Antioxidant Potential of Theaflavins. A Pulse Radiolysis Study

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Abstract: Spectral, acid−base, and redox properties of theaflavin radicals were studied by pulse radiolysis in aqueous solutions. Theaflavin radicals are generated by the azide radical one-electron oxidation of theaflavin, theflavin gallates A and B, and theaflavin digallate. Being relatively strong transient oxidant, $N_3^{\bullet}$ oxidizes more than one phenolic site in the complex polyphenols. The resulting mixture of phenoxyl radicals transforms via an **intra**molecular electron transfer to the hydroxycycloheptenone radical, with apparently lowest reduction potential. The neutral hydroxycycloheptenone radical is more aromatic than the parent compound, which reflects in high rate constant of the formation of the radical. The rate of the reaction of theaflavin with the superoxide radical at pH 7, $k = 1 \times 10^7$ M^{-1} s^{-1}, is an order of magnitude higher than that with epigallocatechin gallate (EGCG), $k = 7.3 \times 10^5$ M^{-1} s^{-1}, in spite of higher reduction potential of the theaflavin radicals ($E_7 = 0.53$ V vs $E_7 = 0.44$ V for EGCG). Even the substitution in the adjacent benzene ring has relatively small effect on the electron density in the radical. Purpurogallin radical, with three hydroxy groups on the benzene ring, has $pK_{r1} = 4.7$ and $E_7 = 0.48$ V, as compared to the theaflavin radical, with only two hydroxy groups on benzene, having $pK_r = 4.3$ and $E_7 = 0.51$ V. The electron donating ability of theaflavins, which are major antioxidants in black tea, is quantitatively assessed on the basis of physicochemical characteristics of daughter radicals and their potential biological action discussed.

Introduction

Flavonoids and polyphenols are major plant antioxidants.[1−3] These compounds are implicated in the prevention of oxyl radical related diseases and pathological disorders.[4−38] A variety of beneficial effects, such as memory stimulation, rejuvenation, and delayed aging, have been ascribed to polyhydroxyaromatic compounds[9,13,19,20,22,23,30,31,39] (often erroneously called bioflavonoids), on the basis of their ability to scavenge certain oxyl radicals. The most often used models are the hydroxyl radical,

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generated by the Haber−Weiss and Fenton chemistry, or the superoxide radical, produced by stimulated neutrophils.[8,11,12,15,34,35,37,38,40,41] Although the results of the scavenging experiments are important preliminary tests for the antioxidant potential, they offer little if any details about the mechanism of antioxidant action. Of special importance is the full physicochemical characterization of the resulting radicals, to put the antioxidant potential of the parent polyphenols on a quantitative scale. The fate of the antioxidant radicals is also of major concern. Although very mild oxidants, antioxidant radicals may oxidize and deplete important physiological antioxidants, such as vitamins E and C.

Tea is one of the most popular beverages in the world. Green tea, where tea leaves are steamed and dried, is more popular in Japan, China, Korea, and some African countries, whereas black tea, obtained upon "fermentation" of tea leaves, is favored by the Western countries. Theaflavins and thearubigins are major phenolic compounds in black tea (approximately 2% of dry tea leaves mass). In addition to the 3,5-dihydroxybenzopyran and/or gallate rings, these complex polyphenols also contain the hydroxybenzocycloheptenone moiety, which is responsible for their characteristic yellow-orange color. In the "fermentation" of tea leaves, gallocatechins, which are major polyphenols in green tea (∼40% of dry tea leaves mass), transparent in the visible, are completely replaced by intensely colored theaflavins and thearubigins in black tea. Given the high content of polyphenols in tea, it is of considerable interest to investigate the difference in the antioxidant potential and action of theaflavins and gallocatechins. In this study we systematically investigated the spectral, acid−base, and redox properties of the theaflavin radicals and a model thearubigin (purpurogallin). The ability of theaflavins to scavenge the superoxide radical is also determined. On the basis of the physicochemical properties of theaflavin radicals, the antioxidant potential of parent polyphenols is quantitatively assessed. The fate of the theaflavin radicals is discussed in light of their possible interaction with vitamin E.

Materials and Methods

All chemicals used in this study were of the highest purity available. Theaflavin, theaflavin gallate A, theaflavin gallate B, and theaflavin digallate were obtained in Mitsui Norin Inc., Food Research Laboratories, according to the in house extraction and purification procedure described elsewhere.[27] Promethazine hydrochloride and purpurogallin were the products of Sigma; methyl gallate, Trolox C, and sodium azide were obtained from Aldrich; potassium bromide, 2-propanol, potassium hydroxide, potassium dihydrogen phosphate, and disodium hydrogen phosphate were obtained from Merck. Water was purified through a Millipore Milli Q ion-exchange columns. Because of possible oxidation of theaflavins by oxygen from air, buffered aqueous solutions containing inorganic solutes were first saturated by high purity N_2O (>99.999%). Then theaflavins and other oxidizable substrates were dissolved, and the solutions were gently purged with N_2O until use. The N_2O:O_2 = 4:1 saturated solutions were generated by mixing the N_2O saturated theaflavin solution with the O_2 saturated one containing all other solutes except theaflavin derivatives at the inlet of the conductivity cell. This was necessary to minimize otherwise rapid thermal oxidation of alkaline aqueous solutions of theaflavin.

The 3 MeV van deGraaff pulse radiolysis apparatus at the Max-Planck-Institut für Strahlenchemie[42] was used for the pulse radiolysis experiments. Optical measurements were performed with a thermostated 2 cm Suprasil quartz cell. The absorbed doses were ∼5 Gy/pulse for the measurements of radical spectra and 0.08−2 Gy/pulse for the electron-transfer kinetics, as determined by the thiocyanate dosimetry, taking $G[(SCN)_2^{\bullet-}]$ and $\epsilon_{480} = 7600\ M^{-1}\ cm^{-1}$. A flow AC-conductivity cell was used in the AC conductivity experiments. The doses were ∼2 Gy/pulse as determined by the DMSO dosimetry at pH 4.

Molecular modeling was done on the Silicon Graphic workstation, using the Spartan program package. The geometries of the theaflavin derivatives and corresponding radicals were optimized by the semiempirical AM1 method, up to a gradient less than 0.01.

Table 1. Reactivities of $N_3^{\bullet}$ with Theaflavins Determined by Pulse Radiolysis in Aqueous Solutions at pH 7, 20 °C

theaflavin, TF	$k(N_3^{\bullet} + TF)$,[a] $M^{-1}\ s^{-1}$
theaflavin	2.0×10^9
theaflavin monogallate A	4.0×10^9
theaflavin monogallate B	4.4×10^9
theaflavin digallate	4.0×10^9
purpurogallin	3.1×10^9

[a] Accurate to ±10%.

Results and Discussion

Antioxidant action of theaflavins is expected to be similar to that of other plant polyphenols, such as catechins, which function as antioxidants by electron donation to free radical oxidants in aqueous solutions.[16−18,43,44] To fully assess antioxidant potential of theaflavins, we investigated the generation and spectral and acid−base properties of theaflavin radicals, one-electron transfer equilibria involving theaflavin radicals and radicals of redox standards, and the ability of theaflavins to scavenge the superoxide radical.

Generation of Theaflavin Phenoxyl Radicals. Theaflavin phenoxyl radicals were generated by the azide radical induced oxidation of theaflavins,[17] as illustrated by eq 1. One-electron oxidation of theaflavins was investigated by pulse radiolysis in aqueous solutions at 20 °C and ionic strength of 26 mM, and the results are summarized in Table 1.

The second-order rate constants for the oxidation of theaflavins by the azide radical are high, $k \sim 10^9\ M^{-1}\ s^{-1}$, similar to those reported earlier for phenols and polyphenols.[17,18] Such high reactivities of the azide radicals with theaflavins ensure rapid generation of the theaflavin phenoxyl radicals for the investigation of their spectral, acid−base, and redox properties.

Spectral Properties. The spectra obtained upon one-electron oxidation of the theaflavins by the azide radical undergo first-order transformation, which is independent of the concentration of theaflavin and azide, but depends on the pH of the solution. This transformation is not seen in the spectra of the purpurogallin radical, a simpler polyhydroxyaromatic compound, with only one type of the phenol moiety. The first-order transformation is illustrated in Figure 1, which shows the spectra of theaflavin digallate and theaflavin radicals recorded immediately upon the reaction with $N_3^{\bullet}$ and after the completion of the subsequent first-order process.

Theaflavin derivatives have several oxidizable phenolic hydroxy groups (see Table 2). Being relatively strong transient oxidant, $E = 1.33$ V,[45] the azide radical apparently attacks more

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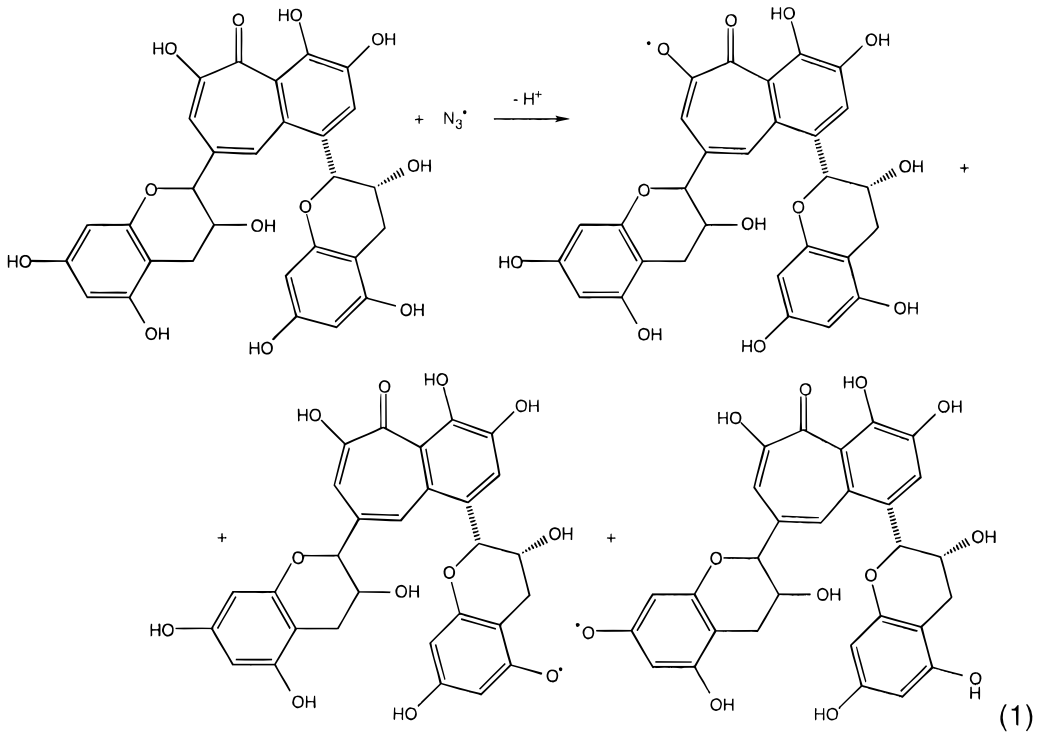


than one site in any theaflavin derivative. Theaflavin has two 3,5,7-trihydroxybenzopyrans (THBP) attached to 3,4,6-trihydroxy-benzocyclohepten-5-one (THBCO) (see Table 2). From previously reported rate constants for phenols and polyphenols[17,18] and the data on purpurogallin (Table 1), it may be suggested that the azide radical reacts with all three parts of theaflavin molecule to generate corresponding phenoxyl radicals. The resulting phenoxyl radicals have different reduction potentials, which causes subsequent intramolecular electron transfer process. Ultimately, the phenoxyl radical with the lowest reduction potential prevails. Similar electron transfer processes were previously observed for the phenoxyl radicals of gallocatechins[17] and other flavonoids.[18] Comparison of the final radical spectra (Figure 1) with the spectra of substituted phenoxyl radicals,[16,17] and that of purpurogallin (Figure 2), indicates that the observed first-order transformation in the radical spectra is due to the **intra**molecular oxidation of THBCO by the THBP phenoxyl radicals. This conclusion is further supported by the measurements of the reduction potentials of various theaflavin radicals (see later Discussion).

Theaflavin gallates A and B and theaflavin digallate have respectively one and two gallate ester moieties in addition to two THBP and one THBCO. Here the attack of the azide radical generates three types of phenoxyl radicals, e.g., THBP, gallate, and THBCO radicals. Being the best electron donor of all phenol groups, THBCO donates an electron to the other phenoxyl radicals with higher reduction potentials. Therefore, the gallate and THBP radicals transform via subsequent **intra**molecular electron transfer to the THBCO radical. This conclusion is supported by the spectra of the radicals (see Figures 1 and 2), which exhibit a strong absorption at ~500 nm regardless of the complexity of constituent phenols, and the reduction potentials of various phenoxyl radicals (see later). It is also noteworthy that the theaflavin monogallate and theaflavin digallate radical spectra differ considerably from the spectra of catechin and gallate radicals.[16,17]

Interestingly, the transformation of the initial mixture of the phenoxyl radicals to the weakest oxidant 3,4,6-trihydroxy-5*H*-benzocycloheptenone radical appears to be independent of the concentration of the theaflavin derivative, which means that the **inter**molecular electron transfer is slow, $k < 10^5$ M^{-1} s^{-1} (the radical decay was not influenced by the change in the polyhydroxyaromatic compound concentration from 0.2 to 1 mM). This is different from the behavior of the gallocatechin radicals, where both inter- and intramolecular electron transfer processes were observed.[17] It is conceivable that the intermolecular electron transfer processes between various theaflavin phenoxyl radicals

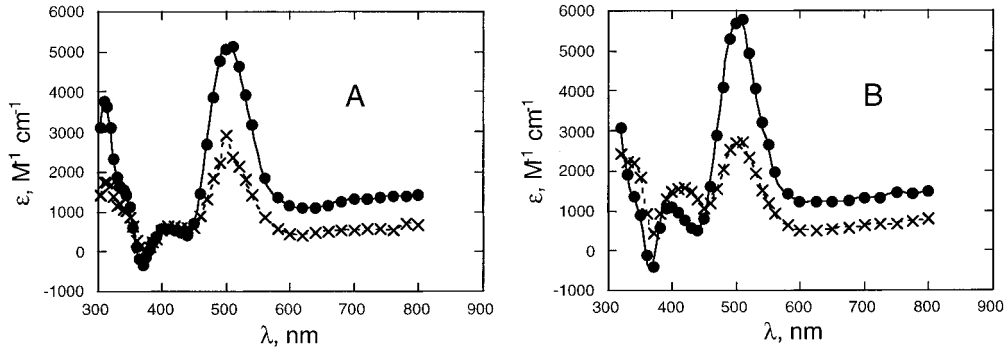
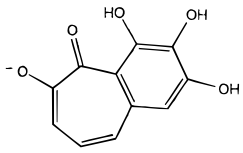
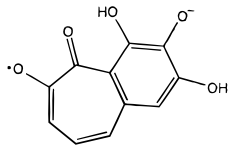
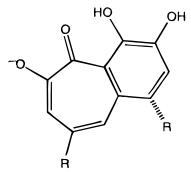
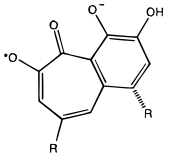
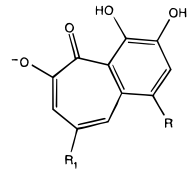
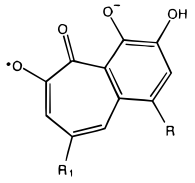
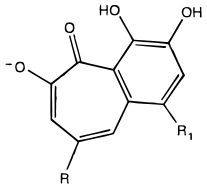
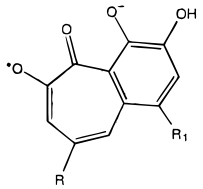
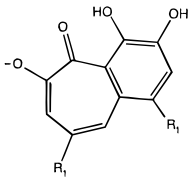
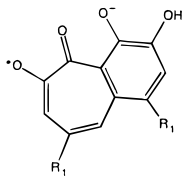


Figure 1. Transient spectra of the phenoxyl radicals obtained by pulse radiolysis of N_2O-saturated aqueous solutions of 10 mM NaN_3, pH 7, 20 °C, (×) upon one-electron oxidation by $N_3^•$, 0.1 mS after the pulse, and (●) upon completion of the first-order process (1 mS for theaflavin and 4 mS for theaflavin digallate): (A) 0.1 mM theaflavin and (B) 0.1 mM theaflavin digallate.

Table 2. Acid−Base Properties of Theaflavins and Theaflavin Phenoxyl Radicals in Aqueous Solutions at 20 °C

Antioxidant	pK_a[a]	Structure of anion[b]	pK_r[c]	Structure of radical anion[d]
Purpurogallin	6.9;10.8		4.7;8.1	
Theaflavin	8.7		4.3	
Theaflavin gallate A	8.6		4.6	
Theaflavin gallate B	8.8		4.5	
Theaflavin digallate	8.8		4.5	

[a] Determined by UV−vis spectroscopy. Estimated to be accurate to ±0.02 pH units. [b] The structures are calculated by the semiempirical AM1 geometry optimization. [c] Determined by the optical pulse radiolysis. Estimated to be accurate to ±0.1 pH unit. R = 3,5,7-trihydroxybenzopyran, R_1 = 5,7-dihydroxybenzopyran-3-*O*-gallate.

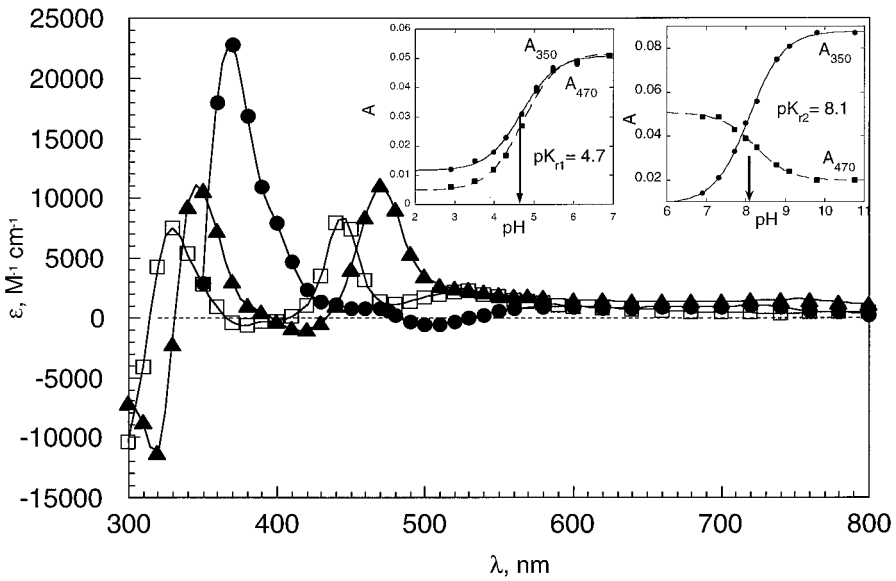


Figure 2. Absorption spectra of the purpurogallin radicals obtained by pulse radiolysis of N_2O-saturated aqueous solutions of 10 mM NaN_3, 0.03 mM purpurogallin, 20 °C. −pH = 3.0; (▲) pH = 7.0; (■) pH 9.2. Insets: titration curves at two absorbancies.

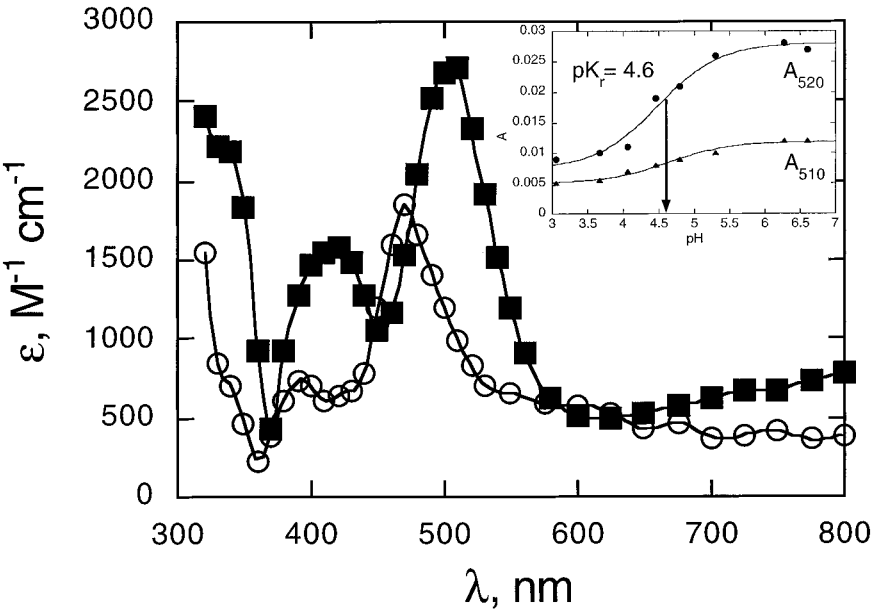


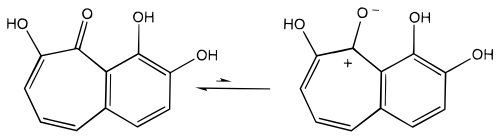
Figure 3. Absorption spectra of the theaflavin digallate radicals obtained by pulse radiolysis of N_2O-saturated aqueous solutions of 10 mM NaN_3, 0.1 mM theaflavin digallate, 20 °C. (○) pH = 3.0; (■) pH = 7.0.

are slowed down because of the size of the radicals and bulky substituents, which hinder direct collisions of the radicals and molecules.

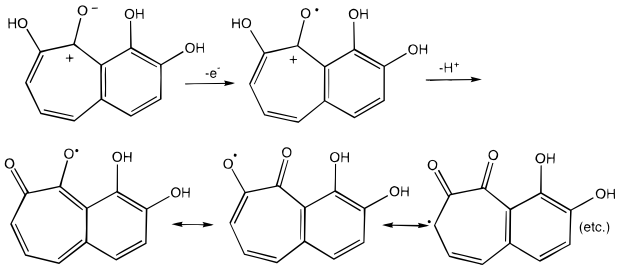
Both rates of the initial one-electron oxidation by $N_3^\bullet$ and of the intramolecular electron transfer increase with the pH of the solution. In the pH range from 6 to 10 (0.18 mM theaflavin, 10 mM NaN_3, 3 mM phosphate buffer), the reactivity of $N_3^\bullet$ increases from 1.8×10^9 M^{-1} s^{-1} to 5×10^9 M^{-1} s^{-1}, while the rate of the subsequent electron transfer increases from 4×10^4 (at pH 6) to 4×10^5 s^{-1} (at pH 9.3). At pH higher than 9.3, the first-order transformation in the radical spectra becomes faster than the initial oxidation and is no longer observable. Theaflavin gallate and theaflavin digallate radicals ($k = 2 \pm 0.4 \times 10^3$ s^{-1} at pH 7) behave similarly. Such increase in the rate of one-electron transfer processes with the pH has been observed for various phenol derivatives,[16−18,45] because the deprotonated phenol (phenoxide ion) group is a much better electron donor than the neutral one.

Acid−Base Properties. The spectra of the theaflavin radicals exhibited pH dependent changes in the pH range from 3 to 12, giving rise to a titration curve. The spectral changes and the titration curve are illustrated in Figures 2 and 3. The pK_r values of the phenoxyl radicals derived from various theaflavins are summarized in Table 2.

The pK_r values of theaflavin, theaflavin gallate, and theaflavin digallate radicals are similar, as may be expected from the similar structure of the radical, which is obviously the 3,4,6-trihydroxy-5*H*-benzocyclohepten-5-one radical in all cases. There is, however, a striking similarity between the first pK_r of theaflavin radical, pK_r = 4.0, and pK_{r1} = 4.7 of the purpurogallin radical in contrast to the significant difference in the deprotonation of the parent compounds (pK_a(theaflavin) = 8.7 vs pK_{a1}-(purpurogallin) = 6.9). In addition, the spectra of neutral and anion phenoxyl radicals derived from 2,3,4,6-tetrahydroxy-5*H*-benzocyclohepten-5-one (purpurogallin) and from 3,4,6-trihydroxy-5*H*-benzocyclohepten-5-one (theaflavin) are similar (Figures 1 and 2), leading to the conclusion that the hydroxycycloheptenone moiety plays a dominant role. Why the benzocycloheptenone moiety is important for the electron transfer processes might be explained by the existence of the resonance charge-separated form in the parent compounds, where the energy needed for charge separation is partially



compensated by the energy gained by resonance of the tropilium cation. One-electron oxidation of the zwitterionic form results in the formation of the phenoxyl radical cation,



This cation radical is very unstable (typically pK_a < 0) and rapidly deprotonates to a neutral radical, stabilized through resonance (as illustrated). The molecular modeling calculation (semiempirical AM1) indicates an **aromatic** neutral phenoxyl radical (with high degree of unpaired electron delocalization on all hydroxy groups), as compared with a nonaromatic parent. Further deprotonation of the hydroxycycloheptenone radical, which can only happen in the benzene ring, gives aromatic radical anion.

As seen from Table 2, theaflavin phenoxyl radicals have pK_a values lower than 7, which means that these radicals are negatively charged in neutral media. This is important for the potential action of various theaflavins as antioxidants-electron donors in biological systems. While the parent theaflavins are neutral in neutral media, which is favorable for their intake through negatively charged cell membranes, the corresponding radicals are negatively charged, which should effectively prevent their passage through and retard their interaction with the membrane.

Reduction Potentials. Reduction potentials of the theaflavin radicals are determined from their electron transfer equilibria with the redox standards at different pH. Because of complex

Table 3. Electron Transfer Reactions of Theaflavins and Theaflavin Radicals Investigated by Pulse Radiolysis of Aqueous Solutions at 20 °C

$$A^{\bullet} + D^{-} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} A^{-} + D^{\bullet}$$

radicals, A$^{\bullet}$, from	donor, D^{-}	pH	$k_{r,}$[a]	k_r[a]	K_{kin}[b]	K_{abs}[b]	ΔE,[c] V
			$M^{-1}\ s^{-1}$				
trolox C	theaflavin	13.5	2.2×10^7			500	0.16
trolox C	theaflavin digallate	13.5	2.5×10^7	5×10^4	500	500	0.16
methyl gallate	purpurogallin	7.0	8.0×10^6	3×10^5	33	33	0.08
promethazine	theaflavin	3.0	1.5×10^8	5×10^6	30	14	0.07
promethazine	theaflavin digallate	3.0	1×10^8			12	0.06
promethazine	pupurogallin	3.0	1.6×10^9			90	0.12

[a] Rate constants of electron transfer reactions, estimated to be accurate to ±10% for forward and to ±20% for reverse process. [b] Equilibrium constants from kinetics, $K_{kin} = k_f/k_r$, and equilibrium absorbances of the radicals, E_{abs}. [c] Redox potential difference calculated from the Nernst equation, $\Delta E = 0.059RT \log K$, where $K = (K_{kin} + K_{abs})/2$.

Table 4. Reduction Potentials (in V, vs NHE) of Theaflavin Radicals at 20 °C

radical from	$E^{0\prime}$ [a]	E_3	E_7	$E_{13.5}$
purpurogallin	(1.02)	0.83	0.48	n.m.[b]
		(0.86)	(0.48)	
theaflavin	(1.10)	0.92	(0.51)	0.03
		(0.92)		(0.03)
theaflavin digallate	(1.10)	0.92	(0.54)	0.03
		(0.93)		(0.04)

[a] Reduction potential at pH = 0, very probably equal to standard reduction potential, assuming that the activities of the radicals are approximately 1. [b] Not measured because aqueous solutions were not stable at such high pH. [c] The values obtained by evaluating the pH dependence of the reduction potential are given in parentheses. For purpurogallin

$$E_{pH} = E^0 + 0.059 \log \frac{(K_{a1}K_{a2} + K_{a1}[H^+] + [H^+]^2)[H^+]}{K_{r1}K_{r2} + K_{r1}[H^+] + [H^+]^2}$$

and for other theaflavin radicals

$$E_{pH} = E^0 + 0.059 \log \frac{(K_a + [H^+])[H^+]}{K_r + [H^+]}$$

transformations involving theaflavin phenoxyl radicals (see previous section) and strong absorption of parent compounds in the visible region of the spectrum, it was impossible to "bracket" the reduction potential of theaflavin radicals at any pH, e.g., to investigate both electron transfer equilibria when the theaflavin derivative is oxidized and when the corresponding radical is reduced. However, in order to get the best possible values, we determined the reduction potentials at different pH and checked the validity of the values by evaluating the pH dependence.[46] We used as redox standards the radicals from promethazine at pH 3 (with $E_3 = 0.92$ V[47]), methyl gallate at pH 7 ($E_7 = 0.56$ V[17]), and Trolox C at pH 13.5 ($E_{13.5} = 0.13$ V[48]). The results are summarized in Tables 3 and 4.

The reduction potentials of the theaflavin radicals, $E_7 = 0.48-0.54$ V, are lower than those of 3,5-dihydroxyanisole, $E_7 = 0.83$ V,[18] a model for 3,5-dihydroxybenzopyran ring, and methyl gallate, $E_7 = 0.56$ V,[17] a model for the gallate esters, radicals. Consequently, the hydroxybenzocycloheptenone moiety is responsible for electron donating ability of theaflavins. It is also interesting to note that the acid−base equilibria of dihydroxybenzopyran and trihydroxybenzene (gallate) substituents to the hydroxycycloheptenone moiety, which must deprotonate above pH 9, have no effect on physicochemical properties of the theaflavin radicals. Neither acid−base (Table 1) nor redox properties of the radicals (Table 4) are affected by the ionization of the hydroxy groups in the substituent rings.

The reduction potentials of the radicals from theaflavin derivatives are favorably low in neutral media, $E_7 = 0.48 - 0.54$ V, which renders parent theaflavins good antioxidants−electron donors. The difference in the reduction potentials of the theaflavin, with two electron-donating hydroxy groups, and purpurogallin radicals, with three hydroxy groups attached to the benzene ring, is only 0.06 V, in accord with the inefficient coupling of the unpaired electron with the benzene ring substituents. The influence of the dihydroxybenzopyran and dihydroxybenzopyran + gallate substituents on the reduction potentials of the theaflavin radicals is indeed minimal. Within a ±0.04 V error of the determination of the reduction potentials, the radicals of various theaflavin derivatives have similar reduction potentials.

The reduction potentials of the theaflavin radicals, $E_7 = 0.48-0.54$ V, are higher than those of the gallocatechin radicals, $E_7 = 0.44$ V.[17] The fact that the black tea antioxidants are inferior electron donors does not necessarily mean that they are not good antioxidants. However, with respect to the ability to damage vitamin E or repair corresponding radical (with $E_7 \sim 0.48$ V),[48] based on their higher reduction potential theaflavin radicals are capable of damaging vitamin E. Conversely, because of their lower oxidation potential, gallocatechins may repair the vitamin E radical. Vitamin E is perhaps the most important lipid-soluble natural antioxidant, which protects cell membranes from peroxidation. Consequently, such potentially damaging effect of the theaflavin radicals may impair their beneficial action in biological systems.

Reduction of the Superoxide Radical. Theaflavin derivatives are intensely colored and sparsely soluble in neutral aqueous solutions. On the other hand, in alkaline solutions where ionized theaflavins can be dissolved in appreciable concentrations, the theaflavin derivatives are rapidly oxidized by oxygen. Because of these difficulties, we chose to measure the rates of the reduction of the superoxide radical by the kinetic conductivity method[16] in moderately alkaline aqueous solutions at pH 10, and by optical pulse radiolysis at pH 7. The results are summarized in Table 5.

The rate constants of the scavenging of the superoxide radical by theaflavins (reaction 2) are an order of magnitude higher in neutral than in alkaline media. The reason for this is very probably the electrostatic repulsion between the negatively charged antioxidative moiety in theaflavins (hydroxybenzocycloheptenone, with p$K_a \sim 9$ (Table 2)) and superoxide (p$K_a \sim$ 4.8)).

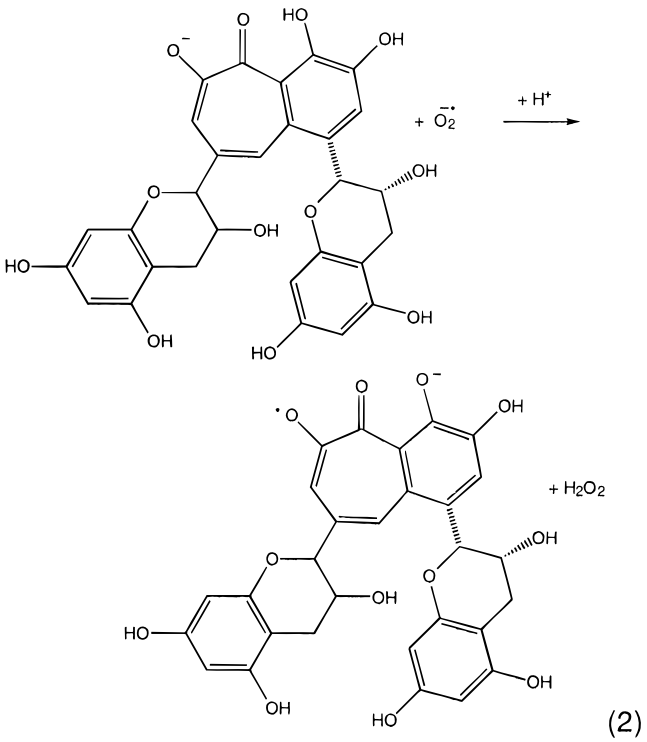
The rates of the reduction of the superoxide radical by theaflavins are very high, $k \sim 10^6 - 10^7\ M^{-1}\ s^{-1}$. This indicates

(46) Clark, W. M. *Oxidation-Reduction Potentials of Organic Systems*; Williams and Wilkins: Baltimore, MD, 1960.
(47) Jovanovic, S. V.; Steenken, S.; Simic, M. G. *J. Phys. Chem.* **1990**, *94*, 3583.
(48) Steenken, S.; Neta, P. *J. Phys. Chem.* **1982**, *86*, 3661.

Table 5. Rate Constants of the Reactions of the Superoxide Radical with Theaflavins, Determined by Kinetic Conductivity at pH 10.0, and by Optical Pulse Radiolysis at pH 7

	$k(O_2^{\bullet -} + TF)$,[a] $M^{-1}\ s^{-1}$	
theaflavin, TF	pH 7	pH 10
purpurogallin	1.8×10^7	n.m.
theaflavin	1.0×10^7	1.0×10^6
theaflavin gallate A	n.m.	2.4×10^5
theaflavin gallate B	n.m.	2.8×10^5
theaflavin digallate	2.1×10^6	3×10^5

[a] Estimated to be accurate to ±10% for optical and to ±20% for AC conductivity measurements.



(2)

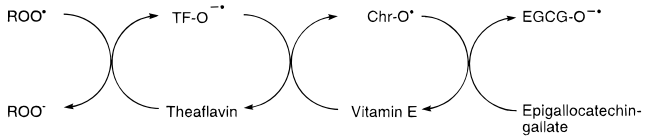
that these tea antioxidants are very good superoxide scavengers. Theaflavin gallates have lower rates of the superoxide scavenging than theaflavin (see Table 5). It may be suggested that the bulkier gallate rings hinder the attack of the superoxide on the hydroxybenzocycloheptenone moiety. In comparison with gallocatechins, the rates of the superoxide scavenging are almost an order of magnitude higher. The reason for high rate constants is probably the charge separation in the benzocycloheptenone ring, which facilitates one-electron abstraction (see earlier discussion on the acid−base properties).

Summary

Theaflavin radicals may conveniently be generated by one-electron oxidation of parent theaflavins with the azide radical in aqueous solutions. Being relatively strong transient oxidant, the azide radical oxidizes more than one moiety in the theaflavins, thus giving rise to a mixture of phenoxyl radicals. These theaflavin radicals undergo intramolecular electron transfer resulting in the hydroxybenzocycloheptenone radical. The charge separation in the parent theaflavins facilitates one-electron abstraction from O2, resulting in the higher rate constants of electron transfer. For example, the rate constant of the superoxide scavenging by theaflavin, $k = 1.0 \times 10^7\ M^{-1}\ s^{-1}$, is more than order of magnitude higher than that of epigallocatechin gallate, $k = 4.3 \times 10^5\ M^{-1}\ s^{-1}$,[17] in spite of higher reduction potential of the theaflavin radicals.

Theaflavins are uncharged in neutral media, which is favorable for their uptake through the negatively charged membranes. Corresponding radicals are negatively charged, which should effectively retard their interaction with the membrane and prevent their uptake.

Both low reduction potentials of theaflavin radicals and the rates of the scavenging of biological oxidant−superoxide radical in neutral media are indicative of high antioxidant potential of theaflavins. However, on the basis of their higher reduction potentials, the theaflavin radicals are potentially harmful to vitamin E, whereas gallocatechins may repair the vitamin E radical (see scheme below).



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