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Excited-State Reactions of Coumarins in Aqueous Solutions. VI. Fluorescence Quenching of 7-Hydroxycoumarins by Chloride Ions in Acidic Solutions

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The fluorescence behavior of 7-hydroxycoumarin and 7-hydroxy-4-methylcoumarin has been studied in acidic aqueous solutions by changing the concentration of chloride ions as a fluorescence quencher. The intensity decrease and lifetime change in fluorescence from neutral and tautomeric forms of photo-excited states are explained by an interaction between protonated molecules generated in excited state and chloride ions. The magnitude of quenching-rate constant is estimated under steady-state conditions and the result is compared with the analysis using time-dependent response.

Complex fluorescent behavior of coumarin derivatives with 7-hydroxyl substituent has been studied in various organic solvents, and in neutral, acidic, and basic aqueous solutions.[1–4] It has been revealed that four fluorescent species typically exist in the photo-excited state according to solvent conditions; they have been identified as neutral, anionic, tautomeric, and protonated species of the excited molecule.

In the acidic region where $[H^+] \approx 0.5\ \mathrm{mol\ dm^{-3}}$, a very interesting situation occurs in the fluorescence of aqueous 7-hydroxycoumarin (7HC) and 7-hydroxy-4-methylcoumarin (7H4MC) solutions. The fluorescence bands from neutral form ($\overset{*}{N}$) and tautomeric form ($\overset{*}{T}$) in their excited states are observable, but those from anionic form ($\overset{*}{A}{}^-$) and cationic form ($\overset{*}{C}{}^+$) are not detected.[4] This means that generated $\overset{*}{A}{}^-$ and $\overset{*}{C}{}^+$, if any, are instantaneously converted to $\overset{*}{N}$ or $\overset{*}{T}$ before they are deactivated radiatively to the ground state. Two fluorescence bands of $\overset{*}{N}$ and $\overset{*}{T}$ are separated in wavelength so enough that the measurement of each band is easily performed. Moreover, with the addition of chloride ions to the solution, these fluorescence bands are effectively quenched; this enables us to confirm detailed mechanisms of acid-base, phototautomerization, and fluorescence-quenching reactions.

The main purpose of this work is to analyze quantitatively the reaction kinetics of the photo-excited 7-hydroxycoumarins in acidic aqueous solutions. The theoretical consideration using rate-equation formulation will be useful to understand the feature of excited-state reactions. The fluorescence data under steady-state conditions and the fluorescence-lifetime measurements will be consistently explained by means of a unified model.

Experimental

Materials. The commercial grade reagents of 7-hydroxycoumarin (7HC) from Wako Pure Chemical Ind. and 7-hydroxy-4-methylcoumarin (7H4MC) from Tokyo Kasei were purified by recrystallization from ethanol and by washing with diethyl ether; 7HC, mp 234 °C; 7H4MC, mp 186 °C. Hydrochloric acid and perchloric acid were of pure quality from Wako Pure Chemical Ind. Water was permeated and distilled.

Methods. UV absorption and fluorescence spectra of the aqueous 7HC and 7H4MC solutions of $\approx 6 \times 10^{-5}\ \mathrm{mol\ dm^{-3}}$ were measured at 20 °C. The pH of the solution was controlled with HCl and $HClO_4$, the chloride ion concentration being adjusted by changing their ratio. Fluorescence-lifetime measurements were performed by means of a time-correlated single-photon-counting fluorometer similar to that described in Ref. 5. Since the lifetime of fluorescers was close to the decay time of a repetitive short-pulsed light at 337 nm from an air-flash lamp, the data were deconvoluted to know the true decay character unaffected by a response function of the measurement system; the typical accuracy of this procedure was ± 0.05 ns.

Results

In a previous paper,[4] the complex pH dependence of absorption and fluorescence spectra of 7HC and 7H4MC in aqueous solutions has been fully investigated. Since the present interest is concentrated on the pH range with $[H^+]$ of $\approx 0.5\ \mathrm{mol\ dm^{-3}}$, the spectral behavior of these fluorophores is summarized as follows. In the electronic ground state, there exists only a neutral molecular species (N), the absorption maxima of which are 324 nm (7HC) and 321 nm (7H4MC). If this neutral species is optically excited at the absorption-peak wavelength, two molecular species formed in the excited state are observable. One is usual neutral molecule ($\overset{*}{N}$), and the other is tautomeric molecule ($\overset{*}{T}$) in which a hydrogen of the 7-hydroxyl group is transferred to the carbonyl group at 2-position. As will be mentioned later, cationic form ($\overset{*}{C}{}^+$) protonated at the carbonyl group and anionic form ($\overset{*}{A}{}^-$) deprotonated at the hydroxyl group can exist in principle, but they are not spectroscopically detected around $[H^+]$ of $0.5\ \mathrm{mol\ dm^{-3}}$. The fluorescence maxima of $\overset{*}{N}$ and $\overset{*}{T}$ are at 397 and 478 nm for 7HC, and at 390 and 475 nm for 7H4MC,

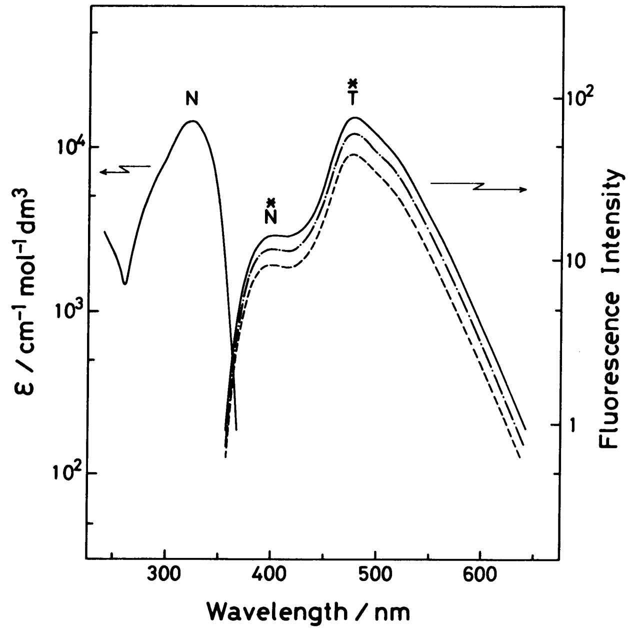


Fig. 1. Absorption and fluorescence spectra of 7HC in the acidic aqueous solution at 20 °C. $[HClO_4]+[HCl]=0.5$ mol dm^{-3}. (——), [HCl]=0; (—·—), [HCl]=0.2 mol dm^{-3}; (---), [HCl]=0.5 mol dm^{-3}. N describes the neutral ground state, and $\overset{*}{\mathrm{N}}$ and $\overset{*}{\mathrm{T}}$ describe the neutral and tautomeric excited state, respectively.

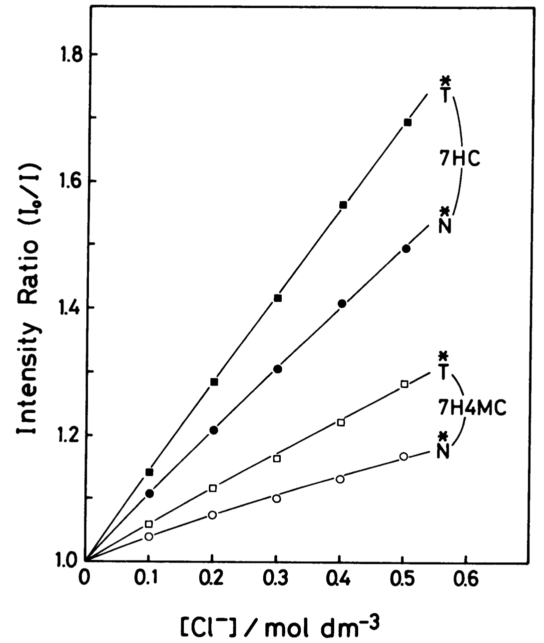


Fig. 2. Dependence of the fluorescence intensity ratio, I_0/I, on [Cl$^-$] in the acidic aqueous solution ([H$^+$]=0.5 mol dm^{-3}) at 20 °C. 7HC (●), (■); 7H4MC (○), (□). $\overset{*}{\mathrm{N}}$ and $\overset{*}{\mathrm{T}}$ describe the excited states of neutral and phototautomeric molecular forms, respectively.

Table 1. Values for the Fluorescence Lifetime Parameters, τ_+, τ_-, and C, of 7HC and 7H4MC in Acidic Aqueous Solutions ([H$^+$]=0.5 mol dm^{-3}) at 20 °C

Substance	[Cl$^-$] mol dm^{-3}	τ_+ ns	τ_- ns	C
7HC	0	4.5_0	0.2_0	2.5
	0.1	3.9_5	0.2_{25}	2.5
	0.2	3.5_5	0.2_5	2.0
	0.3	3.2_0	0.3_0	2.0
	0.4	2.9_0	0.3_{25}	2.0
	0.5	2.6_0	0.3_5	1.5
7H4MC	0	5.0_5	0.1_{25}	13
	0.1	4.7_5	0.1_5	12
	0.2	4.5_0	0.1_5	11
	0.3	4.2_0	0.1_{75}	10
	0.4	4.0_5	0.1_{75}	10
	0.5	3.8_0	0.2_0	8.5

respectively. In Fig. 1, the absorption and fluorescence spectra of an aqueous 7HC solution with 0.5 mol dm^{-3} $HClO_4$ are shown by the solid lines.

When [Cl$^-$] is changed by the amount of an added HCl component with holding pH of the solution as $[HClO_4]+[HCl]=0.5$ mol dm^{-3} ($H_0 \approx 0.20$),⁶⁾ the fluorescence intensity from $\overset{*}{\mathrm{N}}$ and $\overset{*}{\mathrm{T}}$ has been quenched depending on [Cl$^-$] as shown by the dashed curves in Fig. 1. Since the fluorescence bands of $\overset{*}{\mathrm{N}}$ and $\overset{*}{\mathrm{T}}$ are separated far enough to each other, the respective fluorescence yields can be monitored at 397 and 478 nm (7HC), and at 390 and 475 nm (7H4MC), respectively. Figure 2 describes plots of I_0/I for $\overset{*}{\mathrm{N}}$ and $\overset{*}{\mathrm{T}}$ vs. [Cl$^-$] where I_0 and I are the fluorescence intensity for each excited species in the absence and in the presence of chloride ions. Slight deviation from the usual Stern–Volmer linear relationship is observed for both $I_0^{\mathrm{N}}/I^{\mathrm{N}}$ and $I_0^{\mathrm{T}}/I^{\mathrm{T}}$.

Under the pulsed light excitation at 337 nm which generates $\overset{*}{\mathrm{N}}$ at the moment of light absorption, the decay characteristic of fluorescence from neutral and tautomeric fluorescers has been measured at the peak wavelength of each fluorescence band with keeping [H$^+$] at 0.5 mol dm^{-3} but with different [Cl$^-$]. The time-dependent light intensity, $i^{\mathrm{N}}(t)$ and $i^{\mathrm{T}}(t)$, of $\overset{*}{\mathrm{N}}$ and $\overset{*}{\mathrm{T}}$ was fitted satisfactorily for the double-exponential decay character:

$$i^{\mathrm{N}}(t) \propto \exp(-t/\tau_+) + C \cdot \exp(-t/\tau_-) \qquad (1)$$

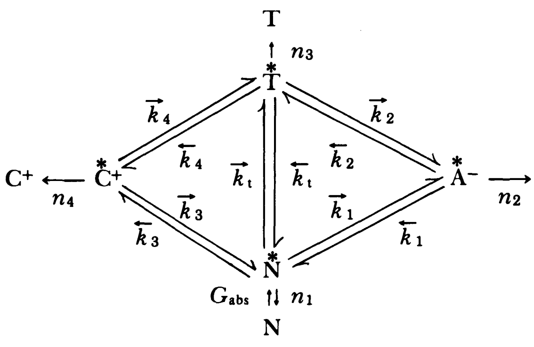
and

$$i^{\mathrm{T}}(t) \propto \exp(-t/\tau_+) - \exp(-t/\tau_-). \qquad (2)$$

The best-fitted values for τ_+, τ_-, and C are given in Table 1.

Discussion

Fluorescence Quenching by Chloride Ions in Acidic Solutions. Based on the generalized scheme for excited-state reactions of coumarins in aqueous solutions confirmed in Ref. 4, we may adopt the following scheme if only the neutral molecule exists in the ground state at [H$^+$] of ≈0.5 mol dm^{-3}:



Scheme 1.

Here, G_{abs} is the generation rate of $\overset{*}{\mathrm{N}}$ and n_j's are the transition rates of the excited species to their ground states. $\overrightarrow{k}_j$'s and $\overleftarrow{k}_j$'s are the protonation and deprotonation rate constants between acids and bases in the excited state. $\overrightarrow{k}_{\mathrm{t}}$ and $\overleftarrow{k}_{\mathrm{t}}$ represent the reaction pathway of tautomerization that does not involve stepwise protonation or deprotonation processes, i.e., the "nondissociative" reaction pathway.[4,7] Because the fluorescence of $\overset{*}{\mathrm{A}}{}^-$ and $\overset{*}{\mathrm{C}}{}^+$, which should appear in the wavelength region between $\overset{*}{\mathrm{N}}$ and $\overset{*}{\mathrm{T}}$ bands, is not detected at [H$^+$] of 0.5 mol dm^{-3}, the following conditions will be satisfied:

$$\overrightarrow{k}_1 \ll \overleftarrow{k}_1[\mathrm{H}^+],\ \overrightarrow{k}_2 \ll \overleftarrow{k}_2[\mathrm{H}^+] \text{ and } \overleftarrow{k}_3[\mathrm{H}^+] \ll \overrightarrow{k}_3,\ \overleftarrow{k}_4[\mathrm{H}^+] \ll \overrightarrow{k}_4. \tag{3}$$

If all decay rates of the excited species, n_j's are of the same order, the ratio of fluorescence intensity of $\overset{*}{\mathrm{T}}$ to that of $\overset{*}{\mathrm{N}}$ in the absence of Cl$^-$ is obtained by means of the rate equation analysis as carried out in Ref. 4 under the conditions of Eq. 3 (cf. Appendix):

$$\frac{I_0^{\mathrm{T}}}{I_0^{\mathrm{N}}} = \frac{n_3^{\mathrm{r}}}{n_1^{\mathrm{r}}} \frac{\overrightarrow{k}'_{\mathrm{t}} + \beta\overleftarrow{k}_3[\mathrm{H}^+]}{n_3 + \overleftarrow{k}'_{\mathrm{t}} + \alpha\overleftarrow{k}_4[\mathrm{H}^+]}, \tag{4}$$

where I_0's represent the fluorescence intensity at [Cl$^-$]=0, n_1^{r} and n_3^{r} are the radiative transition probabilities of $\overset{*}{\mathrm{N}}$ and $\overset{*}{\mathrm{T}}$ to their ground states, respectively, and

$$\overrightarrow{k}'_{\mathrm{t}} = \overrightarrow{k}_{\mathrm{t}} + \overrightarrow{k}_1 \frac{\overleftarrow{k}_2[\mathrm{H}^+]}{n_2 + \overleftarrow{k}_1[\mathrm{H}^+] + \overleftarrow{k}_2[\mathrm{H}^+]},$$

$$\overleftarrow{k}'_{\mathrm{t}} = \overleftarrow{k}_{\mathrm{t}} + \overrightarrow{k}_2 \frac{\overleftarrow{k}_1[\mathrm{H}^+]}{n_2 + \overleftarrow{k}_1[\mathrm{H}^+] + \overleftarrow{k}_2[\mathrm{H}^+]},$$

$$\alpha = \frac{\overrightarrow{k}_3}{n_4 + \overrightarrow{k}_3 + \overrightarrow{k}_4}, \text{ and } \beta = \frac{\overrightarrow{k}_4}{n_4 + \overrightarrow{k}_3 + \overrightarrow{k}_4}. \tag{5}$$

When 0.3 mol dm^{-3} NaCl is added to the acidic solution with 0.5 mol dm^{-3} HClO$_4$, the fluorescence of $\overset{*}{\mathrm{N}}$ and $\overset{*}{\mathrm{T}}$ is decreased, while the addition of 0.3 mol dm^{-3} NaClO$_4$ induces no fluorescence change. This fact shows that Cl$^-$ affects the fluorescence-quenching phenomenon contrary to other inorganic ions such as Na$^+$ and ClO$_4^-$. It is observed in general that halide ions have the quenching ability in order of Cl$^-$<Br$^-$<I$^-$.[8] Further, the fluorescence of $\overset{*}{\mathrm{N}}$, $\overset{*}{\mathrm{T}}$, and $\overset{*}{\mathrm{A}}{}^-$ does not depend on Cl$^-$ quencher over the pH range where $\overset{*}{\mathrm{C}}{}^+$ is not formed in the excited state (pH≧2).[4] All these results lead to the conclusion that $\overset{*}{\mathrm{N}}$ and $\overset{*}{\mathrm{T}}$ fluorescers are quenched only through an interaction of the protonated excited state, $\overset{*}{\mathrm{C}}{}^+$, with Cl$^-$, and that the quenching mechanism will be essentially the same as proposed in Ref. 9:

$$W\colon\ \overset{*}{\mathrm{C}}{}^+ + \mathrm{Cl}^- \xrightarrow{k_{\mathrm{q}}} \mathrm{C}^+ + \mathrm{Cl}^-. \tag{6}$$

Here, the fraction W of $\overset{*}{\mathrm{C}}{}^+$ in Scheme 1 additionally obeys a collisional quenching process where the encounter complex, ($\overset{*}{\mathrm{C}}{}^+\cdot\mathrm{Cl}^-$), decays nonradiatively to C$^+$ and Cl$^-$ with a rate of k_{q}; the remaining fraction $1-W$ of $\overset{*}{\mathrm{C}}{}^+$ obeys the instantaneous or "static" deactivation process. The additional factor W (a function of the quencher concentration [Cl$^-$]) can be approximated by $\exp(-V[\mathrm{Cl}^-])$, where V is the static quenching constant. If the model of Frank and Vavilov[10] is applied to the static quenching process, an instantaneous quenching results when the quencher is within the "sphere of action" having a volume of V/N', where N' is the Avogadro number per millimole.[11]

Since illuminating light is absorbed solely by neutral molecules under the experimental conditions and the fraction $1-W$ of $\overset{*}{\mathrm{C}}{}^+$ is immediately quenched without emitting any light quantum, the effective generation rate of $\overset{*}{\mathrm{N}}$, $\tilde{G}([\mathrm{Cl}^-])$, must be somewhat reduced from G_{abs}.[9] However, this alteration does not appear explicitly in the ratio of fluorescence intensity of $\overset{*}{\mathrm{N}}$ to that of $\overset{*}{\mathrm{T}}$, $I^{\mathrm{T}}/I^{\mathrm{N}}$. In the presence of Cl$^-$, straightforward rate-equation analysis as before gives the relation:

$$\frac{I^{\mathrm{T}}}{I^{\mathrm{N}}} = \frac{n_3^{\mathrm{r}}}{n_1^{\mathrm{r}}} \frac{\overrightarrow{k}'_{\mathrm{t}} + \overleftarrow{k}_3[\mathrm{H}^+]\dfrac{\beta}{1+\gamma[\mathrm{Cl}^-]}}{n_3 + \overleftarrow{k}'_{\mathrm{t}} + \overleftarrow{k}_4[\mathrm{H}^+]\dfrac{\alpha+\gamma[\mathrm{Cl}^-]}{1+\gamma[\mathrm{Cl}^-]}}, \tag{7}$$

where

$$\gamma = \frac{k_{\mathrm{q}}}{n_4 + \overrightarrow{k}_3 + \overrightarrow{k}_4}. \tag{8}$$

From Eqs. 4 and 7, we have

$$\frac{I_0^{\mathrm{T}}/I^{\mathrm{T}}}{I_0^{\mathrm{N}}/I^{\mathrm{N}}} = \frac{1+\{(n_3+\overleftarrow{k}'_{\mathrm{t}}+\overleftarrow{k}_4[\mathrm{H}^+])/(n_3+\overleftarrow{k}'_{\mathrm{t}}+\alpha\overleftarrow{k}_4[\mathrm{H}^+])\}\cdot\gamma[\mathrm{Cl}^-]}{1+\{\overrightarrow{k}'_{\mathrm{t}}/(\overrightarrow{k}'_{\mathrm{t}}+\beta\overleftarrow{k}_3[\mathrm{H}^+])\}\cdot\gamma[\mathrm{Cl}^-]} \tag{9}$$

and finally

$$\frac{1}{\{(I_0^{\mathrm{T}}/I^{\mathrm{T}})/(I_0^{\mathrm{N}}/I^{\mathrm{N}})\}-1} = \{\frac{n_3+\overleftarrow{k}'_{\mathrm{t}}+\overleftarrow{k}_4[\mathrm{H}^+]}{n_3+\overleftarrow{k}'_{\mathrm{t}}+\alpha\overleftarrow{k}_4[\mathrm{H}^+]} - \frac{\overrightarrow{k}'_{\mathrm{t}}}{\overrightarrow{k}'_{\mathrm{t}}+\beta\overleftarrow{k}_3[\mathrm{H}^+]}\}^{-1} \times\{\frac{\overrightarrow{k}'_{\mathrm{t}}}{\overrightarrow{k}'_{\mathrm{t}}+\beta\overleftarrow{k}_3[\mathrm{H}^+]}+\frac{1}{\gamma[\mathrm{Cl}^-]}\}. \tag{10}$$

Since α, $\beta<1$, the first term on the right-hand side of Eq. 10 which is contained in curly brackets is positive. Therefore, the plot of $1/\{(I_0^{\mathrm{T}}/I^{\mathrm{T}})/(I_0^{\mathrm{N}}/I^{\mathrm{N}})-1\}$ vs. $1/[\mathrm{Cl}^-]$ becomes linear and its slope is positive from the theoretical prediction.

The experimental result shown in Fig. 2 is replotted in Fig. 3 in accordance with Eq. 10. It is seen that the data follow very well the predicted linear dependence. By using the intercept and slope of this plot, $\{\overrightarrow{k}'_{\mathrm{t}}/(\overrightarrow{k}'_{\mathrm{t}}+\beta\overleftarrow{k}_3[\mathrm{H}^+])\}\cdot\gamma$ and $\{(n_3+\overleftarrow{k}'_{\mathrm{t}}+\overleftarrow{k}_4[\mathrm{H}^+])/(n_3+\overleftarrow{k}'_{\mathrm{t}}+\alpha\overleftarrow{k}_4[\mathrm{H}^+])\}\cdot\gamma$ are given as 0.406 and 0.729 for 7HC, and 0.120 and 0.324 for 7H4MC, respectively. Although the exact value or γ is not obtained only from this information, its region becomes restricted by the relation:

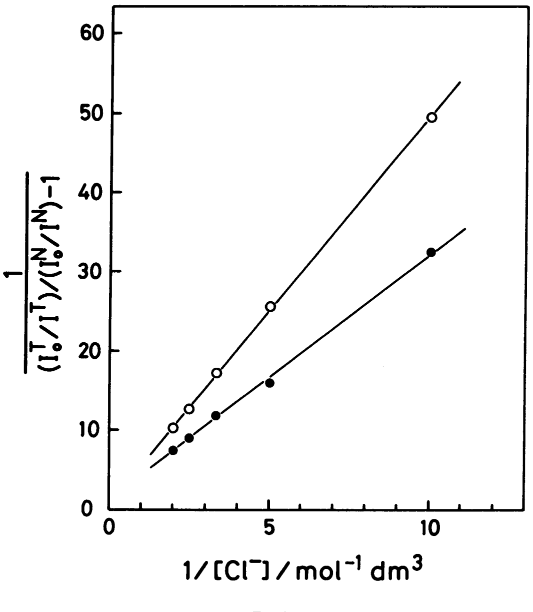
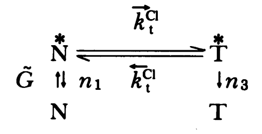


Fig. 3. Plots of $1/\{(I_0^{\mathrm{T}}/I^{\mathrm{T}})/(I_0^{\mathrm{N}}/I^{\mathrm{N}})-1\}$ vs. $1/[\mathrm{Cl}^-]$ for 7HC (●) and 7H4MC (○) in the acidic solution ($[\mathrm{H}^+]=0.5\ \mathrm{mol\ dm^{-3}}$) at 20 °C.

$$\begin{aligned} &0.406<\gamma<0.729 \text{ for 7HC and} \\ &0.120<\gamma<0.324 \text{ for 7H4MC.} \end{aligned} \tag{11}$$

because $\alpha,\beta<1$. Considering the definition of γ (Eq. 8), these results mean that the quenching rate constant k_{q} is less than the decay rate of $\overset{*}{\mathrm{C}}{}^+$ in the absence of quenchers, $n_4+\overrightarrow{k}_3+\overrightarrow{k}_4$, but has the same order of magnitude. From Eq. 11, it is seen that the quenching efficiency of 7H4MC due to Cl⁻ ions is lower than that of 7HC. Possibly, 4-methyl substituent acts protectively on the quenching process, which is conceivable from the general tendency observed in 7-ethoxycoumarins under various conditions.[8,9,12)]

Fluorescence Lifetimes in Acidic Solutions. As described in Scheme 1, the real reactions in the excited state are complicatedly correlated with each other. However, the fundamental aspect of the transient response to a pulsed-light excitation will be approximated by the following simple scheme, if the rate equations are examined in detail under the same situation as discussed in the case of steady-state conditions (cf. Eq. 7):



Scheme 2.

where

$$\overrightarrow{k}_{\mathrm{t}}^{\mathrm{Cl}} = \overrightarrow{k}'_{\mathrm{t}} + \frac{\beta\overleftarrow{k}_3[\mathrm{H}^+]}{1+\gamma[\mathrm{Cl}^-]} \quad \text{and}$$

$$\overleftarrow{k}_{\mathrm{t}}^{\mathrm{Cl}} = \overleftarrow{k}'_{\mathrm{t}} + \frac{(\alpha+\gamma[\mathrm{Cl}^-])\overleftarrow{k}_4[\mathrm{H}^+]}{1+\gamma[\mathrm{Cl}^-]} \tag{12}$$

Then, in accordance with Eqs. 1 and 2, the lifetimes $\tau_\pm$ of $i^{\mathrm{N}}(t)$ and $i^{\mathrm{T}}(t)$ for a light pulse of ideal δ-function are written as[13)]

$$\tau_\pm^{-1} = \tfrac{1}{2}(n_1+\overrightarrow{k}_{\mathrm{t}}^{\mathrm{Cl}}+n_3+\overleftarrow{k}_{\mathrm{t}}^{\mathrm{Cl}}) \mp \tfrac{1}{2}[(n_1+\overrightarrow{k}_{\mathrm{t}}^{\mathrm{Cl}}+n_3+\overleftarrow{k}_{\mathrm{t}}^{\mathrm{Cl}})^2 -4\{(n_1+\overrightarrow{k}_{\mathrm{t}}^{\mathrm{Cl}})(n_3+\overleftarrow{k}_{\mathrm{t}}^{\mathrm{Cl}})-\overrightarrow{k}_{\mathrm{t}}^{\mathrm{Cl}}\overleftarrow{k}_{\mathrm{t}}^{\mathrm{Cl}}\}]^{1/2}. \tag{13}$$

From Table 1, the condition, $\tau_+\gg\tau_-$, is satisfied for both 7HC and 7H4MC, which is equivalent to the case that the second term in the root of Eq. 13 contained in the curly brackets is much smaller than the first term. Then, we have approximate equations:

$$\frac{1}{\tau_+} \approx \frac{n_1n_3+n_3\overrightarrow{k}_{\mathrm{t}}^{\mathrm{Cl}}+n_1\overleftarrow{k}_{\mathrm{t}}^{\mathrm{Cl}}}{n_1+\overrightarrow{k}_{\mathrm{t}}^{\mathrm{Cl}}+n_3+\overleftarrow{k}_{\mathrm{t}}^{\mathrm{Cl}}} \quad \text{and}$$

$$\frac{1}{\tau_-} \approx n_1+\overrightarrow{k}_{\mathrm{t}}^{\mathrm{Cl}}+n_3+\overleftarrow{k}_{\mathrm{t}}^{\mathrm{Cl}} \tag{14}$$

Further, letting the value of τ_+ at $[\mathrm{Cl}^-]=0$ be τ_+^0, the following relation is obtained:

$$\frac{1}{(\tau_+^0/\tau_+)-1} = \left\{ \frac{n_1 + \overrightarrow{k}'_t + (n_1/n_3)\overleftarrow{k}'_t + (n_1/n_3)\overleftarrow{k}_4[\mathrm{H}^+]}{n_1 + \overrightarrow{k}'_t + (n_1/n_3)\overleftarrow{k}'_t + \beta\overleftarrow{k}_3[\mathrm{H}^+] + (n_1/n_3)\alpha\overleftarrow{k}_4[\mathrm{H}^+]} - \frac{\mathrm{n}_1 + \overrightarrow{k}'_t + n_3 + \overleftarrow{k}'_t + \overleftarrow{k}_4[\mathrm{H}^+]}{n_1 + \overrightarrow{k}'_t + n_3 + \overleftarrow{k}'_t + \beta\overleftarrow{k}_3[\mathrm{H}^+] + \alpha\overleftarrow{k}_4[\mathrm{H}^+]} \right\}^{-1} \times \left\{ \frac{n_1 + \overrightarrow{k}'_t + n_3 + \overleftarrow{k}'_t + \overleftarrow{k}_4[\mathrm{H}^+]}{n_1 + \overrightarrow{k}'_t + n_3 + \overleftarrow{k}'_t + \beta\overleftarrow{k}_3[\mathrm{H}^+] + \alpha\overleftarrow{k}_4[\mathrm{H}^+]} + \frac{1}{\gamma[\mathrm{Cl}^-]} \right\}. \quad (15)$$

Equation 15 predicts the linear relationship for the plot of $1/\{(\tau_+^0/\tau_+)-1\}$ vs. $1/[\mathrm{Cl}^-]$. Figure 4 shows the plot where the data in Table 1 are employed. It is seen from the result that the linear relationship is well applicable in the region where $\tau_+ \gg \tau_-$. By using the intercept and slope of the plot, the ratio of complicated combination of various rate constants is again obtained as in the case of steady-state analysis; $\{(n_1+\overrightarrow{k}'_t+n_3+\overleftarrow{k}'_t+\overleftarrow{k}_4[\mathrm{H}^+])/(n_1+\overrightarrow{k}'_t+n_3+\overleftarrow{k}'_t+\beta\overleftarrow{k}_3[\mathrm{H}^+]+\alpha\overleftarrow{k}_4[\mathrm{H}^+]\}\cdot\gamma$ and $[\{n_1+\overrightarrow{k}'_t+(n_1/n_3)\overleftarrow{k}'_t+(n_1/n_3)\overleftarrow{k}_4[\mathrm{H}^+]\}/\{n_1+\overrightarrow{k}'_t+(n_1/n_3)\overleftarrow{k}'_t+\beta\overleftarrow{k}_3[\mathrm{H}^+]+(n_1/n_3)\alpha\overleftarrow{k}_4[\mathrm{H}^+]\}]\cdot\gamma$ are 0.065 and 1.45 for 7HC, and 0.020 and 0.685 for 7H4MC, respectively. Although each rate constant cannot be estimated, the γ values restricted by Eq. 11 are consistent with this result if $n_1 > n_3$. Because of relatively low accuracy of deconvolution for the fast decay component (τ_- and C), the analysis is less fruitful than the consideration on τ_+, whereas the qualitative tendency is predictable according to the same assumption.

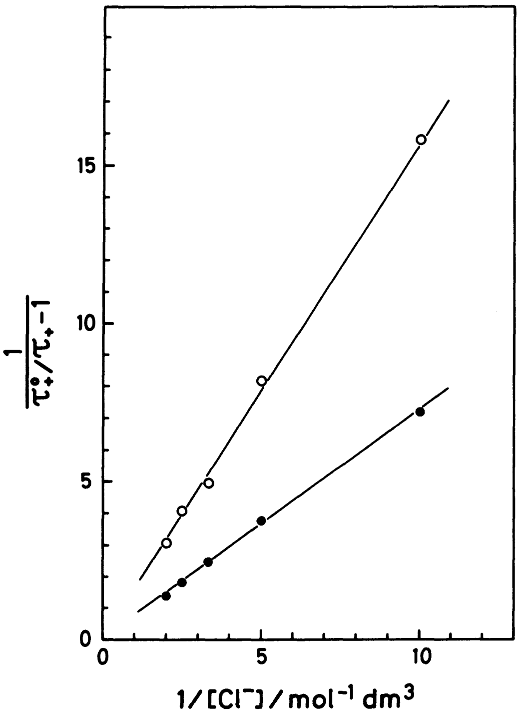


Fig. 4. Plots of $1/\{(\tau_+^0/\tau_+)-1\}$ vs. $1/[\mathrm{Cl}^-]$ for 7HC (●) and 7H4MC (○) in the acidic solution ($[\mathrm{H}^+]=$ 0.5 mol dm^{-3}) at 20 °C.

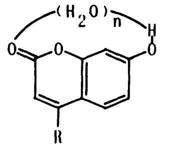
The lifetime analysis based on the fluorescence components from $\overset{*}{\mathrm{N}}$ and $\overset{*}{\mathrm{T}}$ of 7HC and 7H4MC is analogous with the lifetime measurement on monomer and exciplex forms in aromatic compound–quencher systems where an exciplex is generated to result in fluorescence quenching.[13] It should be mentioned here that the excited-state reaction of coumarin derivatives is classified essentially into an acid-base reaction and has time constants of nanosecond order due to high mobility of participating species such as H^+ and Cl^-, whereas the exciplex-formation reaction is a usually slower phenomenon by one or more orders.

The Relationship between Fluorescence Yield and Fluorescence Lifetime Measurements. At the early stage of theoretical analysis on the fluorescence yield, we have assumed that the natural lifetimes, n_j^{-1}, of four molecular species generated in the excited state are of the same order. This is readily conceivable from the experimental result that the ground-state molecules, N, C^+, and A^-, have the similar values of molar extinction coefficients which are closely related to radiative transition probabilities.[14] The absorption spectrum of T cannot be observed in any pH range, because its ground state is energetically unstable in contrast to the other three species. However, theoretical calculations of the energy level and the transition moment of N and T have shown that optical characteristics of these two species are similar, whereas the molecular structure and the energy separation between the highest occupied and the lowest unoccupied orbital are very different.[15] It is thus expected for natural lifetimes of $\overset{*}{\mathrm{N}}$, $\overset{*}{\mathrm{T}}$, $\overset{*}{\mathrm{C}}{}^+$, and $\overset{*}{\mathrm{A}}{}^-$ to be in the range of 1.5—6 ns; it is supported by lifetime measurements over wide pH ranges in aqueous solutions.[4]

Generally, the analysis using fluorescence lifetimes is more tractable than the steady-state analysis in that the instantaneous or "static" quenching process does not appear explicitly. A clear example is presented by Efftink and Ghiron[11] who showed experimentally in an indole–quencher system that τ^0/τ is independent of the factor, $W=\exp(-V[\mathrm{Q}])$, in contrast to I_0/I. In the present case, the fraction W of the excited state $\overset{*}{\mathrm{C}}{}^+$ generated by light illumination is actually quenched by the collisional mechanism corresponding to the rate constant k_q. The remaining fraction $1-W$ is deactivated almost instantaneously after being formed because the quencher Cl^- is accidentally positioned in the proximity of excited molecule and interacts strongly with the latter. Only when such a static quenching mechanism is appropriately included, the fluorescence-yield decrease of 7-hydroxycoumarins as well as of 7-ethoxycoumarins[9] in each fluorescence band under steady-state conditions is reasonably understood. It has been already shown that the static quenching effect is easily taken into account in the framework of rate-equation analysis, if the generation rate G_{abs} is replaced formally by $\tilde{G}(Q)=G_{\mathrm{abs}}\cdot\{(n_1+\overrightarrow{k}_{\mathrm{t}}'+W(Q)\overleftarrow{k}_3[\mathrm{H}^+])/(n_1+\overrightarrow{k}_{\mathrm{t}}'+\overleftarrow{k}_3[\mathrm{H}^+])\}$.[9] Because both I^{N} and I^{T} contain $\tilde{G}(Q)$ as a common multiple factor, it has been an ingenious way for us to examine the ratio of I^{T} to I^{N} in order to avoid the appearance of W in the analytical formulation.

In the case of pulsed excitation, the rate equations for time-variations of $[\overset{*}{\mathrm{N}}]$ and $[\overset{*}{\mathrm{T}}]$ need not include the fraction of excited species that never give fluorescence over an observing interval typically of 50 ns. Namely, the generation rate G_{abs} of $\overset{*}{\mathrm{N}}$ has only to be reduced formally in the first approximation to $\tilde{G}(Q)$ with maintaining the same equational form as $W=1$. Since $\tilde{G}(Q)$ is included only in the intensity amplitude of $i^{\mathrm{N}}(t)$ and $i^{\mathrm{T}}(t)$, the appropriately normalized intensity of them is in principle given by Eqs. 1 and 2 even if $W\neq1$, while the fluorescence yield integrated over a time range is not correctly derived without considering the quencher dependency of W. More rigorous treatment of such transient phenomena has appeared in the serial works of Ware et al.[13] who formulated it by introducing a time-dependent rate constant, instead of W, in the rate equation. However, two different ways of analysis give essentially the same result as pointed out by Effink and Ghiron.[11]

Reaction Pathways for the Phototautomerization. It has been shown in Ref. 4 that the tautomerization of $\overset{*}{\mathrm{N}}$ to $\overset{*}{\mathrm{T}}$ can be promoted by dissociative and nondissociative processes. The dissociative mechanism has two possible pathways: one generates the intermediate protonated species as $\overset{*}{\mathrm{N}}\rightarrow\overset{*}{\mathrm{C}}{}^+\rightarrow\overset{*}{\mathrm{T}}$, and the other generates the intermediate deprotonated species as $\overset{*}{\mathrm{N}}\rightarrow\overset{*}{\mathrm{A}}{}^-\rightarrow\overset{*}{\mathrm{T}}$. In aqueous solutions, there occurs another possibility that $\overset{*}{\mathrm{N}}$ is directly converted to $\overset{*}{\mathrm{T}}$ by means of concerted transfer of hydrogen from hydroxyl site to carbonyl site with the aid of hydrogen bonding of water molecules:



R = H, 7HC; R = $\mathrm{CH_3}$, 7H4MC

Scheme 3.

Since the fluorescence of $\overset{*}{\mathrm{N}}$, $\overset{*}{\mathrm{T}}$, and $\overset{*}{\mathrm{A}}{}^-$ are unaffected by the addition of Cl^-, the reaction pathways $\overset{*}{\mathrm{N}}\rightarrow\overset{*}{\mathrm{A}}{}^-\rightarrow\overset{*}{\mathrm{T}}$ and $\overset{*}{\mathrm{N}}\rightarrow\overset{*}{\mathrm{T}}$ are not disturbed by Cl^- if the ionic strength does not vary largely. On the other hand, the reaction pathway $\overset{*}{\mathrm{N}}\rightarrow\overset{*}{\mathrm{C}}{}^+\rightarrow\overset{*}{\mathrm{T}}$ is extremely disturbed by Cl^- due to the attractive interaction between $\overset{*}{\mathrm{C}}{}^+$ and Cl^-. Probably, the electron transfer from Cl^- to $\overset{*}{\mathrm{C}}{}^+$ within an encounter complex will result in the effective deactivation of $\overset{*}{\mathrm{C}}{}^+$. The same discussion is applicable to three reversed reaction pathways from $\overset{*}{\mathrm{T}}$ to $\overset{*}{\mathrm{N}}$. In the pH region except for $[\mathrm{H}^+]$ of $\approx0.5\ \mathrm{mol\,dm^{-3}}$, the fluorescence band of $\overset{*}{\mathrm{A}}{}^-$ or $\overset{*}{\mathrm{C}}{}^+$ is observable at a wavelength between the fluorescence bands of $\overset{*}{\mathrm{N}}$ and $\overset{*}{\mathrm{T}}$. It is only accidental for 7HC and 7H4MC that the fluorescence from both $\overset{*}{\mathrm{A}}{}^-$ and $\overset{*}{\mathrm{C}}{}^+$ is negligible at $[\mathrm{H}^+]$ of $0.5\ \mathrm{mol\,dm^{-3}}$, and this situation enables us to attain clear-cut understanding of the tautomerization reaction and of the quenching reaction by Cl^-.

Rapid Equilibration Limit. Consideration for the limiting case of rapid equilibration will help understanding the optically induced tautomeric reaction, although the limit is somewhat different from the real case of the present problem, i.e., $\tau_+\gg\tau_-$. If the transformation between $\overset{*}{\mathrm{N}}$ and $\overset{*}{\mathrm{T}}$ is rapid, a quasiequilibrium is realized in the excited state. Such a condition is described by $\overrightarrow{k}_{\mathrm{t}}^{\mathrm{Cl}}$, $\overleftarrow{k}_{\mathrm{t}}^{\mathrm{Cl}}\gg n_1$, n_3. Then, slow and fast decay components of lifetimes corresponding to Eq. 14 become as follows:

$$\frac{1}{\tau_+}\approx\frac{n_3\overrightarrow{k}_{\mathrm{t}}^{\mathrm{Cl}}+n_1\overleftarrow{k}_{\mathrm{t}}^{\mathrm{Cl}}}{\overrightarrow{k}_{\mathrm{t}}^{\mathrm{Cl}}+\overleftarrow{k}_{\mathrm{t}}^{\mathrm{Cl}}}\quad\text{and}\quad\frac{1}{\tau_-}\approx\overrightarrow{k}_{\mathrm{t}}^{\mathrm{Cl}}+\overleftarrow{k}_{\mathrm{t}}^{\mathrm{Cl}}. \tag{16}$$

Equation 16 clearly states that the excited-state equilibration is attained in a short time of τ_- and the total concentration of excited species will decrease rather slowly with the time constant of τ_+ which is the reciprocal of weighted average of decay rates, n_1 and n_3. Although the case of $\tau_+\gg\tau_-$ dose not completely coincide with the case of rapid equilibration, the similar situation will be realized in the present case of 7HC and 7H4MC based on rough order estimation of n_j's and $k_{\mathrm{t}}^{\mathrm{Cl}}$'s using meansured τ_+ and τ_-. From experimental data, $1/\tau_-$ is $\approx5\times10^9\ \mathrm{s^{-1}}$, and n_1 and n_3 are supposed to be in the region of 0.2—$0.5\times10^9\ \mathrm{s^{-1}}$; therefore, $\overrightarrow{k}_{\mathrm{t}}^{\mathrm{Cl}}$ and $\overleftarrow{k}_{\mathrm{t}}^{\mathrm{Cl}}$ are estimated to be $\approx4\times10^9\ \mathrm{s^{-1}}$ by using Eq. 14. Such conditions correspond nearly to the rapid equilibration limit.

Appendix

Derivation of Eqs. 4 and 5. Letting $I_0^{\mathrm{N}}=n_1^{\mathrm{r}}[\overset{*}{\mathrm{N}}]$ and $I_0^{\mathrm{T}}=n_3^{\mathrm{r}}[\overset{*}{\mathrm{T}}]$, $I_0^{\mathrm{T}}/I_0^{\mathrm{N}}=(n_3^{\mathrm{r}}/n_1^{\mathrm{r}})\cdot([\overset{*}{\mathrm{T}}]/[\overset{*}{\mathrm{N}}])$. The ratio, $[\overset{*}{\mathrm{T}}]/[\overset{*}{\mathrm{N}}]$, can be obtained by using Eqs. 2—5 in Ref. 4 under steady-state conditions. Thus, we have

$$\frac{I_0^{\mathrm{T}}}{I_0^{\mathrm{N}}} = \frac{n_3^{\mathrm{r}}}{n_1^{\mathrm{r}}}\ \frac{\overrightarrow{k}_{\mathrm{t}}' + \beta\overleftarrow{k}_3[\mathrm{H}^+]}{n_3 + \overleftarrow{k}_{\mathrm{t}}'' + \alpha'\overleftarrow{k}_4[\mathrm{H}^+]}, \tag{4a}$$

where $\overrightarrow{k}_{\mathrm{t}}'$ and β are given in Eq. 5, and $\overleftarrow{k}_{\mathrm{t}}''$ and α' are given by

$$\overleftarrow{k}_{\mathrm{t}}'' = \overleftarrow{k}_{\mathrm{t}} + \overrightarrow{k}_2 \cdot \frac{n_2 + \overleftarrow{k}_1[\mathrm{H}^+]}{n_2 + \overleftarrow{k}_1[\mathrm{H}^+] + \overleftarrow{k}_2[\mathrm{H}^+]}$$

$$\text{and}\quad \alpha' = \frac{n_4 + \overrightarrow{k}_3}{n_4 + \overrightarrow{k}_3 + \overrightarrow{k}_4}\,. \tag{5a}$$

From Eq. 3, the relations

$$\frac{\overrightarrow{k}_1}{n_2 + \overleftarrow{k}_1[\mathrm{H}^+] + \overleftarrow{k}_2[\mathrm{H}^+]},\quad \frac{\overrightarrow{k}_2}{n_2 + \overleftarrow{k}_1[\mathrm{H}^+] + \overleftarrow{k}_2[\mathrm{H}^+]} \ll 1 \tag{17}$$

and

$$\frac{\overleftarrow{k}_3[\mathrm{H}^+]}{n_4 + \overrightarrow{k}_3 + \overrightarrow{k}_4},\quad \frac{\overleftarrow{k}_4[\mathrm{H}^+]}{n_4 + \overrightarrow{k}_3 + \overrightarrow{k}_4} \ll 1 \tag{18}$$

are automatically satisfied. Since the n_1—n_4 are assumed to be of the same order, we obtain the inequalities:

$$n_3 \gg n_2 \cdot \frac{\overrightarrow{k}_2}{n_2 + \overleftarrow{k}_1[\mathrm{H}^+] + \overleftarrow{k}_2[\mathrm{H}^+]}$$

$$\text{and } n_3 \gg n_4 \cdot \frac{\overleftarrow{k}_4[\mathrm{H}^+]}{n_4 + \overrightarrow{k}_3 + \overrightarrow{k}_4}\,. \tag{19}$$

The application of Eq. 19 to Eqs. 4a and 5a results in Eqs. 4 and 5. It is noted that, under the conditions of Eqs. 17 and 18, the fluorescence intensity from $\overset{*}{\mathrm{C}}{}^+$ and $\overset{*}{\mathrm{A}}{}^-$ (I_0^{C} and I_0^{A}) is negligibly small. If relations $n_2 \ll \overleftarrow{k}_1[\mathrm{H}^+]$ and $n_4 \ll \overrightarrow{k}_3$ are additionally satisfied, which have been assumed in Ref. 4 around pH≈0, $\overrightarrow{k}_{\mathrm{t}}'$, $\overleftarrow{k}_{\mathrm{t}}'$, α, and β in Eq. 5 are simplified as $\overrightarrow{k}_{\mathrm{t}}' = \overrightarrow{k}_{\mathrm{t}} + \overrightarrow{k}_1 \cdot \overleftarrow{k}_2/(\overleftarrow{k}_1 + \overleftarrow{k}_2)$, $\overleftarrow{k}_{\mathrm{t}}' = \overleftarrow{k}_{\mathrm{t}} + \overrightarrow{k}_2 \cdot \overleftarrow{k}_1/(\overleftarrow{k}_1 + \overleftarrow{k}_2)$, $\alpha = \overrightarrow{k}_3/(\overrightarrow{k}_3 + \overrightarrow{k}_4)$, and $\beta = \overrightarrow{k}_4/(\overrightarrow{k}_3 + \overrightarrow{k}_4)$.

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