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Fluorescence Quenching Studies of Curcumin by Hydrogen Peroxide in Acetonitrile Solution

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Summary. Steady state quenching studies of curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, fluorescence by hydrogen peroxide were conducted in acetonitrile solution. A quenching rate constant, k_q, of $1.05 \times 10^{10} M^{-1} \cdot s^{-1}$ was obtained with a short fluorescence lifetime of 347 ps. The reaction rate constant, which is within the diffusion-limited regime, is activation-controlled. The rate constant of deactivation of the thermally excited curcumin was 1.2 orders of magnitude more nonradiative ($2.67 \times 10^9\,s^{-1}$) than radiative ($2.16 \times 10^8\,s^{-1}$). The reaction was exothermic with a ΔG° of -1.97 eV and solvent reorganization energy of 1.37 eV. These values indicate that the electron transfer reaction is solvent-mediated with electron transfer rate constant, k_{ET}, of $2.16 \times 10^{10}\,s^{-1}$.

Keywords. Curcumin; Antioxidant; Fluorescence; Quenching; Electron transfer.

Introduction

Curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione is a yellow pigmented phytochemical isolated from the rhizome of *curcuma longa* L. This compound is known to exist in two main tautomeric forms as shown in Fig. 1 but in solution the principal species is the enol form as shown. Although it is practically insoluble in water, curcumin is quite soluble in both polar aprotic and polar protic solvents and in micelles and liposomes. Curcumin is known to possess numerous pharmacological properties that include antioxidative activity [1–7] and chemo- and phototherapeutic activity [8–13]. It has also been found to induce mitochondrial membrane permeability transition pore (PTP) that ultimately leads to cell apoptosis [14]. It is known to be nontoxic to animals and humans but is toxic to some bacteria at high concentrations [15]. Despite the pharmacological importance of this compound, especially its photoactivity, very minimal fluorescence work has

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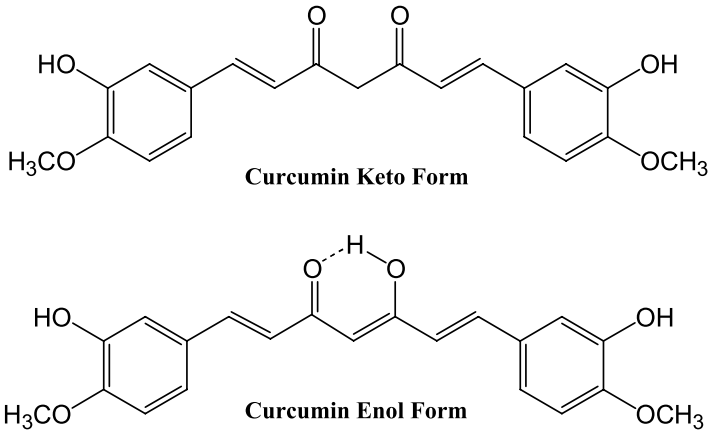

Fig. 1. Structure of curcumin; keto and enol forms

$$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet} \quad \text{(A)}$$

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet} \quad \text{(B)}$$

Scheme 1

$$ROO\bullet + ArOH \rightarrow ArO\bullet + ROOH \quad \text{(C)}$$

$$2\,ArO\bullet \rightarrow \text{Product} \quad \text{(D)}$$

Scheme 2

been performed on it. The minimal work so far done is only in the narrow range of spectral and photophysical properties [16, 17]. The mechanism of the protective activity of curcumin and other related phenolic antioxidants, such as α-tocopherol, as ''chain breaking'' compounds in lipid peroxidation is illustrated in the following Scheme 1: Lipid (*R*H) peroxidation by a peroxyl radical ($ROO^{\bullet}$) proceeds via chain propagating reaction.

In the presence of a phenolic antioxidant (*Ar*OH) the reaction follows Scheme 2.

In reaction (C), a phenolic compound interferes with the peroxyl radical propagation reaction as shown in (B). Such interfering compounds are referred to as ''chain breaking'' compounds. *Ingold* and co-workers have exhaustively elucidated the above mechanism and the stereochemical properties necessary for effective phenolic antioxidative activity [18–20]. This mechanism has been successfully applied to curcumin, solubilized in micellar systems, using azide radical produced by radiolysis [21] and also in aqueous acetonitrile buffered solution in which azide radical was produced by laser photolysis [22]. In this work it was considered to be of more interest and of more significance if the photoreaction of curcumin in the

presence of hydrogen peroxide, a known powerful oxidant produced at a high rate during normal cellular aerobic metabolism, is investigated using fluorescence spectroscopic technique.

Results and Discussion

Figure 2 is the curcumin absorbance spectrum with which the fluorescence lifetime, τ_0, and molar absorptivity, ε, of $4.23 \times 10^4\,\mathrm{M}^{-1} \cdot \mathrm{cm}^{-1}$ were obtained, as described in the Experimental. The spectra of the steady state fluorescence experiments at different concentrations of hydrogen peroxide at constant curcumin concentration are shown in Fig. 3. As indicated by the arrow in this figure, the fluorescence intensity of curcumin is seen to decrease with increasing hydrogen peroxide (quencher, Q) concentration, as it should be. Analysis of the quenching data was carried out in accordance with the *Stern-Volmer* relationship as given in Eq. (1).

$$I^0/I = 1 + K_{\mathrm{SV}}[\mathrm{H_2O_2}] = 1 + k_{\mathrm{q}}\tau_0[\mathrm{H_2O_2}] \tag{1}$$

I^0 and I in this equation are the observed fluorescence intensity in the absence and in the presence of quencher, respectively. K_{SV} is the *Stern-Volmer* quenching constant and k_{q} and τ_0 are the quenching rate constant and natural fluorescence lifetime, respectively. The plot of the observed data, in accordance with Eq. (1) is shown in Fig. 4. As can be seen, the plot obeyed the *Stern-Volmer* equation with a correlation coefficient of better than 0.999 with a slope of $3.57\,\mathrm{M}^{-1}$. This value of this slope was used with the value of τ_0 discussed above in Eq. (1), to obtain a quenching rate constant, k_{q}, of $1.03 \times 10^{10}\,\mathrm{M}^{-1} \cdot \mathrm{s}^{-1}$. The observed experimental parameters for this work are listed in Table 1. As can be seen the value of K_{SV} is surprisingly low. The deactivation process of thermally excited curcumin can be

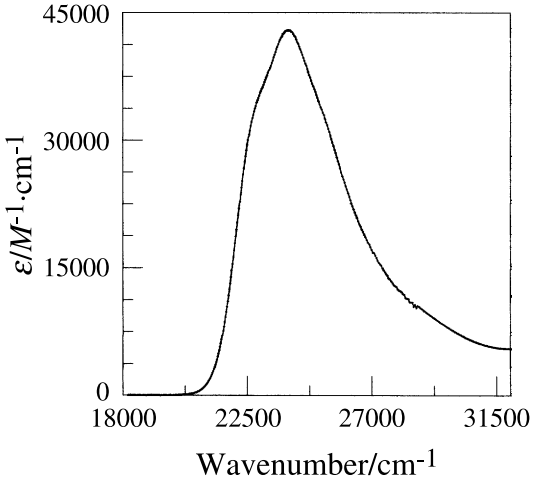

Fig. 2. Absorbance spectrum of $4.34 \times 10^{-5}\,M$ Curcumin

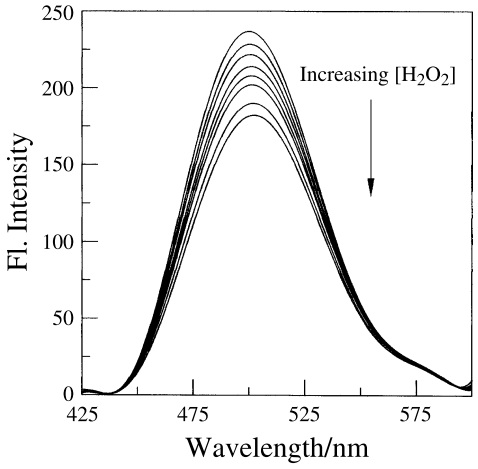

Fig. 3. Fluorescence spectra at increasing $[H_2O_2]$

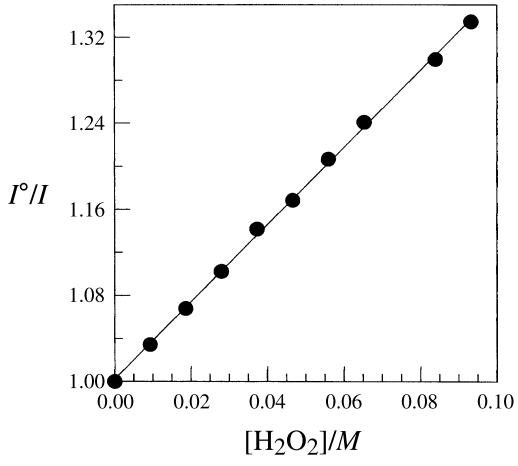

Fig. 4. The *Stern-Volmer* plot of the quenching of curcumin fluorescence by H_2O_2

used to advance some explanation to this low K_{SV} value by considering the radiative and nonradiative deactivation processes in this work using Eqs. (2) and (3).

$$k_r = \Phi/\tau_0 \tag{2}$$

$$k_{nr} = 1 - \Phi/\tau_0 \tag{3}$$

In the above equations, k_r and k_{nr} are radiative and nonradiative rate constants and the rest terms have the meanings described earlier. Using values of 0.104 and 347 ps for Φ and τ, respectively, the k_r and k_{nr} values determined were $2.16 \times 10^8\,s^{-1}$ and $2.67 \times 10^9\,s^{-1}$, respectively. From these values it can be seen that the deactivation of the thermally excited curcumin due to the

Table 1. Parameter values for the fluorescence quenching of curcumin in acetonitrile

Parameter	Value	Parameter	Value
ε	$4.23 \times 10^4\, M^{-1} \cdot \mathrm{cm}^{-1}$	k_{nr}	$2.67 \times 10^9\, \mathrm{s}^{-1}$
R (H_2O_2)	2.3×10^{-8} cm	k_d	$2.23 \times 10^{10}\, M^{-1} \cdot \mathrm{s}^{-1}$
R (Curcumin)	4.85×10^{-8} cm	K	$0.92\, M^{-1}$
K_{SV}	$3.57\, M^{-1}$	k_a	$1.98 \times 10^{10}\, \mathrm{s}^{-1}$
k_q	$1.03 \times 10^{10}\, M^{-1} \cdot \mathrm{s}^{-1}$	k_{et}	$2.16 \times 10^{10}\, \mathrm{s}^{-1}$
τ_0	347 ps	ΔG	-1.97 eV
k_r	$2.16 \times 10^8\, \mathrm{s}^{-1}$	λ_s	1.37 eV

nonradiative process is about 1.2 orders of magnitude more than that of pure fluorescence, k_r. Furthermore, it has been shown that phenols, with no fused chromanol ring react very slowly with the peroxyl radical [18–20]. Curcumin is such a compound, and in these terms the low K_{SV} may be explained. The obtained k_q discussed above was compared with the theoretical bimolecular diffusion-controlled reaction rate constant, k_d, using the *Smoluchowski-Debye* equation for neutral reacting species such as those under consideration in this work.

$$k_d = 4\pi \mathrm{N}/100(D_A + D_D)(R_A + R_D) \tag{4}$$

D and R in Eq. (4) refer to diffusion coefficient and radius, respectively, and N is the *Avogadro* number. Subscripted A and D refer to acceptor and donor species, respectively. The R values were obtained using the *Smoluchowski* relation $R = (3M/4\pi \mathrm{N}\rho)^{1/3}$. While ρ for hydrogen peroxide ($1.11\, \mathrm{g} \cdot \mathrm{cm}^{-3}$) [24] gives an R-value of 2.30×10^{-8} cm, the ρ for curcumin from which an R-value of 4.85×10^{-8} cm was determined, was obtained from the ACD/Lab extension program of the CS ChemDraw. This value was in agreement with that calculated using the incremental atomic volume method by *Edwards* [25]. The diffusion coefficient for the reactants was calculated using the *Stokes-Einstein* relationship $D = \mathrm{k}T/6\pi\eta R$, k is the *Boltzmann* constant, η is the viscosity of the solution (0.34 cP [24]), and $T = 298$ K. The value of k_d thus obtained from these relations is $2.23 \times 10^{10}\, \mathrm{M}^{-1} \cdot \mathrm{s}^{-1}$, a value comparable with the k_q value given above. This indicates that the reaction between curcumin and hydrogen peroxide is diffusion controlled. Noting that the quencher concentration used in this work is less than $0.1\, M$, this observation is therefore consistent with the theory put forward by *Tachiya* and co-workers [26] that for quencher concentrations less than about $0.1\, M$, the quenching mechanism is, in general, diffusion-limited.

Electron Transfer Rate Constant

As stated earlier, the K_{SV} observed in this work is quite low and yet the quenching rate constant was high, within the diffusion-limited regime. Therefore, another possible mechanism for the quenching reaction in this work was considered.

According to *Sutin* [27], k_q and k_d are related to the electron transfer constant, k_{ET}, by Eq. (5).

$$k_q = k_{ET}k_d/k_{ET} + k_{-d} \tag{5}$$

Rearrangement of this equation in accordance with *Darocher*'s method [28], gives Eq. (6).

$$1/k_q = 1/k_d + k_{-d}/k_{ET}k_d = 1/k_d + 1/Kk_{ET} \tag{6}$$

k_{ET} is the first-order electron transfer rate constant and K (equal to k_d/k_{-d}) is the distant dependent association or equilibrium constant of the precursor complex that is obtained using the *Fuoss-Eigen* model [29–31] given in Eq. (7) for uncharged reactants as is the case in this work.

$$K = 4\pi \mathrm{N}R^3/3000 \tag{7}$$

The value of K therefore calculated from Eq. (7) is $0.92\,M^{-1}$ when the R-value is approximated by the sum of the radii of the reactants (7.15 Å). This value is well within the theoretical encounter radius for long-range electron transfer reactions in polar nonviscous solvents, usually $\geq$7 Å [32–34]. A combination of Eqs. (5)–(7) was used to obtain k_{ET} of $2.16 \times 10^{10}\,s^{-1}$. This value is consistent with what is expected for photo induced electron transfer reactions of aromatic molecules within the encounter radius $\geq$7 Å. The activation-controlled rate constant, k_a, of $1.98 \times 10^{10}\,s^{-1}$ for the reaction under study was determined using the *Sutin* formalism [27] $k_a = Kk_{ET}$. These rate constants are listed in Table 1. A comparison of the magnitude of k_a with k_q, k_d, and k_{ET} reveals some relevant facts in this work: a) $k_q \approx k_a$ and $k_{ET} \approx k_d$ and b) K_{SV} is quite low, consistent with the observation of *Ingold* and co-workers [18–20]. The approximate equality in (a) implies an activation controlled reaction, while that in (b) indicates a diffusion-limited electron transfer reaction. In summary, therefore, the reaction mechanism involved in this work may be interpreted as activation controlled electron transfer reaction within a diffusion-limited regime. This conclusion is in agreement with the observation by *Ingold* and co-workers that the rate determining step in peroxyl reactions with phenolic antioxidants (in their case, α-tocopherol) is electron-transfer oxidation and not a hydrogen abstraction reaction.

Solvent Reorganization Energy

For calculating the solvent reorganization energy, λ_s, we have used the *Marcus* dielectric continuum formula [35] given in Eq. (8).

$$\lambda_s = e^2/4\pi\varepsilon_0(1/{\varepsilon_{0p}}^2 - 1/\varepsilon_s)(1/2R_A + 1/2R_D - 1/R_{AD}) \tag{8}$$

In the above equation ε_{0p} and ε_s are the solvent refractive index and the solvent dielectric constant, respectively, these values are taken from Ref. [24], e is the electronic charge, and ε_0 is the permittivity of vacuum. The use of this equation gave a value of 1.37 eV for the solvent reorganization energy for the curcumin-H_2O_2 reaction in acetonitrile. For most diffusion-controlled bimolecular reactions λ_s has been estimated to be within 0.7 and 1.0 eV for solvent separated charge transfer complexes [26], while the *Marcus* theory predicts a value of 1.33 eV in

acetonitrile. The observed λ_s value in this work is within the range of these values and implicates a solvent separated reaction process.

Free Energy Change

In addition to the solvent reorganization energy, the free energy change, ΔG^0, for the electron transfer reaction in this system was also determined using the *Rehm-Weller* relationship given in Eq. (9) [36, 37].

$$\Delta G^0 = E^0{}_{1/2} - E^r{}_{1/2} - E_{0-0} - e^2/4\pi\varepsilon_0\varepsilon_s R_q \tag{9}$$

In this equation $E^0{}_{1/2}$ and $E^r{}_{1/2}$ are the half-wave potentials of the oxidant and reductant species of the reactants, H_2O_2 and curcumin, respectively, E_{0-0} is the zero–zero excitation energy of curcumin, R_q is the effective electron transfer encounter distance between the reactants, which is related to the observed quenching rate constant by Eq. (10) [32]. However, in this work R_q was approximated to $R_A + R_D$.

$$k_q = 4\pi N' D_{AD} R_q \tag{10}$$

Determination of $E_{1/2}$ of Curcumin and H_2O_2

Cyclic voltammograms of curcumin and H_2O_2 were obtained as described in the Experimental. These are shown in Fig. 5. Consistent with the conventional method [38] the current-potential data (E *vs.* $\log(i/(i_d - i))$) were plotted as shown in Fig. 6. The corresponding intercepts at the potential coordinate were taken as $E_{1/2}$ of the respective reactants. In this electrochemical experiment, E is the electrode potential while i is the corresponding current. i_d is the limiting current, the current maximum. The $E_{1/2}$ for H_2O_2, 1.75 V, obtained in this work is in good

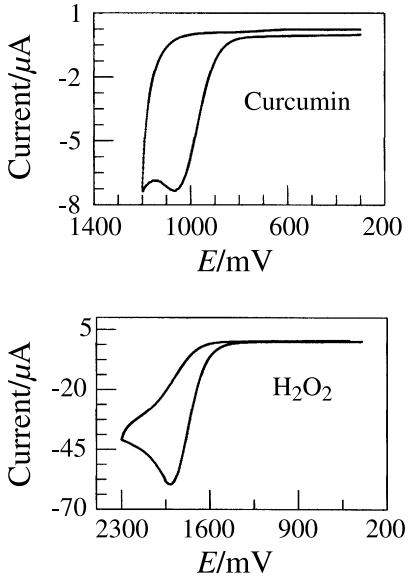

Fig. 5. Voltammetric curves of curcumin and H_2O_2

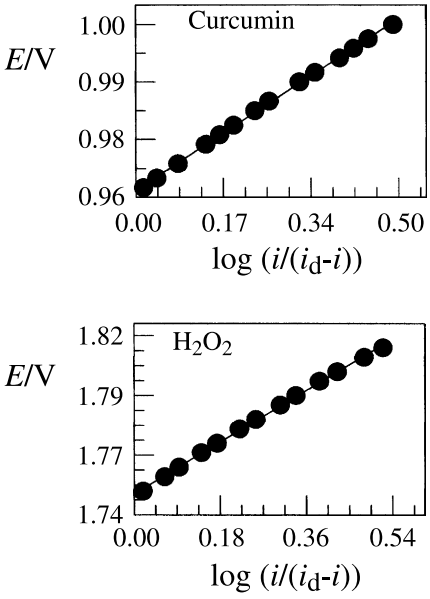

Fig. 6. Plot of potential versus $\log(i/(i_d - i))$ for curcumin and H_2O_2; see Text

agreement with the literature value (1.776 V) [39] and the $E_{1/2}$ value for curcumin was determined as 0.96 V. Using these values, the ΔG^0 calculated from Eq. (9) was -1.97 eV. This value is well within the value consistent with a solvent-separated (or outer sphere) reaction mechanism [40–43].

Conclusion

It has been shown in this work that curcumin fluorescence is quenched by hydrogen peroxide through electron transfer. The quenching rate constant is activation-controlled and is within the diffusion-limited regime. The deactivation of the thermally excited curcumin is due more to a nonradiative process than the fluorescence, which, in part, explains the observed low K_{SV} value. The observed solvent reorganization energy and free energy change implicates a solvent mediated reaction scheme.

Experimental

Materials

Both curcumin and 30% hydrogen peroxide were obtained from Sigma-Aldrich Chemical and acetonitrile was obtained from Fisher Scientific. All the chemicals were of reagent grade quality.

Fluorescence Measurements

All luminescence measurements were performed using a Perkin Elmer's Luminescence Spectrophotometer, Model LS 50B. The excitation and emission wavelengths were 419 and 520 nm, respectively. The excitation and emission spectral band width were each kept constant at 5 nm. In all the experiments, unless otherwise specified, the curcumin concentration was kept constant at 8.57×10^{-7} *M* in acetonitrile solution while the concentration of hydrogen peroxide was allowed to vary from 9×10^{-3} to 0.0932 *M*.

Absorptiometric Experiments

Absorptiometric experiments were performed using a Cary Spectrophotometer, model 1E supplied by Varian Analytical Instruments.

Molar Absorptivity

The molar absorptivity, ε, of curcumin in acetonitrile was determined by plotting the ratio of the observed absorbance, A, *vs*. the concentration, c, as a function of the respective wavelengths, in accordance with *Beer-Lambert*'s law ($A/\mathrm{b}c$). The curcumin concentration used in this experiment was $4.34 \times 10^{-5}\,M$ in 1.0 cm cuvet. The ε was chosen at the peak of the plot at which $\lambda = \lambda_{\max}$.

Fluorescence Lifetime

The fluorescence lifetime, τ_0, for curcumin in acetonitrile was determined using the *Strickler-Berg* relation [23] given in Eq. (11).

$$1/\tau_0 = 2.88 \times 10^{-9}\, n^2 \nu_{\mathrm{f}}{}^3 \phi^{-1} \int \varepsilon \, \mathrm{d}\ln\nu \tag{11}$$

In this equation n, ν_{f}, and ν are the solvent refractive index, the fluorescence emission wavenumber, and the integrated wavenumber of the absorption band, respectively. The refractive index, n, of acetonitrile was taken from Ref. [16]. For the curcumin fluorescence quantum yield, ϕ, values of 0.075 and 0.104 in acetonitrile have been determined [16, 17]. In this work, a value of 0.104 was used. Using this value in Eq. (11) gave a τ_0 value of 347 ps. This is in good agreement with the one (348 ps) obtained by *Khopde* and coworkers [17].

Electrochemical Study

Cyclic voltammetric technique performed with an Electrochemical Analyzer supplied by Cypress Systems was used to obtain the current-voltage data with which the $E_{1/2}$ values for curcumin and H_2O_2 were obtained. The working electrode in this experiment was a glassy carbon electrode of 0.016 cm^2 electrochemical surface area. The counter and reference electrodes were a wound platinum wire and a commercial calomel electrode, respectively. The experiment was performed in acetonitrile solution with $0.12\,M$ tetrabutylammonium perchlorate (*TBAP*) as a supporting electrolyte. The concentration of curcumin and H_2O_2 were $3.3 \times 10^{-3}\,M$ and $3.2 \times 10^{-4}\,M$, respectively. All experiments were performed at room temperature of $t = 25 \pm 0.2^{\circ}\mathrm{C}$.

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