Photochemical Reactions of Antioxidant Sesamol in Aqueous Solution[1]

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ABSTRACT: Antioxidant intermediate radicals generated by continuous ultraviolet (UV) and pulsed UV laser irradiation of sesamol in aqueous solutions were investigated. Radical species with the intensity ratio of 1:4:6:4:1 were observed by using continuous wave electron paramagnetic resonance (EPR) with continuous UV irradiation. The radical was also observed after the irradiation. To observe the intermediate radical right after the excitation, Fourier transform EPR (FT EPR) was used. Trace amounts of the same radical were also detected by FT EPR with pulsed UV laser. Based on the hyperfine coupling constant (2.3 G) and g-value (2.0048), together with FT EPR results, this radical was found to be a benzoquinone anion radical. The radical might have been produced *via* multistep reactions of the excited sesamol in aqueous solution, and it was stable for at least several minutes. In addition to the benzoquinone radical, the formation and dynamics of the sesamolyl radical were investigated by using FT EPR. FT EPR showed the detailed behavior of the radical. Characterization of the intermediate is important to evaluate the antioxidant sesamol.

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KEY WORDS: Antioxidant, EPR, free radical, FT EPR, sesamol.

Antioxidants are useful not only for preserving foods but also for preventing free radical-induced damage, for example, as documented in the field of medicine. Short-lived free radicals (e.g., oxygen radicals) are involved in various diseases such as coronary disease and oxidative stress (1,2). Antioxidants react with short-lived free radicals and become the corresponding intermediates (free radicals) as shown in Scheme 1. If the intermediate is stable or less reactive, the damage induced by the short-lived radical can be minimized. However, the antioxidant intermediates are not yet well understood.

Electron paramagnetic resonance (EPR) is a reliable technique to identify and characterize the intermediates. We utilized two EPR techniques, CW EPR (continuous wave EPR) and FT EPR (Fourier transform EPR), to characterize the intermediate radical of sesamol. Steady-state radicals are mea-
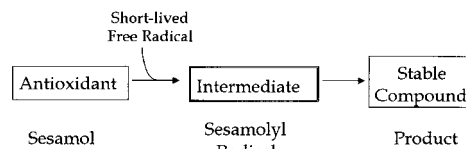
SCHEME 1

sured by conventional CW EPR. The FT EPR technique is a pulsed technique to measure the formation and dynamics of intermediates on an order of nanoseconds to microseconds after excitation.

Antioxidant activity of sesamol from sesame seed was first reported by Budowski *et al.* (3,4). The activity of sesamol in lard, vegetable oil, and hydrogenated oil samples, based on the oil stability, was shown. Our earlier studies on the natural antioxidant sesamol in organic solvents showed that two types of radical species were produced by ultraviolet (UV) irradiation (5). One was sesamolyl radical, and the other was assumed to be a dimer radical. The latter species appeared when the solution was irradiated for a longer time. Furthermore, we showed that antioxidant activity of sesamol might be attributed to the stability (or reactivity) of the intermediate sesamolyl radical. Recently, our FT EPR study showed that photodissociation of OH of sesamol in aqueous solution produced sesamolyl radical and cyclohexadienyl-type radical (6), which was not observed by CW EPR. Despite nearly a half century of investigation, the molecular role of antioxidant sesamol has not been fully revealed.

In this study, the intermediate radical of antioxidant sesamol in aqueous solutions was characterized further by CW EPR and FT EPR. Various intermediate radicals observed by both EPR techniques were described. In addition, an empirical reaction pathway of sesamol excited by UV light is discussed.

EXPERIMENTAL PROCEDURES

Materials. Sesamol, or 3,4-methylenedeoxyphenol, was purchased from Aldrich Chemical Company (Milwaukee, WI). Highest quality obtainable petroleum benzene was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Sesamol was puri-

fied by recrystallization in petroleum benzene (5). Solutions of sesamol with concentrations ranging from 2 to 10 mM were prepared with water from a Millipore Milli-Q purification system (Bedford, MA). The pH of the solutions was adjusted with KOH or NaOH.

Absorption measurements. A Hitachi U-3210 spectrophotometer (Tokyo, Japan) was used to obtain absorption spectra. The quartz absorption cell had a path length of 1 cm.

CW EPR measurements. Prior to EPR measurements, argon gas was bubbled through sesamol solutions for the purpose of deoxygenation. A flat cell (0.3 mm path length) with a 60-μL sample volume was used for the measurement. Sample solutions were flowing during the measurements. EPR signals were measured by FE1X X-band EPR spectrometer (JEOL, Tokyo, Japan). This spectrometer was equipped with a cylindrical TE_{011} mode cavity. Aqueous solutions were illuminated within the EPR cavity with a xenon short arch lamp (Ushio model UXL 500D, 500W; Tokyo, Japan). An interference filter (290 nm maximal transmission) was placed in front of the EPR cavity. Typical EPR conditions were as follows: microwave frequency, 9.35 GHz; microwave power, 10 mW; modulation amplitude, 0.32 Gauss (G); time constant, 1 s; scan rate, 3.125 G/min.

FT EPR measurements. FT EPR measurements were performed with the spectrometer described by Levstein and van Willigen (7). The signal response of the $\pi/2$ microwave pulse (~15 ns) that was applied to the sample was detected in quadrature with a CYCLOPS phase-cycling routine (8). Aqueous solutions were purged with argon gas and pumped through a flat quartz cell held in the microwave cavity. The sample solutions were excited with UV light (308 nm) from a Lambda Physik EMG103 MSC XeCl excimer laser (30-60 mJ/pulse, 40 Hz repetition rate; Göttingen, Germany). The FID (free induction decays) were recorded during a series of delay times (10 ns < τ_d < 50 μs) between laser excitation and microwave pulse. The amplitudes, phases, and linewidths of resonance peaks were derived from the FID with a linear prediction singular value decomposition analysis routine (9). All measurements were performed at ambient temperature.

RESULTS AND DISCUSSION

A CW EPR spectrum, obtained by continuous UV excitation of sesamol in aqueous solution at pH 7, is shown in Figure 1. The spectrum had an intensity ratio of 1:4:6:4:1, which indicated four equivalent hydrogens. The hyperfine coupling constant and *g*-value were 2.3 G and 2.0048, respectively. The EPR constants and intensity ratio of the radical were in good agreement with reported values for benzoquinone anion (BQ) radical (10). The same but weaker signal stayed for several minutes after the UV light was turned off. The BQ radical was also observed in SDS (sodium dodecyl sulfate) micellar solution by the UV irradiation of sesamol. The radical in SDS also stayed for several minutes. Here, detailed analysis showed that the BQ radical in H_2O was not identical to the five-line spectrum previously reported in 1-propanol (5). EPR con-
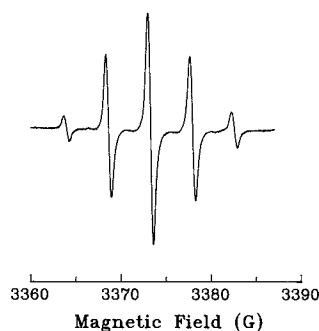

FIG. 1. Continuous wave electron paramagnetic resonance (EPR) spectrum obtained by continuous ultraviolet irradiation of sesamol in H_2O at pH 7. EPR constants are a_H = 2.3 G and *g* = 2.0048.

stants (*g* = 2.001; hyperfine coupling constant ~2.9 G) of the radical in 1-propanol were assumed to represent a dimer radical (5).

To obtain a clue for the process of producing the stable quintet radical by continuous UV irradiation in aqueous solution, we performed FT EPR measurements. CW EPR cannot differentiate the intermediates right after excitation. FT EPR can provide useful information on the formation and dynamics of radicals on an order of nanoseconds to microseconds after the pulsed laser excitation. Sesamol was directly excited by the eximer laser at 308 nm because it showed strong absorption near 308 nm, as shown in Figure 2. This was the ideal system to investigate the intermediates without using a photosensitizer.

The observed FT EPR spectrum of sesamol in H_2O at pH 7 is presented in Figure 3. The signal pattern from high frequency (low magnetic field) to low frequency (high magnetic field) was E (emission) to A (absorption). The intensity change from E to A occurred at the center of the frequency. Thus, the indicated FT EPR spectral pattern was a contribution from the radicals with the same *g*-value. Simulation of
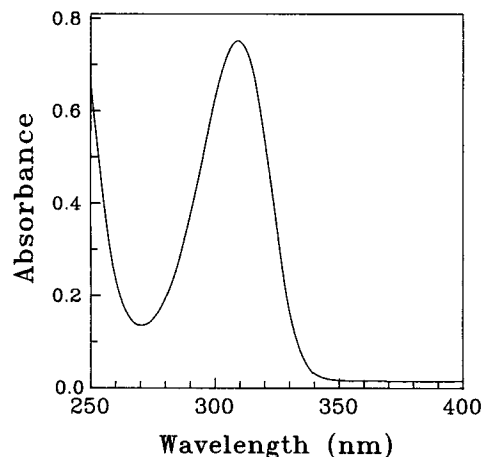

FIG. 2. Absorption spectrum of sesamol in water at pH ~7. Concentration of sesamol was 3.7×10^{-4} M.
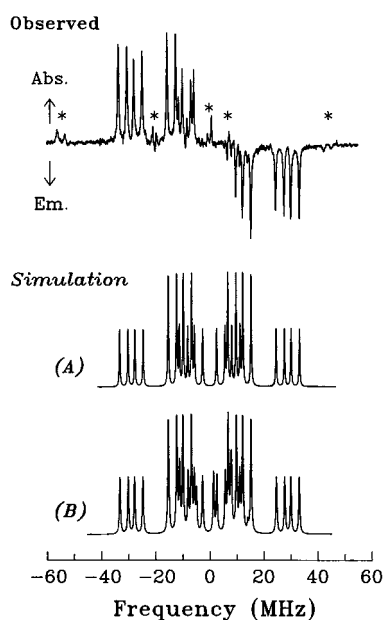
FIG. 3. Top spectrum is Fourier transform (FT) EPR spectrum of sesamol in H_2O (pH 7) observed after 1 μs laser delay. Bottom spectra are simulations: (A) sesamolyl radical and (B) combination of sesamolyl radical and benzoquinone anion radical. Asterisk (*) denotes additional feature in the observed spectrum. For abbreviation see Figure 1.

the observed FT EPR spectrum is presented in Figure 3A. The hyperfine values used for the simulation were as follows: 2-position of H, 1.95 G; methylene 2H, 6.5 G; 5-position of H, 1.1 G; 6-position of H, 8.0 G. These values are in good agreement with the reported value for sesamolyl radical (11). The simulation of the FT EPR spectrum shows that the radicals with the same g-value are sesamolyl radicals. Therefore, the E/A polarization (spectral pattern) is created by the sesamolyl-sesamolyl radical pair.

The hyperfine coupling value (6.5 G) of methylene hydrogen suggests delocalization of unpaired spin onto the fused ring. The electron spin delocalizes due to overlap between the π-cloud of the benzene ring and oxygen $2p_z$ orbitals as depicted in Scheme 2. This delocalization of the unpaired spin makes the intermediate relatively stable.

Intensity change of the sesamolyl radical as a function of laser delay time is plotted in Figure 4. The solid line fits the FT EPR data and is described by the equation:
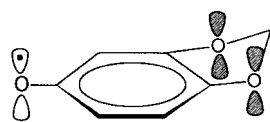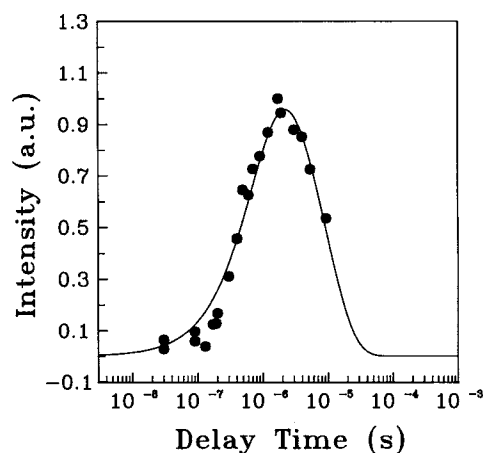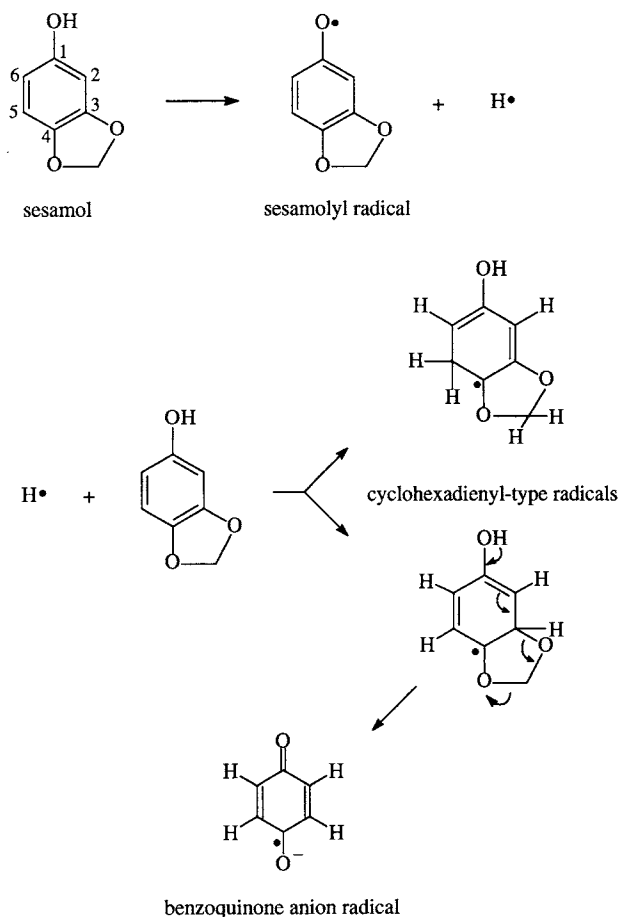

Sesamolyl Radical

SCHEME 2

FIG. 4. FT EPR intensity of sesamolyl radical observed at pH 7 as a function of laser delay time. Solid line indicates the calculated curve for the data. Abbreviation: a.u., arbitrary units; for other abbreviations see Figures 1 and 3.

$$I(t) = C\left[-\exp(-k_f t) + \exp(-k_d t)\right] \qquad [1]$$

where k_f and k_d are the formation and decay rates of the radical, respectively. The formation and decay rates were 1.2×10^6 and 1.1×10^5 s^{-1}, respectively. The intensity reached a maximum at ~2 μs after the laser delay. The relatively slow signal rise was consistent with the radical pair mechanism, which was discussed earlier. The calculated curve predicts that the intensity of the sesamolyl radical becomes nearly zero at ~0.01 s, at which point conventional CW EPR cannot measure the intermediate. The present results suggest that the sesamolyl radical is next to the sesamolyl radical for microseconds. Then, the radicals diffuse and may react with excess sesamol in the solution. It has been suggested that mild oxidation of sesamol with the short-lived free radicals by hydrogen peroxide-peroxidase produced sesamol dimer as a result of sesamol reaction with excess sesamol (12).

Even though the simulation as in Figure 3A indicated the presence of sesamolyl radical in water after the excitation, there were spectral discrepancies between the observed and simulated spectrum. The observed spectrum had additional features, marked with asterisks in Figure 3. In other words, other radical species were detected together with sesamolyl radical. At least two possible features could have overlapped with the signal of the sesamolyl radical. One can be a cyclohexadienyl-type radical (13). The radical results from the addition of H· to sesamol. The H· is produced by photodissociation of sesamol as described by the hypothetical reaction in Scheme 3. The cyclohexadienyl-type radicals are so characteristic that identity of the structure of this radical is unambiguous. The hyperfine structures appear very large as a result of π-conjugation. Thus, the outermost hyperfine splitting of ~96 MHz (~34 G) in Figure 3 can be attributed to CH_2 protons of the cyclohexadienyl-type radical, and ~35 MHz (~12.5 G) is due to CH proton in the ring. These values are in reasonable agreement with reported values for the cyclohexadienyl-type radical (13).

OH

sesamol

O•

sesamolyl radical

+ H•

OH

cyclohexadienyl-type radicals

H• +

OH

OH

benzoquinone anion radical

SCHEME 3

REFERENCES

1. Gale, C., C.N. Martyn, P.D. Winter, and C. Cooper, Vitamin C and Risk of Death from Stroke and Coronary Heart Disease in Cohort of Elderly People, *Br. Med. J. 310*:1563–1568 (1995).
2. Keith, M., A. Germmayegan, M.J. Sole, R. Kurian, A. Robinson, A.S. Omran, and K.N. Jeejeebhoy, Increased Oxidative Stress in Patients with Congestive Heart Failure, *J. Am. Coll. Cardiol. 31*:1352–1356 (1998).
3. Budowski, P., Sesame Oil III. Antioxidant Properties of Sesamol, *J. Am. Oil Chem. Soc. 27*:264–267 (1950).
4. Budowski, P., F.G.T. Menezes, and F.G. Dollear, Sesame Oil. V. The Stability of Sesame Oil, *Ibid. 27*:377–380 (1950).
5. Nakagawa, K., S. Tero-Kubota, Y. Ikegami, and N. Tsuchihashi, EPR and TR EPR Spectroscopic Studies of Antioxidant Sesamolyl and Related Phenoxyl Radicals, *Photochem. Photobiol. 60*:199–204 (1994).
6. Bussandri, A., H. van Willigen, and K. Nakagawa, FT EPR of the pH Dependence of Sesamol in Aqueous Solution, *Appl. Magn. Reson. 17*:577–586 (1999).
7. Levstein, P.R., and H. van Willigen, Fourier Transform-Electron Paramagnetic Resonance Study of the Photochemical Reaction of Acetone with 2-Propanol, *J. Chem. Phys. 95*:900–908 (1991).
8. van Willigen, H., P.R. Levstein, and M.H. Ebersole, Application of Fourier Transform Electron Paramagnetic Resonance in the Study of Photochemical Reaction, *Chem. Rev. 93*:173–197 (1993).
9. de Beer, R., and D. van Ormondt, Resolution Enhancement in Time Domain Analysis of ESEEM, in *Advanced EPR: Applications in Biology and Biochemistry*, edited by A.J. Hoff, Elsevier, Amsterdam, 1989, pp. 135–173.
10. Hales, B.J., Immobilized Radicals. I. Principal Electron Spin Resonance Parameters of the Benzosemiquinone Radical, *J. Am. Chem. Soc. 97*:5993–5997 (1975).
11. Dixon, W.T., and D. Murphy, The Electron Spin Resonance Spectra of Alkyl Aryl Ether Radical Cations, *J. Chem. Soc., Perkin Trans. 2* 1823–1828 (1976).
12. Kurechi, T., K. Kikugawa, and S. Aoshima, Studies on the Antioxidants. XIV. Reaction of Sesamol with Hydrogen Peroxide-Peroxidase, *Chem. Pharm. Bull. 29*:2351–2358 (1981).
13. Jeevarajan, A.S., and R. Fessenden, ESR Study of the Photochemistry of Benzoic Acid Derivatives, *J. Am. Chem. Soc. 114*: 10461–10470 (1992).
14. Cocivera, M., M. Tomkiewicz, and A. Groen, Electron Paramagnetic Resonance and Nuclear Spin Polarization Study of the Photooxidation of Phenol in Water, *Ibid. 94*:6598–6604 (1972).
15. Fukuda, Y., M. Nagata, T. Osawa, and M. Namiki, Contribution of Lignan Analogues to Antioxidative Activity of Refined Unroasted Sesame Seed Oil, *J. Am. Oil Chem. Soc. 63*:1027–1031 (1986).

In addition to the cyclohexadienyl radical, the other possible radical can be a BQ type radical. If the H• attacks the 3-position of sesamol benzene ring as shown in Scheme 3 scheme, BQ radical can be produced following the succession of the fused ring. In particular, continuous UV irradiation of sesamol may easily produce the BQ radical after the succession of the ring because the production is due to multistep reactions. It is noteworthy that a trace amount but clear indication of BQ radical was also detected by FT EPR at pH 11 (6). Even though the signal for FT EPR at the initial stage was weak, there is still an opportunity to progress to the production of the BQ radical. The FT EPR signal is attributed to the electron spin polarization generated by the radicals instead of the actual radical concentration (8). Moreover, formation of the BQ radical was reported by EPR study of photooxidation of phenol in water (14). The combined spectrum of two simulations—sesamolyl radical and benzoquinone anion radical—is presented in Figure 3B. Although FT EPR measurements do not fully account for BQ radical observed by CW EPR, they support the existence of the radical. It is also noted that the present EPR studies do not exclude the existence of a sesamol dimer that showed strong antioxidation (15).