Excited-State Reactions of Coumarins in Aqueous Solutions. V. Fluorescence Lifetimes of 7-Ethoxycoumarin in Acidic Solutions

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Synopsis. The fluorescence lifetime of 7-ethoxycoumarin (7EC) was measured in acidic aqueous solutions while varying the concentration of Cl^- as a fluorescence quencher. The subsequent analysis of the two-component fluorescence decay curves indicated that the protonated species in the excited state led to the fluorescence quenching; this result was quantitatively consistent with the fluorescence-yield study under steady-state conditions.

Since the extensive study of the photophysics of aromatic compounds was carried out by Ware and his co-workers,[1] it has been confirmed that steady-state and time-dependent analyses provide complementary information on various reaction processes and kinetic rate constants. The main concern of this report is to clarify the relation between the fluorescence lifetime and the steady-state quenching measurements of 7-ethoxycoumarin (7EC), which exhibits very interesting excited-state reactions, as has been extensively studied in previous papers.[2,3] Coumarin derivatives are heterocyclic compounds with a ring oxygen and a carbonyl group. Usually they have a very efficient fluorescing ability and show a variety of protonation or deprotonation reactions in the excited state, depending on the solvent conditions. To know the detailed mechanisms of such reactions, we must determine several rate constants which correspond to phenomena in a nanosecond region.

The transient spectroscopy of aqueous 7EC solutions has been conducted as a function of the quencher concentration, and the results have been compared with those of the steady state measurement. Assuming that two reacting species exist in the excited state, only one of which can cause a fluorescence-quenching reaction, the dependence of the lifetimes on the quencher concentration has been analyzed. The proposed model can consistently explain the results of both steady-state and transient studies.

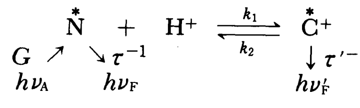
Experimental

The high-purity-grade reagent of 7-ethoxycoumarin (7EC) was obtained from Molecular Probes, Inc., and was used without further purification; mp 84.8 °C. The hydrochloric acid and perchloric acid were of a pure quality and were obtained from Wako Pure Chemical Ind. The water was permeated and distilled.

The usual UV absorption and fluorescence spectra of the aqueous 7EC solution of $\approx 5\times10^{-5}$ 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. The fluorescence lifetime measurements were performed by means of a time-correlated single-photon-counting fluorometer similar to the one described in Ref. 4. Since the lifetime of the fluorescer was close to the decay time of a repetitive short-pulsed light at 337 nm from an air-flash lamp, the result was deconvoluted in order to ascertain the true decay character, unaffected by the response function of the measurement system; the typical accuracy of this procedure was ±0.05 ns.

Results

The fluorescence quenching phenomenon of 7EC by chloride ions in acidic aqueous solutions has already been investigated under steady-state light illumination.[3] When $[H^+]$ was set at 0.5 mol dm^{-3} by the addition of HCl or $HClO_4$, the absorption maximum was at 324 nm, which corresponded to the neutral form (N) of 7EC. On the other hand, the fluorescence spectrum was composed of the emission from the neutral form of excited molecules ($\overset{*}{N}$), peaking at 390 nm, and the weak component from the protonated form ($\overset{*}{C}{}^+$), peaking at 423 nm. The general scheme of the excited state reaction and the fluorescence quenching process was given by:[3]



$$\begin{array}{ccccc} & \overset{*}{N} + H^+ & \underset{k_2}{\overset{k_1}{\rightleftharpoons}} & \overset{*}{C}{}^+ & \\ G \nearrow & \searrow \tau^{-1} & & \downarrow \tau'^{-1} & \\ h\nu_A & h\nu_F & & h\nu'_F & \end{array} \qquad (1)$$

$$\overset{*}{C}{}^+ + Cl^- \xrightarrow{k_q} C^+ + Cl^- \qquad (2)$$

The excited neutral species ($\overset{*}{N}$) is generated by a continuous UV-light illumination at the rate of G, and it is rapidly equilibrated with the excited protonated species ($\overset{*}{C}{}^+$) in the acidic solution. Then, $\overset{*}{C}{}^+$ interacts with the chloride ions and is deactivated to the ground state (C^+). k_1 and k_2 are the forward and backward rate constants for equilibration; τ and τ' are the fluorescence lifetimes of the neutral and protonated species respectively. k_q is the quenching rate constant caused by the collisional interaction between $\overset{*}{C}{}^+$ and Cl^-. By the analysis of the relative fluorescence intensity, I_0/I, depending on $[Cl^-]$ (I_0 and I are the fluorescence intensities in the absence and in the presence of chloride ions), the various rate constants in Eqs. 1 and 2 have already been determined, as cited in Table 1.[3] The ClO_4^- ion showed no observable quenching ability.

Under the pulsed-light excitation at 337 nm which

Table 1. Lifetimes of the Neutral and Protonated 7EC, τ and τ', and Rate Constants Obtained for the Excited State Equilibration, k_1 and k_2, and for the Collisional Quenching Process, k_q[3]

τ	τ'	$k_1\times10^{-9}$	$k_2\times10^{-9}$	$k_q\times10^{-9}$
ns	ns	mol^{-1} dm^3 s^{-1}	s^{-1}	mol^{-1} dm^3 s^{-1}
2.0_0	4.9_0	1.2_5	1.2_4	5.8_0

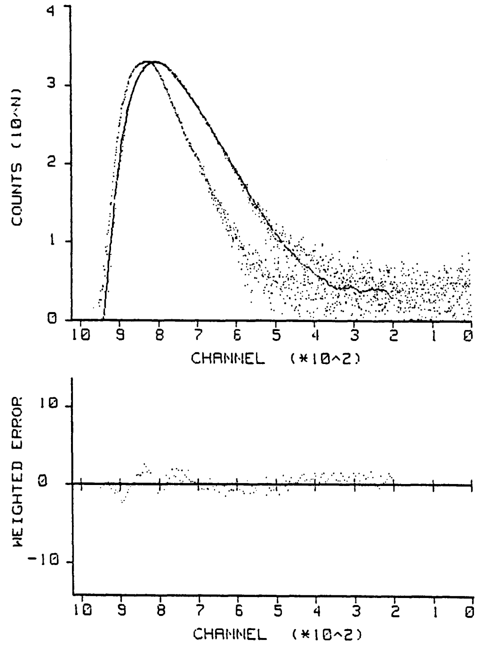


Fig. 1. Decay profiles of a light source and a fluorescence response (dots delayed to the right-hand side) of the aqueous 7EC solution with $[HClO_4]=0.4\,mol\,dm^{-3}$ and $[HCl]=0.1\,mol\,dm^{-3}$. 23.6 channels/ns; the channel number is reversed to the time progress. The solid line shows the fitted theoretical curve for the fluorescence decay; $\tau_+=1.85\,ns$, $\tau_-=0.35\,ns$, $C=0.3$. The weighted residual, $(f_i^{obs}-f_i^{calc})/(f_i^{obs})^{1/2}$, is also shown.

Table 2. Experimental Values for the Fluorescence Lifetime Parameters (τ_+, τ_-, and C) of the Neutral Form of 7EC at 20 °C and Those Calculated by Using the Constants in Table 1

$[Cl^-]$ / $mol\,dm^{-3}$	τ_+ / ns	τ_- / ns	C	τ_+^{cal} / ns	τ_-^{cal} / ns	C^{cal}
0	2.7_0	0.4_0	0.4_0	2.56	0.46	0.70
0.1	1.8_5	0.3_5	0.3_0	1.71	0.39	0.38
0.2	1.5_0	0.3_0	0.2_5	1.40	0.33	0.22
0.3	1.3_0	0.2_5	0.2_0	1.25	0.29	0.14
0.4	1.2_0	0.2_0	0.1_5	1.16	0.25	0.09
0.5	1.1_5	0.1_5	0.1_0	1.11	0.22	0.07

generates $\overset{*}{N}$, the decay characteristic of fluorescence from the neutral fluorescer has been measured at 390 nm in the acidic aqueous solution while varying the concentration of Cl^-. The pH is kept constant by satisfying $[HClO_4]+[HCl]=0.5\,mol\,dm^{-3}$, and $[Cl^-]$ is controlled by the amount of the HCl component added. The time-dependent light intesity, $i^N(t)$, of $\overset{*}{N}$ could be analysed by means of a decay curve of a double exponential character:

$$i^N(t) \propto \exp(-t/\tau_+) + C\cdot\exp(-t/\tau_-) \tag{3}$$

The best-fitted values for τ_+, τ_-, and C are shown in Table 2. The typical decay profiles of a light source, a fluorescence response, and the fitted curve are shown in Fig. 1. The total fluorescence spectrum of 7EC at $[H^+]\approx 0.5\,mol\,dm^{-3}$ includes two components which

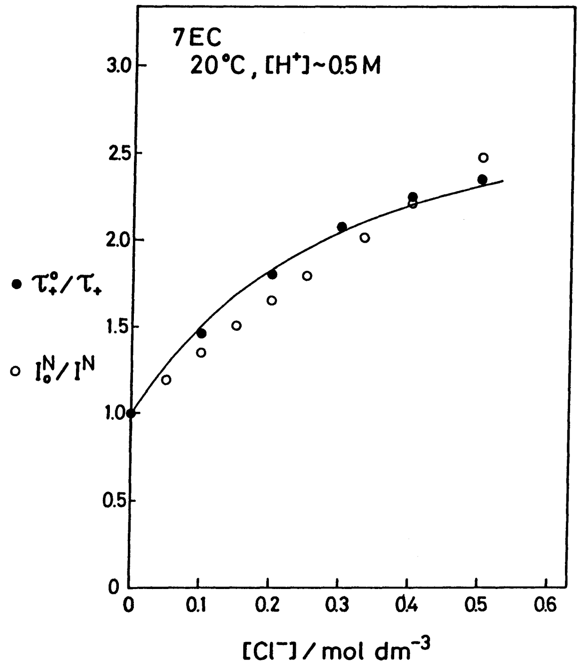


Fig. 2. Dependences of the relative fluorescence intensity, I_0^N/I^N (○), and the relative lifetime, τ_+^0/τ_+ (●), of 7EC on $[Cl^-]$ in the acidic aqueous solution at 20 °C. The solid line shows the theoretical prediction for τ_+^0/τ_+ vs. $[Cl^-]$ presented in the text.

result from the $\overset{*}{N}$ and $\overset{*}{C}{}^+$ in the solution. Since the concentration of $\overset{*}{C}{}^+$ is lower than that of $\overset{*}{N}$ by about one order of magnitude at $[H^+]\approx 0.5\,mol\,dm^{-3}$, the fluorescence contribution of $\overset{*}{C}{}^+$ at 390 nm is negligible in the lifetime measurement. Equation 2 describes how the fluorescence quenching by Cl^- is operative only for $\overset{*}{C}{}^+$, as was proved previously.[3] This has been reaffirmed by the fact that the time-variation in the $\overset{*}{N}$ fluorescence in a simple neutral solution, where no $\overset{*}{C}{}^+$ is formed, is not changed by the further addition of 0.5 $mol\,dm^{-3}$ NaCl.

In Fig. 2, the dependences of the relative fluorescence intensity, I_0^N/I^N, and the relative lifetime, τ_+^0/τ_+, on $[Cl^-]$ are shown, where τ_+^0 is a slow-decay component of the fluorescence lifetime of $\overset{*}{N}$ in the absence of Cl^-. These plots do not obey the linear Stern-Volmer relationship. The nonlinear dependency of the steady state result (I_0^N/I^N vs. $[Cl^-]$) was clearly explained in a previous paper.[3]

Discussion

Based on the scheme described by Eqs. 1 and 2, the time-dependent rate equation of the excited species can be derived. Subsequently, the response of $i^N(t)$ for a light pulse with an ideal δ-function is easily obtained:

$$\tau_\pm^{-1} = \frac{1}{2}\,[\tau^{-1} + \tau'^{-1} + k_1[H^+] + k_2 + k_q[Cl^-] \mp \sqrt{\{(\tau^{-1}+k_1[H^+]) - (\tau'^{-1}+k_2+k_q[Cl^-])\}^2 + 4k_1[H^+]k_2}\,] \tag{4}$$

$$C = -\frac{\tau'^{-1} + k_2 + k_q[\mathrm{Cl}^-] - \tau_-^{-1}}{\tau'^{-1} + k_2 + k_q[\mathrm{Cl}^-] - \tau_+^{-1}} \qquad (5)$$

From Eqs. 4 and 5, τ_+, τ_-, and C are theoretically predictable using the constants given in Table 1. The calculated values are shown in the last three columns of Table 2; the dependence of $\tau_+^{0,\mathrm{cal}}/\tau_+^{\mathrm{cal}}$ on $[\mathrm{Cl}^-]$ is drawn in Fig. 2 with a solid line. The quencher-concentration dependence of τ_+^0/τ_+ agrees very well with the theoretical prediction. It is possible in principle to determine the rate constants, k_1, k_2, and k_q, from the obtained set of τ_+, τ_-, and C values by using $1/\tau_+ + 1/\tau_- \propto \text{const.} + k_q[\mathrm{Cl}^-]$, etc. Because of the relatively low accuracy of deconvolution for the fast-decay component (τ_- and C, ±10—30%) compared with that of τ_+ (±2—5%), the rate constants to be determined are less reliable than those given by steady-state measurements (±1%). The lifetime analysis based on observing the fluorescence component from $\overset{*}{\mathrm{N}}$ of 7EC is analogous to the lifetime measurement of a monomer form in aromatic compound-quencher molecule systems, where an exciplex is generated to result in fluorescence quenching.1) The excited-state reaction of coumarin derivatives is classified essentially as an acid-base reaction and has time constants of a nanosecond order because of the very high mobilties of the participating species, such as H^+ and Cl^-, whereas the exciplex-formation reaction is a usually slower phenomenon by one or more orders.

The fluorescence-lifetime study of the aqueous solution of 7EC is characteristic in that the instantaneous or 'static' quenching process, which makes the steady state analysis complex, does not appear in the first approximation if the quencher concentration is not very high. A similar example has been presented by Efftink and Ghiron,5) who have shown experimentally, in an indole-quencher system, that τ^0/τ is independent of the $W = \exp(-V[\mathrm{Q}])$ factor, in contrast to I_0/I, where V is the static quenching constant. After pulsed-light illumination, the fraction, W, of the generated excited state, $\overset{*}{\mathrm{C}}{}^+$, 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 $\overset{*}{\mathrm{C}}{}^+$ and interacts directly with it. Including such a static quenching mechanism, the fluorescence-yield decrease of 7-ethoxycoumarins by Cl^- in acidic solutions under steady state conditions has been successfully explained.3) In the rate equations for the time-variations of $[\overset{*}{\mathrm{N}}]$ and $[\overset{*}{\mathrm{C}}{}^+]$ under pulsed excitation, the fraction of excited species that never give fluorescence over an observing interval of, typically, 50 nanoseconds and which result in the static quenching must be treated separately from a reaction system described with time-independent rate constants. That is, the generation rate, G, of $\overset{*}{\mathrm{N}}$ should be reduced effectively to $G \cdot \{(\tau^{-1} + Wk_1[\mathrm{H}^+])/(\tau^{-1} + k_1[\mathrm{H}^+])\}$, maintaining the same equational form as $W=1$.3) Such an approximation is valid for a weak static quenching case ($V \ll k_q\tau'$) and for rapid protonation and deprotonation reactions. A more rigorous treatment has appeared in the serial works of Ware et al.1) and Weller,6) who formulate the transient nature of the diffusing reactants by introducing a time-dependent rate constant into the rate equation and who show that the static quenching mentioned here is essentially equivalent to the transient effect. The replacement of the quenching-reaction term with $k_q(t)[\mathrm{Cl}^-][\overset{*}{\mathrm{C}}{}^+]$ will be required in the present problem, where $k_q(t)$ decreases to the usual constant k_q at a large t. However, except for a very short interval after pulsed excitation, the two different ways of analysis which utilize W and $k_q(t)$ as the key parameters give approximately the same results for the case of a weak transient effect.1,5,6) It may be concluded that, under appropriately chosen conditions, the normalized intensity for $i^{\mathrm{N}}(t)$ is given by Eq. 3—5, even if $W \neq 1$, whereas the integrated yield of the fluorescence is not correctly derived without taking account of the static quenching effect.

It has been made clear that, in the steady state study, the static quenching parameter, W, is a very important factor in analyzing the nonlinear behavior of I_0/I vs. $[\mathrm{Cl}^-]$, but, in the transient study, W is less important for the time variation in the fluorescence from $\overset{*}{\mathrm{N}}$. Experimentally, the quencher dependence of τ_+^0/τ_+ behaves very differently from $I_0^{\mathrm{N}}/I^{\mathrm{N}}$, as is shown in Fig. 2. The parameters determined by the steady state measurement could consistently reproduce the data from the transient spectroscopy, although these two methods differ in the treatment of the static quenching factor, W. Therefore, the validity of the fundamental scheme of Eqs. 1 and 2 for the excited state reactions of 7EC has been well confirmed by independent experiments.

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