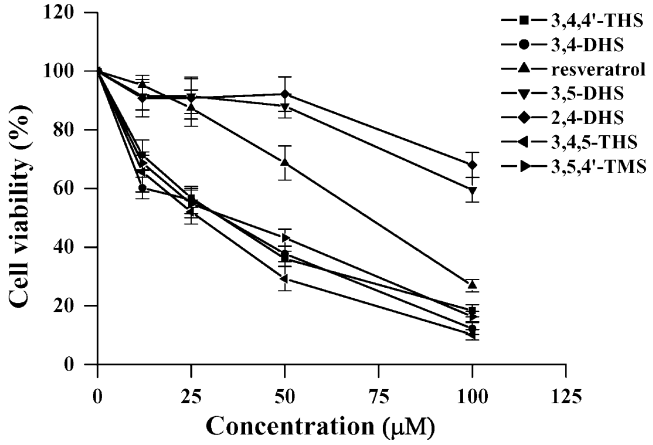


Fig. 19 Inhibitory effect of resveratrol and its analogs on the proliferation of HL-60 cells.

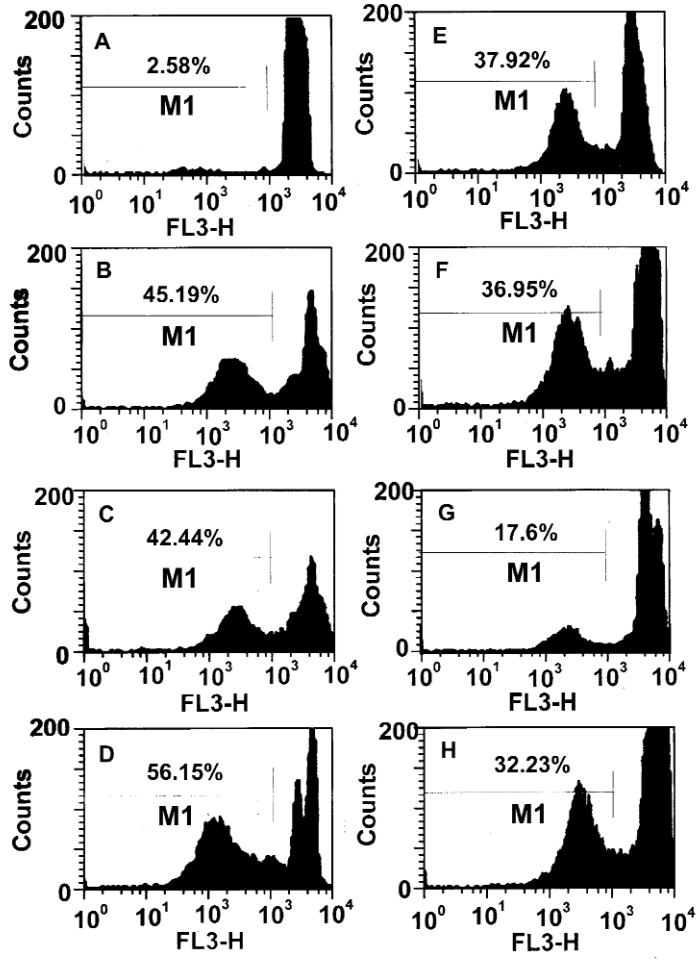


Fig. 20 Flow cytometry analysis of apoptosis of HL-60 cells. Cells were treated with resveratrol and its analogs for 48 h. The M1 marker shows the sub-G1 cell fraction that corresponds to the apoptotic cells. (a) medium alone; treated with: (b) 50 μM of 3,4-DHS; (c) 50 μM of 3,4,4'-THS; (d): 50 μM of 3,4,5-THS; (e): 150 μM of resveratrol; (f): 200 μM of 3,5-DHS; (g): 200 μM of 2,4,-DHS; (h): 50 μM of 3,5,4'-TMS.

Table 1 Comparison of oxidation potential, antioxidant activity, and apoptosis-inducing activity of resveratrol analogs [43,46][a].

Compound	Oxidation potential (V vs. SCE)	Antioxidant activity	$ApoEC_{50}$ (μM) (Jurkat cells)	$ApoEC_{50}$ (μM) (HL-60 cells)
Resveratrol	0.67	1.00	>200	85 ± 5.6
3,4-DHS	0.36	1.60	20 ± 4.4	33 ± 3.5
3,4,5-THS	0.23	1.30	20 ± 4.2	31 ± 7.2
3,4,4'-THS	0.34	1.78	27 ± 5.7	38 ± 4.3
4,4'-DHS	0.43	0.94	>200	>200
3,5-DHS	0.79	0.87	>200	>200
4-HS	0.66	0.66	>200	>200

[a]Oxidation potentials were determined in CTAB micelle. Antioxidant activity is expressed as the relative inhibition period in CTAB micelle. Apoptosis-inducing activity is expressed as $ApoEC_{50}$, which is the concentration required to induce 50 % apoptosis of the cancer cells as determined by flow cytometry.

CONCLUSIONS

GOHs and resveratrol and its analogs are good antioxidants against free radical-induced lipid peroxidation and possess cytotoxitic and apoptosis-inducing activity on HL-60 cancer cells. Significant correlation exists between their antioxidant activity in micelles and in biological systems, between the antioxidant activity and the cyctotoxic and apoptotic activity in HL-60 cancer cells. Compounds bearing *ortho*-dipheonoxyl groups generally exhibit higher activity than those bearing no such functionalities.

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