How Curcumin Works Preferentially with Water Soluble Antioxidants

Slobodan V. Jovanovic,*,† Charles W. Boone,‡ Steen Steenken,§ Manuela Trinoga,§ and Robert B. Kaskey⊥

Contribution from Helix International, 381 Viewmount Drive, Nepean, ON, Canada K2E 7R9, Department of Pathology, Georgetown University Medical School, Washington, DC, Max-Planck-Institut für Strahlenchemie, 34-36 Stiftstrasse, Mülheim, Germany, and Gene Print, Inc., Bala Cynwyd, Pennsylvania

Received October 30, 2000. Revised Manuscript Received February 2, 2001

Abstract: In this study we investigated physicochemical characteristics of the curcumin radical by pulse radiolysis and laser flash photolysis. Two methylated curcumin derivatives, methylcurcumin and trimethylcurcumin, were synthesized to explore the role of phenol hydroxy and β-diketone moieties in the free radical chemistry of curcumin. Our results show that the initially generated β-oxo-alkyl transforms rapidly, probably via an intramolecular H-atom shift, into the phenoxyl-type curcumin radical. This phenoxyl does not react with oxygen, $k < 10^5$ M^{-1} s^{-1}, and can be repaired by any water-soluble antioxidant with appropriate redox potential, $E_6 < 0.83$ V, for example, with vitamin C, $k = (6 \pm 1) \times 10^6$ M^{-1} s^{-1}. A molecular mechanism of cancer chemoprevention by curcumin is proposed, with special emphasis on the synergism with water-soluble antioxidants.

Introduction

Numerous studies have demonstrated the remarkable cancer preventive properties of curcumin.[1−10] This natural antioxidant inhibits cancerous growth in various cancer models alone and in combinations with other "plant phenolics". The chemopreventive effects of curcumin have been attributed to various properties, including its anti-angiogenesis action,[11,12] which limits the blood supply to rapidly growing malignant cells, its stimulation of Phase I and Phase II detox systems, e.g. inhibition of COX-1 and COX-2 enzymes, and stimulation of glutathione *S*-transferase,[13,14] its interference with cell growth by inhibition of protein kinases,[8] and especially its neutralization of carcinogenic free radicals.[9,15−25] It is possible that any one, more than one, or all of these biological, biochemical, and chemical mechanisms are responsible for the anticarcinogenic potential of curcumin. While biological and biochemical mechanisms tend to be tissue and organ specific, chemical anti-carcinogenesis, e.g. free radical neutralization, works at all levels of a biological system.

Of particular interest is the ability of curcumin to intercept and neutralize potent chemical carcinogens, such as ROS (superoxide, peroxyl, hydroxy radicals) and NOS (nitric oxide, peroxynitrite).[9,15−25] The inactivation of carcinogenic free radicals, which induce and propagate chemical and biochemical processes involved in the inflammatory response, could be the major *chemical* anti-carcinogenic mechanism of curcumin and curcuminoids. It is usually assumed that the phenol moiety is responsible for antioxidant properties of any plant phenolic compound. Consequently, the free radical chemistry of curcumin (an *o*-methoxyphenol derivative) has focused on its phenol rings.[15,17−19] Other studies have pointed to the possible involvement of the β-diketone moiety in the antioxidant action of curcumin and its derivatives.[9,25,26] A recent report by some of us[24] describes the H-atom donation from the β-diketone moiety

† Helix International.
‡ Georgetown University Medical School.
§ Max-Planck-Institut für Strahlenchemie.
⊥ Gene Print, Inc.

(1) Cheng, A. L.; Lin, J. K.; Hsu, M. M.; Shen, T. S.; Ko, J. Y.; Lin, J. T.; Lin, B. J.; Wu, M. S.; Yu, H. S.; Jee, S. H.; Chen, G. S.; Chen, T. M.; Chen, C. A.; lai, M. K.; Pu, Y. S.; Pan, M. H.; Wang, Y. J.; Tsai, C. C.; Hsieh, C. Y. *American Society of Clinical Oncology, 1998 Annual Meeting*; Abstract 2140.

(2) Singh, S. V.; Hu, X.; Srivastava, S. K.; Singh, M.; Xia, H.; Orchard, J. L.; Zaren, H. A. *Carcinogenesis* **1998**, *19*, 1357.

(3) Khafif, A.; Schantz, S. P.; Chou, T. C.; Edelstein, D.; Sacks, P. G. *Carcinogenesis* **1998**, *19*, 419.

(4) Reddy, B. S.; Kawamori, T.; Rao, C. V.; Lubet, R. A.; Steele, V. E.; Kelloff, G. J. *Proc. Am. Assoc. Cancer Res.* **1979**, *39*, 126.

(5) Kawamori, T.; Lubert, R.; Steele, V. E.; Keloff, G. J.; Kaskey, R. B.; Rao, C. V.; Reddy, B. S. *Cancer Res.* **1999**, *59*, 597.

(6) Samaha, H. S.; Keloff, G. J.; Steele, V.; Rao, C. V.; Reddy, B. S. *Cancer Res.* **1997**, *57*, 1301.

(7) Wargovich, M. J.; Chen, C.-D.; Jimenez, A.; Steele, V. E.; Velasco, M.; Stephens, L. C.; Price, R.; Gray, K.; Keloff, G. J. *Cancer Epid. Biom. Prev.* **1996**, *5*, 355.

(8) Reddy, B. S. http://aacr.edoc.com/1999_proceedings/Abstracts/6508001.html.

(9) Masuda, T.; Hidaka, K.; Shimohara, A.; Maekawa, T.; Takeda, Y.; Yamaguchi, H. *J. Agric. Food Chem.* **1999**, *47*, 71.

(10) Verma, S. P.; Salamone, E.; Goldin, B. *Biochem. Biophys. Res. Commun.* **1997**, *233*, 692.

(11) Thaloor, D.; Singh, A. K.; Sidhu, G. S.; Prasad, P. V.; Kleinman, H. K.; Maheswari, R. K. *Cell Growth Differ.* **1998**, *9*, 305.

(12) Arbiser, J. L.; Klauber, N.; Rohan, R.; Vanleeuwen, R.; Huang, M. T.; Fisher, C.; Flynn, E.; Byers, H. R. *Mol. Med.* **1998**, *4*, 376.

(13) Venkatesan, N. *Br. J. Pharmacol.* **1998**, *124*, 425.

(14) Rajakrishnan, V.; Viswanathan, P.; Rajasekaran, K. N.; Gunashekaran, G.; Menon, V. P. *Med. Sci. Res.* **1998**, *26*, 715.

(15) Anto, R. J.; Kuttan, G.; Babu, K. V. D.; Rajasekharan, K. N. *Int. J. Pharmaceut.* **1996**, *131*, 1.

(16) Chignell, C. F.; Bilski, P.; Reszka, K. J.; Motlen, A. G.; Sik, R. H.; Dahl, T. A. *Photochem. Photobiol.* **1994**, *59*, 295.

(17) Gorman, A. A.; Hamblett, I.; Srinavasan, V. S.; Wood, P. D. *Photochem. Photobiol.* **1994**, *59*, 389.

(18) Priyadarsini, K. I. *Free Radicals Biol. Med.* **1997**, *23*, 1997.

(19) Priyadarsini, K. I.; Devasagayam, T. P. A.; Rao, M. N. A.; Guha, S. N. *Radiat. Phys. Chem.* **1999**, *54*, 551.

(20) Sreejayan, N.; Rao, M. N. A. *Arzneim.-Forsch.* **1996**, *46*, 169.

(21) Sreejayan, N.; Rao, M. N. A. *J. Pharm. Pharmacol.* **1997**, *49*, 105.

(22) Unnikrishnan, M. K.; Rao, M. N. A. *Mol. Cell. Biochem.* **1995**, *146*, 35.

(23) Souza, C. R. A.; Osme, S. F.; Gloria, M. B. A. *J. Food Proc. Preserv.* **1997**, *21*, 353.

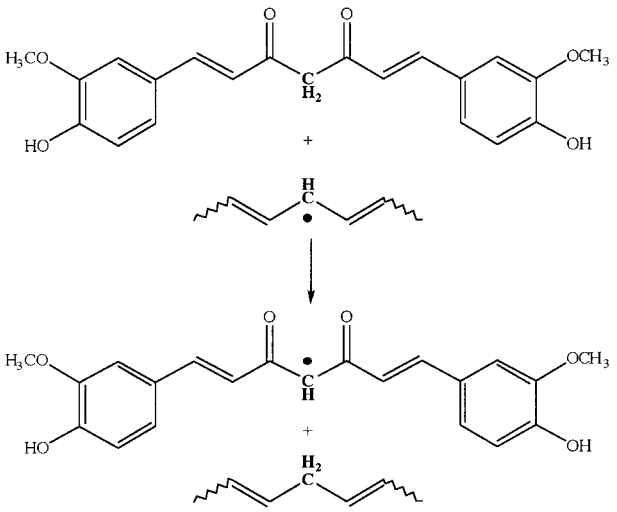
(24) Jovanovic, S. V.; Steenken, S.; Boone, C. W.; Simic, M. G. *J. Am. Chem. Soc.* **1999**, *121*, 9677.

(25) Sugiyama, Y.; Kawakishi, S.; Osawa, T. *Biochem. Pharmacol.* **1996**, *52*, 519.

10.1021/ja003823x CCC: $20.00
Published on Web 03/09/2001

to a lipid alkyl or a lipid peroxyl radical as a potentially more important antioxidant action of curcumin.

In the case of the H-atom donation to a bis-allylic radical (e.g. the linoleic acid radical) the following reaction occurs:

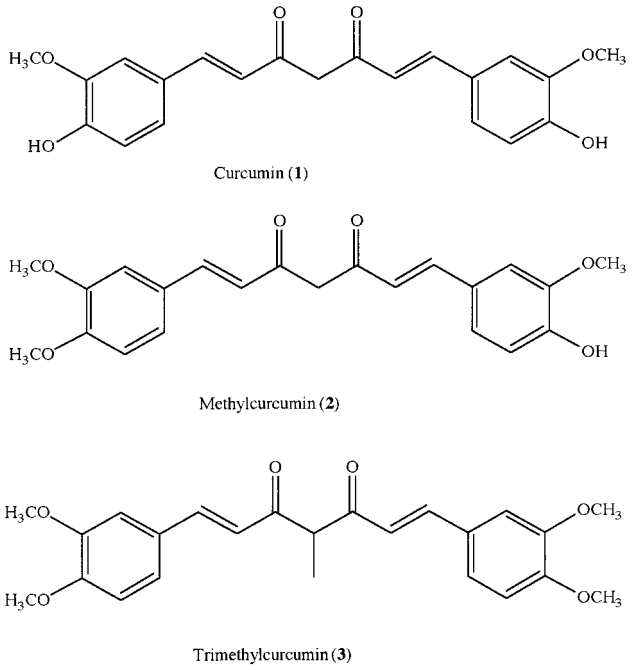


It is possible that the resulting resonance-stabilized β-oxo-alkyl curcumin radical,[24] with unpaired electron density distributed between three C and two O atoms, adds oxygen at the central C atom to become a peroxyl radical. This would be an undesirable reaction because peroxyl radicals propagate lipid peroxidation, which is highly damaging of cell membranes.[27] In comparison, when the addition of oxygen is inefficient, the curcumin radicals may react with each other or with other free radicals to yield stable products, such as vanillin, ferrulic acid, and curcumin dimers.[9] The chemical and antioxidant properties of these stable products are considerably different from those of curcumin.[28,29]

The question arises, what is the molecular basis of the reported synergism[3] of curcumin with catechin and epigallocatechin gallate? The water-insoluble curcumin molecule residing in the central lipid layer of cell membranes is probably not even close to the aqueous milieu adjacent to the membranes in which the water-soluble catechins are located. Assuming a mechanism similar to the well-known synergism between lipid-soluble (water-insoluble) vitamin E and water-soluble ascorbate (vitamin C),[30] it may be postulated that the curcumin radical, generated by antioxidant action, positions itself at the border of the cell membrane adjacent to the aqueous milieu, in short "pops out" of the membrane, to be repaired by the catechins. However, for this mechanism to operate, the curcumin radical must be more polar than curcumin itself and, in addition, be able to oxidize catechins, a requirement that is satisfied by the curcumin phenoxyl radical.

In this study we used pulse radiolysis of aqueous and laser flash photolysis of acetonitrile solutions of curcumin and two methylated curcumin derivatives to determine the properties of the curcumin radical. Our results indicate that the phenoxyl is the predominant curcumin radical. This radical is formed either by direct one-electron oxidation of a methoxyphenol moiety or by an intramolecular H-atom transfer from any methoxyphenol ring to the incipient β-oxo-alkyl radical. The implications of these findings for the chemoprevention of cancer by naturally occurring curcumin, demethoxycurcumin, and bisdemethoxycurcumin are discussed below. A model for the molecular basis of the synergism between curcumin and water-soluble antioxidants is presented.

Chart 1



Materials and Methods

Chemicals used in this study were of the highest purity available. Curcumin (**1**, 99.95% purity) was the product of R-Kane Inc., N.J. The structures of curcumin and methylated curcumin derivatives are presented in Chart 1. Acetonitrile, dimethyl sulfoxide, methyl iodide, potassium carbonate, sodium hydrogenphosphate, and potassium dihydrogenphosphate were the products of Merck. Bis(azo-methylamidine) dihydrochloride and *tert*-butyl peroxide were obtained from Aldrich. Water used to prepare all solutions was purified through a Millipore Milli-Q system to a resistivity better than 18 MΩ/cm. Prior to irradiation, the solutions were saturated with high-purity gases (Ar, N_2O, O_2) by gentle bubbling for 30 min. The pH of aqueous solutions was adjusted with a phosphate buffer.

Fully computerized laser flash photolysis at the Max-Planck-Institut für Strahlenchemie[31] was used for photochemical investigations. A 248 nm Lambda Physik excimer laser was used to irradiate the samples contained in the 0.1 cm flow-cell. The flow rate of ~1 mL/min, which ensured a constant supply of fresh solution, was controlled by a peristaltic pump. The absorbance of the solution to be irradiated was adjusted so that less than 10% of the laser light is absorbed by curcumin derivatives to minimize their photolysis.

The 3 MeV Van-de-Graaff pulse radiolysis equipment with optical detection at the Max-Planck-Institute für Strahlenchemie[32] was used for the pulse radiolysis studies. A 2 cm supra-sil quartz cell with temperature variation through a thermostatically controlled liquid jacket was used for sample irradiation.

The rate constants were determined at ~1−2 Gy/pulse to minimize interference from radical−radical reactions. Thiocyanate dosimetry was

(26) Schaich, K. M.; Fisher, C.; King, R. In *Food phytochemicals for cancer prevention II. Teas, Spices, and Herbs*; Ho, C.-T., Osawa, T., Huang, M.-T., Rosen, T. R., Eds.; ACS Symp. Ser. No. 547; American Chemical Society: Washington, DC, 1994.

(27) von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor and Francis: London, 1987.

(28) Jovanovic, S. V.; Steenken, S.; Tosic, M.; Marjanovic, B.; Simic, M. G. *J. Am. Chem. Soc.* **1994**, *116*, 4846.

(29) Jonsson, M.; Lind, J.; Reitberger, T.; Eriksen, T. E.; Merényi, G. *J. Phys. Chem.* **1993**, *97*, 11278.

(30) Doba, T.; Burton, G. W.; Ingold, K. U. *Biochim. Biophys. Acta* **1985**, *835*, 298.

(31) Anklam, E.; Steenken, S. *J. Photochem. Photobiol.* **1988**, *43A*, 233.

(32) Jagannadham, V.; Steenken, S. *J. Am. Chem. Soc.* **1984**, *106*, 6542.

used in dose determinations, assuming $G[(SCN)_2^{\bullet -}] = 6.2 \times 10^{-7}$ mol J^{-1} in N_2O-saturated 10 mM KSCN aqueous solutions.

Synthesis and Characterization of Methylcurcumin (2). Methylcurcumin, 1-(3,4-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, was synthesized by a mild methylation of curcumin. Curcumin (0.1 M) was refluxed with 1.9 M methyl iodide in the presence of 0.024 M sodium carbonate in acetone at 60 °C for 40 h. The resulting mixture contained ~35% **2** (according to HPLC analysis). The mixture was evaporated to dryness on a rotary evaporator.

2 was separated by a preparative reverse phase HPLC in acetonitrile/water. Evaporation of the HPLC fraction yielded dark yellow viscous oil. The compound was identified by the 500 MHz 2D NMR spectroscopy. The mass spectrum confirmed the structure of **2**, M^+ at 382, and additional peaks at 364, 191, and 181. The purity of the compound according to the HPLC and mass spectroscopy was higher than 99%.

Synthesis and Characterization of Trimethylcurcumin (3). Trimethylcurcumin, 1,7-di(3,4-dimethoxyphenyl)-4-methyl-1,6-heptadiene-3,5-dione (**3**), was synthesized by a prolonged mild methylation of curcumin. Curcumin (0.05 M) was stirred with 1.9 M methyl iodide in the presence of 0.024 M sodium carbonate in acetone at 20 °C for 80 h. The resulting mixture, which contained more than 90% of **3**, was evaporated to dryness on a rotary evaporator and dissolved in ethyl acetate. The ethyl acetate solution was washed with three volumes of water and evaporated to dryness. The orange powder was further purified by a preparative reverse-phase HPLC. The less polar component was collected and characterized by the 500 MHz 2D NMR and mass spectroscopy. The mass spectrum of the powder confirmed the presence of trimethylcurcumin, M^+ at 410, and additional peaks at 219, 191, etc. The purity of the compound according to the mass spectra was higher than 99%.

Results and Discussion

The curcumin radical has been generated by the pulse radiolysis of an N_2O-saturated 40% aqueous DMSO at pH 5.4 by the following set of reactions,[24,33]

$$H_2O \rightsquigarrow H, {}^{\bullet}OH, e^-_{aq}, H_3O^+, \text{etc.} \quad (1)$$

$$e^-_{aq} + N_2O + H_3O^+ \rightarrow {}^{\bullet}OH + H_2O + N_2 \quad (2)$$

$${}^{\bullet}OH + (CH_3)_2SO \rightarrow {}^{\bullet}CH_3 + CH_3SO_2^- + H_3O^+ \quad (3)$$

$${}^{\bullet}CH_3 + (\mathbf{1}) \rightarrow CH_4 + {}^{\bullet}(\mathbf{1}); k = 3.5 \times 10^9\ M^{-1}\ s^{-1} \quad (4)$$

At 0.65 mM curcumin (**1**), the curcumin radical is generated within 2 μs. Upon addition of ascorbate in concentrations from 0.3 to 3.4 mM, the spectrum of the curcumin radical decayed with an apparent pseudo-first-order kinetics, as monitored at 510 nm. From the pseudo-first-order rate, the second-order rate of $k = (6 \pm 1) \times 10^6\ M^{-1}\ s^{-1}$ is derived.

This rate is slower than $k = 2.5 \times 10^7\ M^{-1}\ s^{-1}$ reported[17] for the same reaction in acetonitrile/aqueous buffer (1:4) at pH 7. The reason for this discrepancy may be our choice of lower pH 5.4. The reaction may be slower because of higher reduction potential of the ascorbate radical at this pH ($E_{5.4} = 0.40$ V, vs $E_7 = 0.28$ V).[34]

Regardless of the method of generation (pulse radiolysis or laser flash photolysis), the decay of the curcumin radical was not affected by oxygen in concentrations from 0.2 to 2 mM. From the second-order rate of the radical decay the upper limit of the rate can be set, $k < 10^5\ M^{-1}\ s^{-1}$, in full agreement with the earlier study.[17]

(33) Veltwisch, D.; Janata, E.; Asmus, K.-D. *J. Chem. Soc., Perkin Trans. 2* **1980**, 146.

(34) Steenken, S.; Neta, P. *J. Phys. Chem.* **1982**, *86*, 3661.

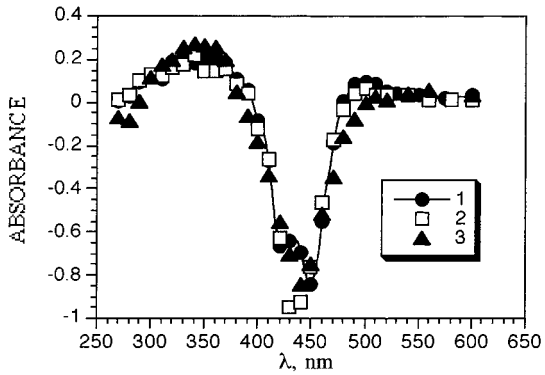


Figure 1. Transient absorption spectra upon completion of the reaction of $(CH_3)_3CO^{\bullet}$ with curcumin derivatives obtained by 248 nm laser flash photolysis of Ar-saturated acetonitrile solution containing 10% water, 0.8 M *tert*-butylperoxide, and 0.05 mM curcumin (**1**) and its methyl derivatives (**2** and **3**).

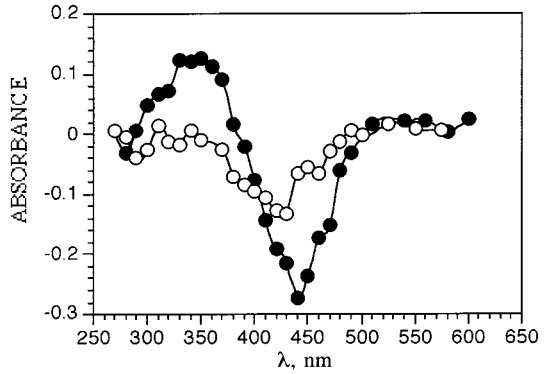


Figure 2. Transient absorption spectra of radicals from **3** generated by laser flash photolysis of acetonitrile solutions containing 10% water, 0.8 M *tert*-butylperoxide, and 0.05 mM **3**: (●) Ar-saturated solution; (○) O_2 -saturated solution. Laser power was kept at ~15% to supress second-order processes.

Oxidation of ascorbate and the lack of reactivity with oxygen suggest that the curcumin radical is an oxyl rather than a carbon-centered radical. The oxyl curcumin radical(s) are very likely a mixture of the phenoxyl, where the unpaired electron is delocalized over the phenol ring and the adjacent allyl chain, and the β-oxo-alkyl, where the unpaired electron is delocalized within the β-diketone linkage. We used the *tert*-butoxyl and methylamidino peroxyl radicals to selectively generate alkoxyl radicals from **1**, **2**, and **3** and study their spectral and kinetic characteristics.

The *tert*-butoxyl radical is well-known alkoxyl radical, which reacts preferentially by an H-atom abstraction. The *tert*-butoxyl radical was generated by photolysis of 0.8 M *tert*-butylperoxide in Ar-saturated acetonitrile. It reacts rapidly with the curcumin derivatives, $k \sim 5 \times 10^9\ M^{-1}\ s^{-1}$, as derived from the "bleaching" of strongly yellow-colored solutions. The resulting transient spectra are presented in Figure 1.

As seen from Figure 1, the absorption spectra of radicals from **1**, **2**, and **3** are similar, the slight differences in "bleaching" originating from different UV−vis absorption spectra. The disappointing lack of any significant difference between the spectra of radicals from **1** and **2**, where both phenoxyl and β-oxo-alkyl radicals may be generated in different proportions, and **3**, where only β-oxo-alkyl radicals are possible, is a consequence of an apparent overlap of strongly absorbing parent compounds and weak spectral bands of the daughter radicals.

However, in the presence of oxygen (at 2 and 20 mM), the decay of radicals from **1** and **2** (with typical $\tau_{1/2} \sim 100$ μs)

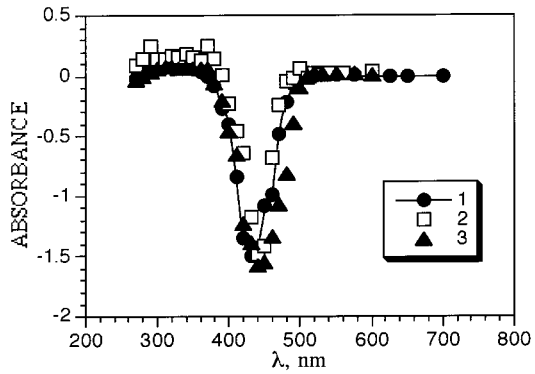
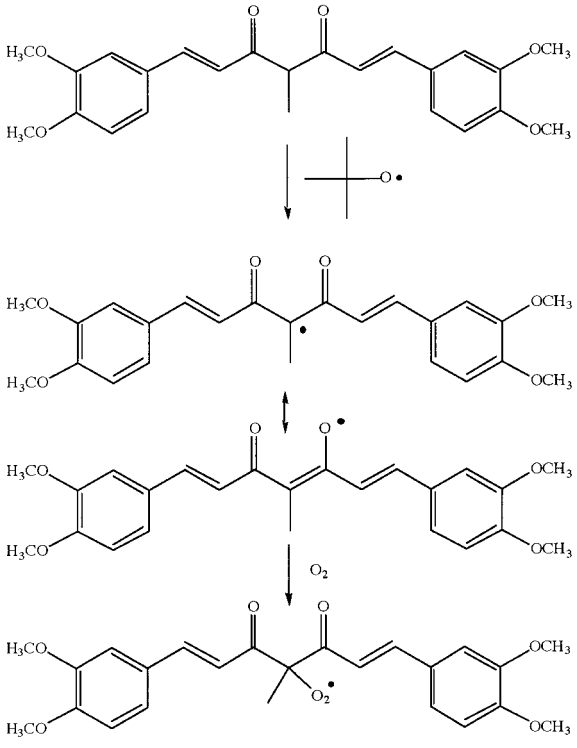


Figure 3. Transient absorption spectra upon completion of the reaction of the methylpropionamidine peroxyl radical with curcumin derivatives obtained by 248 nm laser flash photolysis of an air-saturated solvent mixture (1:1 water/acetonitrile) containing 50 mM 2,2′-azobis(2-methylpropionamidine)·2HCl and 0.05 mM curcumin (**1**) and its methyl derivatives (**2** and **3**).

remains unaffected in the studied spectral range, whereas the 360 nm band in the spectrum of the radical derived from **3** rapidly decays. Such decay indicates the reaction of the β-oxo-alkyl radical from **3** with oxygen, as shown below. From the

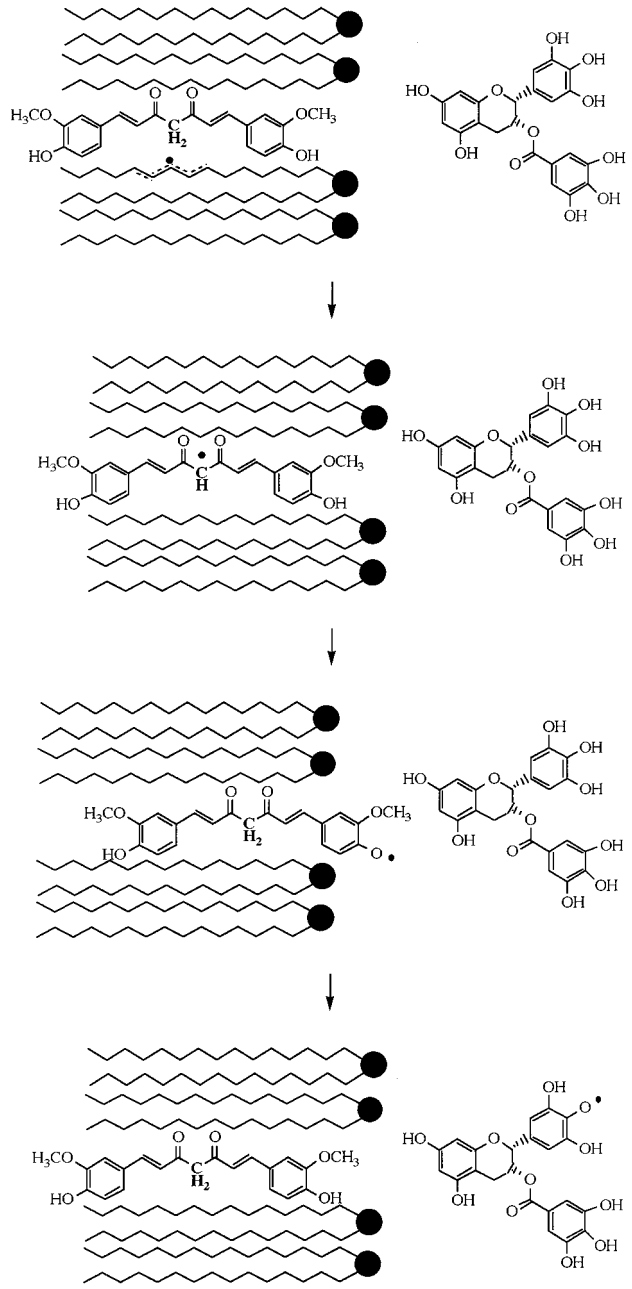


pseudo-first-order decay, the reaction rate constant of the radicals from **3** with oxygen can be estimated as $k \sim 10^8$ M^{-1} s^{-1} in pure acetonitrile and $k \sim 10^7$ M^{-1} s^{-1} in acetonitrile/water (9:1). The spectrum of the radicals from **3** in Ar-saturated acetonitrile and upon saturation of the same solution with oxygen is shown in Figure 2.

The addition of oxygen to the β-oxo alkyl curcumin radical, at $k \sim 10^7$ M^{-1} s^{-1}, is slow in comparison with the diffusion-controlled reactions of alkyl radicals ($k \sim 10^9$ M^{-1} s^{-1}).[27] The slow reaction with oxygen is suggested to be caused by resonance-stabilization of the β-oxo alkyl radical. Similar behavior was reported for the linoleic acid allylic radical, with $k = 1.8 \times 10^8$ M^{-1} s^{-1}.[35]

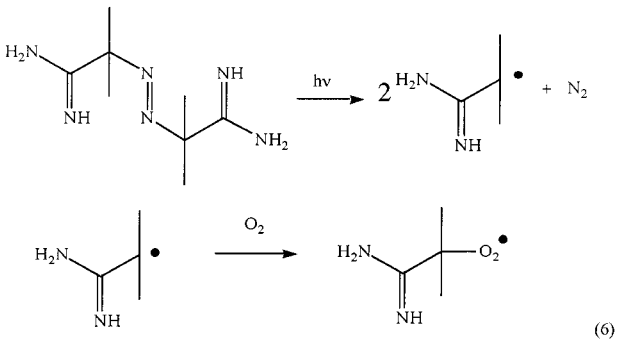
(35) AlSheikhly, M.; Simic, M. G. *J. Phys. Chem.* **1989**, *93*, 3103.

Scheme 1



We further studied the reactions of the alkyl peroxyl radicals with curcumin and its methylated derivatives in physiologically more relevant aqueous solutions.

The methylpropionamidine radicals were generated by photodecomposition of 50 mM 2,2′-azobis(2-methylpropionamidine)·2HCl in acetonitrile/water (1:1) at pH 5.0, i.e.

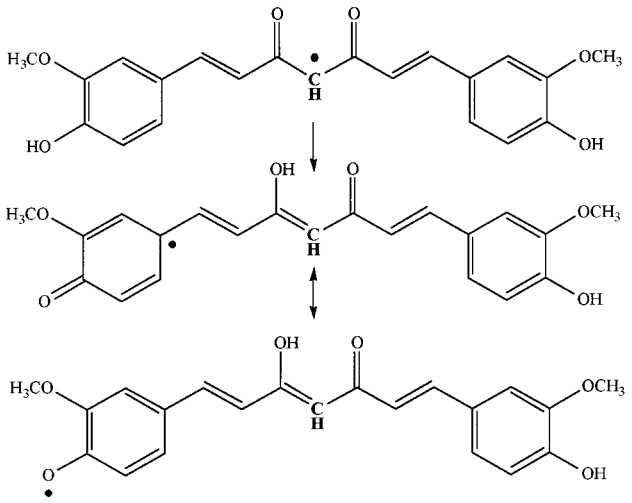


In air-saturated solutions ($[O_2] \sim 0.2$ mM), the initially generated methyl propionamidine radicals add oxygen at close to a diffusion controlled rate of $k \sim 10^9$ M^{-1} s^{-1} to become peroxyl radicals. These peroxyl radicals readily react with curcumin and its methylated derivatives in concentrations from 0.1 to 0.6 mM at $k \sim 10^8$ M^{-1} s^{-1}. The spectra of the transients from these reactions are presented in Figure 3.

As seen from Figure 3, the spectra of radicals derived from curcumin and its methylated derivatives are similar to those generated by the *tert*-butoxyl reaction, with the notable exception of **3**, where the 360 nm band is missing from the spectrum generated in oxygenated aqueous solutions. The initially generated β-oxo-alkyl radical of **3** reacts with oxygen to generate the corresponding peroxyl radical. The similarity of spectra of the radicals from **3** generated by the butoxyl radical in the presence of oxygen and the alkyl peroxyl radical (see Figures 2 and 3) is taken to support this conclusion.

Curcumin and its derivatives efficiently and rapidly react with the methyl propionamidine peroxyl radicals, which are the model for lipid peroxyl radicals. Such antioxidant action of curcumin and methylcurcumin may be expected from the favorable reduction potential of the phenol moiety, $E_6 = 0.83$ V, e.g., there is a $\sim$0.23 V or $E \sim 5$ kcal/mol driving force for the reduction of methyl peroxyl radicals.[36] Trimethylcurcumin, **3**, reacts rapidly with alkyl peroxyl radicals by an apparent H-atom donation. However, the latter reaction generates a radical that reacts with oxygen, leading to the possible propagation of the peroxidation. Therefore, the β-diketone moiety alone does not have antioxidant properties. Apparently, the presence of both β-diketone and phenol is necessary for optimal antioxidant function of curcumin.

We propose that as a result of higher resonance stabilization afforded in the phenoxyl radical, the initially generated curcumin alkoxyl radical undergoes rapid intramolecular H-shift, as presented below.



Conclusions

Our results indicate that one of the curcumin oxyl radicals generated by its antioxidant action undergoes "molecular reorganization", i.e., the initially generated curcumin β-oxo alkyl is transformed into the phenoxyl radical. When the molecular reorganization is not possible, as in trimethylcurcumin, the curcumin β-oxo alkyl radical adds oxygen at $k = 10^7 - 10^8$ M^{-1} s^{-1} in aqueous and acetonitrile solutions, respectively, to generate potentially damaging peroxyl radical.

The reduction potential of the phenoxyl curcumin radical is $\sim$0.8 V (0.83 V at pH 6)[24] at physiological pH 7. Consequently, curcumin may be fully repaired by electron donors with favorable oxidation potentials, that is with E_7 lower than $\sim$0.8 V, such as epigallocatechin gallate ($E_7 = 0.43$ V), catechin ($E_7 = 0.55$ V), or vitamin C (0.28 V). Such electron transfer (with accompanying proton transfer) reaction will maintain optimal concentration of curcumin in the cell membrane at the expense of a water-soluble antioxidant. Therefore, highly beneficial effects of curcumin, which are otherwise lost because of its fast turnover and low physiological uptake, may be successfully maintained with the more bioavailable and water-soluble catechins.

If generated by one-electron oxidation, the phenoxyl radicals from demethoxycurcumin and bisdemethoxycurcumin, which are present with curcumin in natural extracts of Curcuma Longa, will be more reactive and damaging than that of curcumin, because of their considerably higher reduction potentials. The reduction potential of the *p*-styryl-phenoxyl at pH 7 can be estimated as 1.00 V[37](the phenoxyl present in bis-demethoxycurcumin), which is $\sim$0.2 V higher than the corresponding potential of the curcumin radical. Moreover, this is higher than the 0.90 V needed to oxidize tyrosine,[38] an ubiquitous amino acid, often crucial for enzyme activity. Therefore, demethoxy- and bisdemethoxycurcumin are considerably inferior physiological antioxidants to curcumin.

Pure curcumin is an extremely potent lipid-soluble antioxidant. It positions itself within the cell membrane, where it intercepts lipid (peroxyl) radicals and becomes a phenoxyl radical. Being more polar than curcumin, the phenoxyl radical may "travel" to the surface of the membrane, where it may be repaired by any water-soluble antioxidant with $E_7 < 0.80$ V.

Scheme 1 illustrates the physiological antioxidant mechanism of curcumin and synergism with a water-soluble antioxidant such as epigallocatechin gallate (EGCG).

Acknowledgment. S.V.J. would like to thank Prof. Z. Stojanac for helpful discussions on the synthesis of natural curcuminoids.

JA003823X

(36) Jovanovic, S. V.; Jankovic, I.; Josimovic, L. *J. Am. Chem. Soc.* **1992**, *114*, 9018.

(37) Jovanovic, S. V.; Tosic, M.; Simic, M. G. *J. Phys. Chem.* **1991**, *95*, 10824.

(38) Jovanovic, S. V.; Steenken, S.; Simic, M. G. *J. Phys. Chem.* **1991**, *95*, 684.