The Solubilization Equilibrium of 7-Ethoxycoumarins in Ionic Micelles

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Synopsis. The solubilization behavior of 7-ethoxycoumarin (7EC) and 7-ethoxy-4-methylcoumarin (7E4MC) in aqueous micellar solutions of sodium dodecyl sulfate (SDS) and hexadecyltrimethylammonium chloride (HDTC) was studied by fluorescence spectroscopy. The equilibrium constants for the binding of the fluorophores into micelles and their fluorescence yields in the micellar phase were determined.

Several authors[1,2] have studied the characteristics of micelles and vesicles formed of surfactants or phospholipids using hydroxy- and aminocoumarins as the pH indicator, since the absorption spectra of these compounds, when bound to the molecular aggregations, sensitively change reflecting the shift of acid-base equilibria. Recently, it has been revealed that 7-ethoxycoumarins are also solubilized into SDS micelles in aqueous solutions, and their fluorescence is greatly reduced above the critical micelle concentration (cmc) whereas the absorption spectra show little observable change.[3] Since the microenvironment around electronically excited molecules is detectable by means of fluorescence measurements, the fluorescence-probing technique is complementary to the absorption measurement that detects ground-state molecules. The fluorescence method using the coumarin derivatives is advantageous over the absorption method reported, because the latter must use buffer solutions or acids for the control of pH;[1,2] this introduces an inevitable complexity such as the problem of ionic-strength change into the analysis of data. Although several aromatic fluorescent probes have already been proposed,[4,5] heterocyclic compounds such as coumarins are very different from those in regard to interactions with the surroundings, and have interesting solubilization properties for anionic and cationic micelles.

Experimental

High-purity grade reagents of 7-ethoxycoumarin (7EC) and 7-ethoxy-4-methylcoumarin (7E4MC) were obtained from Molecular Probes Inc. and used without further purification. Sodium dodecyl sulfate (SDS, Wako) was of biochemical grade. Hexadecyltrimethylammonium chloride (HDTC, Wako) was treated with charcoal in ethanol, recrystallized, and then washed with ether. Sodium chloride (Kanto) was of special grade. Water was permeated and distilled.

The UV absorption spectra were obtained on a Hitachi 323 spectrophotometer and measurements of the fluorescence spectra and fluorescence yields were made on a Hitachi MPF 4 spectrofluorometer with an S-5 type photomultiplier tube. The temperature of the sample cuvette was thermostated with circulating water from a constant-temperature bath (25±0.1 °C). The wavelengths of excitation light were chosen to be 325 nm for 7EC and 322 nm for 7E4MC, which nearly coincided with their absorption maxima in various solutions; there was no disturbing absorption of surfactants or salts at these wavelengths. The dependence of the absorption spectra on the surfactant concentration was small, while that of the fluorescence spectra was so large that the whole spectrum of the fluorescence was carefully recorded under various conditions. The concentration of 7EC and 7E4MC in the sample solution was $4—5\times10^{-5}$ mol dm^{-3}.

Results

Fluorescence properties of 7-ethoxycoumarin derivatives in simple aqueous solutions have been fully investigated.[6,7] When 7EC or 7E4MC was dissolved in the aqueous SDS solution with a concentration higher than cmc,[8] the absorption spectra were changed slightly from those in the absence of SDS with a peak position red-shifted by ≈1.5 nm and with a peak value of molar extinction coefficient decreased by a few percent. On the other hand, the fluorescence behavior strongly depended on the SDS concentration. When SDS was added to the solution, the fluorescence intensity began to decrease drastically as soon as the cmc of SDS was reached and tended to be constant at higher SDS concentrations than 0.025 mol dm^{-3} for both 7EC and 7E4MC, while the peak position of a fluorescence band blue-shifted by ≈4 nm maintaining the spectral shape.

7-Ethoxycoumarins were also solubilized into cationic micelles such as hexadecyltrimethylammonium chloride (HDTC). The peak shifts of absorption and fluorescence spectra at 0.025 mol dm^{-3} HDTC were similar to those in the SDS solution. The HDTC solution, however, showed an even larger variation in the fluorescence intensity than the SDS solution. Figure 1

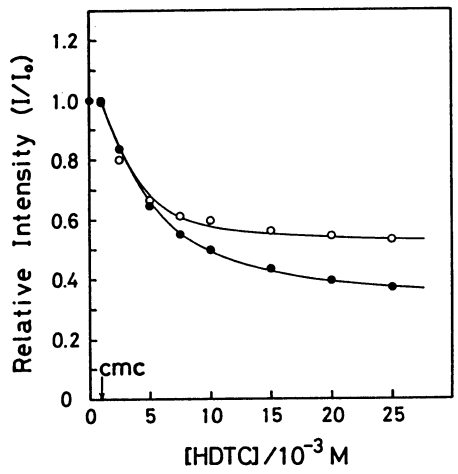

Fig. 1. Plots of the relative fluorescence intensity, I/I_0, vs. the concentration of HDTC at 25 °C. 7EC (●); 7E4MC (○). Solid lines are the theoretical curve predicted by Eq. 3; the aggregation number, n, is assumed to be 80.

presents a plot of the relative fluorescence intensity, I/I_0 vs. [HDTC], where I and I_0 are the fluorescence intensities in the presence and in the absence of HDTC. Since the cmc of HDTC is smaller than the cmc of SDS by about one order of magnitude,[8] a decrease in the fluorescence intensity by the addition of HDTC begins at a lower concentration of the surfactant. The fluorescence intensity tended to be constant for [HDTC]>0.025 mol dm^{-3}.

Since ionic surfactants have their counterions, the fluorescence-quenching phenomena due to various inorganic ions in the solution must be taken into account.[9] The quenching effect for 7EC and 7E4MC due to Na^+ and Cl^-, being the counterions of SDS and HDTC, was negligible in a simple aqueous solution.[6] When NaCl was added to SDS micellar solutions, the fluorescence of 7EC and 7E4MC decreased slightly (≈3% at [NaCl]=0.1 mol dm^{-3}). Considering that micellar parameters such as the aggregation number (n) or the degree of ionization (α) are delicately influenced by the addition of salts, this small change in the fluorescence intensity is, possibly, not due to quenching by Na^+ and Cl^- but due to a micellar parameter change. In either case, the fluorescence quenching by Na^+ and Cl^-, if any, is negligibly small because the employed concentration of SDS and HDTC is not more than 0.025 mol dm^{-3}. This is consistent with the result obtained by phosphorescence measurements of naphthalene in frozen SDS micelles with varying amounts of added NaCl, up to ≈0.2 mol dm^{-3}.[10]

Discussion

To analyze the fluorescence property of 7EC and 7E4MC in aqueous micellar solutions, we may adopt the two-reactive-state model for the light-emitting processes in the aqueous and micellar phases

$$\mathrm{N} \xrightarrow{\varepsilon} \overset{*}{\mathrm{N}} \xrightarrow{\eta} \mathrm{N} + h\nu \qquad (1)$$

$$\mathrm{D}_n\mathrm{N} \xrightarrow{\varepsilon'} \mathrm{D}_n^*\mathrm{N} \xrightarrow{\eta'} \mathrm{D}_n\mathrm{N} + h\nu'$$

and

$$\mathrm{D}_n + \mathrm{N} \overset{K}{\rightleftharpoons} \mathrm{D}_n\mathrm{N}. \qquad (2)$$

Here, D_n represents the micelle formed of n surfactants. N and D_nN are the fluorescent molecules in the aqueous phase and in the micellar phase, respectively; $\overset{*}{N}$ and D_n^*N are their excited states generated on light illumination. $h\nu$ and $h\nu'$ are the emitted light quanta from the respective molecular species. ε and ε' are the molar extinction coefficients of N and D_nN, respectively. η and η' are the quantum efficiencies for the radiative transition of $\overset{*}{N}$ and D_n^*N to N and D_nN, respectively. Assuming that the fluorophore in an aqueous phase is in equilibrium with the fluorophore in a micelle (Eq. 2), the equilibrium constant is given by $K=[D_nN]/[D_n][N]$. The total concentration of the fluorescent molecule is defined by $C_N=[N]+[D_nN]$, and the total density of the micellar particle is given by $C_M=[D_n]+[D_nN]$. C_N is fixed throughout the measurement. C_M depends on both the added content of a surfactant (C_S) and n as $C_M=(C_S-\mathrm{cmc})/n$ above cmc.

When N and D_nN are excited by the light at a fixed wavelength, the generation rates of $\overset{*}{N}$ and D_n^*N are in proportion to ε and ε' at the wavelength, respectively. Then, the relative fluorescence intensity, I/I_0, is given by

$$\frac{I}{I_0} = \frac{1+(\eta'/\eta)(\varepsilon'/\varepsilon)K[\mathrm{D}_n]}{1+(\varepsilon'/\varepsilon)K[\mathrm{D}_n]}, \qquad (3)$$

where $[D_n]$ is obtained from the definitions of K, C_N, and C_M:

$$[\mathrm{D}_n]=[\{(1+KC_\mathrm{N}-KC_\mathrm{M})^2+4KC_\mathrm{M}\}^{1/2} -(1+KC_\mathrm{N}-KC_\mathrm{M})]/2K. \qquad (4)$$

The detailed derivation of this result appeared in Ref. 3. For usual measurements, C_N was fixed around 4—5×10^{-5} M. If $1+KC_N \ll KC_M$, Eq. 4 predicts $[D_n]\approx C_M-C_N$. In contract, a full expression of Eq. 4 must be employed at small C_M in order to estimate $[D_n]$. Based on Eqs. 3 and 4, the theoretically predicted variation in I/I_0 can be compared with the fluorescence data obtained.

Since almost all fluorophores become solubilized into the micelle at $C_S \gg$ cmc, the ε' values of 7EC and 7E4MC in the micelle can be experimentally determined. ε'/ε for SDS at the excitation wavelength was 0.972 (7EC) and 0.965 (7E4MC); for HDTC, 0.953 (7EC) and 0.960 (7E4MC). The aggregation number of micelles was taken from independent experiments as n=62 (SDS)[11,12] and n=80 (HDTC).[13] Then, unknown parameters to be fixed in Eqs. 3 and 4 are η'/η, K, and cmc, which can be determined by a best-fit analysis for the data points. The theoretical curve derived is plotted with a thin solid line in Fig. 1. The theory explained very well the entire behavior of the data; the determined parameters are cited in Table 1. The inaccuracy in K is brought about by the n value employed; the scattering of n from experiment to experiment was about 10%, so that the same order of uncertainty exists in K, whereas η'/η and cmc are insensitive to such inaccuracy.

For the SDS micelle, K differs by an order of magnitude between 7EC and 7E4MC (Table 1), in spite of the small difference in the molecular structure. A larger K means a stronger binding of the fluorophore to the micelle. The change in the substituent group from hydrogen to methyl at the 4-position is supposed to

Table 1. Ratios of the Quantum Efficiency, η'/η, Equilibrium Constants, K, and Critical Micelle Concentrations, cmc, in Aqueous SDS and HDTC Solutions at 25 °C

Surfactant	Substance	η'/η	K / mol^{-1} dm^3	cmc / mol dm^{-3}
SDS[a]	7EC	0.74	2.8×10^4	6.0×10^{-3}
	7E4MC	0.88	2.6×10^5	
HDTC	7EC	0.30	3.5×10^4	1.0×10^{-3}
	7E4MC	0.51	9.0×10^4	

a) The values for SDS are taken from Ref. 3.

induce such a large influence on the solubilization, probably due to a hydrophobic interaction of the methyl group. This difference in K between 7EC and 7E4MC becomes rather smaller for the HDTC micelle (Table 1). SDS and HDTC form typical anionic and cationic micelles, having different carbon numbers in the alkyl chain (C_{12} and C_{16}, respectively). Although K will be affected by the charge on the micelle, the surfactant length, the solubilization site within the micelle, etc., it seems that the substituent effect would overcome the other factors to induce the higher K value for 7E4MC in both SDS and HDTC solutions.

The fluorescence intensity change in the mixed solvent of water-ethanol was examined as a function of the water content in a previous study.[3] If the quantum efficiency, η', appearing in Eq. 3 is essentially determined by the polarity around the site in the micelle occupied by the fluorophore, this result is conveniently related with η'/η in Table 1. When the fluorescence-yield in the mixed solvent is compared with η'/η=0.74 (7EC) and 0.88 (7E4MC) obtained in the SDS solution, the polarity of the solubilized site is revealed to resemble the polarity in the mixed solvent of 30 wt% aqueous ethanol, having the effective dielectric constant, D_{eff}=62±1 (25 °C).[14] No meaningful difference has been found between the D_{eff} values of 7EC and 7E4MC within an experimental error. Considering that the dielectric constant of a typical aliphatic compound is about 2 and that of water is 78.5, the solubilized fluorophore seems to be located not in the core region of the micelle but near its surface and may be partly exposed to the aqueous atmosphere around the micelle (in the palisade layer). This effective polarity ($D_{eff}\approx$62) is larger than 16—49 obtained for benzene derivatives and naphthalene.[5] Since 7-ethoxycoumarins are heterocyclic compounds with oxygen in the ring and in the substituent, and are insoluble in nonpolar solvents, their affinity to the alkyl chain in micelles is thought to be lower than aromatic compounds. Therefore, it is natural that the solubilized 7EC and 7E4MC is embedded at a shallower site than aromatic molecules.

For the HDTC micelle, D_{eff}=39±1 (25 °C). As is mentioned earlier, the aliphatic chain of HDTC is longer than SDS, so the flexibility of this longer chain may make easier the movement of the fluorophore toward the micelle core. Then, the location of the fluorophore in the HDTC micelle becomes deeper than in the SDS micelle. The interior of n-alkyltrimethylammonium bromide micelles is more polar than sodium n-alkyl sulfate micelles with the same n.[15] If the SDS (n=12) micelle is compared with the HDTC (n=16) micelle as in this work, the interior of the HDTC micelle may be less polar due to the longer alkyl chain of HDTC. By the use of naphthalene as a fluorescent molecule, it has been shown that its solubilized site within n-alkyltrimethylammonium bromide micelles is not close to the polar region of the micelle surface and, also, does not reside in the micelle core.[15] These results are consistent with D_{eff} for the HDTC micelle which is an intermediate value between D of pure water and typical aliphatic solvents. The K value difference between 7EC and 7E4MC may decrease in the HDTC micelle compared with that in the SDS micelle because the hydrophobic interaction of a methyl substituent is supposed to be less effective to distinguish 7EC from 7E4MC at a deeper part in the micelle.

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