H-Atom Transfer Is A Preferred Antioxidant Mechanism of Curcumin

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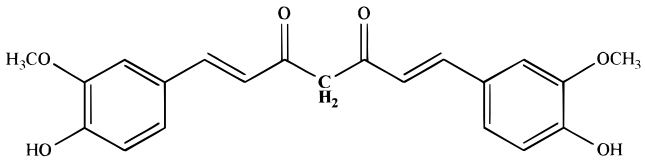
Abstract: Antioxidant mechanisms of curcumin, bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, have been studied by laser flash photolysis and pulse radiolysis. The keto−enol−enolate equilibrium of the heptadienone moiety of curcumin determines its physicochemical and antioxidant properties. In neutral and acidic aqueous solutions (from pH 3 to 7), the keto form dominates, and curcumin acts as an extraordinarily potent H-atom donor. The reaction rate constant with the methyl radical $(3.5 \pm 0.3) \times 10^9$ M^{-1} s^{-1} is close to diffusion control in 40% aqueous DMSO at pH 5. The *tert*-butoxyl radical reacts with curcumin in acetonitrile solutions at a diffusion controlled rate, $k = (7.5 \pm 0.8) \times 10^9$ M^{-1} s^{-1}. The apparent site of reaction is the central CH_2 group in the heptadienone link, which has two labile hydrogens. This is supported by comparing the reaction patterns of curcumin and dehydrozingerone (DHZ) ("half-curcumin", 4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one). DHZ does not react with the methyl radical, indicating that the presence of the labile hydrogens is crucial for the H-atom donating ability of curcumin. The *tert*-butoxyl radical reacts with DHZ at almost an order of magnitude lower rate $(1.1 \pm 0.1) \times 10^9$ M^{-1} s^{-1}, clearly abstracting an H-atom from the phenolic OH group. The reaction mechanism of curcumin changes dramatically above pH 8, where the enolate form of the heptadienone link predominates. As a consequence, the reaction of the methyl radical diminishes completely in alkaline media, and the phenolic part of the molecule takes over as (electron donor) reaction site. The electron donating ability of curcumin is assessed from the measurements of one-electron-transfer equilibria of DHZ radicals. Reduction potential of the DHZ phenoxyl radical, $E(\text{pH} = 6.5) = 0.83 \pm 0.06$ V, and $E(\text{pH} = 13.0) = 0.47 \pm 0.06$ V vs NHE, which may be expected for an ortho-methoxy-substituted phenoxyl radical, indicate only moderate electron-donating ability. The importance of H-atom donation vs electron donation in free radical scavenging and antioxidant mechanisms of curcumin is discussed.

Introduction

Curcumin (**1**) is a yellow pigment of turmeric, a spice manufactured from the root of *Curcuma longa*. Turmeric is one of the major spices in certain Asian cuisines, notably Indian. It has been argued that increased dietary curcumin leads to improved chemoprevention of cancer,[1−6] possibly through inhibition of the growth of blood capillaries in the cancerous tissue.[7,8] Curcumin also has bactericidal action[9] and may minimize oxidative damage through free radical scavenging.[6,9−14]

In view of the importance of dietary antioxidants in chemoprevention of degenerative diseases, such as cancer, Alzheimer's, Parkinson's, and cardiovascular diseases, antioxidant and chemopreventive mechanisms of curcumin have been thoroughly investigated. Bactericidal action has been linked to a photodynamic effect.[9,10] Low photochemical yield of singlet oxygen in benzene, $\phi = 0.12$,[9] is characteristic for an n,π^* triplet precursor.[15] On the other hand, the triplet energy 191 kJ/mol in benzene[9] is fairly low and consistent with a π,π^* triplet. Quenching of singlet oxygen by curcumin is surprisingly slow, $k = 2.5 \times 10^5$ M^{-1} s^{-1}.[9] Free radical scavenging ability[2,6,9−14]



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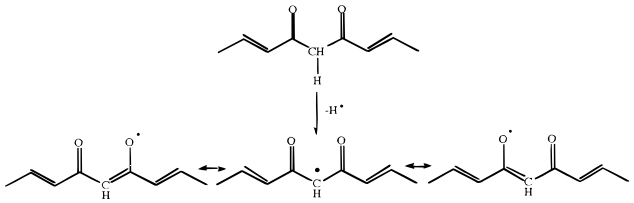
and inhibition of lipid peroxidation have been attributed to electron donation from the phenolic part of curcumin.

Phenolic antioxidants usually scavenge damaging free radicals by an electron-transfer mechanism. The electron-donating ability is determined by the one-electron oxidation potential of the parent antioxidants, expressed by definition as the reduction potential of the corresponding phenoxyl radicals. Using empirical linear-energy relations,[16−18] the oxidation potential of curcumin can be estimated at pH 7 as $E_7 = 0.77$ V (similar to that of hesperidin).[19] This is considerably higher than E_7(vitamin E) ≈ 0.48 V or E_7(vitamin C) $= 0.28$ V,[20] which means that the phenoxyl radical of curcumin can oxidize vitamin E or vitamin C. It is also higher than the potential of some other lipid soluble antioxidants, such as methyl gallate ($E_7 = 0.52$ V) or quercetin ($E_7 = 0.33$ V).[21] Surely, such high oxidation potential cannot be the basis for the observed excellent antioxidant properties of curcumin.

What makes curcumin a superb antioxidant? In the keto form of curcumin, the heptadienone linkage between the two methoxyphenol rings contains a highly activated carbon atom (highlighted in **1**). It is obvious that the C−H bonds on this carbon should be very weak, due to delocalization of the unpaired electron on the adjacent oxygens:



If this is the case, this group can serve as an H-atom donor. We have investigated by laser flash photolysis and pulse radiolysis in aqueous and acetonitrile solutions the ability of curcumin to donate an H-atom. Our results clearly show that the H-atom donation is a preferred reaction of curcumin at pH ≤ 7 and in nonprotic solvents. The antioxidant mechanisms of curcumin in vivo, and its interactions with other physiological and dietary antioxidants are discussed in the light of this novel possibility of action.

Materials and Methods

Curcumin (99.5% purity) was a generous gift of Mr. R. Kaskey of R-Kane, Inc, Pennsauken, NJ. All other chemicals were of the highest purity commercially available. 4-(4-hydroxy-3-methoxybenzene)-buten-3-one(= DHZ, i.e., "half-curcumin"), benzophenone, and diethylamine were obtained from Aldrich, promethazine hydrochloride from Sigma, *N*,*N*,*N*′,*N*′-tetramethylene-*p*-phenylenediamine hydrochloride from Fluka, dimethyl sulfoxide, acetonitrile, 2-propanol, acetone, and phosphate buffer were the products of Merck. Water was purified through a Millipore MilliQ system to a resistivity better than 18 MΩ/cm. Prior to pulse radiolysis or flash photolysis experiments, the solutions were either deaerated by passing high purity Ar for 20 min or saturated with N_2O to convert hydrated electrons to OH radicals.

UV−vis spectra were measured on a Shimadzu UV−vis, and a HP 8450A diode array spectrophotometer. Supra-sil quartz cuvettes (1 and 10 mm) were used.

The 3 MeV van-de-Graaff pulse radiolysis equipment with optical detection at the Max-Planck-Institut für Strahlenchemie[22] 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 spectra of the radicals were measured at 5−10 Gy/pulse, whereas the rate constants were determined at considerably lower 1−2 Gy/pulse to minimize interference from radical−radical reactions. Thiocyanate dosimetry was used in dose determinations, assuming $G[(SCN)_2^{\bullet-}] = 6.0$ in N_2O-saturated 10 mM KSCN aqueous solutions.

Fully computerized laser photolysis ($\lambda = 248$ nm) at the Max-Planck-Institut[23] was used for photochemical investigations.

Results and Discussion

H-Atom Transfer Reactions of Curcumin. Curcumin, bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dienone, undergoes proton-transfer equilibria with $pK_{a1} = 8.55 \pm 0.05$ and $pK_{a2} = 10.41 \pm 0.05$ (the pK_a values were determined from the pH dependent spectral changes). The ionized curcumin is more water soluble and expected to be a better electron donor than the nonionized form. On the other hand, the nonionized curcumin may exist in the keto form, which may be an excellent H-atom donor as discussed above. It is conceivable that the keto form of curcumin predominates in acidic and neutral aqueous solutions and in the cell membrane.

The methyl radical, $^{\bullet}CH_3$, is generated from $(CH_3)_2SO$ by pulse radiolysis of N_2O-saturated aqueous solutions of 5 M dimethyl sulfoxide at pH = 5.0 in the following series of reactions,[24]

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

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

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

The methyl radical was found to react rapidly with curcumin in the concentration range from 0.09 to 0.28 mM. The spectrum of the resulting curcumin radical is shown in Figure 1.

The rate of the methyl radical reaction is found to be dependent on the concentration of curcumin, from which the reaction rate constant is derived as $k = (3.5 \pm 0.3) \times 10^9$ M^{-1} s^{-1}. To the best of our knowledge, this is the highest rate of the reaction of the methyl radical with any substrate. It is considerably higher than the rates of H-abstraction[25,26] from either aliphatic $k(^{\bullet}CH_3 + GSH$, at pH = 7) $= 5 \times 10^7$ M^{-1} s^{-1}, aromatic $k(^{\bullet}CH_3 +$ 4-methoxythiophenol, at pH = 3) $= 1.3 \times 10^8$ M^{-1} s^{-1} or heterocyclic thiols, $k(^{\bullet}CH_3 +$ 1-methyl-5-ethyl-4-mercaptoimidazole, at pH = 7.0)$= 1.5 \times 10^5$ M^{-1} s^{-1}.

The reaction of the methyl radical with curcumin is pH-dependent. The rate of the reaction decreases with the increase in the pH from 5 to 9. This is interpreted as due to concomitant decrease in the concentration of the keto form. At pH 9.5, the build up of the curcumin radical is completely suppressed.

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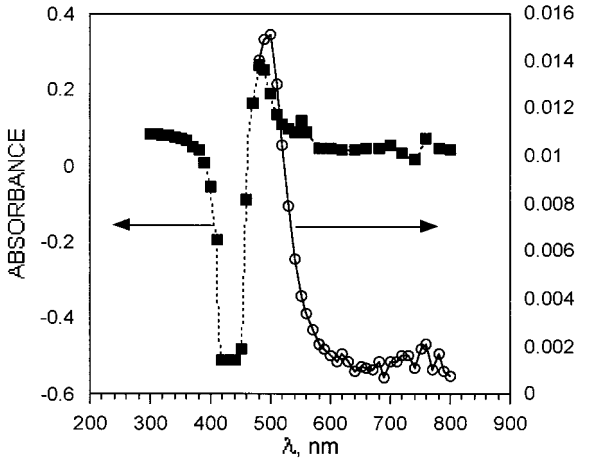
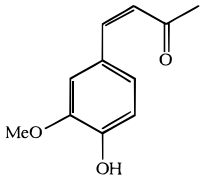


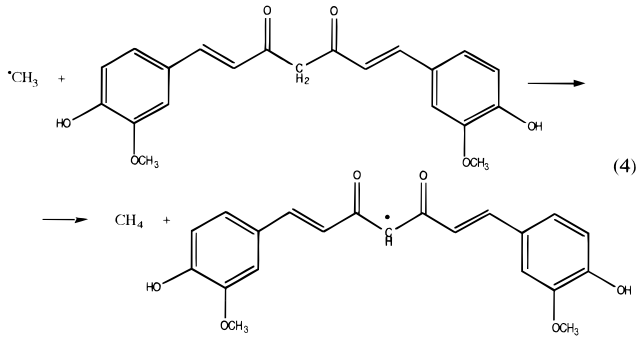
Figure 1. Spectra of curcumin transients obtained by -O- pulse radiolysis of 0.087 mM curcumin in N_2O-saturated aqueous solution of 40% DMSO at pH 4.80, $D = 2$ Gy/pulse, and −■− 248 nm laser photolysis of Ar-saturated acetonitrile solution of 0.074 mM curcumin and 1.27 M *tert*-butylperoxide.

The methyl radical did not react ($k < 10^5$ M^{-1} s^{-1} at pH 5.0) with dehydrozingerone, DHZ, 4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one:



At this pH DHZ ("half-curcumin") is in the keto form, but the terminal CH_3 group is apparently a considerably poorer H-atom donor than the central CH_2 in curcumin.

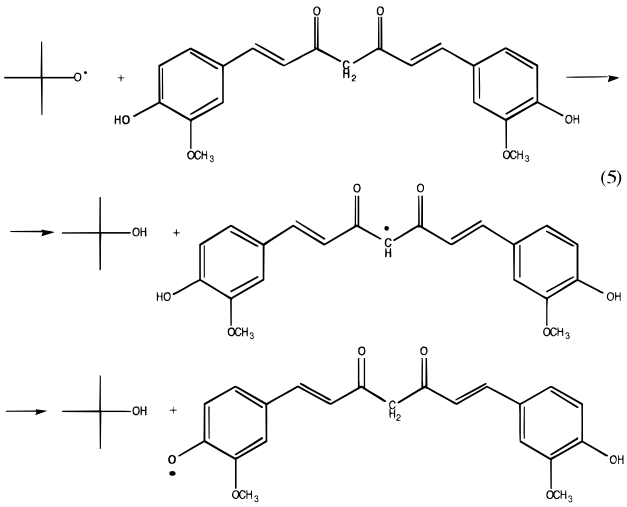
We conclude that the methyl radical abstracts an H-atom from the central CH_2 group in curcumin, as indicated in reaction 4,



(4)

Photochemically generated (by 248 nm laser photolysis of Ar-saturated acetonitrile solutions of 1.3 M *tert*-butylperoxide) *tert*-butoxyl radical, $(CH_3)_3CO^•$, was found to react with curcumin by abstracting the H-atoms from the central CH_2 group and, to a considerably smaller extent if at all, from the phenolic OH group as indicated below (spectrum of the resulting curcumin radicals is shown in Figure 1).

The reaction rate constant, $k = (7.5 \pm 0.8) \times 10^9$ M^{-1} s^{-1}, is close to diffusion controlled. This should be compared with almost an order of magnitude slower H-abstraction from the phenolic O−H group in DHZ, $k[(CH_3)_3C-O^• + DHZ] = (1.1 \pm 0.1) \times 10^9$ M^{-1} s^{-1}. From the difference between the rates of reaction with curcumin and DHZ ("half-curcumin"), H-abstraction from the phenolic O−H in curcumin would account for only ∼15% of the reaction.



(5)

H-atom transfer reactions of curcumin were also investigated using the benzophenone ketyl and 2-propanol radicals.

The photochemically generated (by 248 nm laser photolysis of 1 mM benzophenone, 0.1 M diethylamine, 0.1 M HCl in acetonitrile) benzophenone ketyl radical, $Ph_2C^•OH$, reacted with curcumin at $k = (1.5 \pm 0.1) \times 10^{10}$ M^{-1} s^{-1} in acetonitrile solutions.

The radiolytically generated (pulse radiolysis of N_2O-saturated aqueous solution of 40% 2-propanol, 10% acetone at pH 5.0) 2-propanol radical, $(CH_3)_2C^•-OH$, reacted with curcumin at $k = (7.1 \pm 0.7) \times 10^7$ M^{-1} s^{-1}.

The spectra of the resulting curcumin radicals from the ketyl and 2-propanol radical reactions are shown in Figure 2. As seen from Figure 2, in addition to the 490 nm band characteristic of the carbon centered curcumin radical, there is a broad band at ∼750 nm. Clearly, the spectra of curcumin radicals generated by the ketyl and 2-propanol radicals indicate that the H-atom transfer from the central CH_2 group is a predominant mechanism of reaction (see Figures 1 and 2). However, on the basis of the similarity of the spectra in Figure 2 with the spectra of benzophenone[27,28] and naphthoketone[29] ketyl radicals, and the well-known reducing nature of 2-propanol radical,[30] we hypothesize that the 750 nm band is due to the curcumin ketyl radical, generated in an apparently minor reaction by the reduction of a carbonyl group followed by rapid protonation of incipient curcumin radical anion.

Electron-Transfer Reactions. Electron-transfer reactions of curcumin have been the focus of interest of free radical chemists.[9−11] The methoxyphenol moiety was singled out as the most probable antioxidant reaction site. It is a common prejudice that a phenolic part of any molecule is *always* responsible for the antioxidant activity. Actually, the two ortho-methoxyphenols in curcumin can only be very moderate electron donors on the basis of the reduction potentials of the corresponding ortho-phenoxyl radicals. Taking the empirical formula[21]

$$E_7\,(\text{in V vs NHE}) = 0.95 + 0.31\Sigma\sigma^+$$

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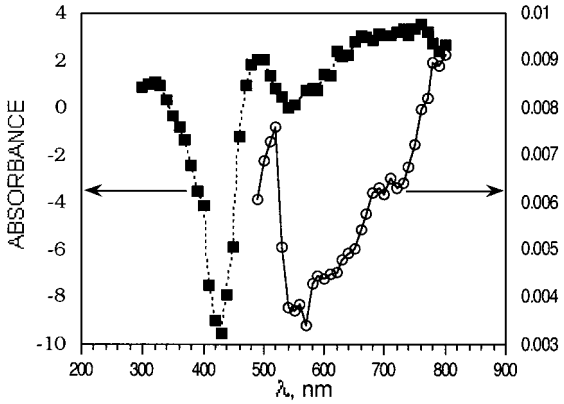

Figure 2. Spectra of curcumin transients obtained by -○- pulse radiolysis of N_2O-saturated aqueous solution of 0.73 mM curcumin, 35% 2-propanol, and 6% acetone at pH 5.0, $D = 2$ Gy/pulse and −■− 248 nm laser photolysis of Ar-saturated acetonitrile solution of 0.029 mM curcumin, 1 mM benzophenone, 1 M Diethylamine.HCl.

where σ^+ is the Brown substituent constant,[31] the calculated reduction potential of the orthomethoxyphenoxyl curcumin radical is

$$E_7 = 0.95 + 0.31(+0.39 - 0.96) = 0.77 \text{ V}$$

The actual reduction potential of the curcumin phenoxyl radicals is very difficult to determine from the equilibrium kinetics, given the very low solubility of curcumin in aqueous solutions, intensely colored solutions at high pH and the possibility of generating more than one curcumin radical. We have, therefore, used DHZ ("half-curcumin"), from which the phenoxyl radical can be generated exclusively and which is somewhat more soluble in aqueous solutions.

The reduction potential of the DHZ phenoxyl radical, $E_{6.5} = 0.86$ V vs NHE, was determined from the electron-transfer equilibrium with promethazine radical cation at pH 6.5. The promethazine radical cation was generated by pulse radiolysis in an N_2O-saturated aqueous solution of 3.11−4.18 mM promethazine·HCl, 0.1 M KBr, and 3 mM phosphate buffer. Upon addition of DHZ in concentrations from 0.016 to 0.17 mM, the promethazine radical cation, $Pz^{\bullet+}$, oxidized DHZ, MeO−Ph-OH, to the corresponding phenoxyl radical in an apparent electron-transfer reaction

$$Pz^{\bullet+} + \text{MeO}-\text{Ph-OH} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} Pz + \text{MeO}-\text{Ph-O}^{\bullet} + H^+ \quad (6)$$

From the measured pseudo-first-order rate constants, using the standard procedure,[32] the following values are obtained:

$$k_f = (1.8 \pm 0.2) \times 10^8 \text{ M}^{-1}\text{ s}^{-1},$$
$$k_r = (7.0 \pm 1.4) \times 10^5 \text{ M}^{-1}\text{ s}^{-1}, \quad \text{and} \quad K_{kin} = 260$$

We choose the more accurate equilibrium constant, which is derived from the absorbances of the promethazine radical at equilibrium, $K_{abs} = 146$, to calculate the redox potential difference

$$\Delta E = 0.059 \log 146 = 0.13 \text{ V}$$

From the redox potential difference, and the literature value of the reduction potential of the promethazine radical cation,[32] $E = 0.99$ V, we calculate the reduction potential of the DHZ phenoxyl radicals as

$$E_{6.5} = 0.99 - 0.13 = 0.86 \text{ V}$$

which is in a fair agreement with the calculated $E_{6.5} = 0.80$ V.[33]

Our value of the reduction potential of the DHZ phenoxyl radical is considerably lower than the recently published $E_6 = 1.09$ V.[34] The latter value was determined against the azide redox couple as a reference, with an equilibrium constant of $\sim 10^4$. We believe that our value is more accurate because the promethazine radical cation oxidizes half-curcumin, which means that the reduction potential of corresponding phenoxyl radicals must be lower than 0.99 V. In addition, the accuracy of any equilibrium measurements in the azide-phenol system, where both azide and phenoxyl radicals decay at $k \approx 10^9$ M^{-1} s^{-1}, is bound to large experimental error.

We have also studied the one-electron reduction of the DHZ phenoxyl radicals at pH 13.0 by *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine· (TMPD), $E_{13}(\text{TMPD}^{\bullet+}/\text{TMPD}) = 0.26$ V.[20] The DHZ phenoxyl radical was generated in an N_2O-saturated aqueous solution containing from 1 to 2.1 mM half-curcumin, 0.1 M KOH, and 0.1 M KBr. The DHZ phenoxyl radical oxidized TMPD to the corresponding radical

$$\text{MeO}-\text{Ph-O}^{\bullet} + \text{TMPD} \rightarrow \text{TMPD}^{\bullet+} + \text{MeO}-\text{Ph-O}^- \quad (7)$$

From the TMPD concentration dependent build-up of absorbance at 560 nm

$$k_f = (2.6 \pm 0.3) \times 10^9 \text{ M}^{-1}\text{ s}^{-1}$$

The reduction potential difference is calculated from the equilibrium constant derived from the absorbances of the TMPD radical cation at equilibrium, $K_{abs} = 375$

$$\Delta E = 0.059 \log 375 = 0.15 \text{ V}$$

Taking $E_{13}(\text{TMPD}^{\bullet+}/\text{TMPD}) = 0.26$ V[20] we calculate the reduction potential of the DHZ radicals as $E_{13} = 0.41$ V.[35]

$pK_a = 8.0 \pm 0.1$ of DHZ, determined by UV−vis spectroscopy, cannot account for a ~0.4 V drop in the reduction potential of the radical from pH 6.5 to pH 13. However, we have not investigated further the pH dependence of the reduction potential of the DHZ phenoxyl radical.

The reduction potential of the phenoxyl radical of DHZ is relatively high in comparison with well-known antioxidants-electron donors, such as vitamin E ($E_7 = 0.48$ V; $E_{13} = 0.19$ V),[20] gallocatechins ($E_7 = 0.44$ V)[36] or vitamin C ($E_7 = 0.28$ V).[20] In fact, it was reported[9] that curcumin phenoxyl was

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(33) While our manuscript was being reviewed we found that 4-methoxyphenoxyl radical, generated by pulse radiolysis in N_2O-saturated aqueous solution of 0.1 M KBr, oxidizes DHZ with $k_f = 2.2 \times 10^8$ M^{-1} s^{-1} and $k_r = 8 \times 10^7$ M^{-1} s^{-1} ($K_{kin} = 3$; $K_{abs} = 3$). Based on $E_{6.5}$(4-methoxyphenol) = 0.80 V/NHE, $E_{6.5} = 0.77$ V/NHE is derived for DHZ radical. Taking this value and adding $E_{6.5} = 0.86$ V vs Pz, we derive $E_{6.5} = 0.83 \pm 0.06$ V.

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

(35) While our manuscript was being reviewed, we found that DHZ radical oxidized 3,5-dihydroxyanisole at pH 13 in N_2O-saturated aqueous solution of 0.1 M KBr, with $k_f = 8.6 \times 10^8$ M^{-1} s^{-1} and $k_r = 1.5 \times 10^8$ M^{-1} s^{-1} ($K_{kin} = 5.7$; $K_{abs} = 4.6$). Based on E_{13}(3,5-DHA) = 0.46 V, we calculate E_{13}(DHZ) = 0.5 V. Taking this as a more accurate and adding 0.41 V determined against TMPD, E_{13}(DHZ) = 0.47 ± 0.06 V.

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capable of oxidizing vitamin E and vitamin C, in complete agreement with the reduction potentials of corresponding radicals.

The oxidation potential of DHZ (which is very similar to that of curcumin) is lower than $E_7 = 1.05$ V of alkyl peroxyl radicals,[37] which means that curcumin can neutralize alkyl peroxyl radicals, such as lipid peroxyl, by donating an electron.

Such moderate electron donating ability and relative insolubility in water, which is an ideal medium for electron-transfer processes involving peroxyl radicals,[38] are likely to diminish the importance of electron transfer in antioxidant mechanisms of curcumin.

Conclusions

Curcumin is practically insoluble in water at neutral pH. In slightly acidic media and possibly in the interior of cell membranes, it is likely to exist in the keto form. This form appears to favor H-atom transfer reactions.

On the basis of the rate constants of H-atom transfer reactions, curcumin is a superb H-atom donor. It appears to be better than such well-known, "classical" H-atom donors as thiols. $k(^{\bullet}CH_3 + \text{curcumin}) = 3.5 \times 10^9\ M^{-1}\ s^{-1}$ is more than an order of magnitude higher than the fastest known $k(^{\bullet}CH_3 + \text{4-methoxythiophenol, at pH 3}) = 1.3 \times 10^8\ M^{-1}\ s^{-1}$. It may, therefore, be concluded that H-atom transfer plays a crucial role in the antioxidant action of curcumin.

The one-electron reduction potential of the methoxyphenol moiety in curcumin, as estimated from the measurements of the reduction potential of the DHZ ("half-curcumin") phenoxyl radical, is relatively high, $E_{6.5} = 0.83 \pm 0.06$ V.[33] Although this enables scavenging of alkylperoxyl radicals by electron transfer, it is much higher than $E_7 = 0.48$ V of vitamin E and $E_7 = 0.28$ V of vitamin C. In agreement with this, curcumin transients can oxidize vitamin E and C to the corresponding free radicals.

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