

Selected Paper

Kinetic Study of the Mechanism of Free-Radical Scavenging Action in Curcumin: Effects of Solvent and pH

Keishi Ohara,* Wataru Mizukami, Aiko Tokunaga, Shin-ichi Nagaoka, Hidemitsu Uno,[1] and Kazuo Mukai

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

[1]Department of Molecular Science, Integrated Center for Science (INCS), Ehime University, Matsuyama 790-8577

Received October 14, 2004; E-mail: ohara@chem.sci.ehime-u.ac.jp

A kinetic study was performed for the free-radical scavenging actions of curcumin and half-curcumin, which has a half-structure of curcumin, in order to clarify the mechanism of free-radical-scavenging in curcumin. The second-order rate constants for radical-scavenging reactions of curcumin and half-curcumin were measured by a stopped-flow spectrophotometer in several organic solvents (methanol, ethanol, acetonitrile, chloroform, and benzene) and in aqueous Triton X-100 (5.0 wt %) micelle solutions at various pH. The difference in the rate constant and solvent dependence between curcumin and half-curcumin suggests that the enol structure with the intramolecular hydrogen-bond of curcumin strongly enhances the radical-scavenging activity. Furthermore, notable pH dependences were observed for the rate constants of curcumin and half-curcumin in micelle solutions, suggesting that the acid–base dissociation equilibrium of phenol-protons in curcumin and half-curcumin affects their radical-scavenging activities.

In recent years, many scientific reports on curcumin (Fig. 1a), which is a yellow pigment contained in the Indian spice turmeric, have been available in journals of various fields: medicine, pharmacy, agriculture, biology, and chemistry.[1–20] Remarkable pharmacological activities of curcumin in living bodies and tissues have been found one after another, and these important biological and medical aspects still activate many scientists. The antioxidant activity is considered to be one of the most important factors in pharmacological activities, because it would protect living bodies and tissues from diseases and injuries caused by active-oxygen species (AOS).[1–5] In fact, inhibitions of lipid-peroxidation[3–5] and DNA-cleavage,[5] and cancer-preventive effects[1,2] have been reported for curcumin, and are considered to result from its high antioxidant activity against AOS, such as singlet oxygen and free radicals.[1–9] The antioxidant mechanism for curcumin has been of great interest because of its relatively high activity compared with other polyphenol compounds, such as catechins and flavonoids.

Recently, several contradictory reports on active sites in the curcumin molecule for the hydrogen abstraction of free-radical species were published.[10–16] In general, the free-radical-scavenging action of curcumin has been considered to be performed by a hydrogen atom transfer (HAT) from its phenolic OH-groups to radicals, being similar to that of other well-known natural antioxidants, such as tocopherols, catechins, and flavonoids. This mechanism is reasonable because curcu-

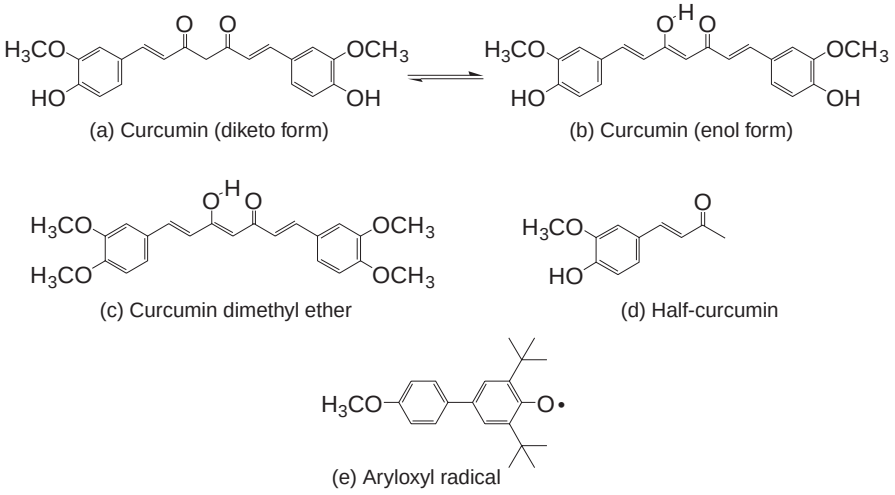


Fig. 1. Molecular structures of curcumin (β-diketo and enol forms), curcumin dimethyl ether, half-curcumin, and aryloxyl radical (ArO$^\bullet$).

Published on the web April 8, 2005; DOI 10.1246/bcsj.78.615

min has a structure in which two ferulic acids are condensed, and thus has two *o*-methoxyphenol moieties that should have HAT activity. However, remarkable high activity of curcumin compared with the other polyphenols observed in biological and pharmacological systems could not be explained only in terms of additional activity as a dimer of ferulic acid. This high activity of curcumin might come from the unique π-conjugated structure, including two *o*-methoxyphenols, that is, the enol form of curcumin (see Fig. 1b). Recently, Jovanovic et al. proposed a novel mechanism for the HAT activity of curcumin, in which it is given by the methylene hydrogens (–CH_2–) at the molecular center of curcumin, and is responsible for the high antioxidant activity of curcumin.[10,11] Barclay et al.[12] and Priyadarsini et al.[13–15] independently demonstrated the importance of the phenolic OH-groups for antioxidant actions from investigations of curcumin derivatives whose phenolic OH-groups are protected by methylation (Fig. 1c). Wright has reported a systematic theoretical study on the HAT activity of curcuminoids, and has also concluded that this activity mainly comes from the phenolic OH-groups, and that the methylene hydrogens of curcumin have little activity for HAT.[16]

Curcumin is often represented as an α,β-unsaturated β-diketone (heptadiene-dione, diketo form) structure (Fig. 1a), but it should exist as a conjugated β-hydroxy-α,β-unsaturated ketone (enol form) (Fig. 1b) in many media. Crystallographic,[17–19] NMR,[20] and theoretical[16] studies have suggested that curcumin exists predominantly in the enol form in nonpolar organic solvents because of strong stabilization due to the intramolecular hydrogen bond between the enol-proton and its neighboring carbonyl-oxygen. On the other hand, in polar protic solvents, hydrogen bonding between curcumin and solvent molecules may become important because it can inhibit the intramolecular hydrogen bond in curcumin. Although these keto–enol tautomerism and solvation effect are considered to largely affect the antioxidant activity of curcuminoids, few reports related to these points have been available. Consequently, further investigations are necessary for understanding the antioxidant mechanism and high activity of curcumin.

In the present study, a kinetic approach to the antioxidant mechanism of curcumin was performed in view of the solvent and pH effects. A model molecule, "half-curcumin" (4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one) (Fig. 1d), which has the half-structure of curcumin, was used to compare its radical-scavenging activity with that of curcumin in various solvents. Since half-curcumin has a shorter π-conjugation than curcumin, it will become a bench mark for the activity of *o*-methoxyphenol moiety in curcumin lacking π-conjugation. The second-order rate constant (k_s) for the reaction of a curcuminoid with an aryloxyl radical (2,6-di-*t*-butyl-4-(4-methoxyphenyl)phenoxyl, ArO$^\bullet$, Fig. 1e) (Scheme 1) has been measured by a stopped-flow spectrophotometer in several solvents and aqueous Triton X-100 (TX-100) micelle solutions at various pH values. The effects of the keto–enol tautomerism and the dissociation of phenolic protons in the HAT action of curcumin are discussed on the basis of results obtained by measurements of the solvent and pH effects on the reaction rate (k_s).

$$\mathrm{ArO}\cdot + \mathrm{Curcuminoid} \xrightarrow{k_s} \mathrm{ArOH} + \mathrm{Curcuminoid}\cdot$$

Scheme 1.

Experimental

Curcumin is a commercially available reagent from Wako Pure Chemicals, and was used as received. Half-curcumin was synthesized from vanillin. ArO$^\bullet$ was prepared according to a method reported before.[21] Methanol and ethanol were dried with NaH and purified by distillation. Chloroform, benzene, and acetonitrile were obtained from Wako, and purified by distillation. Triton X-100 was an extra-pure grade reagent commercially available from Nacalai Tesque, and was used as received. All buffer solutions were prepared using deionized water purified by a Millipore Q system. The pH of the solutions was adjusted using the following buffers, whose concentrations were 0.1 mol dm^{-3} (M): pH 2.0–3.0, CH_3COONa–HCl; pH 4.0–5.0, CH_3COONa–CH_3COOH; pH 6.0–8.0, Na_2HPO_4–KH_2PO_4; pH 9.0–11.0, Na_2CO_3–$NaHCO_3$.[21,22] The concentration of TX-100 in the buffer solutions was kept at 5.0 wt %.

The kinetic data were obtained with a Unisoku Model RS-450 stopped-flow spectrophotometer by mixing equal volumes of an antioxidant solution and ArO$^\bullet$ solution under a nitrogen atmosphere.[21,22] The reactions were studied under pseudo-first-order conditions, and the absorption decay of ArO$^\bullet$ was well-characterized by a single exponential decay. The decay-rate constants were estimated in the usual way using a standard least-squares analysis. All of the experiments were performed at 25 °C. The detailed experimental procedures were reported in previous papers.[21,22]

Results and Discussion

Solvent Dependence of the Aryloxyl-Radical Scavenging Rate Constants (k_s) for Curcumin and Half-curcumin. The pseudo-first-order rate constant (k_{obsd}) for the scavenging reaction of ArO$^\bullet$ by curcuminoid was obtained from the decrease in the absorbance at 580 nm of ArO$^\bullet$ radical (Fig. 2). The rate constant (k_{obsd}) is given by[21]

$$k_{obsd} = k_0 + k_s[\mathrm{curcuminoid}], \quad (1)$$

where k_0 is the rate constant for the natural decay of ArO$^\bullet$ in the medium. The second-order rate constant (k_s) was obtained as a slope of plots of k_{obsd} versus the concentration of curcuminoid ([curcuminoid]). The k_s values obtained for the scavenging reaction of ArO$^\bullet$ by curcumin and half-curcumin in meth-

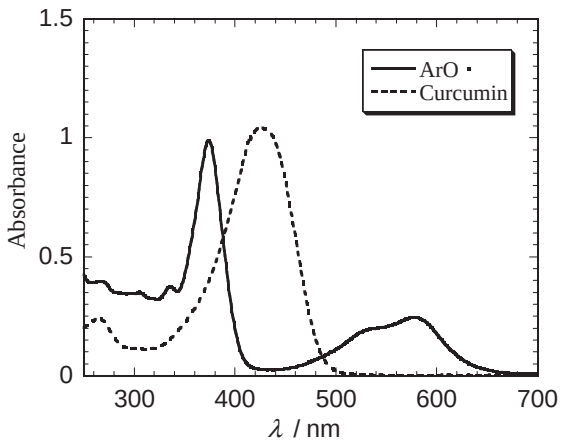


Fig. 2. Absorption spectra of aryloxyl radical and curcumin.

Table 1. Second-Order Rate Constants for the Scavenging Reaction of ArO• with Curcumin (k_s^{CR}) and Half-curcumin ($k_s^{Half\text{-}CR}$) in Organic Solvents and the Aqueous Triton X-100 Micelle Solutions (5.0 wt %, pH 7.0) at 25.0 °C

Solvent	$k_s^{CR}/M^{-1}\,s^{-1}$	$k_s^{Half\text{-}CR}/M^{-1}\,s^{-1}$	$k_s^{CR}/k_s^{Half\text{-}CR}$	$E_T(25)$[a)]
Methanol	3.53×10	1.27×10	2.77	55.5
Ethanol	4.01×10	1.53×10	2.62	51.9
Acetonitrile	1.71×10^2	3.51×10	4.86	46.0
Chloroform	4.02×10^2	7.04×10	5.71	39.1
Benzene	5.52×10^2	1.24×10^2	4.46	34.5
TX-100 at pH 7	1.27×10^3	1.12×10^2	11.3	—

a) Parameter which take into account of solvent polarity and hydrogen-bonding ability.[8,23]

anol, ethanol, acetonitrile, chloroform, benzene, and aqueous TX-100 micelle solutions (5.0 wt %) at pH 7.0 are listed in Table 1.

The k_s values for curcumin (k_s^{CR}) in ethanol (4.01×10 $M^{-1}\,s^{-1}$) were much smaller than those of α-tocopherol (5.12×10^3 $M^{-1}\,s^{-1}$) and ubiquinol-10 (5.19×10^3 $M^{-1}\,s^{-1}$), and the same order as that of BHT (butylated hydroxytoluene, 2,6-di-*t*-butyl-4-methylphenol, $k_s = 3.5 \times 10$ $M^{-1}\,s^{-1}$) reported in a previous paper.[22] The k_s value for half-curcumin ($k_s^{Half\text{-}CR}$) in ethanol (1.53×10 $M^{-1}\,s^{-1}$) was 38% of k_s^{CR}. This result seems to be reasonable from the view that curcumin would act as a dimer of half-curcumin. A similar result was obtained in methanol. In acetonitrile, benzene, and chloroform, the k_s^{CR} values were 4–14 times as large as that obtained in ethanol. On the other hand, the $k_s^{Half\text{-}CR}$ values in these solvents were 2–8 times as large as that obtained in ethanol. In these solvents, the k_s^{CR} values were rather larger than twice the $k_s^{Half\text{-}CR}$ values. For a detailed discussion, the ratios of ($k_s^{CR}/k_s^{Half\text{-}CR}$) are also listed in Table 1. The $k_s^{CR}/k_s^{Half\text{-}CR}$ values seem to include important information. The $k_s^{CR}/k_s^{Half\text{-}CR}$ values were expected to be around 2 on the basis that curcumin has a dimer-structure of half-curcumin. The fact that the $k_s^{CR}/k_s^{Half\text{-}CR}$ values in methanol and ethanol were around 2.7 suggests that curcumin would act as a dimer of half-curcumin in alcohols. On the other hand, the $k_s^{CR}/k_s^{Half\text{-}CR}$ values observed in acetonitrile, benzene, and chloroform are more than 4, clearly suggesting that the activity of curcumin was enhanced in these solvents. The radical-scavenging activity of curcumin was shown to be strongly dependent on the solvents.

Figure 3 shows semi-logarithm plots of k_s^{CR} (circle) and $k_s^{Half\text{-}CR}$ (square) obtained in this study versus $E_T(25)$, which is a parameter taking into account of solvent polarity and hydrogen-bonding ability.[8,23] The obtained linear relation of logarithms of $k_s^{Half\text{-}CR}$ to $E_T(25)$ supports that the radical-scavenging reaction by half-curcumin progresses as typical HAT from phenolic OH group. On the other hand, plots for curcumin separated into two groups of solvents: i.e., (i) methanol and ethanol, (ii) acetonitrile, chloroform, and benzene. The linear relation of logarithms of k_s^{CR} to $E_T(25)$ for a group of (ii) solvents also supports that the radical-scavenging reaction by curcumin progresses as typical HAT. However, a difference in the plots of curcumin between (i) alcohols and (ii) the other solvents suggests that curcumin has a different way to act in alcohols from that in the other solvents. According to these results, the difference in the solvent effect on k_s between curcumin and half-curcumin can be explained as follows.

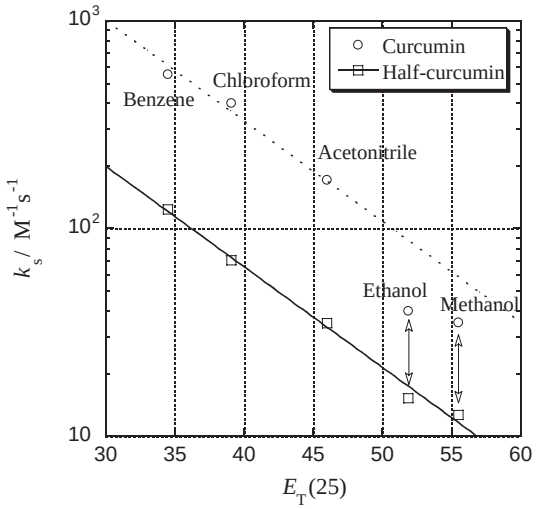


Fig. 3. Semi-logarithm plots of k_s^{CR} (circle) and $k_s^{Half\text{-}CR}$ (square) versus $E_T(25)$ for solvents.

Curcumin is considered to exist dominantly as the enol form (*cis*-enol, Fig. 1b) in acetonitrile, chloroform, and benzene because of a strong intramolecular hydrogen-bond between the enol-proton and its neighboring carbonyl-oxygen. One of the reasons for this is that these aprotic solvents have little ability to make a hydrogen bond with the carbonyl-oxygen of curcumin. A NMR spectrum of curcumin in deuterated chloroform ($CDCl_3$) also indicates the enol structure of curcumin by the observation of an enol-proton around 17 ppm. Moreover, a symmetrical equivalency of methylene protons and two phenol groups in the NMR spectrum suggests that a fast exchange (migration) of the enol-proton occurs between two oxygen sites (Fig. 4a). The enol structure and its intramolecular proton-migration can strongly stabilize its corresponding phenoxyl radical, because the delocalization of an unpaired electron can spread into every part of the curcumin radical (Fig. 4b). In addition, it is considered that the enol form of curcumin should have a lower oxidation potential coming from its extended π-conjugation compared with half-curcumin. The notable stabilization in the radical and the lower oxidation potential would raise the HAT activity of curcumin. This is the reason why the k_s^{CR} value is larger than twice that of $k_s^{Half\text{-}CR}$ in group (ii) solvents (see Table 1).

On the other hand, in alcohols, alcohol molecules would easily form hydrogen bonds with the carbonyl oxygen of curcumin using alcoholic OH protons. This type intermolecular

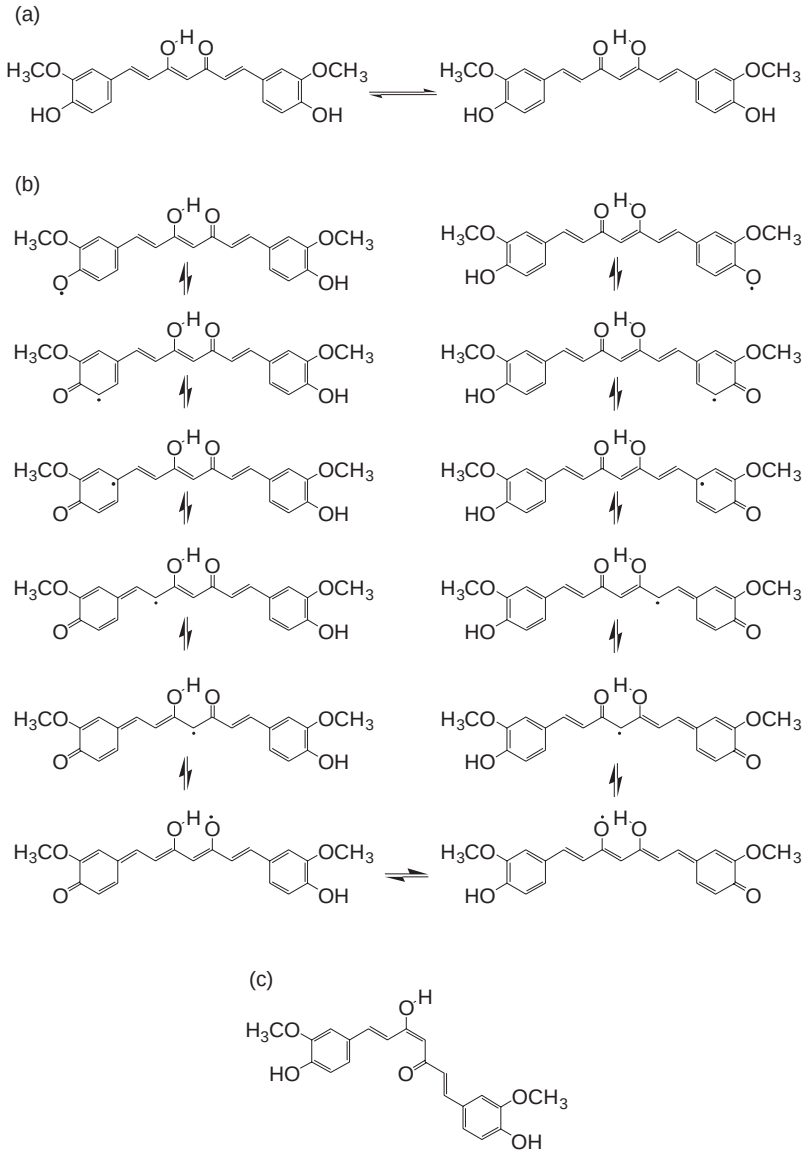


Fig. 4. (a) Intramolecular hydrogen-migration in curcumin. (b) Resonance structure of curcumin radical. (c) Molecular structure of *trans*-enol form of curcumin.

hydrogen bond might inhibit the formation of intramolecular hydrogen bonds in curcumin. As a result, curcumin may exist as several forms including *trans*-enol (Fig. 4c) and diketo forms (Fig. 1a). Such a solvation might restrict delocalization of an unpaired electron in the curcumin radical, and might reduce the stabilization of the curcumin radical. Therefore, curcumin would almost act as a dimer of half-curcumin in alcohols. The spectroscopic results in previous studies also suggest intermolecular hydrogen bonding between curcumin and the solvent alcohol.[8,24,25] This intermolecular hydrogen bonding becomes important in protic solvents, shifting the equilibrium in favor of the *trans*-enol and diketo forms.

Interestingly, in the TX-100 micelle at pH 7.0, the k_s^{CR} value was 11-times as large as the $k_s^{Half\text{-}CR}$ value. This fact suggests that curcumin should exist as an activated *cis*-enol form with an intramolecular hydrogen bond in the aqueous TX-100 micelle system, similarly to in chloroform and benzene, despite of existence of many water molecules that can inhibit intramolecular hydrogen-bonding. This may be one of the important factors for understanding the high activity of curcumin in biological systems, such as in membranes and various tissues. The detailed radical-scavenging behavior of curcuminoids in micelle solutions is discussed in the next section.

pH Dependence of the Aryloxyl Radical Scavenging Rate (k_s) for Curcumin and Half-curcumin. In previous work, kinetic studies of the radical-scavenging reaction of vitamin C and flavonoids in aqueous TX-100 micelle solutions at various pH values were performed using a stopped-flow spectrophotometry.[21,22,26] Their second-order rate constants showed notable pH dependences, according to the variation of the mole fraction; also the activity of some species coexisted by the acid–base dissociation equilibrium.

In this work, the second-order rate constants of the scavenging reaction of $ArO^{\bullet}$ by curcumin and half-curcumin in TX-100 micelle solutions were measured by varying the pH. The rate constants obtained for curcumin and half-curcumin (k_s^{CR}

Table 2. pH Dependence of the Second-Order Rate Constants for the Scavenging Reaction of ArO• with Curcumin (k_s^{CR}) and Half-curcumin ($k_s^{Half\text{-}CR}$) in the Triton X-100 Micelle Solutions (5.0 wt %) at 25.0 °C

pH	$k_s^{CR}/M^{-1}\,s^{-1}$	$k_s^{Half\text{-}CR}/M^{-1}\,s^{-1}$
4.0	1.25×10^3	1.13×10^2
5.0	1.22×10^3	1.13×10^2
6.0	1.26×10^3	1.16×10^2
7.0	1.27×10^3	1.12×10^2
8.0	1.44×10^3	1.16×10^2
8.5	1.87×10^3	—
9.0	2.05×10^3	8.94×10
9.5	2.18×10^3	—
10.0	2.07×10^3	4.89×10
11.0	1.67×10^3	2.01×10
12.0	8.55×10^2	$<10^{-1}$

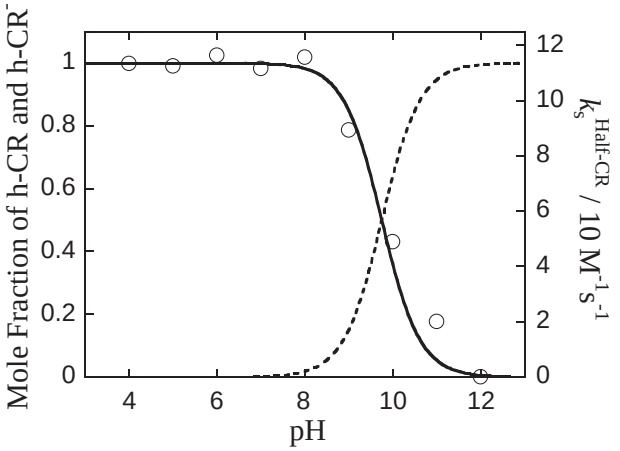
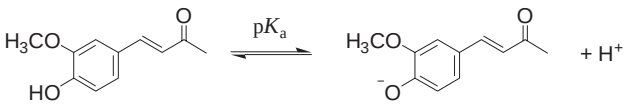


Fig. 5. Plots of second-order rate constant ($k_s^{Half\text{-}CR}$) for the scavenging reaction of ArO• by half-curcumin versus pH (open circle). Solid and broken lines show mole-fraction of two half-curcumin species (h-CR and h-CR$^-$) versus pH calculated by assuming p$K_a = 9.75$.

and $k_s^{Half\text{-}CR}$) are listed in Table 2. The k_s^{CR} values in the micelle solutions were around 10^3 $M^{-1}\,s^{-1}$, and one to two orders of magnitude larger than those obtained in homogeneous solutions, as listed in Table 1. On the other hand, the $k_s^{Half\text{-}CR}$ values in micelle solutions were equal to, or less than, 10^2 $M^{-1}\,s^{-1}$, the same order as that in benzene, and larger than those in alcohols and chloroform. The rate constants for both curcumin and half-curcumin showed remarkable pH dependences.

Figure 5 shows plots of the second-order rate constants ($k_s^{Half\text{-}CR}$) for the scavenging reaction of ArO• by half-curcumin versus pH. The observed rate constants were constant in pH 4–8, and suddenly decrease at pH 9 to pH 12 with an increase of the pH. This pH-dependent behavior of $k_s^{Half\text{-}CR}$ was analogous to those observed for vitamin C and rutin previously reported.[21,22,26] Half-curcumin has a phenolic hydroxy proton that can be released according to an acid–base equilibrium. This equilibrium should be represented by the following scheme (Scheme 2). The undissociated form (h-CR) and dissociated monoanion form (h-CR$^-$) of half-curcumin were considered to have different rate constants for radical-scavenging. The pH dependence of $k_s^{Half\text{-}CR}$ may be represented as the sum of the contributions for both species (Eq. 2),



Scheme 2.

$$k_s^{Half\text{-}CR} = k_s^{a} f(\text{h-CR}) + k_s^{b} f(\text{h-CR}^-), \quad (2)$$

where k_s^a and k_s^b are second-order rate constants (independent of pH) for h-CR and h-CR$^-$, f(h-CR) and f(h-CR$^-$) are the pH-dependent mole fractions of h-CR and h-CR$^-$. The k_s^a value was determined to be 1.13×10^2 $M^{-1}\,s^{-1}$ by averaging the observed $k_s^{Half\text{-}CR}$ values at pH 4–7, because only the undissociated form (h-CR) exists in solution in this pH region. The k_s^b value was determined to be 0 from the $k_s^{Half\text{-}CR}$ value (less than 10^{-1} $M^{-1}\,s^{-1}$) at pH 12, where only the mono-anion form (h-CR$^-$) was considered to exist in solution. The pK_a value for half-curcumin was determined to be 9.75 from the best fit using Eq. 2 and the k_s^a and k_s^b values. The value of pK_a (= 9.75) is reasonable compared with other *o*-methoxyphenols. The solid and broken lines in Fig. 5 show the pH-dependent mole-fractions of two half-curcumin forms (h-CR and h-CR$^-$), calculated by assuming p$K_a = 9.75$. Good accordance between the measured k_s and the calculated mole-fraction curve of the undissociated form was obtained. It is important that the monoanion form of half-curcumin (h-CR$^-$) scarcely has any scavenging activity versus ArO•. The present result suggests that a phenolic –OH proton is essential for the antioxidant action of half-curcumin, and the present free-radical scavenging reaction progresses as HAT.

Figure 6 shows plots of the second-order rate constants (k_s^{CR}) for the scavenging reaction of ArO• by curcumin versus the pH. The observed rate constant was almost constant at 1.25×10^3 $M^{-1}\,s^{-1}$ in pH 4–7, and gradually increased to a maximum value 2.18×10^3 $M^{-1}\,s^{-1}$ at pH 9.5, and decreased to 8.55×10^2 $M^{-1}\,s^{-1}$ at pH 12 with an increase of the pH. This pH-dependent behavior of k_s^{CR} is analogous to that observed for vitamin C.[21] Therefore, the present result might be explained by taking account of the pH-dependent contributions of three species (CR, CR$^-$, and CR^{2-}) produced by the acid–base dissociation equilibrium of curcumin, which has different antioxidant activity (Scheme 3). The pH dependence of the rate constant (k_s^{CR}) can be represented as the sum of the contributions for three species,

$$k_s^{CR} = k_s^{a} f(\text{CR}) + k_s^{b} f(\text{CR}^-) + k_s^{c} (\text{CR}^{2-}), \quad (3)$$

where k_s^a, k_s^b, and k_s^c are the second-order rate constants (independent of pH) for the CR, CR$^-$, and CR^{2-} forms, respectively. f(CR), f(CR$^-$), and f(CR^{2-}) are the pH-dependent mole-fractions of CR, CR$^-$, and CR^{2-}, respectively. Using a similar procedure to the case of half-curcumin, the determination of these rate constants (k_s^a, k_s^b, and k_s^c) and the dissociation constants (pK_{a1} and pK_{a2}) for curcumin was tried. The k_s^a value was determined to be 1.25×10^3 $M^{-1}\,s^{-1}$ by averaging the observed $k_s^{Half\text{-}CR}$ values at pH 4–6. The k_s^c value was as-

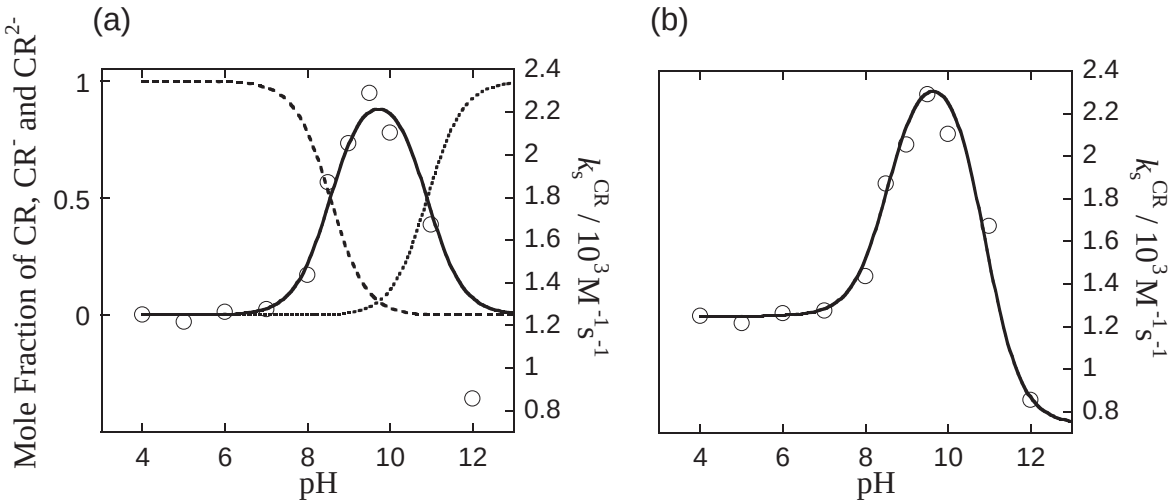
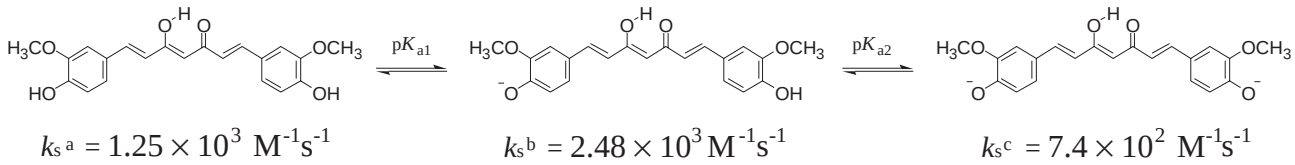


Fig. 6. Plots of second-order rate constants (k_s^{CR}) for the scavenging reaction of ArO• by curcumin versus pH (open circle). Broken, solid, and dotted lines in Fig. 6a show mole fractions of three curcumin species (CR, CR^-, and CR^{2-}) versus pH, calculated by assuming $pK_{a1} = 8.55$ and $pK_{a2} = 10.9$. Solid line in Fig. 6b shows plots of the calculated k_s^{total} versus pH according to Eq. 3 using the obtained k_s^a, k_s^b, and k_s^c values and the calculated mole fractions.



Scheme 3.

sumed to be slightly less than the k_s^{CR} value at pH 12 ($8.55 \times 10^2\ M^{-1}\,s^{-1}$), because the mole fraction of the di-anion form (CR^{2-}) would be more than 0.9 at pH 12. The k_s^b value is considered to be similar to the maximum k_s^{CR} value at pH 9.5 ($2.18 \times 10^3\ M^{-1}\,s^{-1}$) because the mono-anion form (CR^-) shows the largest activity in three species and the largest contribution at pH 9.5. The fitting simulations to the observed pH-dependence curve of k_s^{CR} were performed by varying the k_s^b, k_s^c pK_{a1}, and pK_{a2} values. From the best fitting result, each value for curcumin was estimated to be $pK_{a1} = 8.55$, $pK_{a2} = 10.9$, $k_s^b = 2.48 \times 10^3\ M^{-1}\,s^{-1}$, and $k_s^c = 7.4 \times 10^2\ M^{-1}\,s^{-1}$. These obtained pK_a values agree with those reported.[14,27] The broken, solid, and dotted lines in Fig. 6a show the mole fractions of three curcumin species (CR, CR^-, and CR^{2-}), respectively, versus the pH, calculated using $pK_{a1} = 8.55$ and $pK_{a2} = 10.9$. The solid line in Fig. 6b shows a plot of the calculated k_s^{CR} versus pH using the obtained k_s^a, k_s^b, k_s^c, pK_{a1}, and pK_{a2} values. Good accordance between the measured rate constants and the simulated curve in Fig. 6b was obtained.

The present result for the pH dependence indicates that the monoanion form of curcumin (CR^-) has the highest activity for a radical-scavenging reaction in three forms of curcumin. Under the standard biological conditions (pH ~ 7), curcumin does not show its full ability because it takes the undissociated form (CR), whose k_s^a value is half of k_s^b for CR^-. The activity of the di-anion form (CR^{2-}) was further less than those of the other forms, but rather larger than that of half-curcumin. As a result, the mechanism of the radical-scavenging reaction by curcumin can be explained in the following way. The radical-scavenging reaction by curcumin progresses as HAT, and its activity is responsible for both (i) the existence of the phenolic –OH proton, and (ii) its oxidation potential.[22,26] Because the negative charge in the mono-anion form might reduce the oxidation potential of phenolic –OH, the mono-anion form shows a larger activity than the undissociated form. The di-anion form (CR^{2-}) has no phenolic proton as a result of two-proton dissociation. However, its radical-scavenging activity cannot be negligible. This fact suggests that the electron-transfer process partially contributes to the radical-scavenging reaction by curcumin at a high pH. There might be another explanation that the di-anion form activity is responsible for the activity of the methylene protons or enol-proton. However, the rate constant obtained for curcumin dimethyl ether (Fig. 1c), which has no phenolic –OH group, was less than $10^{-2}\ M^{-1}\,s^{-1}$ in the TX-100 micelle system at pH 7.0. Therefore, we concluded that the activity of the di-anion form was responsible for the conjugated phenolate structure and the contribution of the electron-transfer processes.

In TX-100 micelle systems, the values of k_s^{CR} were always more than 10-times as large as those of $k_s^{Half\text{-}CR}$ at pH 4–12. This is very important for understanding the remarkably high activity of curcumin in biological systems, such as in membranes, various tissues, and so on. It is natural to consider that the remarkable high activity for the radical-scavenging reaction by curcumin is due to the longer π-conjugation in its structure compared with other *o*-methoxyphenols, including half-curcumin. Considering an investigation of the solvent ef-

fect of k_s^{CR} and $k_s^{Half\text{-}CR}$, curcumin should exist as activated *cis*-enol form in aqueous TX-100 micelle system, similarly to in chloroform and benzene, despite the existence of many water molecules that can inhibit the intramolecular hydrogen bond in curcumin. It is probable that the enol form and the intramolecular hydrogen-bonding around the molecular center of curcumin are stabilized in the hydrophobic area of the micelle by protecting from water molecules. As described above, the enol form with the intramolecular hydrogen bond of curcumin would show remarkable activity because of the stabilization of its corresponding radical by delocalization of an unpaired electron.

Conclusion

A kinetic study was performed on the free-radical scavenging actions of curcumin and half-curcumin in order to clarify the mechanism of free-radical-scavenging in curcumin. The second-order rate constants for radical-scavenging reactions of curcumin and half-curcumin were measured in several organic solvents and in aqueous TX-100 micelle solutions at various pH. The difference in the rate constant and solvent dependence between curcumin and half-curcumin suggests that the enol structure with the intramolecular hydrogen-bond of curcumin strongly enhances the radical-scavenging activity. Furthermore, notable pH dependences were observed for the rate constants of curcumin and half-curcumin in micelle solutions, suggesting that the acid–base dissociation equilibrium of phenol-protons in curcumin and half-curcumin affects their radical-scavenging activities. These solvent and pH dependences suggest that the radical-scavenging reaction by curcumin progresses mainly as HAT, and its activity is almost responsible for the phenolic –OH protons and the oxidation potential of curcumin. Because curcumin exists as the enol form in the oil–water interface region of membranes, curcumin acts as a good free-radical scavenger as well as rutin and vitamin C in biological systems.

We thank Professor L. R. C. Barclay and Dr. M. R. Vinqvist of Mt. Allison University for their kindness and useful discussions. This work was supported by Grants-in-Aid for the Scientific Research C (15550123, 16550016) and Scientific Research on Priority Areas "Application of Molecular Spins: Nanomagnets to Biological Spin Systems" (Area No. 769, 15087104) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT).

References

1 "Phenolic Compounds in Food and Their Effects on Health II," ed by M.-T. Huang, C.-T. Ho, and C. Y. Lee, American Chemical Society, Washington DC (1992).

2 M.-T. Huang, R. C. Smart, C. Q. Wong, and A. H. Conney, *Cancer Res.*, **48**, 5941 (1988).

3 O. P. Sharma, *Biochem. Pharmacol.*, **25**, 1811 (1976).

4 E. Kunchundy and M. N. A. Rao, *Int. J. Pharm.*, **57**, 173 (1989).

5 E. Kunchundy and M. N. A. Rao, *Int. J. Pharm.*, **58**, 237 (1990).

6 H. H. Tønnesen, *Int. J. Pharm.*, **50**, 91 (1989).

7 K. C. Das and C. K. Das, *Biochem. Biophys. Res. Commun.*, **295**, 62 (2002).

8 F. Ortica and M. A. J. Rodgers, *Photochem. Photobiol.*, **74**, 745 (2001), and references therein.

9 T. Masuda, Y. Toi, H. Bando, T. Maekawa, Y. Takeda, and H. Yamaguchi, *J. Agric. Food Chem.*, **50**, 2524 (2002).

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

11 S. V. Jovanovic, C. W. Boone, S. Steenken, M. Trinoga, and R. B. Kaskey, *J. Am. Chem. Soc.*, **123**, 3064 (2001).

12 L. R. C. Barclay, M. R. Vinquist, K. Mukai, H. Goto, Y. Hashimoto, A. Tokunaga, and H. Uno, *Org. Lett.*, **2**, 2841 (2000).

13 K. I. Priyadarsini, *Free Radical Biol. Med.*, **23**, 838 (1997).

14 K. I. Priyadarsini, D. K. Maity, G. H. Naik, M. S. Kumar, M. K. Unnikrishnan, J. G. Satav, and H. Mohan, *Free Radical Biol. Med.*, **35**, 475 (2003).

15 S. M. Khopde, K. I. Priyadarsini, P. Venkatesan, and M. N. A. Rao, *Biophys. Chem.*, **80**, 83 (1999).

16 J. S. Wright, *J. Mol. Struct.: THEOCHEM*, **591**, 207 (2002).

17 H. H. Tønnesen, J. Karlsen, and A. Mostad, *Acta Chem. Scand.*, **B36**, 475 (1982).

18 H. H. Tønnesen, J. Karlsen, A. Mostad, U. Pedersen, P. B. Rasmussen, and S. O. Lawesson, *Acta Chem. Scand.*, **B37**, 179 (1983).

19 J. Karlsen, A. Mostad, and H. H. Tønnesen, *Acta Chem. Scand.*, **B42**, 23 (1988).

20 A. A. Gorman, I. Hamblett, S. V. Srinivasan, and P. D. Wood, *Photochem. Photobiol.*, **59**, 389 (1994).

21 K. Mukai, M. Nishimura, and S. Kikuchi, *J. Biol. Chem.*, **266**, 274 (1991).

22 K. Mukai, W. Oka, K. Watanabe, Y. Egawa, S. Nagaoka, and J. Terao, *J. Phys. Chem. A*, **101**, 3746 (1997).

23 K. Dimroth, C. Reichardt, T. Siepmann, and F. Bohlmann, *Liebigs Ann. Chem.*, **661**, 1 (1963).

24 C. F. Chignell, P. Bilski, K. J. Reszka, A. G. Motten, R. H. Silk, and T. A. Dahl, *Photochem. Photobiol.*, **59**, 295 (1994).

25 M. O. Iwunze, *Tenside, Surfactants, Deterg.*, **40**, 96 (2003).

26 K. Mukai, Y. Kanesaki, Y. Egawa, and S. Nagaoka, "Phytochemicals and Phytopharmaceuticals," ed by F. Shahidi and C.-T. Ho, AOCS Press, Champaign, Illinois (2000), Chap. 20.

27 M. Borsari, E. Ferrari, R. Grandi, and M. Saladini, *Inorg. Chim. Acta*, **328**, 61 (2002).