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Excited-State Reactions of Coumarins in Aqueous Solutions. III. The Fluorescence Quenching of 7-Ethoxycoumarins by the Chloride Ion in Acidic Solutions

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The fluorescence yield of 7-ethoxycoumarins in acidic aqueous solutions becomes reduced with an increase in the chloride-ion concentration. In acidic solutions, two types of excited molecules, a neutral molecule and a protonated molecule with a positive charge, are generated by a UV light illumination. Only the excited state of a protonated molecule is quenched by a chloride ion; the quenching reaction proceeds via both a diffusional and a static process. The rate constant for the diffusional process is $(3.7—5.8)\times10^9$ mol^{-1} dm^3 s^{-1}. The static quenching component can be approximately described by the $V[\mathrm{Q}]$ expression and the static quenching constant, V, is 1.2—1.7 mol^{-1} dm^3. One possible physical interpretation of the quenching mechanism is discussed in relation to the charge of colliding species and the electron transfers between them.

In a previous paper[1)] interactions between 7-ethoxycoumarins and halide ions in a neutral aqueous solution were discussed. It has been revealed that bromide and iodide ions cause a very pronounced quenching effect on the fluorescence of 7-ethoxycoumarins. On the other hand, chloride ions show no indication of quenching, even at considerably high quencher concentrations. This has been explained in terms of the oder of the quenching ability of the halide ions, i.e., $\mathrm{I}^- > \mathrm{Br}^- \gg \mathrm{Cl}^-$.

When a solution is changed from neutral to acidic, 7-ethoxycoumarins can take two forms of the photo-excited state, neutral and protonated. It was recently found that in an acidic solution, even chloride ions have a remarkable quenching effect on the fluorescence of the two molecular species.

To quantitatively analyze this phenomena, chloride ions were chosen as the quencher. Then, the fluorescence intensities of 7-ethoxycoumarin (**1**) and 7-ethoxy-4-methylcoumarin (**2**) in acidic aqueous solutions were measured in detail as a function of the quencher concentration. In this paper a reasonable scheme for the reaction kinetics is proposed and several reaction-rate constants are derived by means of the obtained data. The quenching mechanism is discussed while taking account of the ionic nature of the interacting species.

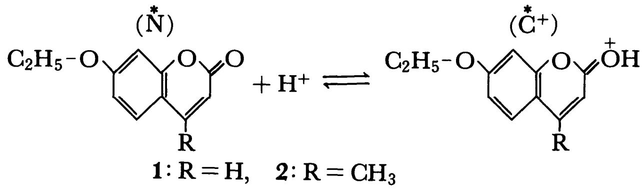
Experimental

Material. High-purity grade reagents of 7-ethoxycoumarin (**1**) and 7-ethoxy-4-methylcoumarin (**2**) were obtained from Molecular Probes Inc. and used as purchased. Their melting points were determined by means of DTA:[2)] **1**, mp 84.8°; **2**, mp 115.5°. Sodium chloride, hydrochloric acid, and perchloric acid were of pure-quality from Wako Pure Chemical Ind. Water was permeated and distilled.

Methods. The UV absorption spectra were obtained on a Hitachi 323 spectrophotometer and fluorescence intensity measurements were made on a Hitachi MPF 4 spectrofluorometer with an S-5 type photomultiplier tube. Spectral measurements were performed at 20 °C unless otherwise mentioned. For fluorescence measurements, the wavelengths of the excitation light were chosen to be 324 nm for **1** and 321 nm for **2**, corresponding to the absorption maximum of the respective compounds. The concentrations of the fluorescent molecules in a solution were $\approx5\times10^{-5}$ mol dm^{-3} for **1** and $\approx4\times10^{-5}$ mol dm^{-3} for **2**. These provided for the most reliable recordings of the fluorescence intensity using the spectrofluorometer.[3)] The pH or H_0 value[4)] of the solution was controlled with $\mathrm{HClO_4}$ and HCl. In the quenching experiment, the fluorescence of a series of samples containing a constant amount of a fluorescent compound and a graduated amount of quencher was measured. Fluorescence lifetime measurements were performed by means of a time-correlated single-photon-counting fluorometer, similar to the one described in Ref. 5. When the lifetime was close to the decay time of repetitive short-pulsed light at 337 nm from an air-flash lamp, the deconvolution procedure was performed assuming the exponential decay of the fluorescence intensity. The resulting accuracy was within ±0.05 ns.

Results and Discussion

pH Dependence of Absorption and Fluorescence Spectra. In the electronically ground state of 7-ethoxycoumarins, only one molecular species was spectroscopically detectable in an aqueous solution of neutral and acidic regions (pH 7 to H_0 −3) where fluorescence-quenching measurements have been carried out. In the excited state, a neutral molecule, $\overset{*}{\mathrm{N}}$ and a protonated molecule, $\overset{*}{\mathrm{C}}{}^{+}$ appear.[6)] The interconversion of these species can be described by the reaction:



1: R = H, **2**: R = $\mathrm{CH_3}$

Figure 1 shows the UV absorption spectrum and the visible fluorescence spectra of 7-ethoxycoumarin (**1**). The absorption maxima of **1** and **2** in the neutral solution were 324 and 321 nm respectively. These absorption spectra did not vary over the wide pH range

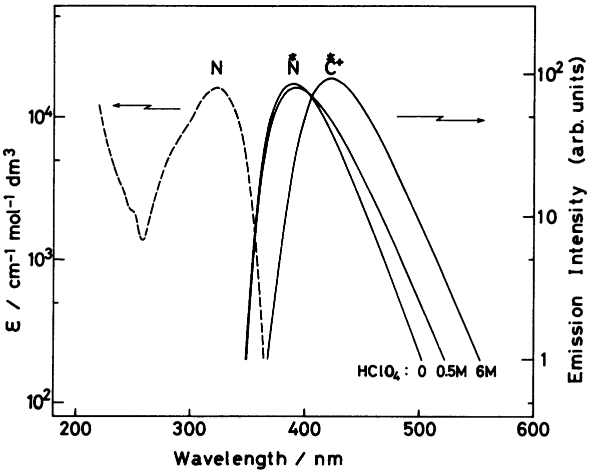


Fig. 1. Absorption and fluorescence spectra of 7-ethoxycoumarin (**1**) in aqueous solutions. N indicates the absorption spectrum of the neutral molecule. $\overset{*}{N}$ and $\overset{*}{C}^+$ are the fluorescence spectra from the excited state of the neutral and the protonated molecules respectively; the typical fluorescence spectra for the neutral solution and for the solution with 0.5 and 6 mol dm^{-3} HClO$_4$ added are shown.

of interest ($H_0 > -3$). The new absorption band of a protonated molecule barely appeared in a solution of concentrated sulfric acid ($H_0 < -6$) at longer wavelengths. Such absorption bands have been observed by others for similar coumarin derivatives;[18,19] the peaks are at 362 and 353 nm for **1** and **2**, respectively. In contrast, the fluorescence spectra began to change in shape and wavelength position for moderately acidic solutions. The fluorescence peak at 390 nm of Compound **1** in a neutral solution shifted to at last 423 nm at $H_0 \approx -3$; in the case of Compound **2**, these peaks were at 382 and 412 nm, respectively. The fluorescence peaks of these compounds at shorter wavelengths correspond to a neutral molecule in an excited state. The peaks at longer wavelengths correspond to a protonated molecule in an excited state.[6]

In moderately acidic solutions, these two types of fluorescence bands overlapped each other; therefore, it is necessary to separate the spectral bands in order to obtain a quantitative value for the relative fluorescence intensities of the two molecular species. For this procedure, it is very convenient to plot the emission intensity of the fluorescence spectrum with a logarithmic scale (Fig. 1). This is done because the contribution from each molecular species is readily recognized by adding the respective spectral bands and reconstructing the data in the graph at a given pH.

Fluorescence Quenching by Halide Ions. The fluorescence quenching by halide ions in a neutral aqueous solution was fully investigated in a previous paper and the quenching process was clarified.[1] From the experimentally obtained results, it has been concluded that chloride ions have no effect on the fluorescence intensity of a neutral molecule. Figure 2 shows plots of I_0/I vs. [Cl$^-$], where I_0 and I are the fluorescence intensities in the absence and in the presence of chloride ions. At pH≈7, a neutral molecule is the only species both in ground and excited states and Cl$^-$ has no effect on the fluorescence intensity for [Cl$^-$]$\leq$0.5 mol dm^{-3}.

Fluorescence Quenching by Chloride Ions in a Strongly Acidc Solution. When 6 mol dm^{-3} of HClO$_4$ was added beforehand to the solution ($H_0 = -2.84$), protonated molecules became the unique species in the excited state and fluorescence was considerably quenched by the Cl$^-$ (contained as a controlled amount of NaCl up to 0.25 mol dm^{-3}). In Fig. 2 data are shown for an aqueous solution with 6 mol dm^{-3} HClO$_4$ added; it can be seen from the figure that I_0/I depends linearly on [Cl$^-$]. In such case, the Stern-Volmer relationship derived under steady state conditions is apllicable:[7]

$$\frac{I_0}{I} = 1 + K_{sv}[Q]. \tag{1}$$

K_{sv} is the collisional quenching constant and [Q] is the quencher concentration. The data in Fig. 2 show the linear dependence of I_0/I on the concentration of chloride ions; the quenching constant, K_{sv}, of a protonated molecule (due to Cl$^-$) is estimated to be 6.80 mol^{-1} dm^3 for **1** and 4.21 mol^{-1} dm^3 for **2**. Using the rate constant, k_q, and the lifetime of the excited state of a protonated molecule, τ', the quenching constant is given by $K_{sv} = k_q\tau'$. The k_q values were estimated to be 1.39×10^9 mol^{-1} dm^3 s^{-1} for **1** and 8.77×10^8 mol^{-1} dm^3 s^{-1} for **2**, respectively, with the lifetimes shown in Table 2. The occurrence of this quenching effect is attributable to an increase in the interaction between positively charged molecules and negatively charged chloride ions through an attractive electrostatic force in comparison with the interaction between a neutral molecule and an ion.

Fluorescence Quenching by Chloride Ions in a Moderately Acidic Solution. When the same quenching experiment was performed in a moderately acidic solution, the dependence of I_0/I (for neutral and protonated molecules) on the Cl$^-$ concentration did not obey the usual Stern-Volmer relationship, as can be clearly seen in Fig. 2 (a plot of I_0/I vs. [Cl$^-$]). The curve for the fluorescence of the neutral molecule curved downward away from a linear dependence; the curve for the protonated molecule curved upward. Since the sum of the concentrations of HClO$_4$ and HCl was made to be constant at 0.5 mol dm^{-3}, the ionic concentration,[8] Γ, and the acidity strength,[4] H_0, did not change for various concentrations of [Cl$^-$] (Γ=1 mol dm^{-3} and H_0=0.20). $\Gamma=\sum_i c_i z_i^2$, where c_i and z_i are the molar concentration and the valence of the ions in a solution. Thus, the unexpected behavior (mentioned above) cannot be related to a change in these values.

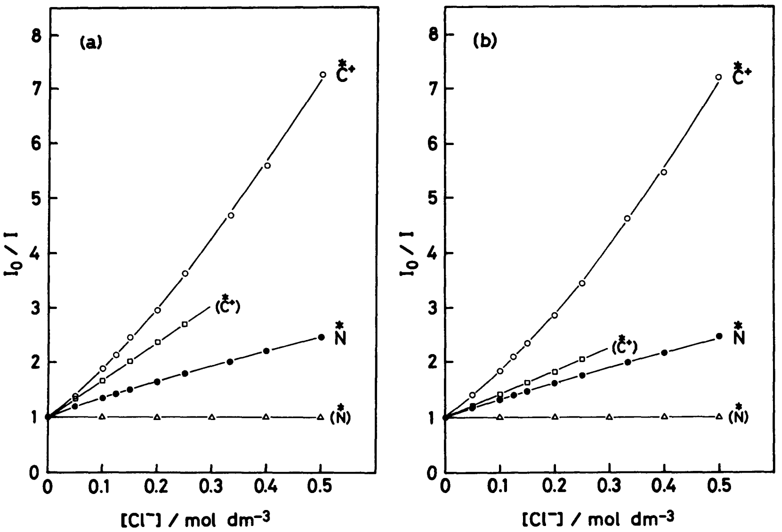


Fig. 2. Dependence of I_0/I on the concentration of the chloride ion in the neutral (△), the moderately acidic (○; ●), and the strongly acidic (□) solutions. (a), 7-ethoxycoumarin (**1**); (b), 7-ethoxy-4-methylcoumarin (**2**).

In order to analyze such phenomena from a general point of view, the following scheme for the chemical reaction and the fluorescence-quenching process is given:

$$N + h\nu_A \xrightarrow{G_{abs}} \overset{*}{N}, \tag{2}$$

$$\begin{tikzcd} & \overset{*}{N} + H^+ \arrow[dl, "n_1"'] \arrow[d, "n_2"] \arrow[r, shift left, "k_1"] & \overset{*}{C}{}^+ , \arrow[l, shift left, "k_2"] \arrow[d, "n_1'"'] \arrow[dr, "n_2'"] & \\ N + h\nu_F & N & C^+ + h\nu_F' & C^+ \end{tikzcd} \tag{3}$$

$$W : \overset{*}{C}{}^+ + Q \underset{k_2'}{\overset{k_1'}{\rightleftharpoons}} (C^+Q)^* \xrightarrow{k_3'} C^+ + Q, \tag{4}$$

$$1 - W : \overset{*}{C}{}^+ + Q \longrightarrow C^+ + Q. \tag{5}$$

Here, N and C^+ represent neutral and protonated molecules, respectively. $\overset{*}{N}$ and $\overset{*}{C}{}^+$ are their excited states and Q and $h\nu$ represent the quencher and the light quantum. $(C^+Q)^*$ is the excited state of the encounter complex between $\overset{*}{C}{}^+$ and the quencher. G_{abs} is the generation rate of $\overset{*}{N}$; the k's and n's are the reaction-rate constants and the transition probabilities, respectively. Equation 3 describes an excited-state reaction of neutral molecules with protons which generates a protonated molecule. n_1 and n_1' are the radiative-transition probabilities that cause the fluorescence; n_2 and n_2' are the nonradiative-transition probabilities toward the electronic ground state. Equation 4 shows that the fraction, W, of $\overset{*}{C}{}^+$ obeys a collisional quenching process in which the encounter complex, $(C^+Q)^*$, decays nonradiatively to C^+ and Q with a rate of k_3'; Equation 5 shows that a fraction, $1-W$, of $\overset{*}{C}{}^+$ obeys the instantaneous deactivation process discussed in Ref. 1.

It is an essential idea for the explanation of experimental data that only a certain fraction, W, of the excited states is actually quenched by the collisional mechanism. The remaining fraction, $1-W$, is deactivated almost instantaneously after being formed since a quencher is accidentally positioned in the proximity of the molecule at the time of light-absorption and interacts very strongly with the molecule. Generally, this additional factor, W (a function of the quencher concentration, [Q]), can be approximated by $\exp(-V[Q])$, where V is the static quenching constant. If the model of Frank and Vavilov[9] is adopted for the instantaneous or "static" quenching process, an instantaneous quenching results when a quencher happens to reside within a "sphere of action" having a volume of V/N', where N' is the Avogadro's number per millimole.[10]

Since illuminating light is absorbed solely by neutral molecules under experimental conditions and the fraction, $1-W$, of the protonated molecule in the excited state is immediately quenched, the effective generation rate of neutral molecules in the excited state, $\tilde{G}(Q)$, is as a first approximation:

$$\begin{aligned}\tilde{G}(Q) &= G_{abs} - \{1 - W(Q)\}G_{abs}\frac{k_1[H^+]}{n_1+n_2+k_1[H^+]} \\ &= G_{abs}\frac{n_1+n_2+W(Q)k_1[H^+]}{n_1+n_2+k_1[H^+]},\end{aligned} \tag{6}$$

where

$$W(Q) = \exp(-V[Q]) \text{ and } W(0) = 1. \tag{7}$$

The rate equations for each molecular species, therefore, become:

$$\frac{d[\overset{*}{N}]}{dt} = \tilde{G} - (n_1+n_2+k_1[H^+])[\overset{*}{N}] + k_2[\overset{*}{C}{}^+], \tag{8}$$

$$\frac{d[\overset{*}{C}{}^+]}{dt} = k_1[H^+][\overset{*}{N}] - (n_1'+n_2'+k_1'[Q]+k_2)[\overset{*}{C}{}^+] + k_2'[(C^+Q)^*], \tag{9}$$

$$\frac{d[(C^+Q)^*]}{dt} = k_1'[Q][\overset{*}{C}{}^+] - (k_2'+k_3')[(C^+Q)^*]. \tag{10}$$

If the steady-state condition is satisfied, we may obtain the densities of the excited molecules by letting the left-hand side of Eqs. 8—10 be zero. Then, the densities of $\overset{*}{N}$ and $\overset{*}{C}{}^+$ are:

$$[\overset{*}{N}] = \tilde{G}\tau/\left(1+\frac{1+k_q\tau'[Q]}{1+k_2\tau'+k_q\tau'[Q]}k_1\tau[H^+]\right), \tag{11}$$

$$[\overset{*}{C}{}^+] = \left(\tilde{G}\tau'\frac{k_1\tau[H^+]}{1+k_2\tau'+k_q\tau'[Q]}\right)\Big/ \left(1+\frac{1+k_q\tau'[Q]}{1+k_2\tau'+k_q\tau'[Q]}k_1\tau[H^+]\right), \tag{12}$$

where

$$\tau = \frac{1}{n_1+n_2} \text{ and } \tau' = \frac{1}{n_1'+n_2'}, \tag{13}$$

$$k_q = \gamma k_1' = \frac{k_3'}{k_2'+k_3'}k_1'. \tag{14}$$

τ and τ' correspond to the lifetimes of neutral and protonated molecules. k_q and γ are the apparent rate constant for the collisional quenching process and the efficiency of the quenching reaction, respectively. The total fluorescence intensities from $\overset{*}{N}$ and $\overset{*}{C}{}^+$ are defined by:

$$I^N = n_1[\overset{*}{N}] \text{ and } I^C = n_1'[\overset{*}{C}{}^+]. \tag{15}$$

Since the value of $\tilde{G}$ at [Q]=0 is G_{abs}, the fluorescence intensities at [Q]=0 for $\overset{*}{N}$ and $\overset{*}{C}{}^+$, i.e., I_0^N and I_0^C, are written as:

$$I_0^N = G_{abs}n_1\tau/\left(1+\frac{k_1\tau[H^+]}{1+k_2\tau'}\right) \text{and} \tag{16}$$

$$I_0^C = \left(G_{abs}n_1'\tau'\frac{k_1\tau[H^+]}{1+k_2\tau'}\right)\Big/\left(1+\frac{k_1\tau[H^+]}{1+k_2\tau'}\right). \tag{17}$$

Then, the relative fluorescence intensities, in comparison with the intensity at [Q]=0, of each excited molecule are given by:

$$\frac{I_0^N}{I^N} = \left\{\left(1+\frac{1+k_q\tau'[Q]}{1+k_2\tau'+k_q\tau'[Q]}k_1\tau[H^+]\right)\Big/ \left(1+\frac{k_1\tau[H^+]}{1+k_2\tau'}\right)\right\}\frac{1+k_1\tau[H^+]}{1+W(Q)k_1\tau[H^+]}, \tag{18}$$

$$\frac{I_0^C}{I^C} = \frac{I_0^N}{I^N}\,\frac{1+k_2\tau'+k_q\tau'[Q]}{1+k_2\tau'}. \tag{19}$$

From Eqs. 18 and 19, it is easily shown that $I_0^N/I^N \to 1$ and $I_0^C/I^C \to 1$ if $[Q]\to 0$, and $I_0^C/I^C > I_0^N/I^N > 1$ for $[Q]>0$. These results can reasonably explain, in principle, the dependence of I_0/I on the concentration of the quencher. The experimental data in Fig. 2 show that I_0^C/I^C is always larger than I_0^N/I^N.

Tabel 1. Typical Values of I_0^N/I^N Shown in Fig. 2

Substance	[Cl⁻]			
	0	0.05 mol dm^{-3}	0.1 mol dm^{-3}	0.125 mol dm^{-3}
1	1	1.19$_3$	1.34$_7$	1.42$_2$
2	1	1.17$_0$	1.32$_3$	1.39$_4$

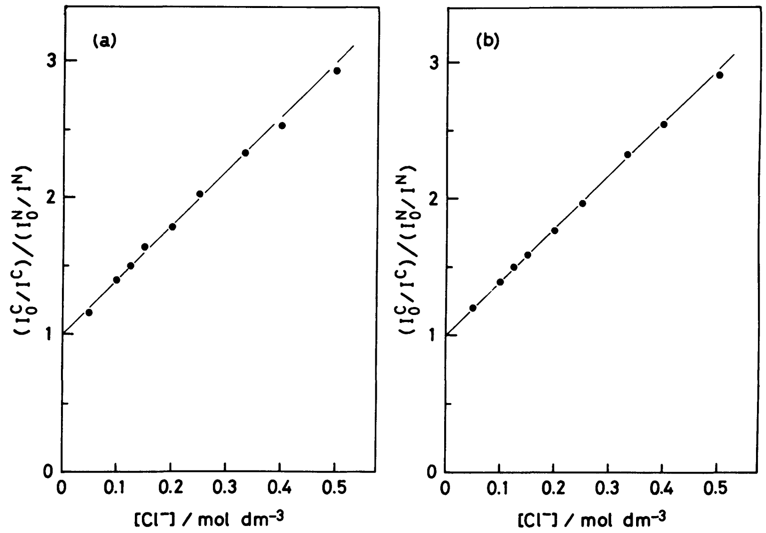


Fig. 3. Plots of $(I_0^C/I^C)/(I_0^N/I^N)$ vs. [Cl⁻]. (a), **1**; (b), **2**.

Table 2. Lifetimes of the Two Molecular Species, τ and τ', and Values of $k_q\tau'/(1+k_2\tau')$ and $k_1\tau[H^+]/(1+k_2\tau')$

Substance	$\tau/\text{ns}^{a)}$	$\tau'/\text{ns}^{a)}$	$k_q\tau'/(1+k_2\tau')/\text{mol}^{-1}\,\text{dm}^3$	$k_1\tau[H^+]/(1+k_2\tau')^{b)}$
1	2.0_0	4.9_0	4.0_0	0.17_6
2	2.2_5	4.8_0	3.9_2	0.26_1

a) The lifetime was measured at a suitably chosen pH value where only one molecular species was detectable. b) The value at $[H^+]=0.5\ \text{mol}\,\text{dm}^{-3}$.

Table 3. Rate Constants Obtained for the Excited-State Reactions and for the Collisional Quenching Process, Collisonal Quenching Constants, Static Quenching Constants, and Radii of the Sphere of Action in the Aqueous Solution with $[H^+]=0.5\ \text{mol}\,\text{dm}^{-3}$ and $\Gamma=1\ \text{mol}\,\text{dm}^{-3}$

Substance	$k_1\times10^{-9}$	$k_2\times10^{-9}$	$k_q\times10^{-9}$	K_{sv}	V	r
	$\text{mol}^{-1}\,\text{dm}^3\,\text{s}^{-1}$	s^{-1}	$\text{mol}^{-1}\,\text{dm}^3\,\text{s}^{-1}$	$\text{mol}^{-1}\,\text{dm}^3$	$\text{mol}^{-1}\,\text{dm}^3$	Å
1	1.2_5	1.2_4	5.8_0	28.4	1.2	7.7
2	1.0_4	0.72_1	3.6_5	17.5	1.7	8.8

Reaction-Rate Constants for the Quenching Process. To quantitatively analyze the quenching process in an acidic solution, we can derive from Eq. 19:

$$\frac{I_0^C/I^C}{I_0^N/I^N}=1+\frac{k_q\tau'}{1+k_2\tau'}[Q]. \qquad (20)$$

This relationship predicts the linear dependence of $(I_0^C/I^C)/(I_0^N/I^N)$ on the concentration of the quencher; its slope gives the value of $k_q\tau'/(1+k_2\tau')$. The experimental data of $(I_0^C/I^C)/(I_0^N/I^N)$ vs. $[Cl^-]$ are plotted in Fig. 3. The dependence is a linear function of $[Cl^-]$ and the values of $k_q\tau'/(1+k_2\tau')$ are $4.00\ \text{mol}^{-1}\,\text{dm}^3$ for **1** and $3.92\ \text{mol}^{-1}\,\text{dm}^3$ for **2**, respectively.

It is helpful, at this stage, to define the fluorescence intensity of excited molecules at both $[Q]=0$ and $k_1\tau[H^+]\ll1$ by $\bar{I}_0^N$. From Eq. 16 we obtain $\bar{I}_0^N=G_{abs}n_1\tau$, and

$$\frac{\bar{I}_0^N}{I_0^N}=1+\frac{k_1\tau[H^+]}{1+k_2\tau'}. \qquad (21)$$

The values of $\bar{I}_0^N/I_0^N$ and, of course, $k_1\tau[H^+]/(1+k_2\tau')$ are obtainable from the fluorescence data of a neutral solution and an acidic solution with $[H^+]=0.5\ \text{mol}\,\text{dm}^{-3}$. The resulting values of $k_1\tau[H^+]/(1+k_2\tau')$ at $[H^+]=0.5\ \text{mol}\,\text{dm}^{-3}$ are 0.176 for **1** and 0.261 for **2**, respectively. Since the original form of $W(Q)$ as an exponential function of $[Q]$ is not tractable for an analytical calculation, the same approximation as was adopted in the former work[1] may be used again for a relatively low quencher concentration:

$$W(Q)\approx1-V[Q]. \qquad (22)$$

Here, V is the static quenching constant. It is noted that this approximation has meaning only for $V[Q]<0.15$ when estimating the unknown parameter. Thus, the numerical values of I_0^N/I^N for the low concentration region of $[Cl^-]$ are cited in Table 1.

By using Tables 1 and 2 as well as the approximation given by Eq. 22, values of $k_q\tau'(=K_{sv})$ and V can be obtained from a best-fit analysis of the data points and the theoretical prediction. Then, K_{sv}, V, k_1, k_2, and k_q are given as cited in Table 3. Incidentally, the radius of the sphere-of-action for the quenching process, r, is estimated and cited in the same table, with the relation, $V/N'=4\pi r^3/3$, where N' is Avogadro's number per millimole. Qualitatively speaking, it is shown with the theoretical prediction that both I_0^N/I^N and I_0^C/I^C increase monotonically with an increase in the quencher concentration. The increase in I_0^N/I^N saturates at the higher concentration range and the value of I_0^N/I^N reaches asymptotically to $(1+k_2\tau')(1+k_1\tau[H^+])^2/(1+k_2\tau'+k_1\tau[H^+])$. The increase in I_0^C/I^C becomes linear with a slope of $(1+k_1\tau[H^+])^2k_q\tau'/(1+k_2\tau'+k_1\tau[H^+])$ in the higher concentration range. In general, I_0^N/I^N and I_0^C/I^C change in a complicated manner; thus, an analytical treatment has been possible only in the low concentration range where Eq. 22 is valid.

Equilibrium Constants in the Excited State. Since we know the values of k_1 and k_2, the reaction kinetics of Eq. 3 can be dealt with quantitatively. If equilibrium is reached in the excited state, analogous to the case in the ground state, the equilibrium constant for the conjugate acid of the neutral molecule, $\overset{*}{C}{}^+$, is described by $\overset{*}{K}_{C^+}=\overset{*}{a}_N a_{H^+}/\overset{*}{a}_{C^+}$. $\overset{*}{a}_N$ and $\overset{*}{a}_{C^+}$ are the activities of the neutral and protonated molecules in excited states, and a_{H^+} is the activity of a hydrogen ion. By using the difinition of the acidity function,[4] $H_0=-\log(a_{H^+}\overset{*}{f}_N/\overset{*}{f}_{C^+})$, where $\overset{*}{f}_N$ and $\overset{*}{f}_{C^+}$ are the activity coefficients of $\overset{*}{N}$ and $\overset{*}{C}{}^+$, and by assuming the relation, $k_1[\overset{*}{N}][H^+]=k_2[\overset{*}{C}{}^+]$, the following relation is obtainable for the $p\overset{*}{K}$ value:

$$p\overset{*}{K}_{C^+}=-\log\overset{*}{K}_{C^+}=-\log\frac{k_2}{k_1[H^+]}+H_0. \qquad (23)$$

With the aid of Eq. 23, the $p\overset{*}{K}_{C^+}$ values were determined to be -0.10 for **1** and 0.06 for **2**, respectively.

Table 4. Comparison between the Collisional Quenching-Rate Constants and the Static Quenching Constants under Various Conditions

Substance	Quencher-Species	Condition	$k_q\times10^{-9}$ / $\mathrm{mol^{-1}\,dm^3\,s^{-1}}$	V / $\mathrm{mol^{-1}\,dm^3}$
1	$I^--\overset{*}{N}$	Neutral	$10._2$	1.6_2
	$Br^--\overset{*}{N}$	Neutral	4.3_0	0.0_8
	$Cl^--\overset{*}{C}{}^+$	$HClO_4+HCl$, 0.5M[a]	5.8_0	1.2
	$Cl^--\overset{*}{C}{}^+$	$HClO_4$, 6M	1.3_9	0
2	$I^--\overset{*}{N}$	Neutral	9.6_5	1.8_1
	$Br^--\overset{*}{N}$	Neutral	3.7_6	0.0_4
	$Cl^--\overset{*}{C}{}^+$	$HClO_4$-HCl, 0.5M	3.6_5	1.7
	$Cl^--\overset{*}{C}{}^+$	$HClO_4$, 6M	0.87_7	0

a) 1 M=1 mol dm^{-3}.

This shows that the forward and backward reactions described by Eq. 3 become competitive when the acidity function of a solution is in the proximity of zero.

Efficiency of the Collisional Quenching Process and Static Quenching Constant. The rate constant for the generation of the encounter complex, $(C^+Q)^*$, in Eq. 4 can be roughly estimated by the relation, $k_1'\approx4\pi N'RD$. Here, R is the encounter distance between the reactants and D is the sum of the individual diffusion coefficients of the reactants.[11,12] If the values of R and D are reasonably assigned as 7 Å and $1.5\times10^{-5}\,\mathrm{cm^2\,s^{-1}}$ (based on molecular and ionic volumes and the Stokes-Einstein relation[7]), the diffusion-limited value of the quenching-rate constant, k_1', is estimated to be about $8\times10^9\,\mathrm{mol^{-1}\,dm^3\,s^{-1}}$. The apparent rate constants for the collisional quenching, k_q, under various conditions are given in Table 4, together with the static quenching constants, V. k_q and k_1' are related by Eq. 14 and different by a factor, γ, representing the efficiency of the collisional quenching process.

Since k_q with $\overset{*}{N}$ quenched by iodide ions are comparable or slightly greater than $8\times10^9\,\mathrm{mol^{-1}\,dm^3\,s^{-1}}$, the efficiency of collisional quenching is supposed to be nearly unity, as has been concluded in previous work.[1] Such an effective collisional quenching has been attributed to a charge-transfer interaction between $\overset{*}{N}$ and I^- since the lower ionization energy of I^- (compared to other halide ions) increases the probability of a transfer of an electron from I^- to $\overset{*}{N}$.[13,14] However, the external heavy-atom effect through a spin-orbit coupling in the encounter complex between $\overset{*}{N}$ and I^- might also play a role in the collisional quenching process.[15] From the static quenching constant, V, for an interaction between $\overset{*}{N}$ and I^-, the radius of the sphere-of-action was calculated to be 8—9 Å. This is slightly larger than the value (6—7 Å) estimated as the sum of the radii of the coumarin and an iodide ion. Excited molecules generated very near to or in contact with iodide ions are subjected to an instantaneous deactivation toward the ground state due to the very strong interaction among the reactants.

When Br^- is used as a quencher, the efficiency of the collisional quenching process in a neutral solution becomes lower and, consequently, k_q decreases. This phenomenon is attributable to an increased ionization energy of Br^-, compared with I^-. This leads to a decrease in the strength of the charge-transfer interaction. Of course, a decrease in the heavy-atom effect of bromide ions might explain a part of this phenomenon. In parallel with this tendency, the static quenching constant, V in Table 4, decreases drastically and the encounter distance, R, for the collisional process is effectively shortened to γR. Thus, the radius of the sphere-of-action, being a measure of the interaction strength, is also considered to be shortened by the same order of magnitude as represented by the small value of V. Incidentally, Cl^- has no effect on fluorescence quenching for an excited neutral molecule, $\overset{*}{N}$, over the examined range of the quencher concentration.

In addition to the above-mentioned usual type of fluorescence quenching, we have recently found that $\overset{*}{C}{}^+$ is also quenched, even by the chloride ions in a moderately acidic solution, where $[H^+]=0.5\,\mathrm{mol\,dm^{-3}}$, while $\overset{*}{N}$ does not suffer quenching to any extent; k_q becomes 3.7—$5.8\times10^9\,\mathrm{mol^{-1}\,dm^3\,s^{-1}}$, comparable in magnitude to the value for Br^- in a neutral solution. Since the heavy-atom effect of chloride ions is negligibly small compared to that from iodide and bromide ions, the occurrence of such intermediate quenching of $\overset{*}{C}{}^+$ by Cl^- (examined here) can be attributed to the existence of an attractive coulomb interaction between the reactants. Since $\overset{*}{N}$ does not have any charge on it, the interaction between $\overset{*}{N}$ and halide ions will be a contact interaction (a hard-core type interaction) in nature. Interactions between $\overset{*}{C}{}^+$ and Cl^-, on the other hand, are based on a long-ranged coulomb force, as long as the ionic concentration is low. However, the high concentration of dissolved ions tends to screen the interaction force. This attractive coulomb interaction will increase the probability of an electron being transferred from Cl^- to $\overset{*}{C}{}^+$ in the encounter complex, and, consequently, deactivating the complex to C^+ and Cl^- (Eq. 4). k_q is less than $8\times10^9\,\mathrm{mol^{-1}\,dm^3\,s^{-1}}$. One reason for this may be that the efficiency of the collisional-quenching process becomes less than unity; then, every encounter event with Cl^- does not lead to a nonradiative decay of the excited protonated molecule. The other one is that k_1' may be affected by the ionic concentration, Γ, the effect of which would not be negligible under the experimental conditions. It is well known that the rate constant of a chemical reaction between particles with opposite charges becomes smaller if the ionic concentration of the solution would increase.[16] It has been very difficult to decide from only the data obtained here which factor for the collisional quenching process is essential to cause a smaller k_q value compared with the diffusion-limited value.

The static quenching constants, V (Table 4), for the quenching of $\overset{*}{C}{}^+$ by Cl^- in a moderately acidic solution is close to that for the quenching of $\overset{*}{N}$ by I^- in a neutral solution. The accuracy of V is not as high as that of k_q because of the approximation used here. However, it is sufficient for a qualitative discussion of the static quenching process. The estimated radius of the sphere-of-action for static quenching by Cl^- is 8—9 Å. This is, again, slightly larger than 6—7 Å that was estimated as the sum of the radii of the coumarin and a chloride ion. It may be considered strange that the interaction between $\overset{*}{N}$ and Br^- and the interaction between $\overset{*}{C}{}^+$ and Cl^- have similar k_q values but very different V values. The interaction between $\overset{*}{C}{}^+$ and Cl^- originates from an attractive coulomb force and extends rather widely over the solvent media; that between $\overset{*}{N}$ and Br^- is a contact interaction. This situation determines the great difference in V for the two cases.

Moreover, solvation effects and ion-pair generation[17] are considered to be important in a static-quenching event. The drastic decrease in V from I^- to Br^- (mentioned above) might be partly due to the strength of solvation ordering: $I^- < Br^- < Cl^-$. The solvation by water molecules will intervenes with the contact interaction and the static quenching of Br^- decreases in consequence. This point could not be checked for Cl^- since it shows no sign of a quenching effect in a neutral solution. Ion-pair generation is expected in the case of an interaction between $\overset{*}{C}{}^+$ and Cl^- if the both ionic species reside accidentally very near each other at the time the excited state of the protonated molecule is generated. There are, in general, two types of ion-pairs: the solvent-separated ion-pair and the contact-type ion-pair. The former is so loosely coupled that the electronic character of the species is almost the same as that of free ions. Considering the unchanged shape of the fluorescence spectrum of $\overset{*}{C}{}^+$ for a high concentration of Cl^- and the estimated sphere-of-action value, a solvent-separated ion-pair may be formed. This leads to the occurrence of an effective static quenching. In conclusion, static quenching may be effectively initiated in an atmosphere where reactants are caged by surrounding solvated water molecules. Ion-pair formation will strongly increase the probability of a deactivation of excited molecules.

Effect of the Ionic Concentration on the Rate Constant. When the ionic concentration, Γ, is 1 mol dm^{-3}, the Debye's screening length[8] is 4.3 Å. This is comparable to the mean radius of a molecule. The coulomb force between $\overset{*}{C}{}^+$ and Cl^-, being extended over the molecular dimension, is effective for static quenching. In the case of a solution with 6 mol dm^{-3} $HClO_4$ added, the Γ is almost determined by the amount of $HClO_4$ and $\Gamma \approx 12$ mol dm^{-3}. In this case, Debye's screening length becomes 1.2 Å and the electrostatic attraction is strongly screened by the ionic atomosphere. Thus, the interaction is limited to a region near the surface of the ionic species and the static quenching constant, V, becomes effectively zero, being inferred by the fact that the usual Stern-Volmer relation is apllicable to a plot of I_0/I vs. $[Cl^-]$.

As can be seen from Table 4, k_q for an interaction between $\overset{*}{C}{}^+$ and Cl^- in a solution of $[H^+]=0.5$ mol dm^{-3} is about four times larger than for a solution with an added 6 mol dm^{-3} $HClO_4$. As mentioned earlier, the reaction rate between particles with opposite charges becomes smaller if the ionic concentration is increased. Because of the very high value of the ionic concentration, the Debye-Hückel theory does not hold in its fundamental form, while the tendency of the change in the rate constant in ionic solutions is qualitatively correct from the point of view of a more detailed analysis.[16] The qenching effect of I^- and Br^- for $\overset{*}{C}{}^+$ in a solution containing 6 mol dm^{-3} $HClO_4$ was very large and the fluorescence was almost completely quenched in spite of the high ionic concentration. A quantitative experiment could not be performed in these cases.

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