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Free Radical Studies of Ellagic Acid, a Natural Phenolic Antioxidant

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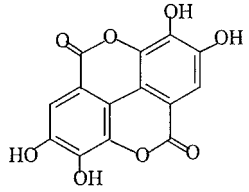
Ellagic acid, a plant-derived polyphenol, inhibits γ-radiation (hydroxyl radical) induced lipid peroxidation in rat liver microsomes in a dose- and concentration-dependent manner. Its antioxidant capacity has been estimated using the 1,1-diphenyl-2-picrylhydrazyl radical assay. To understand the actual mechanisms involved in antioxidant activity and the free radical scavenging ability,a nanosecond pulse radiolysis technique has been employed. The rate constants for the reactions of several reactive oxygen species and reactive nitrogen species such as hydroxyl, peroxyl, and nitrogen dioxide radicals have been found to be in the range of 10^6-10^9 M^{-1} s^{-1}. The ellagic acid radicals have been characterized by the absorption spectra and decay kinetics. Studies on the reactions of ellagic acid with the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) radical and the radicals of ellagic acid with ascorbate have been used to estimate its one-electron reduction potential. Ellagic acid has also been found to be a good scavenger of peroxynitrite. Using stopped-flow reaction analyzer with absorption detection, the rate constant for this reaction has been determined to be 3.7×10^3 M^{-1} s^{-1}. The electron spin resonance spectra of the oxidized ellagic acid radicals have been recorded by horseradish peroxidase and hydrogen peroxide method.

KEYWORDS: Polyphenols; antioxidants; free radicals; pulse radiolysis

INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated inside cells by exposure to several endogenous and exogenous agents, causing damage to many important biomolecules that have been implicated in several human diseases (*1*). These pro-oxidants are kept in check by endogenous antioxidants, but under diseased conditions, the balance is shifted in favor of pro-oxidants, leading to oxidative stress. Hence, exogenous dietary antioxidants capable of scavenging free radicals may have potential in preventing diseased conditions (*2*, *3*). Ellagic acid is a polyphenol (structure given below) found in a wide variety of fruits and nuts such as raspberries, strawberries, walnuts, grapes, and black currants (*4*−*7*). It is the major phenolic constituent present in distilled beverages (*8*). It exhibits antimutagenic, antioxidant, and anti-inflammatory activity in bacterial and mammalian systems (*4*, *9*−*14*). Epidemiological studies indicate that there is an inverse association between the incidence of coronary heart diseases and fruit consumption, and this has been largely attributed to the antioxidant action of phenolic compounds (*15*, *16*). According to the German national food consumption survey in the Bavarian subgroup, the daily intake of phenolic acids (222 mg) per person, through fruits and vegetables, contains 5.2 mg/day of ellagic acid (*17*). Ellagic acid exhibited cardioprotective properties on a model of neoepinephrine myocarditis in rats (*18*). On oral administration, ellagic acid exhibited hepatoprotective activity against carbon tetrachloride both in vitro and in vivo (*19*, *20*). Ellagic acid induced G1 arrest, inhibited overall cell growth, and caused apoptosis in tumor cells (*21*). One of the studies reports better protection of ellagic acid than vitamin E against oxidative stress (*22*). Using flow linear dichroism experiments, it was found that ellagic acid binds to double-stranded calf thymus DNA (*23*). Formation of 8-hydroxydeoxyguanosine by 2-nitropropane in rat liver nuclear DNA was inhibited by ellagic acid (*24*). It reduced cytogenetic damage induced by radiation, hydrogen peroxide, and mitomycin C in bone marrow cells of mice (*25*, *26*).

Cozzi et al. proposed that the observed cytogenetic protection against hydrogen peroxide by ellagic acid could be through



Ellagic acid

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scavenging reactive free radicals (*26*). The protective effects of ellagic acid are thus attributed to several factors including DNA binding, inhibition of the production of ROS, scavenging of ROS, and protection of DNA from alkylating injury. From the structure−function relationship, it has been suggested that both phenolic hydroxy groups and the lactone are necessary for its activity at different conditions (*27*). Although a lot of research work on different physiological and pharmacological aspects of ellagic acid has been carried out, there are not many studies on the actual free radical reactions, especially their direct reactions with ellagic acid. Hence, it is important to study the antioxidant activity of ellagic acid and to determine its reaction kinetics in terms of rate constants, including some physicochemical properties. In the present investigations, we have carried out studies on its ability to scavenge reactive oxygen and nitrogen free radicals using a pulse radiolysis technique and a stopped-flow spectrometer in microsecond to millisecond time scales. The antioxidant activity of ellagic acid is reported in terms of its ability to inhibit radiation-induced lipid peroxidation in rat liver microsomes and reaction with DPPH radicals.

EXPERIMENTAL PROCEDURES

Materials and Methods: Ellagic acid, thiobarbituric acid (TBA), and butylated hydroxytoluene (BHT) were purchased from Sigma Chemical Co. All other reagents, obtained from reputable local suppliers, were of the highest purity. Nitrous oxide (N_2O) used was of IOLAR grade. DPPH was from BDH.

Pulse radiolysis experiments were carried out on a 7 MeV linear electron accelerator described elsewhere (*28*). All of the experiments were done using a 50-ns electron pulse with an absorbed dose of 10−12 Gy per pulse. The absorbed dose was measured using thiocyanate dosimetry monitoring $(SCN)_2^{\bullet-}$ at 500 nm (*29*). On radiolysis, water generates reactive radicals such as e^-_{aq}, $^\bullet OH$, and $^\bullet H$ and less reactive molecular species such as H_3O^+, H_2, and H_2O_2 (*30*):

$$H_2O \rightarrow e^-_{aq}, {}^\bullet OH, {}^\bullet H, H_3O^+, H_2, H_2O_2 \quad (1)$$

The concentration of hydroxyl radicals was doubled by irradiating an N_2O-saturated aqueous solution where e^-_{aq} was quantitatively converted to the $^\bullet OH$ radical:

$$e^-_{aq} + N_2O \rightarrow {}^\bullet OH + OH^- + N_2 \quad (2)$$

Generation of different oxidizing radicals has been extensively covered in the literature, and hence only a brief mention is made here (*30*). Azide radicals ($N_3^\bullet$) were generated by irradiating an N_2O-saturated aqueous solution containing 1×10^{-2} M sodium azide, where all of the $^\bullet OH$ radicals were exclusively converted to N_3 ($^\bullet OH + N_3^- \rightarrow OH^- + N_3^\bullet$) with the radiation chemical yield of 0.56 μmol J^{-1}. Halocarbonperoxyl radicals ($CCl_3O_2^\bullet$) were generated (radiation chemical yield of 0.65 μmol J^{-1}) by pulse radiolysis of aerated aqueous solutions of 48% (v/v) 2-propanol and 4% (v/v) carbon tetrachloride (*31*). Nitrogen dioxide radicals ($NO_2^\bullet$) were generated by the reaction of $^\bullet OH$ radicals with nitrite ions ($^\bullet OH + NO_2^- \rightarrow OH^- + NO_2^\bullet$). For this aqueous solutions containing 0.01 M KNO_2, at pH 7, saturated with N_2O were pulse radiolyzed. For all of these studies, the ellagic acid concentration was kept at 50−100 μM. To determine the rate constant for the reaction of a specific radical with ellagic acid, the rate of formation (k_{obs}) of the ellagic acid radical was monitored at a suitable wavelength as a function of ellagic acid concentration and the bimolecular rate constant was determined from the linear plot of k_{obs} versus ellagic acid concentration.

Lipid peroxidation was carried out by the γ-radiolysis of rat liver microsomes. Microsomes were prepared by sacrificing male albino Wistar strain rats (180−200 g) by decapitation. The livers were quickly removed and washed with cold isolation medium (0.25 M sucrose containing 10 mM Tris-HCl, pH 7.4). A 10% liver homogenate was made in isolation medium, and microsomes were isolated by differential centrifugation as described elsewhere (*31*, *32*). All of the operations were done at 0−4 °C.

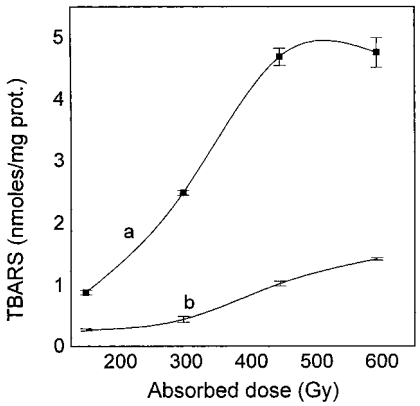

Figure 1. Inhibition of radiation-induced lipid peroxidation by ellagic acid in N_2O-purged rat liver microsomes, assessed by TBARS as a function of absorbed dose in the absence (a) and presence (b) of 20 μM ellagic acid.

Steady state γ-radiolysis was carried out using a ^{60}Co γ-source with a dose rate of 15 Gy min^{-1} as measured by standard Fricke dosimetry. γ-Radiation-induced lipid peroxidation at different doses was studied in aerated and N_2O-purged microsomal solution in the absence and presence of ellagic acid at the physiological pH of 7.4 (phosphate buffer). The detailed methodology used in the lipid peroxidation is given in references *31* and *32*. Protein was estimated according to the Lowry method (*33*). The extent of lipid peroxidation was estimated in terms of thiobarbituric acid reactive substances (TBARS) using 15% w/v trichloroacetic acid, 0.375% w/v TBA, 0.25 N hydrochloric acid, and 0.05% w/v BHT as TBA reagent and measuring the absorbance at 532 nm ($\epsilon_{532} = 1.56 \times 10^5$ M^{-1} cm^{-1}).

Peroxynitrite was synthesized by the ozonolysis of alkaline sodium azide solution, at 0−4 °C according to the procedure given in ref *34*. The peroxynitrite solution thus prepared was stored at −20 °C and used within 3−4 weeks. The concentration of peroxynitrite was determined by measuring its absorbance at 302 nm, using the extinction coefficient of 1650 M^{-1} cm^{-1}.

Stopped-flow experiments were carried out using an SX-18.MV multimixing stopped-flow reaction analyzer from Applied Photo Physics Ltd. It was used in single mixing mode, and the reaction was monitored using absorption detection. In this, syringe I contained peroxynitrite in an alkaline buffer (solution A) and syringe II contained ellagic acid in Na_2HPO_4 buffer (solution B). The final pH of the solution was measured after equal volumes of solutions A and B had been mixed.

RESULTS AND DISCUSSION

Inhibition of Lipid Peroxidation. γ-Radiolysis of N_2O-saturated aqueous microsomal solution produces mainly $^\bullet OH$ radicals, which can induce peroxidation in the membrane lipids. **Figure 1a** represents an increase in the $^\bullet OH$-induced lipid peroxidation in microsomes measured in terms of TBARS, with radiation doses starting from 100 to 600 Gy. However, addition of 20 μM ellagic acid significantly suppressed TBARS level as indicated by **Figure 1b**.

Figure 2 shows the effect of ellagic acid on TBARS formation at different concentrations at a constant radiation dose of 296 Gy. The concentration of ellagic acid was varied from 2 to 30 μM. The extent of inhibition in lipid peroxidation was found to increase with an increase in the concentration of ellagic acid. At 2 μM ellagic acid concentration, 42% inhibition was obtained, whereas 24.8 μM ellagic acid exhibited 76% protection. From **Figure 2**, the IC_{50} value (amount of ellagic acid required to inhibit peroxidation by 50%) was determined to be

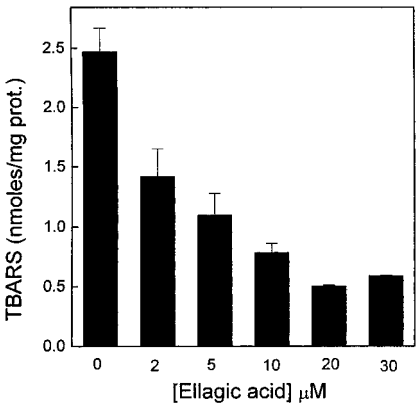

Figure 2. Effect of concentration of ellagic acid on radiation-induced lipid peroxidation, estimated as TBARS produced when N_2O-purged microsomes were exposed to a dose of 296 Gy.

3.14 μM. These results indicate that ellagic acid acts as a good antioxidant even at very low concentrations.

Free Radical Scavenging Ability and DPPH Assay. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay is a well-known method to estimate antioxidant activity (*35*−*37*) of different compounds. Earlier Zafrilla et al. (*6*) also studied the DPPH scavenging ability of ellagic acid and determined the Trolox equivalent capacity (TEAC) of ellagic acid to be 2.6 mM. In the present paper, we have also determined the free radical scavenging activity of ellagic acid by following the absorbance of DPPH radical at 517 nm. The decrease in absorbance was monitored at different concentrations of the ellagic acid, from which the amount required to bring down the absorbance to half its original value is determined. Thus, at a DPPH concentration of 227 μM, the half-reduction value for ellagic acid was found to be 15 μM. This study supports the earlier observation by Zaffrilla et al. that ellagic acid possesses free radical scavenging ability and the antioxidant action is mainly due to the scavenging of the free radicals. Detailed studies on actual ROS and RNS scavenging activity of ellagic acid are discussed in the following sections.

Ground State Optical Absorption Studies. Ellagic acid has four phenolic OH groups with a fused benzofuran structure. Hence, it is expected to have at least four pK_a values corresponding to the various prototropic equilibria (**Scheme 1**).

Scheme 1

$$EG(OH)_4 \underset{pK_a}{\rightleftharpoons} EG(OH)_3(O^-) + H^+ \underset{pK_a}{\rightleftharpoons} EG(OH)_2(O^-)_2 + H^+ \underset{pK_a}{\rightleftharpoons} EG(OH)(O^-)_3 + H^+ \underset{pK_a}{\rightleftharpoons} EG(O^-)_4 + H^+$$

Accordingly, the absorption spectrum changed when the pH was varied from 4.5 to 12. By following the changes in absorbance at 360 nm as a function of pH, two distinct pK_a values could be evaluated as 6.3 and 11.2. Other pK_a values were probably unresolvable but are expected to be in this pH range only.

Pulse Radiolysis Studies. *Azide Radical Reactions.* Azide radicals are known to react by electron transfer only and generally produce radical cations of the substrates with which they react. In the case of phenols, the radical cations, produced by electron transfer, lose a proton as the pK_a of the radical is <0, to finally produce phenoxyl radicals. Hence, we studied the reactions of azide radicals with ellagic acid at widely different pH values to understand exactly the characteristic absorption spectrum of its phenoxyl radicals. The reaction of

Table 1. Bimolecular Rate Constant for the Reaction of N_3 and $CCl_3O_2^\bullet$ Radicals with Ellagic Acid at Different pH Values

	bimolecular rate constant (M^{-1} s^{-1}) with	
pH	$N_3^\bullet$	$CCl_3O_2^\bullet$
4.5		4.5×10^7
7	3.7×10^9	1.4×10^8
8.5	4.6×10^9	3.2×10^8
10.7	8.1×10^9	4.2×10^8

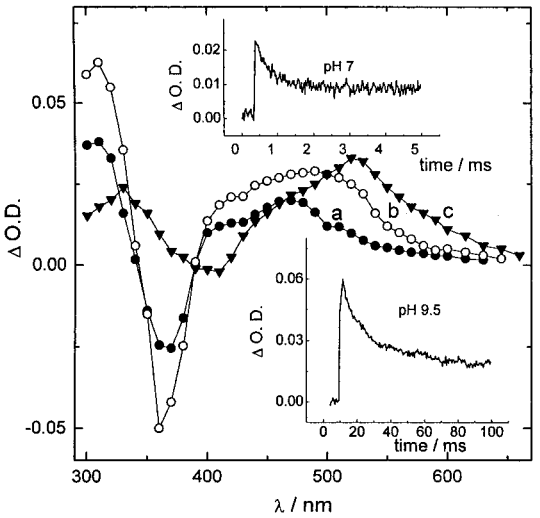

Figure 3. Difference absorption spectra of the transient obtained on pulse radiolysis of N_2O-saturated aqueous solution containing 0.025 M sodium azide and 50 μM ellagic acid at pH 7 (a), pH 8.5 (b), and pH 10.5 (c). Absorbed dose was 10 Gy per pulse. Inset shows decay traces at pH 7 and 9.5 at 470 and 520 nm, respectively.

$N_3^\bullet$ radicals with ellagic acid (reaction 3) was studied at pH 7, 8.5, 10.3, and 10.8.

$$EG(OH)_4 + N_3^\bullet \rightarrow N_3^- + [EG(OH)_4]^{\bullet +} \rightarrow EG(OH)_3O^\bullet + H^+ \quad (3)$$

The bimolecular rate constants for this reaction at different pH values were found to vary from 3.7×10^9 to 8.0×10^9 M^{-1} s^{-1} (**Table 1**). The radicals in this pH range absorb in the 300−600 nm wavelength region with absorption maximum varying from 470 to 520 nm. **Figure 3** gives the transient spectra at these pH values. From **Figure 3** it can be noticed that the absorption spectrum varies significantly with the pH. We therefore made an attempt to determine the pK_a of the radicals. The absorbance changes at 480 and 520 nm were followed in the pH range from 4 to 13. The absorbance at these wavelengths was corrected for the loss in the parent absorption at each pH. **Figure 4** shows the changes in the corrected absorbances at 480 and 520 nm at different pH values, from which two different pK_a values of 5.2 and 10.3 were deduced for the one-electron oxidized radicals of ellagic acid (**Scheme 2**).

Scheme 2

$$EG(OH)_3O^\bullet \underset{pK_a}{\rightleftharpoons} EG(OH)_2O^-O^\bullet + H^+ \underset{pK_a}{\rightleftharpoons} EG(OH)(O^-)_2O^\bullet + H^+$$

One-electron oxidation of ellagic acid produces phenoxyl radicals, and the two pK_a values of the radicals indicate subsequent loss of proton from the remaining OH groups

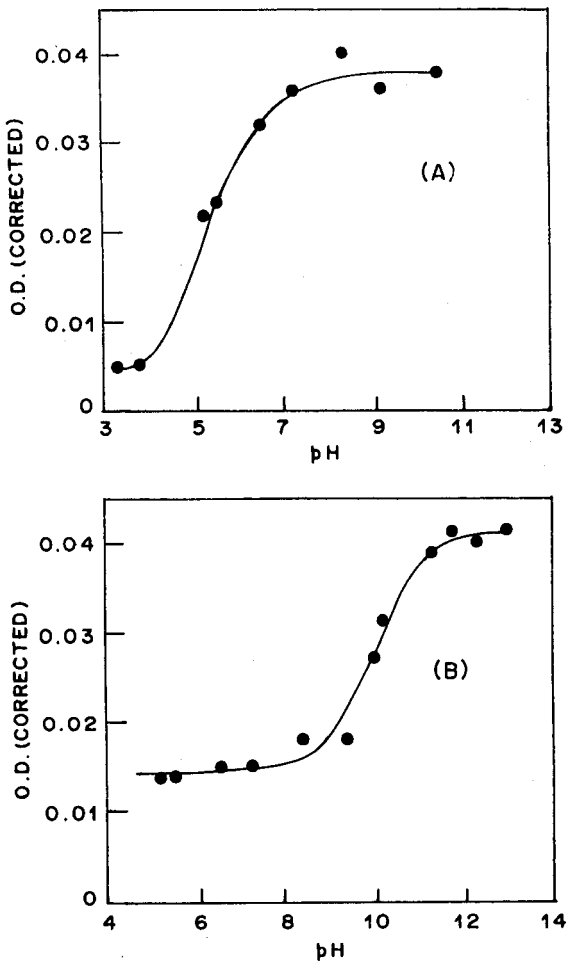

Figure 4. pK_a curves showing the variation in the absorbance of the radicals of ellagic acid at 480 nm (A) and 520 nm (B) as a function of pH. The radicals were produced by electron transfer to azide radicals using pulse radiolysis. Dose = 11 Gy per pulse.

(**Scheme 2**). The radical decay was also found to depend on the pH. The inset of **Figure 3** shows the decay of oxidized ellagic acid radicals at pH 7 and 9.5. At pH 9.5, the radical lifetime increased significantly due to deprotonation of the hydroxyl group.

Hydroxyl Radical Reactions. Hydroxyl radicals are known to be one of the most important biologically damaging species and have been implicated in the etiology of several diseases (*15*). Many important phenolic antioxidants have been found to be good scavengers of hydroxyl radicals. We studied the reactions of $^{\bullet}OH$ radical with ellagic acid at pH 7; the rate of the reaction was found to be diffusion controlled (8.9×10^9 M^{-1} s^{-1}). The ellagic acid radicals at this pH absorb in the wavelength region from 300 to 600 nm with absorption maximum in the 450−470 nm region. The absorption spectrum closely matches with that observed for the azide radical reaction (**Figure 3**), suggesting that the OH radical brings about oxidation only and generates phenoxyl radicals (reaction 4) as in the case of azide radicals.

$$EG(OH)_4 + {}^{\bullet}OH \rightarrow OH^- + [EG(OH)_4]^{\bullet+} \rightarrow EG(OH)_3O^{\bullet} + H^+ \quad (4)$$

Reaction of CCl_3O_2 Radicals. Peroxyl radicals are important oxidants produced during the peroxidation of lipids. In the present study, halocarbon peroxyl radicals are used as model peroxyl radicals. The reactions of $CCl_3O_2^{\bullet}$ radicals were studied at pH 4.5, 7, 8.5, and 10.7. The transient spectra at different pH values looked similar to those produced by the azide radical reaction. The rate constants for the reactions were found to vary from 4.5×10^7 to 4.2×10^8 M^{-1} s^{-1} when the pH was varied from pH 4.5 to 10.7 (**Table 1**). The reaction of $^{\bullet}OH$ and $N_3^{\bullet}$ radicals with ellagic acid could not be studied at pH <7 due to solubility limitations. However, in the CCl_4/2-propanol matrix, the solubility of ellagic acid increased even at pH <7, and rate constant values could be measured accurately. These results suggest that the peroxyl radicals react via similar reaction as $^{\bullet}OH$ radicals (reaction 4). Low rate constant values may also suggest the reaction occurs by hydrogen abstraction (reaction 5).

$$EG(OH)_4 + CCl_3O_2^{\bullet} \rightarrow EG(OH)_3O^{\bullet} + CCl_3O_2H \quad (5)$$

In both the cases, the phenoxyl radical is produced and the nature of the spectrum is similar. At pH <4.5, all of the phenolic OH groups on ellagic acid are protonated and the reaction is by hydrogen abstraction, and hence the reaction rate constants are low. At pH >10, the anionic form is easily available for oxidation. These results suggest that ellagic acid is a good scavenger of peroxyl radical, and at physiological pH, the partially protonated form is moderately reactive.

Reaction of $NO_2^{\bullet}$ Radicals. The $NO_2^{\bullet}$ radical is one of the most important free radical constituents of cigarette smoke and is a major atmospheric pollutant (*15*, *38*). Reaction of the $NO_2^{\bullet}$ radical with ellagic acid was studied at pH 7 and 8.5, and the rate constants for the reaction of $NO_2^{\bullet}$ with elllagic acid were determined to be 2×10^7 and 8×10^7 M^{-1} s^{-1}, respectively. The transient absorption spectrum of the radical compared well with that by azide radical reaction, suggesting that this reaction too produced the oxidation product. The rate constants are comparable to those of many well-known antioxidants such as vitamin E and β-carotene (*39*).

Reaction with $ABTS^{\bullet-}$ Radicals. $ABTS^{\bullet-}$ radicals were generated by the reaction of azide radicals with $ABTS^{2-}$. They absorb at 600−650 nm and do not show any appreciable decay even in several seconds. The one-electron potential for the formation of $ABTS^{\bullet-}$ from $ABTS^{2-}$ is 0.68 V versus NHE at pH 7, and its potential remains constant over a wide pH range (*40*). We studied electron transfer between $ABTS^-$ and ellagic acid at pH 7 and 8.5. For these studies the concentration of $ABTS^{2-}$ was kept at 1 mM and that of ellagic acid was varied from 25 to 150 μM. The rate of the reaction was monitored by following the decay at 600 nm due to the $ABTS^-$ radical and formation of ellagic acid radical at 480 nm. **Figure 5** gives the absorption−time profiles for the decay of $ABTS^{\bullet-}$ in the presence of ellagic acid and formation of ellegic acid radicals (parts a and b, respectively) on reaction of $ABTS^{\bullet-}$ with ellagic acid. From the slope of the linear plot for the rate of decay at 600 nm versus concentration of ellagic acid, the rate constants were determined to be 1.8×10^8 and 4.8×10^8 M^{-1} s^{-1} at pH 7 and 8.5, respectively. Due to limited solubility of ellagic acid, no clear equilibrium and reversibility of electron transfer could be established between the two. However, the overall reaction indicates that there is electron transfer from $ABTS^{\bullet-}$ to ellagic acid and the one-electron potential of ellagic acid is <0.68 V versus NHE.

Reaction of Ellagic Acid Radical with Ascorbic Acid. Now that the upper limit for the one-electron potential of ellagic acid radical is fixed as 0.68 V, we studied its reactivity with ascorbic acid. Ascorbic acid is easily oxidizable with the one-electron potential of 0.320 V versus NHE and is very often considered

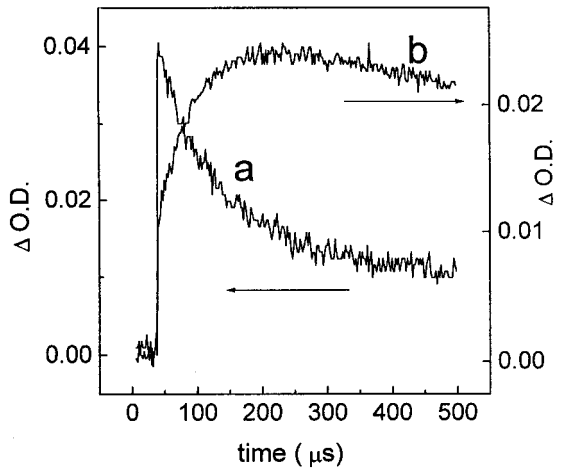

Figure 5. Absorption–time plots showing (a) decay of $ABTS^{-}$ radicals at 600 nm in the presence of 50 μM ellagic acid generated by pulse radiolysis of N_2O-saturated aqueous solutions containing 1 mM $ABTS^{2-}$, 25 mM azide, and 50 μM ellagic acid at pH 7 and (b) formation of ellagic acid radicals at 480 nm in the same system.

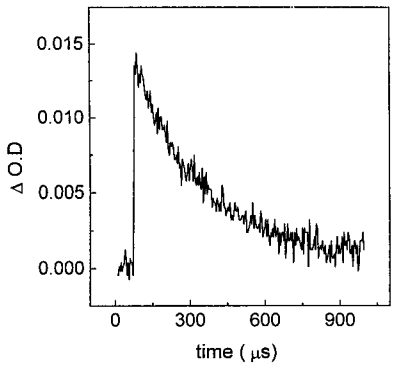

Figure 6. Decay of ellagic acid radicals at 480 nm in the presence of ascorbate produced after pulse radiolysis of N_2O-saturated aqueous solutions containing 100 μM ellagic acid, 25 mM azide, and 300 μM ascorbate at pH 7.

to be a terminal oxidant (*15*). This reaction was studied at pH 7 and 9. For these studies, the concentration of ellagic acid was fixed at 1×10^{-4} M and that of ascorbic acid was varied from 1×10^{-4} to 5×10^{-4} M. The rate of this reaction was monitored by following the decay of ellagic acid radicals at 480 nm as a function of ascorbic acid concentration. In the absence of ascorbic acid, ellagic acid radicals do not show any appreciable decay, but in its presence, the decay increased and changed to pseudo-first-order kinetics. **Figure 6** gives the absorption−time plot for the decay of ellagic acid radicals at 480 nm in the presence of ascorbic acid. From this, the rate constant for this reaction was determined to be 9.8×10^6 M^{-1} s^{-1}. The above two reactions suggest that the one-electron potential of the ellagic acid at pH 7 may be between 0.68 and 0.32 V versus NHE.

Reaction with Peroxynitrite. Peroxynitrite is produced by the diffusion-controlled reaction of NO with $O_2^{\bullet -}$ (*41*). It is a very powerful oxidant and is known to cause both one- and two-electron oxidation reactions (*42*−*44*). It has been found to be responsible for the NO-mediated toxicity. It is known to cause damage to cellular biomolecules, leading to cell death. Antioxidants such as vitamin E, vitamin C, and glutathione are known to inhibit peroxynitrite-induced damage to lipids, proteins, DNA, etc. To test the efficacy of ellagic acid to scavenge peroxynitrite, the reaction of peroxynitrite with ellagic

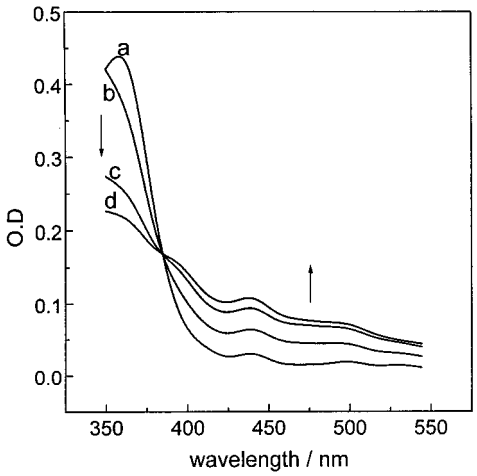

Figure 7. Time-resolved spectra obtained after mixing 200 μM peroxynitrite with 50 μM ellagic acid at pH 8.5 after 0.5 s (a), 3.5 s (b), 10 s (c), and 20 s (d).

acid was studied at pH 8.5, using a stopped-flow spectrometer. **Figure 7** gives the time-resolved spectra showing the decrease in the absorbance at 370 nm due to ellagic acid and a newly forming species absorbing in the 400−500 nm region. The rate constant for this reaction was determined to be 2×10^3 M^{-1} s^{-1}. The rate constant is comparable to that of many well-known antioxidants such as glutathione and Trolox. Peroxynitrite reactions involve both NO_2 and $^{\bullet}OH$ radicals. The reaction with ellagic acid may take place both at phenolic OH group and also at the lactone carbonyl group, and the products of reaction could be nitration, oxidation, and hydroxylation. The formation of products having red-shifted absorption indicates that nitration is one of the important reactions with peroxynitrite.

ESR Spectrum of Ellagic Acid Radical. The ESR spectrum of the phenoxyl radicals of ellagic acid was recorded in alkaline solutions (pH 9.5) after oxidation by horseradish peroxidase (HRP)/H_2O_2. Ellagic acid was dissolved in methanol and mixed with HRP/H_2O_2 in the ESR cell just before the ESR spectrum was recorded. The recording was completed immediately after mixing. The ESR spectrum is structureless, and hence no hyperfine coupling constants could be determined (figure not given). A similar spectrum was obtained upon autoxidation of the ellagic acid. Using the DPPH radical as a marker, the g value of the ellagic acid radical was determined to be 2.0044.

Conclusions. Ellagic acid is a plant polyphenol gifted with antimutagenic, anti-inflammatory, anticlastogenic, anticarcinogenic, and antioxidant activities. However, there are scant reports in the literature about its free radical reactions and the physicochemical properties of its radicals. Therefore, in the present paper, we first tested its antioxidant activity by following its ability to inhibit lipid peroxidation induced by γ-radiation in microsomes. Our results clearly show that ellagic acid is very effective even at micromolar concentrations in inhibiting lipid peroxidation. One of the factors responsible for the antioxidant action is its ability to scavenge free radicals. The radicals of ellagic acid were produced by the reaction with biologically important radicals in the microsecond to millisecond time scales using pulse radiolysis and stopped-flow spectrometry. Ellagic acid was found to scavenge ROS and RNS such as hydroxyl radicals, perxoyl radicals, NO_2 radicals, and peroxynitrite with rate constants comparable to those of many well-known antioxidants such as vitamin E and vitamin C. The one-electron reduction potential, a quantity indicating the energy required to produce ellagic acid radicals from ellagic acid by electron

transfer, was found to be between 0.68 and 0.32 V versus NHE. The one-electron potential of several phenolic antioxidants such as flavonoids and tocopherol are in this potential range. The lifetimes of the radicals were found to be pH dependent, suggesting that there may be a possible exchange of proton between the phenoxyl radical and the adjacent hydroxyl group. The phenoxyl radicals were also characterized by the ESR spectrum. No hyperfine splitting could be resolved for the radicals.

Ellagic acid exhibits minimum solubility in water, but its solubility increases in organic solvents such as methanol and DMSO. This suggests that ellagic acid can act as a good lipophilic antioxidant. This property along with its ability to scavenge peroxyl radicals makes it a probable candidate for the chain-breaking antioxidant. Recent studies indicate that ellagic acid binds to DNA by intercalating with the minor groove because of its planar structure. All of these important and unique characteristics of ellagic acid support the observed fact that it inhibits lipid peroxidation at much lower concentrations than vitamin E. However, detailed studies on the bioavailability of ellagic acid and its absorption capacity from diet after consumption need to be further addressed. Low dietary intake of natural antioxidants has been associated with significantly increased risk of chronic diseases such as cancer. The search for new natural antioxidants, especially from dietary origin, is therefore the need of the hour. Because polyphenols are important constituents of dietary food, ellagic acid is a promising antioxidant with potential applications.

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