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Excited-State Reactions of Coumarins in Aqueous Solutions. IV. The Fluorescence Quenching of 7-Ethoxycoumarins by Inorganic Ions in Micellar Solutions of Sodium Dodecyl Sulfate

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The effect of added salts (NaCl, NaBr, CsCl) on the fluorescence behavior of 7-ethoxycoumarin (7EC) and 7-ethoxy-4-methylcoumarin (7E4MC) has been studied in aqueous micellar solutions of sodium dodecyl sulfate (SDS). Data on the fluorescence quenching as a function of the salt concentration are analyzed in terms of a proposed two-reactive-state (aqueous and micellar phases) model. The results are in good accord with the model and permit the evaluation of the quenching-rate constants attributable to Br^- and Cs^+ respectively in the two phases. Quenching mechanisms influencing the fluorescence yield are discussed on the basis of the electrostatic force among micelles and quenchers, the electron-transfer reaction from anion to fluorescer, and the hydration degree of the quencher ions.

Since most coumarin derivatives in solutions have a strong fluorescence which depends on interactions with the surroundings, they can be used as fluorescent probes for the investigation of various reactions among organic molecules, inorganic ions, and molecular aggregations. In previous papers,[1,2] the fluorescence quenching of 7-ethoxycoumarin (7EC) and 7-ethoxy-4-methylcoumarin (7E4MC) by halide ions has been studied in aqueous solutions. Generally, halide ions are able to quench the fluorescence of dissolved fluorophores by the external heavy-atom effect[3] or the charge-transfer interaction.[4,5] It has been revealed that bromide and iodide ions in neutral aqueous solutions are very efficient fluorescence quenchers of 7EC and 7E4MC, whereas a chloride ion showed no such effect.[1] In acidic aqueous solutions, however, a chloride ion, as well as bromide and iodide ions, had a pronounced quenching effect on the fluorescence, because the photoexcited state of 7EC and 7E4MC preferred to associate with a proton in the solution, and, subsequently, the generated cationic fluorophore interacted attractively with the chloride ion.[2] In addition to these interactions of the fluorescers with inorganic ions, it has recently been found that 7EC and 7E4MC are easily solubilized into the micelle formed in an aqueous solution of sodium dodecyl sulfate (SDS).[6] The solubilization property of fluorescers and their microenvironment in micelles will be made clear by a spectroscopic investigation of the fluorophores distributed in the aqueous and micellar phases respectively.

In the present work, the absorption and fluorescence spectra of aqueous micellar solutions containing 7EC and 7E4MC are measured precisely upon the addition of several inorganic fluorescence quenchers. Since SDS forms anionic micelles, the solubilized fluorophore is protected from the quenching reaction with anionic quenchers. On the other hand, the quenching by cationic ions is enhanced because the quencher concentration near the surface of SDS micelles becomes higher as a result of electrostatic attraction. Based on a generalized scheme for quenching reactions in aqueous micellar systems, these aspects will be proved quantitatively through the determination of the quenching-rate constants for typical inorganic ions.

Experimental

Materials. The high-purity-grade reagents of 7-ethoxycoumarin (7EC) and 7-ethoxy-4-methylcoumarin (7E4MC) were obtained from Molecular Probes, Inc., and were used without further purification. The sodium dodecyl sulfate (SDS, Wako) was of a biochemical grade. The sodium chloride, sodium bromide, and cesium chloride were pure in quality (Wako). The water was permeated and distilled.

Methods. The UV-absorption spectra were obtained on a Hitachi 323 spectrophotometer, while the measurements of the fluorescence spectra and the fluorescence yields were made on a Hitachi MPF 4 spectrofluorometer with an S-5 type photomultiplier. The temperature of the sample cuvette was thermostated with circulating water from a constant-temperature bath (25±0.1 °C).

The wavelengths of the excitation light were chosen to be 326 nm for 7EC and 323 nm for 7E4MC; these values nearly coincided with their absorption maxima under various conditions, and there was no disturbing absorption of surfactants or ions at these wavelengths. The aqueous micellar solutions in high ionic concentrations were so labile that ultrasonic agitation and warming-up procedures of the solution were necessary to ensure homogeneity, and the fluorescence measurement was done as quickly as possible so as not to change the equilibrium conditions of the sample. The concentration of 7EC and 7E4MC was $4—5\times10^{-5}$ mol dm^{-3} unless otherwise mentioned.

Results

When 7EC and 7E4MC are dissolved in aqueous SDS solutions with concentrations higher than the critical micelle concentration (cmc),[7] they become distributed between the micellar and aqueous phases. The absorption spectra of 7EC and 7E4MC are changed from those in the absence of SDS with a peak-position red-shifted by $\approx$1.5 nm and with a slightly decreased peak-value of the molar extinction coefficient (ε). Compared to this, the dependence of the fluorescence spectra on the SDS concentration becomes much larger, as may be seen from Fig. 1. By the addition of 0.025 mol dm^{-3} SDS, the fluorescence intensities are decreased to 74% (7EC) and 88% (7E4MC) at 25 °C, and the peak position of a fluorescence band is blue-shifted by $\approx$4 nm without changing the original spectral shape.[6] The 7EC and 7E4MC solubilized in the micellar phase are believed to have lower fluorescence yields than in the aqueous phase, because those in less polar water-organic mixed solvents such as water-dioxane are also low; in them a microenvironment similar to the micellar interior may be realized.[6]

The fluorescence quenching phenomena of these fluorophores by halide ions in aqueous solutions have been investigated in a previous paper.[1] The addition of NaCl to the solution did not result in any noticeable change in the fluorescence spectrum (at least for [NaCl]$\leqq$0.5 mol dm^{-3}), while NaBr could strongly quench the fluorescence, whose dependence on the salt concentration approximately followed the linear Stern–Volmer relation in the concentration range of interest, if we neglect a small deviation (a few percent) which becomes a discernible amount only for [NaBr]>0.3 mol dm^{-3}. The order of the quenching ability of the halide ions homogeneously distributed in the aqueous phase was shown to be: $I^- > Br^- \gg Cl^- \approx 0$.[4] CsCl also quenches the fluorescence, but the degree of quenching is very low compared to that in the case of NaBr. Taking into consideration the fact that NaCl (the pair of Na$^+$ and Cl$^-$) does not contribute to the quenching in the aqueous phase, Br$^-$ is classified as a very efficient quencher, and Cs$^+$, as a very weak one. Usually the quenching ability of halide ions for coumarin derivatives of a neutral form is much greater than that of alkali ions with similar atomic weights, e.g., $I^- \gg Cs^+$ (I=53, Cs=55). Therefore, the fluo-

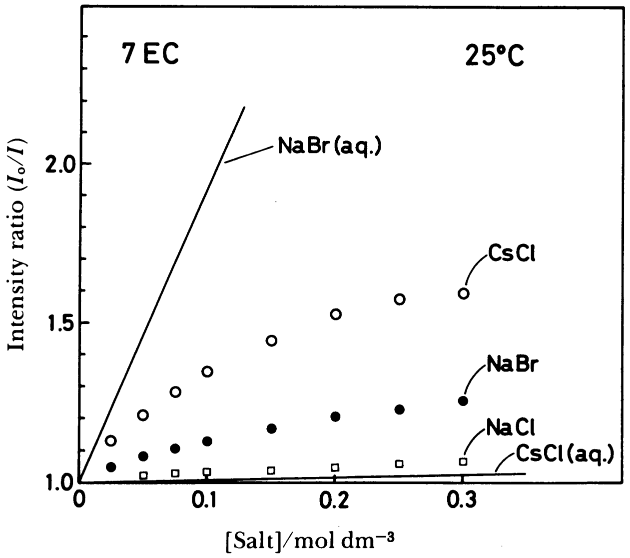


Fig. 2. Dependence of the fluorescence intensity ratio, I_0/I, of 7EC on the concentration of salts in aqueous solutions (solid lines) and in 0.025 mol dm^{-3} SDS solutions (data points) at 25 °C.

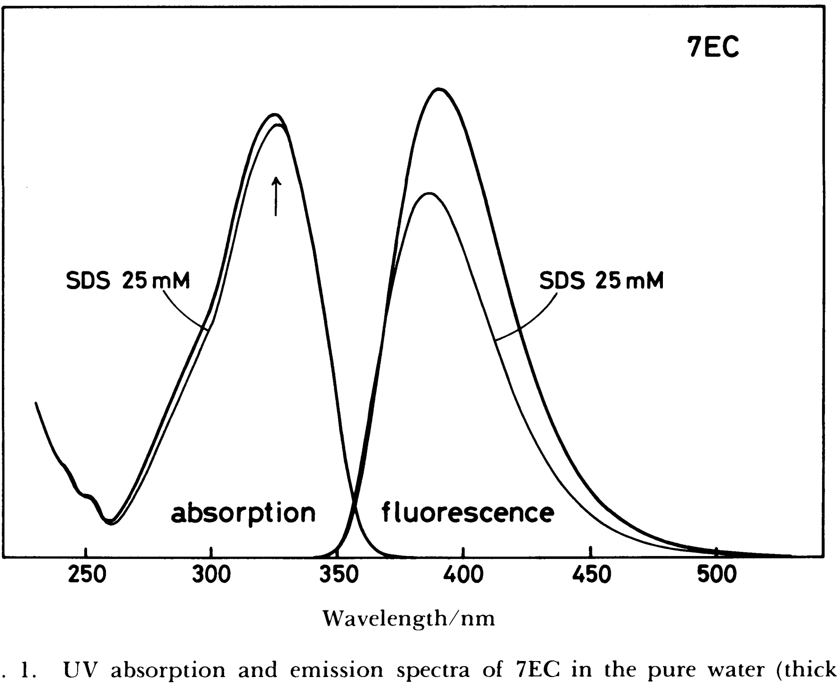


Fig. 1. UV absorption and emission spectra of 7EC in the pure water (thick lines) and in the aqueous solution of 0.025 mol dm^{-3} SDS (thin lines), respectively. The arrow indicates the wavelength of excitation for the emission spectra.

rescence quenching attributable to these inorganic ions cannot be explained consistently by the external heavy-atom effect; rather, it is possibly attributable to a charge-transfer interaction or an electrically induced perturbation among fluorophores and ions. Figures 2 and 3 show, by thin solid lines, the Stern–Volmer plots for I_0/I vs. [NaBr] and [CsCl], where I and I_0 are the fluorescence intensities in the presence and in the absence of added salts respectively. The quenching constants, K_{sv}, evaluated for Br^- and Cs^+ in the aqueous phase at 25 °C are cited in Table 1.

The solubilization phenomena of fluorophores combined with the fluorescence quenching due to inorganic ions bring about additional complexities in the fluorescence analysis, but these complexities will permit the study of various reaction mechanisms occurring in the solution. When NaCl, NaBr, and CsCl are added to the aqueous 7EC and 7E4MC solutions in which 0.025 mol dm^{-3} SDS has been dissolved, the absorption spectra do not change very much except for a slight decrease in ε (a few percent). In spite of the similar absorption spectra, the fluorescence intensity is appreciably decreased by the addition of NaBr and CsCl, which have the fluorescence quenching ability attributable to Br^- and Cs^+ in aqueous solutions without any surfactant. Figures 2 and 3 show plots of I_0/I vs. the concentration of salts in the presence of SDS micelles at 25 °C. It can readily be seen, by comparing the data with the thin solid line (upper), that the quenching ability of NaBr is suppressed by the micelle formation, while, on the contrary, that of CsCl is enhanced. These results can be understood qualitatively, considering that the electrostatically attractive force between the anionic SDS micelle and Cs^+ catalyzes the fluorescence-quenching reaction of the fluorophore embedded in the micelle interior, whereas the repulsive interaction between the micelle and Br^- decreases the probability of a quenching reaction. For Br^- and Cs^+ quenchers in micellar solutions, the Stern–Volmer relation does not hold any more to show nonlinear downward deviations (Figs. 2 and 3).

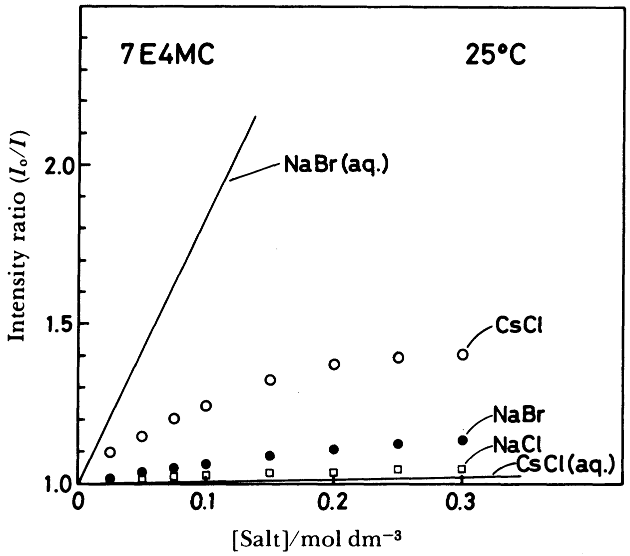


Fig. 3. Dependence of the fluorescence intensity ratio, I_0/I, of 7E4MC on the concentration of salts in aqueous solutions (solid lines) and in 0.025 mol dm^{-3} SDS solutions (data points) at 25 °C.

The addition of NaCl has also induced a decrease of several percent in the fluorescence intensity, probably caused by the change in such micelle parameters as cmc, the aggregation number (n), and the degree of ionization of a micelle (α). These factors are subtly affected by the ionic strength of the solution and will determine the micelle density and the microenvironment around the solubilization site of fluorophores. Since Cl^- has no quenching ability in the aqueous phase and is electrically repelled from the negatively charged head-group of SDS on the surface of sphere-like micelles, we need not think of the quenching from Cl^-. While Na^+ also has no quenching ability in the aqueous phase, there is a possibility that the quenching of the solubilized fluorophore may take place as a consequence of a number of Na^+ ions being attracted onto the negatively charged micelle surface. However, by using the optical detection of magnetic resonance (ODMR), Ghosh et al.[8] proved the absence of perturbation on the π-electron distribution of the fluorophore due to added Na^+ in a naphthalene–sodium n-alkyl sulfate micelle system, in spite of the

Table 1. Quenching Constants, K_{sv} and K'_{sv}, and Quenching-Rate Constants, k_q and k'_q, in the Aqueous and Micellar Phases Respectively of the 0.025 mol dm^{-3} SDS Solution at 25 °C

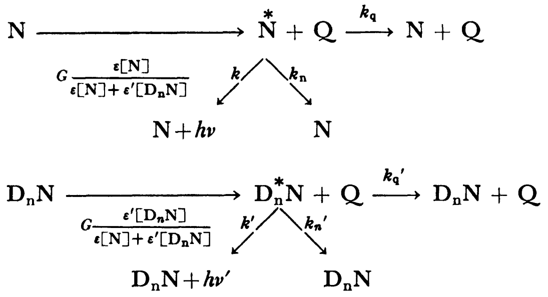
Quencher	Substance	K_{sv} / $mol^{-1}\,dm^3$	K'_{sv} / $mol^{-1}\,dm^3$	$k_q \times 10^{-9}$ [a] / $mol^{-1}\,dm^3\,s^{-1}$	$k'_q \times 10^{-9}$ [a] / $mol^{-1}\,dm^3\,s^{-1}$
Br^-	7EC	9.24	0.21	5.13	0.16
	7E4MC	8.38	0.24	3.99	0.13
Cs^+	7EC	0.08	9.84	0.04	7.39
	7E4MC	0.07	8.36	0.03	4.52

a) $\tau = 1.8$ ns (7EC) and $\tau = 2.1$ ns (7E4MC) at 25 °C were used,[1] and τ' was estimated by the use of $\tau' = \tau \cdot (\eta'/\eta)$.

large effect from Cs^+. Furthermore, the addition of NH_4Cl, $NaClO_4$, and Na_2SO_4 with the same ionic strength as NaCl, all of which are, in many cases, thought to have little or no quenching efficiency against coumarin derivatives, induced small changes in the fluorescence intensity, very similar to the case of NaCl. Therefore, Na^+, NH_4^+, Cl^-, ClO_4^-, and SO_4^{--} are not 'fluorescence quenchers,' although a small effect attributable to the ionic-strength change is found for all these ions and cannot be neglected.

Discussion

Model for the Fluorescence Change. To analyze the fluorescence quenching phenomena of fluorophores in an aqueous micellar solution containing various salts as quenchers, we may adopt the following general scheme of the light-emitting mechanism (two-reactive-state model):



(1)

$$D_n + N \overset{K_{eq}}{\rightleftharpoons} D_nN \tag{2}$$

Here, D_n represents the micelle formed of n surfactants. N and D_nN are the ground states of the fluorophore in the aqueous and micellar phases respectively; $\overset{*}{N}$ and $D_n\overset{*}{N}$ are their excited states generated on light illumination. G is the flux of illuminating light, and ε and ε' are the molar extinction coefficients of N and D_nN respectively at the excitation wavelength. $h\nu$ and $h\nu'$ are the light quanta emitted from each molecular species. k's are the rate constants for the radiative transition of $\overset{*}{N}$ and $D_n\overset{*}{N}$ to their ground states, and k_n's are the rate constants for the nonradiative transition. k_q's are the bimolecular quenching-rate constants resulting from the collision with added quencher ions, Q. Assuming that the fluorophore in the aqueous phase is in equilibrium with that in the micellar phase (Eq. 2), the equilibrium constant is given by $K_{eq}=[D_nN]/[D_n][N]$.[6] It may be noted that the fluorophore cannot be transferred between the aqueous and micellar phases in its lifetime of the optically excited state because the time constant needed to attain such equilibriation is of the order of microseconds,[9] while the fluorescence lifetime is about 2 ns, as will be discussed later.

When N and D_nN are excited by the light at a fixed wavelength with a flux, G, the generation rates of $\overset{*}{N}$ and $D_n\overset{*}{N}$ are in proportion to ε and ε' respectively. Therefore, based on the fact the fluorescence spectrum of $\overset{*}{N}$ bears a close resemblance to that of $D_n\overset{*}{N}$, the total fluorescence intensity is given by the summation of the respective intensities at the peak position:

$$I = I_N + I_{D_nN} = \frac{G}{\varepsilon[N]+\varepsilon'[D_nN]} \times \left\{\frac{\varepsilon\eta[N]}{1+K_{sv}[Q]} + \frac{\varepsilon'\eta'[D_nN]}{1+K'_{sv}[Q]}\right\} \tag{3}$$

where the quantum efficiencies of the fluorescers in the aqueous and micellar phases are defined by $\eta=k/(k+k_n)$ and $\eta'=k'/(k'+k'_n)$ respectively; their fluorescence lifetimes are $\tau=1/(k+k_n)$ and $\tau'=1/(k'+k'_n)$, and the quenching constants are given by $K_{sv}=k_q\tau$ and $K'_{sv}=k'_q\tau'$. Using Eq. 2 and letting the value of I at $[Q]=0$ be I_0, the relative fluorescence intensity is written as:

$$\frac{I}{I_0} = \frac{1+\frac{\varepsilon'}{\varepsilon}K^0_{eq}[D_n]^0}{1+\frac{\varepsilon'}{\varepsilon}K_{eq}[D_n]} \cdot \frac{1}{1+\frac{\varepsilon'\eta'}{\varepsilon\eta}K^0_{eq}[D_n]^0} \times \left\{\frac{1}{1+K_{sv}[Q]} + \frac{\frac{\varepsilon'\eta'}{\varepsilon\eta}K_{eq}[D_n]}{1+K'_{sv}[Q]}\right\} \tag{4}$$

where K^0_{eq} and $[D_n]^0$ are the equilibrium constant and the density of micelles at $[Q]=0$. Apparently, $I_0/I\to1$ at $[Q]\to0$. If the dependence of $K_{eq}[D_n]$ on $[Q]$ is small, the first term on the right-hand side of Eq. 4 reaches to unity. Then, at $K_{sv}[Q]$, $K'_{sv}[Q]\ll1$, $I_0/I\approx1+aK_{sv}[Q]+bK'_{sv}[Q]$, where $a=1/(1+\frac{\varepsilon'\eta'}{\varepsilon\eta}K_{eq}[D_n])$ and $b=\frac{\varepsilon'\eta'}{\varepsilon\eta}K_{eq}[D_n]/(1+\frac{\varepsilon'\eta'}{\varepsilon\eta}K_{eq}[D_n])$. A more careful inspection of Eq. 4 leads to the prediction that the plot of I_0/I vs. [Q] curves nonlinearly downward apart from the linear relation, $I_0/I=1+aK_{sv}[Q]+bK'_{sv}[Q]$.

If the effect of the ionic strength, i.e., the dependence of $K_{eq}[D_n]$ on [Q], cannot be ignored, the reference of the fluorescence intensity must be replaced, in order to get a tractable formula, by the value under a hypothetical condition, $K_{sv}=K'_{sv}=0$, where no quenching reaction occurs. Letting this reference intensity be $\bar{I}_0$, Eq. 4 is, therefore, rewritten as:

$$\frac{I}{\bar{I}_0} = \frac{1}{1+\frac{\varepsilon'\eta'}{\varepsilon\eta}K_{eq}[D_n]} \cdot \left\{\frac{1}{1+K_{sv}[Q]} + \frac{\frac{\varepsilon'\eta'}{\varepsilon\eta}K_{eq}[D_n]}{1+K'_{sv}[Q]}\right\} \tag{5}$$

Finally, we attain:

$$\frac{1}{(I/\bar{I}_0)(1+K_{sv}[Q])-1}=\frac{1+\frac{\varepsilon'\eta'}{\varepsilon\eta}K_{eq}[D_n]}{\frac{\varepsilon'\eta'}{\varepsilon\eta}K_{eq}[D_n]}\cdot \times\left(\frac{K'_{sv}}{K_{sv}-K'_{sv}}+\frac{1}{K_{sv}-K'_{sv}}\cdot\frac{1}{[Q]}\right) \qquad (6)$$

Equation 6 shows that the plot of $1/\{(I/\bar{I}_0)(1+K_{sv}[Q])-1\}$ vs. $1/[Q]$ is linear if the first term on the right-hand side of Eq. 6 is insensitive to [Q]. Therefore, the quenching constants can be known by means of the slope and intercept of this plot.

Interpretation of the Fluorescence-Quenching Data. The concentrations of 7EC and 7E4MC (C_N) for fluorescence measurements are fixed around 4—5×10^{-5} mol dm^{-3}. Above the cmc, it can reasonably be assumed that a spherical micelle of n surfactant molecules with a diameter of about 4 nm is generated in the solution and that the surfactant-monomer concentration in the bulk-water phase remains constant. Then, the number-density of micelles (C_M) is estimated by using the total concentration of the added surfactant (C_S) as $C_M=(C_S-\text{cmc})/n$. For the aqueous solution of SDS at 25 °C, the cmc and n are 8×10^{-3} mol dm^{-3} and 62 respectively.[10,11] As $C_S=2.5\times10^{-2}$ mol dm^{-3}, C_M is estimated to be $\approx3\times10^{-4}$ mol dm^{-3}, which is greater than the added fluorophore concentration (C_N) by one order of magnitude. Under such conditions, almost all fluorophores are solubilized into the micelle,[6] and the concentration of empty micelles is given simply by $[D_n]\approx C_M-C_N\approx2.5\times10^{-4}$ mol dm^{-3}. The molar extinction coefficients of 7EC and 7E4MC in the micelle (ε') and in the bulk water (ε) are independently measurable at $C_S\gg$cmc and at $C_S=0$ respectively. The ε'/ε values in an aqueous SDS micellar solution at the excitation wavelength are 0.972 (7EC) and 0.965 (7E4MC). The ratio of the quantum efficiencies, η'/η, can be estimated, by the use of a method described earlier,[6] as 0.74 (7EC) and 0.88 (7E4MC). Anyhow, if the salt effect does not seriously change the situation, the factor, $\varepsilon'\eta'/\varepsilon\eta$, in Eq. 4 should be a constant slightly less than unity.

The fluorescence-yield change shown in Figs. 2 and 3 as a function of the concentration of added salts is essentially explained by Eq. 4. However, such nonlinear plots for micelle–quencher systems are so complex to analyze that a more convenient expression to argue quantitatively (Eq. 6) has been proposed. First, the reference intensity ($\bar{I}_0$) corresponding to the case of $K_{sv}=K'_{sv}=0$ must be determined in accordance with the experiments. As has been mentioned earlier, the addition of NaCl to the micellar system induces a slight decrease in the fluorescence intensity. Since Cl^- has no quenching ability in the aqueous phase and is repelled from the negatively charged SDS micelle, this ion, apparently, cannot contribute to the fluorescence quenching in the micellar phase. In spite of the attractive interaction with SDS micelles, Na^+ has been proved to cause no electronic reaction against the molecule embedded in SDS micelles; this is in contrast to the case of Cs^+, which is confirmed by means of the ODMR technique.[8] Therefore, the fluorescence decrease upon the addition of NaCl is not caused by the quenching effect, but by the change in ionic strength, forcing micelles to alter their characteristic parameters (cmc, n, and α). Thus, $\bar{I}_0$ can be determined as the fluorescence intensity of the solution in which NaCl of the same ionic strength as the quenchers employed (NaBr and CsCl) is dissolved. Experimentally, C_M and, consequently, $[D_n]$ do depend on the amount of salt added through the change in cmc and n. Moreover, the salt effect on K_{eq} may also be important.[12] It is very difficult, at present, to know quantitatively the exact effect from the ionic-strength changes. Irrespective of all such complexities, however, it should be noted that the standard of the fluorescence intensity has always been taken from the solution with an appropriate amount of NaCl.

As the $I/\bar{I}_0$ appearing on the left-hand side of Eq. 6 can thus be calculated at each [Q] by using the experimentally determined $\bar{I}_0$ value, and since K_{sv} has already been known from an independent experiment in a simple aqueous solution (Table 1), it now becomes possible to plot $1/\{(I/\bar{I}_0)(1+K_{sv}[Q])-1\}$ vs. $1/[Q]$. These plots are shown for Br^- in Fig. 4 and for Cs^+ in Fig. 5. The signs on both sides of Eq. 6 are reversed for the Cs^+ quencher because the sign of $K_{sv}-K'_{sv}$ depends on the charge of the quencher ions. The data points in Figs. 4 and 5 obey very well the expected linear relationship, thus showing the good applicability of the proposed model irrespective of the charge type of the inorganic quenchers. The quenching constants (K'_{sv}) of the fluorescer solubilized in SDS micelles due to Br^- and Cs^+ are calculated by dividing the intercept by the slope of the plots in Figs. 4 and 5; they are cited in Table 1. For Br^-, K'_{sv} is much less than K_{sv}. This reduction is caused by the repulsive force between the anionic micelle and the anionic quencher. For Cs^+, on the contrary, K'_{sv} becomes larger by about two orders of magnitude than K_{sv}, the change being more drastic than in the case of Br^-. The associative interaction of the positively charged Cs^+ ion with the anionic micelle catalyzes to quench the fluorescence. Similar phenomena were observed by Eftink and Ghiron[13] for indole derivatives in a SDS micelle–Cs^+ system, but their analysis was only a primitive one insufficient for discussing the quenching mechanism in detail.

The lifetimes (τ) of 7EC and 7E4MC in the aqueous solution have been measured in Ref 1. The lifetimes (τ') in the micelle can be calculated by the use of $\tau'=\tau\cdot(\eta'/\eta)$. Then the quenching-rate constants k_q

($=K_{sv}/\tau$) and k_q' ($=K_{sv}'/\tau'$) are obtained as is shown in Table 1. It is reasonable that 7EC and 7E4MC have the same order of k_q values, for these molecules are different only in the substituent at the 4-position. The k_q and k_q' values of 7EC are always larger than those of 7E4MC. Since this tendency is independent of the charge on quencher ions, and also of the environmental conditions in the aqueous and micellar phases, the quenching reaction seems to be partly screened off by the 4-methyl substituent. The ratio of k_q values for 7EC and 7E4MC in the aqueous phase is nearly equal to the ratio in the micellar phase, irrespective of whether the quencher is Br^- or Cs^+. If the screening effect of the 4-methyl substituent is equally operative in both phases, this parallelism shows that the solubilization sites of the fluorophore should be essentially identical in position. It has already been shown that the polarities felt by these fluorophores

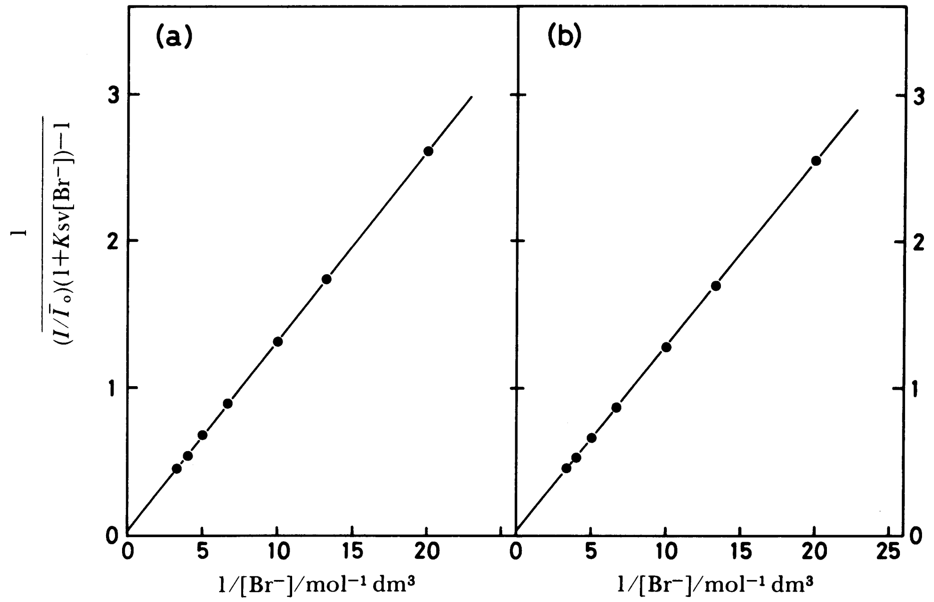


Fig. 4. Plots of $1/\{(I/\bar{I}_0)(1+K_{sv}[Br^-])-1\}$ vs. $1/[Br^-]$ in the 0.025 mol dm^{-3} SDS solution at 25 °C. (a), 7EC; (b), 7E4MC.

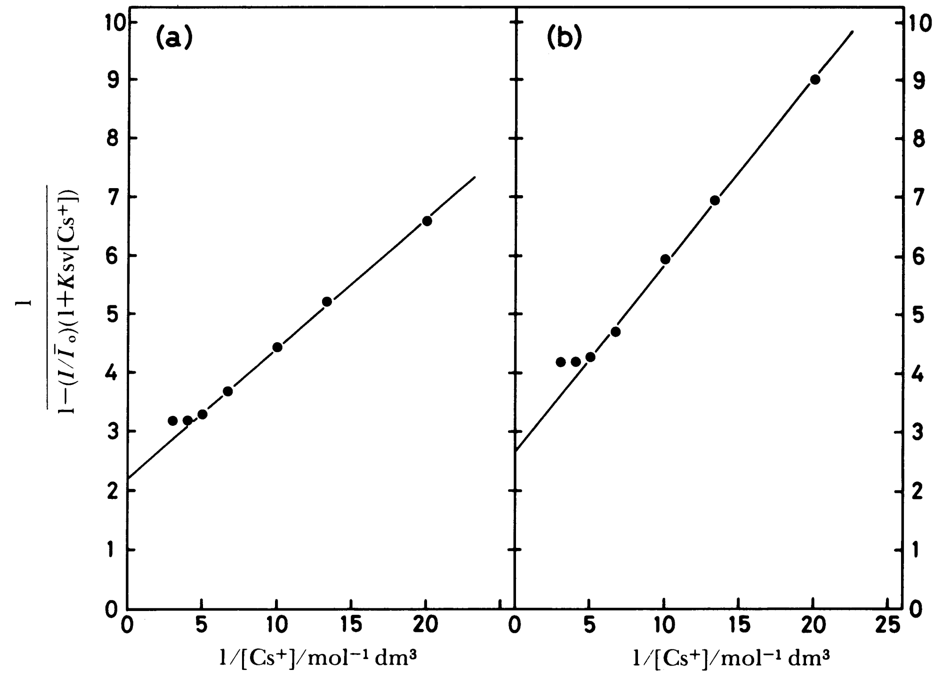


Fig. 5. Plots of $1/\{1-(I/\bar{I}_0)(1+K_{sv}[Cs^+])\}$ vs. $1/[Cs^+]$ in the 0.025 mol dm^{-3} SDS solution at 25 °C. (a), 7EC; (b), 7E4MC.

within the SDS micelle are very similar,[6] a finding which supports the conclusion presented above.

The most probable quenching mechanism for Br^- is the charge-transfer interaction between the ion and the fluorophore, as has been pointed out in earlier works.[1,4,5] If the encounter distance between the fluorescer and quencher, R, and the sum of the diffusion coefficients of each species in aqueous solutions, D, at 25 °C are reasonably assigned as 0.7 nm and $1.5\times10^{-5}\,cm^2\,s^{-1}$ by means of the Stokes-Einstein relation (fluorophore radius ≈0.47 nm; van der Waals radius of Br^-≈0.23 nm), the diffusion-limited value of the quenching-rate constant (k_1) for Br^- is estimated to be $\approx8\times10^9\,mol^{-1}\,dm^3\,s^{-1}$, with $k_1=4\pi NRD$, where N is the Avogadro number. If all collisional events among fluorescers and quenchers result in the quenching reaction, k_q will coincide with k_1 and the quenching efficiency (γ) will become unity. Since the experimentally obtained k_q for Br^- (Table 1) is about one-half of k_1, $\gamma\approx0.5$. In contrast to this, k_q' for Br^- is only 1.5—2% of k_1 (Table 1). Such a situation can readily be understood on the basis of the idea that Br^- impinging on the micelle surface is repelled instantaneously by the negatively charged SDS head-groups as a result of electrostatic repulsion and the quenching reaction with the solubilized fluorophore rarely occurs. When I^- is employed as a quencher instead of Br^-, large quenching effects have been observed in both the aqueous and micellar phases. Because the quenching ability of I^- against 7EC and 7E4MC is very high, and because the Stern-Volmer relation does not hold even in a simple aqueous solution,[1] it has been impossible to treat the case of I^- quantitatively within the framework developed here. Quina et al.[14] have investigated the I^- quenching of a pyrene derivative in SDS micelles; they have analyzed it in terms of a two-reactive-state model in which only one of the states (the aqueous phase) is quenchable. This assumption, which represents a special case of the generalized scheme given by Eq. 1, is insufficient for the analysis of a coumarin derivative-SDS micelle system, as is apparent from the investigation of Br^-.

The fluorescence-quenching mechanism due to inorganic cations in aqueous solutions has not been so clear as in the case of inorganic anions, such as halides and pseudohalides.[4,5] As is cited in Table 1, the k_q values for Cs^+ in the aqueous phase are smaller than the values for Br^- by about two orders of magnitude. 7EC and 7E4MC are neutral molecules, and the cations and anions of interest (e.g., Rb^+, Cs^+, Br^-, and I^-) have similar mobilities in aqueous solutions.[15] Therefore, the collisions between the fluorescer and the various quenchers will be nearly equal in probability; if so, the heavy-atom effects from Rb^+ and Br^- and from Cs^+ and I^- should be of the same order. Nevertheless, the experimentally determined quenching-ability order is $I^->Br^-\gg Cs^+>Rb^+$, irrespective of the order of the atomic weights, which are rough measures of the spin-orbit coupling. Further, the ionic radii and the enthalpy changes for the hydration of Br^- and Cs^+ are so similar that there is no positive explanation for the widely different quenching abilities of Cs^+ and Br^- in terms of the participation of heavy-atom effect. The charge-transfer mechanism may, instead, be operative for the cation-quenching. The quenching ability of halide ions is known to increase as the ionization energy of the ion ($X^-\rightarrow X$) is decreased; therefore, the origin of quenching was concluded to be the electron transfer from the quencher to the fluorescer.[4,5] Since the electron-affinity order of alkali ions ($M^+\rightarrow M$) is $Na^+>K^+>Rb^+>Cs^+$, the quenching ability caused by the charge-transfer from the fluorescer to the quencher, if possible, must align in the same order. This idea is not applicable to the present problem, however, because the quenching ability is ordered experimentally as $Cs^+>Rb^+>K^+\approx Na^+\approx0$. The electron-transfer from the cation to the fluorescer that generates dication is energetically more improbable. Although these contradictions will be partly mitigated by the subtle hydration effect that depends on the ionic radius, it is unreasonable to think that either the heavy-atom effect or the charge-transfer interaction is mainly responsible for the weak cationic quenching in the aqueous phase. The most natural explanation possible at present is that the weaker hydration of Cs^+ than of the other cations, indicated by the smaller Stokes' radius for hydrated ions,[15] enables it to come in closer contact with the fluorescer; consequently, an induced electronic perturbation increases the non-radiative deactivation of the fluorescer.

The quenching-rate constant of Cs^+ for the fluorescer in micelles (k_q') becomes larger than k_q by more than two orders of magnitude. This increase in k_q' is qualitatively expected from the attractive interaction between the SDS micelle and Cs^+. However, there occurs the problem which of 'static' and 'bimolecular' quenching mechanisms will really operate in the solution. When the Na^+ of the original counterion of SDS is gradually replaced by Cs^+ by adding CsCl, the concentration of Cs^+ near the micelle surface steadily becomes higher than that in the bulk water. Under such circumstances, it is predictable for a ground-state complex like D_nN-Cs^+ to be generated, thus leading to an instantaneous deactivation of the optically excited state. However, it has not been detected by the measurement of the absorption spectra at any high concentration of added Cs^+. The 'static' or ground-state-complex induced quenching, therefore, can be discarded or need not be taken into account. It can be justified, on the basis of this result, that, at the beginning of analysis, the quenching effect predicted by Eq. 1 is based on the bimolecular interaction between fluorescers and quenchers. Using the van der Waals radius of Cs^+ ($r_v \approx 0.2$ nm), the diffusion-limited value of the quenching constant, k_1, becomes $\approx 9 \times 10^9$ $mol^{-1}\,dm^3\,s^{-1}$. The k_q values are so small compared to k_1 that the Cs^+ in the aqueous phase is only a weak quencher. Since the SDS micelle is a spherical particle with a radius of about 2 nm, the collisional rate between the micelle and ion amounts to $\approx 2 \times 10^{10}$ $mol^{-1}\,dm^3\,s^{-1}$. Considering that the k'_q values for Cs^+ are several tenths of this value, the collisions between Cs^+ and the micelle would lead to a nonradiative deactivation of the excited state only when Cs^+ has come close to the solubilized fluorophore, possibly residing in the micelle interior, but very near the surface. Among bimolecular mechanisms, a contact-type interaction of Cs^+ such as a heavy-atom effect cannot be effective for a fluorescer embedded in the micelle interior across the palisade layer with an interfacial potential barrier. As has been mentioned in the case of the bulk-water phase, there is also no physical reason for the effective occurrence of charge-transfer interaction between the fluorescer and Cs^+. Ghosh et al.[8] have shown that the association of Cs^+ ions with SDS micelles can serve to decrease the extent of the hydration of the micelle, making a less polar environment for the fluorophore. Since the fluorescence yield of 7EC and 7E4MC is sensitively dependent on the polarity of the microenvironment,[6] the polarity change due to the cation becomes a possible candidate for a reasonable explanation. The quenching ability of Cs^+ in the aqueous phase is very low, but it becomes very large for the solubilized fluorophore by virtue of the incident flux of Cs^+ ions toward the micelle surface. Cs^+ impinges against the surface by thermal agitation and electrostatic attraction, and will be exchanged with loosely bounded counterions. Since Cs^+ has a smaller hydrated Stokes' radius than Na^+,[15] it can approach closer to the micelle interface to induce the environmental-polarity change around the fluorophore. It is instructive to notice that the photoionization yields of perylene in SDS micelles was decreased by the addition of monovalent inorganic salts,[16] although the electron-liberation process occurring under a high-energy UV excitation has no direct correlation with the quenching process of the present problem. The binding degree of the counterion at the micelle interface seems to depend on the size of the hydrated ions, and the change in the photoionization efficiency has been understood in terms of the expectation that smaller hydrated ion radii favor a closer approach to the surface and decrease the interfacial electric potential extensively. Considering these results and the difficulty that hydrated inorganic ions have in penetrating deep into the hydrophobic core region of micelles, the solubilization site of the fluorophore must be near to the micelle interface rather than in the micelle core in order that the Cs^+ quenching caused by the collision of Cs^+ against the micelle surface operates efficiently. In addition, it may be possible to generate an activated encounter-complex described by a structure like $D_n^*N \cdots Cs^+ \cdots (H_2O)_n$, where D_n^*N is the fluorescer in the micelle, H_2O is solvated water, and the dots describe loose couplings as presented in the 'outer-sphere' reaction between a metal ion and a ligand of metal-complexes. The role of such a cation-bridged complex was emphasized by Shporer et al.[17] to explain the electron-transfer reaction between cyano-metal complexes in a solution in which cations have been dissolved as catalysts; they found that the reaction rate increases from Li^+ to Cs^+. In the present case, although it seems unlikely that the electron transfer really occurs between the fluorescer and solvated water, the cation-assisted complexation may contribute effectively to the nonradiative deactivation of excited fluorophores. It is believed that the polarity change and the formation of the excited-state complex will act in concert to enhance the quenching ability of Cs^+ in the anionic micellar system.

Limitation on the Applicability of the Proposed Model. The discussion has hitherto proceeded by assuming that the first term on the right-hand side of Eq. 6, $(1+\frac{\varepsilon'\eta'}{\varepsilon\eta}K_{eq}[D_n])/\frac{\varepsilon'\eta'}{\varepsilon\eta}K_{eq}[D_n]$, is independent of the added salt concentration. If $(\varepsilon'\eta'/\varepsilon\eta)K_{eq}[D_n] \gg 1$, this assumption is valid no matter how much the term depends on the salt concentration. By means of the slope of the plots in Fig. 4 and the obtained K_{sv} and K'_{sv} values shown in Table 1, the value for Br^- can be estimated to be $(\varepsilon'\eta'/\varepsilon\eta)K_{eq}[D_n] \approx 6.1$ (7EC) and ≈ 38.6 (7E4MC), which satisfy the assumption. Further, the C_M $(=(C_S-\mathrm{cmc})/n)$ necessary for the estimation of $[D_n]$ is only weakly dependent on the ionic strength under the experimental condition employed, as cmc is decreased while, on the contrary, n is increased by the addition of salts. These two reasons for the insensitiveness of the first term in Eq. 6 to the ionic strength confirm that the data follow very generally the theoretically predicted linear relationship. Since the counterion of an aqueous SDS micelle, Na^+, is not changed by the addition of NaBr, the factor of $\varepsilon'\eta'/\varepsilon\eta$ can be approximated by the value with no added salt (salt-free conditions).[6] Then, the $K_{eq}[D_n]$ values are 8.4 (7EC) and 45.7 (7E4MC). When the 'solubilization ratio' of the fluorophore is defined by $p=[D_nN]/([D_nN]+[N])$, p is rewritten as $p=K_{eq}[D_n]/(K_{eq}[D_n]+1)$; its values are estimated to be 0.89 (7EC) and 0.98 (7E4MC). These p values are very close to those in a salt-free SDS micelle[6] and show that the majority of fluorophores exist in the micelle even in NaBr is added to the solution. If $[D_n]$ should be approximated by $[D_n] \approx C_M - C_N \approx 2.5 \times 10^{-4}$ $mol\,dm^{-3}$, as carried out before under salt-free conditions, the K_{eq} values can be calculated as 3.4×10^4 $mol^{-1}\,dm^3$ (7EC) and 1.8×10^5

$\text{mol}^{-1}\,\text{dm}^3$ (7E4MC); these values are in good accord with the value based on a different method⁶⁾ in spite of the approximations used here and the existence of the additive.

From Fig. 5, the $(\varepsilon'\eta'/\varepsilon\eta)K_{\text{eq}}[\text{D}_\text{n}]$ values for Cs^+ are calculated to be 0.85 (7EC) and 0.61 (7E4MC), smaller than the values for Br^- by one or more orders of magnitude. If the first term of Eq. 6 varies through the dependence of $K_{\text{eq}}[\text{D}_\text{n}]$ on $[\text{Cs}^+]$, the applicability of the linear relationship is restricted. As may be seen in Fig. 5, data have deviated systematically from the theory at high concentrations of CsCl (at small $1/[\text{Cs}^+]$). The region where the proposed model seems valid is, at most, from $[\text{Cs}^+]=0.05$ to $0.2\,\text{mol}\,\text{dm}^{-3}$. There may be another possibility that the static quenching process mentioned earlier begins to contribute at higher Cs^+ concentrations, although no sign of the ground-state complex formation has yet been detected spectroscopically. When the counterions of the micelle are changed from Na^+ to Cs^+, ε' shows no observable change. η' will decrease with this change through the generation of a less polar environment by the Cs^+ attachment to the micelle surface. However, the amount of decrease is believed to be not very large, for the fluorescence yields of 7EC and 7E4MC are a smoothly varying function of the environmental polarity,⁶⁾ which cannot explain the drastic decrease in $(\varepsilon'\eta'/\varepsilon\eta)K_{\text{eq}}[\text{D}_\text{n}]$. Possibly, $K_{\text{eq}}[\text{D}_\text{n}]$ or p is affected by the polarity change around the solubilization site, which serves to make the state of the solubilized fluorophores unstable. The latter may be the main reason for the observed decrease in the solubilization ratio. It must be mentioned in this connection that, if η' is decreased by adding CsCl instead of NaCl or NaBr, the k_q' value for Cs^+ in Table 1 understimates the quenching reaction, for η' under salt-free conditions is employed in the estimation.

Hautala et al.¹⁸⁾ have reported large and complex effects of oxygen on the fluorescence lifetimes of naphthalene in aqueous micellar solutions in addition to the fluorescence-quenching effects attributable to inorganic ions. The situation is different from the case of coumarin derivatives because the fluorescence lifetimes of 7EC and 7E4MC ($\tau\approx2$ ns) are too short to be affected by a quenching process caused by oxygen in comparison with the case of naphthalene ($\tau\approx40$ ns). Experimentally, no quenching phenomenon caused by dissolved oxygen has been found through the examination of deaerated samples.

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