Bull. Chem. Soc. Jpn. Vol. 82, No. 6, 689–691 (2009)

Kinetic Study of Singlet-Oxygen Quenching by Caffeic Acid and Related Phenols

Keishi Ohara,* Yoko Ichimura, and Shin-ichi Nagaoka

Department of Chemistry, Faculty of Science, Ehime University, Matsuyama 790-8577

Received September 25, 2008; E-mail: ohara@chem.sci.ehime-u.ac.jp

The second-order rate constants (k_Q) of singlet-oxygen quenching by caffeic acid and related phenols were measured in ethanol and toluene. Caffeic acid derivatives deactivate singlet-oxygen largely by physical quenching at $k_Q = 10^6$–$10^7\,M^{-1}\,s^{-1}$ through the charge-transfer transition state.

4-Hydroxycinnamic acid derivatives (HCAs), such as caffeic acid (CA, 3,4-dihydroxycinnamic acid) and ferulic acid (FA, 4-hydroxy-3-methoxycinnamic acid) (Figure 1), are plant-origin polyphenols, and are contained in beans, fruits, herbs, and vegetables.[1–3] A considerable amount of HCAs are ingested from dietary sources into animals and humans. The health effects of HCAs have been attractive research targets and many reports have been published.[1–3] It has been reported that like other natural polyphenols HCAs show certain antioxidant and pharmacological activities.[1–3] Our previous kinetic study on free-radical scavenging by HCAs clarified their abilities as antioxidants, which were less than those of tocopherols and catechins, but sufficient to protect biological systems from pathologies caused by free radicals such as lipid-peroxyl radicals.[1]

Singlet oxygen ($^1O_2$) is molecular oxygen in the electronically excited $^1\Delta_g$ state, which is recognized as a reactive-oxygen species.[4–6] $^1O_2$ production through photosensitization or photobiological processes often induces oxidative injuries in biological systems and also in industrial products. HCAs should show $^1O_2$ quenching activity because of the presence of reactive phenolic hydroxy (OH) groups, as well as many other $^1O_2$ quenchers.[2,4–9] Hence, HCAs may be very valuable for humans and other animals, and moreover they can be obtained easily from plants. Accordingly, systematic studies on structure–activity relationships for $^1O_2$ quenching by HCAs are desirable.

In the present work, the second-order rate constants (k_Q) of $^1O_2$ quenching by CA and its related phenols (Figure 1) were measured by kinetic competition-reaction and time-resolved EPR (TR-EPR).[10,11] The mechanism and the structure–activity relationship for $^1O_2$ quenching by HCAs are discussed on the basis of the k_Q values thus obtained.

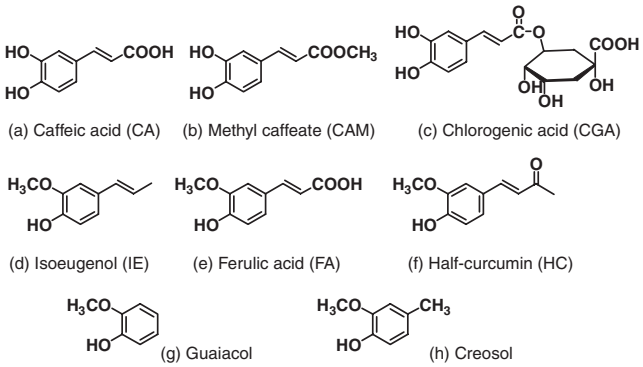

Figure 1. Structures of CA and its related phenols.

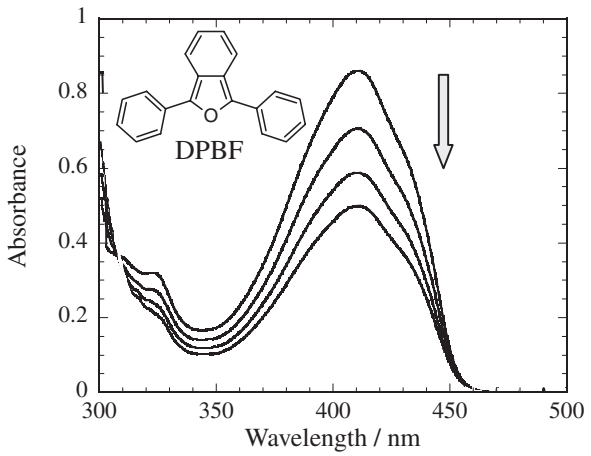

Figure 2. Absorption spectra recorded at 20 min time-intervals in ethanol at 35 °C. $[EP] = 8.1 \times 10^{-5}$ M and $[DPBF] = 4.3 \times 10^{-5}$ M.

Experimental

The structures of the molecules studied in this work are shown in Figure 1. Caffeic acid (CA), chlorogenic acid (CGA), ferulic acid (FA), isoeugenol (IE), guaiacol, and creosol were commercially available reagents from Nacalai Tesque, and were used as received. Half-curcumin (HC, 4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one) and methyl caffeate (caffeic acid methyl ester, CAM) were prepared according to reported methods.[1,12] 3-(1,4-Epidioxy-4-methyl-1,4-dihydro-1-naphthyl)propionic acid (EP) as a $^1O_2$ generating reagent was prepared according to a published procedure.[7,8] 2,5-Diphenyl-3,4-benzofuran (DPBF), 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO), and tetraphenylporphine (TPP) were commercially available reagents from Wako Pure Chemicals and were used as received. Ethanol (Wako) and toluene (Wako) were dried and purified by distillation.

The k_Q measurement by kinetic competition-reaction was performed for an ethanol solution of EP, DPBF, and an antioxidant at 35.0 °C with a Shimadzu UV-2100S spectrophotometer.[7,8] $^1O_2$ was generated by thermal decomposition of EP. The decay curve of the DPBF absorption at 410 nm (Figure 2) was analyzed with a reported procedure.[7,8] In the analysis, the value $k_0 = 8.3 \times 10^4\,s^{-1}$ was used as the natural decay rate constant of $^1O_2$ in ethanol.[6–8]

The k_Q measurements by TR-EPR were performed in toluene at room temperature with a JEOL JES-FA100 EPR spectrometer.[13] Detailed theory of chemically induced dynamic electron polarization (CIDEP) phenomena and the experimental method have

Published on the web June 12, 2009; doi:10.1246/bcsj.82.689

Table 1. k_Q Values and Peak Oxidation Potentials (E_p vs. Ag/Ag^+) of CA and Related Phenols

	$k_Q/10^6\,M^{-1}\,s^{-1}$		E_p/mV[a)]
	Ethanol	Toluene	Acetonitrile
Caffeic acid (CA)	1.23	—	900
Methyl caffeate (CAM)	0.90	—	937
Chlorogenic acid (CGA)	0.97	—	881
Isoeugenol (IE)	2.22	16.3	844
Ferulic acid (FA)	3.46	11.9	966
Half-curcumin (HC)	10.4	12.2	953
Guaiacol	0.36	0.37	1050
Creosol	2.60	1.53	986

a) The values from Ref. 1.

been described in the literature.[10,11] An air-saturated sample solution containing 0.1 mM TPP as a photosensitizer for $^1O_2$ and 0.1 mM TEMPO as a probe for $^1O_2$ was passed through a quartz tube (dia.: 1 mm) and irradiated with a Nd-YAG laser (Continuum Surelight-I, SHG: 532 nm, 9.7 Hz). The decay curve of the EPR signal for TEMPO was recorded by a digital oscilloscope (HP-54510B) and was analyzed according to a reported procedure.[10,11]

When $^1O_2$ quenching progresses by a bimolecular process between $^1O_2$ and a quencher (Q) (reaction 1), the $^1O_2$ decay rate-constant (k_d) is expressed as follows:[9–11]

$$^1O_2 + Q \xrightarrow{k_Q} \text{Products} \quad (1)$$

$$k_d = k_0 + k_Q[Q] \quad (2)$$

The k_Q value can be determined as a slope of the plot of k_d versus the concentration of Q ([Q]).

Results and Discussion

The k_Q values for CA and its related phenols obtained in ethanol and toluene are listed in Table 1, together with their anodic peak oxidation potential (E_p) values in acetonitrile.[1] The k_Q measurements for CA, CAM, and CGA in toluene were unsuccessful because of poor solubility. The k_Q values of these HCAs are spread over 10^6 to $10^7\,M^{-1}\,s^{-1}$, but are 1 or 2 orders smaller than those reported for epigallocatechin gallate ($k_Q = 1.47 \times 10^8\,M^{-1}\,s^{-1}$) and α-tocopherol ($2.06 \times 10^8\,M^{-1}\,s^{-1}$).[8] The k_Q values of CA and FA are close to those reported in acetonitrile.[2] From the present results, it is clear that HCAs show certain $^1O_2$ quenching activity, but they are less significant than other natural antioxidants. However, taking into account their large daily intakes, HCAs are considered to act as $^1O_2$ quenchers to protect biological systems. In ethanol, the k_Q values of FA and HC are larger than those of CA, CGA, and CAM. This fact is in contrast to the results for the second-order rate constant of free-radical-scavenging (k_s) and for vitamin E regeneration (k_r).[1] The k_s values of CA and CGA were reported to be 4 times as large as those of FA and HC. The k_r values showed a similar trend. These tendencies of k_s and k_r can be explained by the difference in the E_p values of CA and CGA and FA and HC. The E_p values of CA and CGA were 50–80 mV smaller than those of FA and HC, and as a result, in free-radical scavenging, the hydrogen-atom transfer reactions of CA and CGA to the radical progress faster than those of FA and HC.

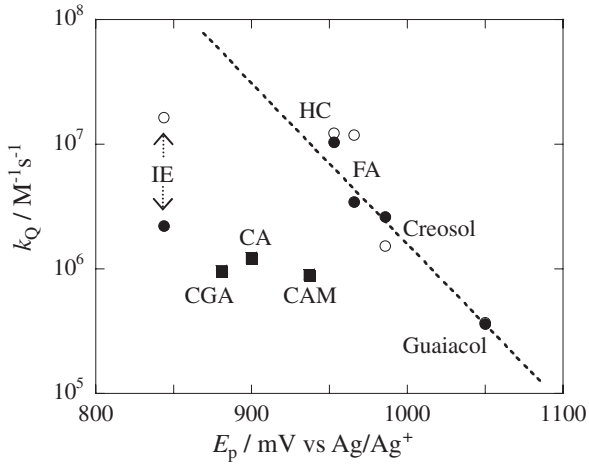

Figure 3. Semi-logarithm plot of k_Q vs. E_p for CA, CAM, CGA (■: in ethanol), and *o*-methoxyphenols (●: in ethanol, ○: in toluene).

Figure 3 shows a semi-logarithm plot of k_Q versus E_p for CA and related phenols. In *o*-methoxyphenols (FA, HC, guaiacol, and creosol), a pseudo-linear relationship between $\log k_Q$ and E_p was obtained. The $\log k_Q$ value increases with decrease of E_p. Similar correlations between $\log k_Q$ and E_p were reported for $^1O_2$ quenching by tocopherols, flavonoids, and catechins.[7,8] This behavior indicates that these *o*-methoxyphenols deactivate $^1O_2$ through a charge-transfer (CT) transition state, as well as other phenolic quenchers.[7–9] On the basis of this CT type $^1O_2$ quenching mechanism, k_Q values in toluene were expected to be larger than those in ethanol.[4,5] However, solvent dependence of k_Q was not clear in the results for HC, FA, creosol, and guaiacol. The k_Q value for IE followed a different trend from those for the other *o*-methoxyphenols. The k_Q value for IE was very small for its low E_p value. It is difficult to give a suitable explanation for this result. The $^1O_2$ quenching mechanism for IE might be different from the other *o*-methoxyphenols. On the other hand, in CA, CAM, and CGA, the change of $\log k_Q$ with E_p was very small, and the plots for them were different from those for *o*-methoxyphenols. This behavior might suggest a difference in $^1O_2$ quenching mechanism between *o*-methoxyphenols and *o*-hydroxyphenols. The existence of strong intramolecular hydrogen-bonding between two phenolic OH groups in CA and CGA is known.[1,14] This intramolecular hydrogen-bonding might reduce the $^1O_2$ quenching activity by locking a proton at the OH group or by changing the charge distribution around the OH groups. The k_Q values of CA and CGA in D_2O were reported to be larger than that of FA.[2] This result might come from the fact that the intramolecular hydrogen-bonds in *o*-hydroxyphenols dissociate in water.

It has been considered that $^1O_2$ quenching consists of "physical quenching" and "irreversible chemical reaction."[4–9] $^1O_2$ quenching by chemical reaction decomposes the quencher, whereas that by physical quenching does not. Figure 4 shows the absorption spectrum monitored at 20 min time-intervals in a solution containing EP and CA ($[EP] = 1.3 \times 10^{-4}$ M and $[CA] = 6.1 \times 10^{-5}$ M) at 35 °C. The rise of the absorption at 290 nm with time indicates the generation of the EP precursor

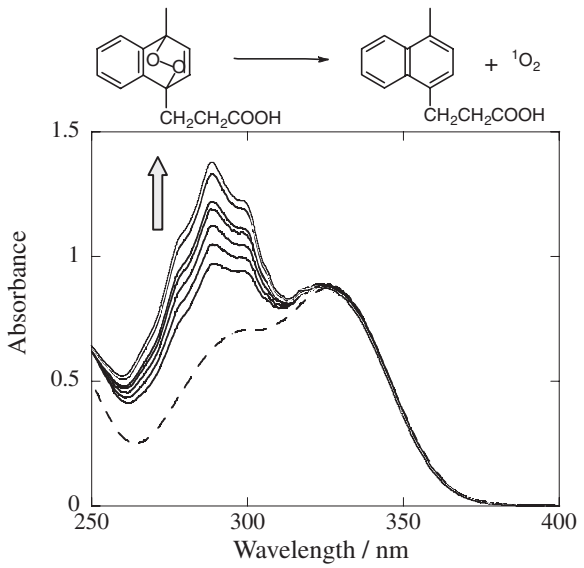

Figure 4. Absorption spectra recorded at 20 min time-intervals in ethanol at 35 °C. $[EP] = 1.3 \times 10^{-4}$ M and $[CA] = 6.1 \times 10^{-5}$ M. The broken line shows the spectrum of CA.

together with $^1O_2$ by decomposition of EP (Figure 4).[7,8] Although $^1O_2$ was generated and quenched by CA in this system, the change of the absorption due to CA (335 nm) was negligibly small. Similar results were obtained for the other HCAs and *o*-methoxyphenols. The results show that the contribution of the chemical reaction in $^1O_2$ quenching by HCAs is very small (<3%), and that their $^1O_2$ quenching progresses largely by physical quenching. Thus, HCAs are expected to be robust toward $^1O_2$ quenching.

In conclusion, from the present study, it is clarified that HCAs have been demonstrated to show activity towards $^1O_2$ quenching, and that they deactivate $^1O_2$ largely by physical quenching through a charge-transfer transition state. Activities of CA, CAM, and CGA less than FA might be explained by the effects of intramolecular hydrogen-bonding.

This work was supported by Grant-in-Aid for Scientific Research C (Nos. 16550016 and 19559919) from the Japan Society for the Promotion of Science (JSPS) and Scientific Research on Priority Areas "Application of Molecular Spins: Nanomagnets to Biological Spin Systems" (Area No. 769, 15087104) from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT). K.O. is grateful to T. Origuchi and Professor Emeritus K. Mukai.

References

1 K. Ohara, Y. Ichimura, K. Tsukamoto, M. Ogata, S. Nagaoka, K. Mukai, *Bull. Chem. Soc. Jpn.* **2006**, *79*, 1501, and references cited therein.

2 S. Foley, S. Navaratnam, D. J. McGarvey, E. J. Land, T. G. Truscott, C. A. Rice-Evans, *Free Radical Biol. Med.* **1999**, *26*, 1202, and references cited therein.

3 M. R. Olthof, P. C. H. Hollman, M. B. Katan, *J. Nutr.* **2001**, *131*, 66, and references cited therein.

4 C. Schweitzer, R. Schmidt, *Chem. Rev.* **2003**, *103*, 1685, and references cited therein.

5 *Singlet O_2*, ed. by A. A. Frimer, CRC Press, Boca Raton, FL, **1985**, Vols. I–IV.

6 F. Wilkinson, W. P. Helman, A. B. Ross, *J. Phys. Chem. Ref. Data* **1995**, *24*, 663.

7 S. Nagai, K. Ohara, K. Mukai, *J. Phys. Chem. B* **2005**, *109*, 4234, and references cited therein.

8 K. Mukai, S. Nagai, K. Ohara, *Free Radic. Biol. Med.* **2005**, *39*, 752, and references cited therein.

9 S. Nagaoka, A. Fujii, M. Hino, M. Takemoto, M. Yasuda, M. Mishima, K. Ohara, A. Masumoto, H. Uno, U. Nagashima, *J. Phys. Chem. B* **2007**, *111*, 13116.

10 M. Mitsui, K. Takeda, Y. Kobori, A. Kawai, K. Obi, *J. Phys. Chem. A* **2004**, *108*, 1120.

11 A. Kawai, K. Shibuya, *J. Photochem. Photobiol., C* **2006**, *7*, 89, and references cited therein.

12 K. Ohara, W. Mizukami, A. Tokunaga, S. Nagaoka, H. Uno, K. Mukai, *Bull. Chem. Soc. Jpn.* **2005**, *78*, 615.

13 K. Ohara, Y. Hashimoto, C. Hamada, S. Nagaoka, *J. Photochem. Photobiol. A* **2008**, *200*, 239.

14 L. Kong, Z.-L. Sun, L.-F. Wang, H.-Y. Zhang, S.-D. Yao, *Helv. Chim. Acta* **2004**, *87*, 511.