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Fluorometric Analysis of Micelle Formation of Sodium Dodecyl Sulfate[1]

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The micelle formation of sodium dodecyl sulfate (SDS) was studied using pyrene as a fluorescence probe. The critical micelle concentration (CMC) of SDS was estimated to be 8 mM from the changes in monomer fluorescence intensity and ratio of fluorescence intensities at 392 and 375 nm, in good agreement with the reported values obtained from different methods. The results of measurements of excimer fluorescence and fluorescence polarization suggested that the small aggregates of SDS molecules are formed in their low concentrations below the CMC and that the flexibility of hydrocarbon chains in soap molecules is remarkably reduced by growing from aggregates to micelles. The difference in arrangement of hydrocarbon chains between the premicellar and micellar states were manifested also by the different responses of fluorescence parameters of pyrene to $MgCl_2$ and temperature in low and high concentrations of SDS.

Keywords——SDS micelle; premicellar state of SDS; micelle formation; fluorometric analysis; pyrene

Sodium dodecyl sulfate (SDS) is an anionic surfactant which is widely used to solubilize the proteins of biological membranes. It gives also a useful model system for the study of the physicochemical properties of natural and synthetic micelles in appropriate conditions.

In this decade a variety of physical techniques such as electron spin resonance,[3] nuclear magnetic resonance[4] and Raman spectroscopy[5] have been applied to that study.

Recently fluorometric methods using two parameters, fluorescence polarization[6] and excimer fluorescence,[7] have been developed for research of the physical states of the hydrocarbon cores of synthetic micelles. Fluorescence polarization and excimer fluorescence measurements are based on the viscosity dependence of the rates of rotational and translational diffusion of a hydrocarbon fluorophore[8] in lipophilic mileu, respectively. These methods are useful particularly in measuring the magnitude of arrangement of the hydrocarbon chains of the constitutive molecules of synthetic micelles as well as model and biological membranes.

In this paper we present an application of pyrene to the fluorometric analysis of micelle formation of SDS and suggest the existence of a premicellar state or a transient state of structural organization of SDS molecules in the process of completion of the micelle with increasing SDS concentration.

1) The results were partly presented at 97th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1977.
2) Location: *Kanagawa-machi, Kanazawa, Ishikawa-ken 920-11, Japan.*
3) M.J. Povich, J.A. Mann, and A. Kawamoto, *J. Colloid Interface Soc.*, **41**, 145 (1972); F.H. Kirkpartrick and H.F. Sandberg, *Arch. Biochem. Biophys.*, **156**, 635 (1975).
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Materials and Methods

Fluorescence Measurement——Fluorescence of pyrene was measured using a Hitachi fluorescence spectrophotometer MPF-4 equipped with a rhodamine B quantum counter at 25° unless otherwise stated. Fluorescence spectra were corrected for instrumental response. Pyrene was used in a final concentration of 3.3×10^{-6} M unless specified otherwise in the Results. Excitation wavelength used was 340 nm in all fluorescence determinations. Excimer to monomer ratio (I_E/I_M) was calculated from the fluorescence intensities at 470 nm (for excimer) and 392 (for monomer) with excitation at 340 nm. Fluorescence polarization was defined as the ratio, $(I_V-I_H)/(I_V+I_H)$, where I_V and I_H are the fluorescence intensities of vertically and horizontally polarized emission with vertically polarized light, respectively.

Chemicals——Sodium dodecyl sulfate (SDS) was purchased from Wako Pure Chemical Co. Pyrene obtained from Wako Pure Chemical Co. was recrystalized from ethanol before use and dissolved in ethanol in a concentration of 1 mM as a stock solution.

Results

Fluorescence spectra of pyrene in SDS solution

Figure 1 shows the emission spectra of pyrene in the presence and absence of SDS.

The emission spectrum of pyrene alone (2×10^{-6} M) exhibits a superposition of the intense monomer fluorescence band from 375 to 420 nm with resolved peaks and the structureless broad band with maximum at about 470 nm due to the excimer.

When 20 mM SDS was mixed with pyrene, the emission maxima of the dye did not change but the monomer fluorescence intensity was remarkably increased with an inversion of intensities at 375 and 392 nm. On the contrary, the excimer fluorescence intensity of the dye was decreased by addition of 20 mM SDS.

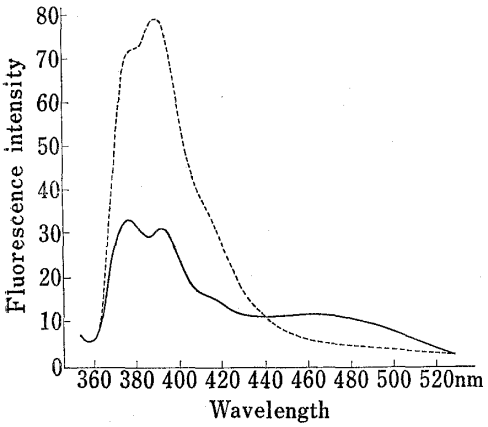

Fig. 1. Emission Spectra (Corrected) of Pyrene in the Presence (------) and Absence (——) of SDS (20 mM)

Fluorescence intensity is expressed in arbitrary units.

Fluorescence Characteristics of Pyrene

Figure 2 shows the effect of solvent polarity and viscosity on the fluorescence parameters of pyrene itself.

As can be seen in Fig. 2A, the ratio of fluorescence intensity at 392 nm to that at 375 nm (I_{392}/I_{375}) increased markedly with decreasing solvent polarity of ethanol/water binary mixture. Viscosity variation of medium did not affect on this parameter (see the curve for glycerol in Fig. 2A).

On the other hand, the ratio, I_E/I_M, showed a strong dependence on both solvent polarity and viscosity of medium as shown in Fig. 2B. With decreasing solvent polarity or with increasing viscosity, a proportional decrease in I_E/I_M was induced up to about 20% of either solvent.

Another difference between the behavior of the I_E/I_M and I_{392}/I_{375} values was observed in varied concentrations of the dye. The I_E/I_M ratio of pyrene itself was remarkably increased by increasing its concentration, but I_{392}/I_{375} was not (Fig. 3).

These results suggest that I_{392}/I_{375} is sensitive to environmental polarity around the probe molecules, whereas the excimer formation strongly depends on the collisional process of the fluorophore.

Changes in Fluorescence Parameters of Pyrene associated with Micelle Formation of SDS

The changes in some of fluorescence parameters of pyrene as a function of SDS concentration are illustrated in Fig. 4.

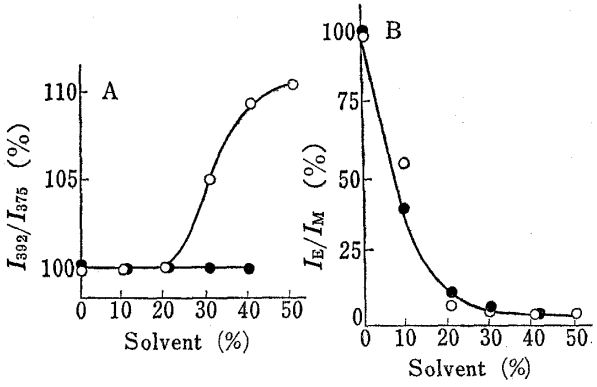

Fig. 2. Effects of Solvents on I_{392}/I_{375} (A) and I_E/I_M (B) of Pyrene alone

The values are expressed as relative to those without addition of solvents, ethanol (○) and glycerol (●). Consult "Materials and Methods" and text for the significance and calculation of these parameters.

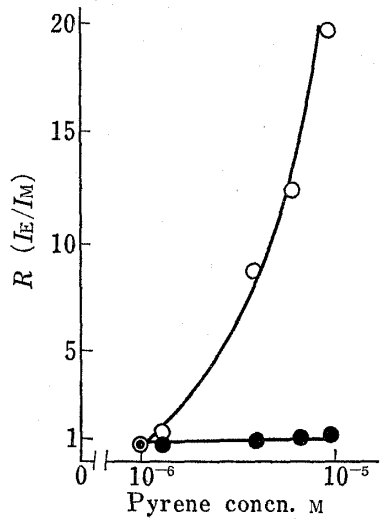

Fig. 3. Dependence of I_E/I_M and I_{392}/I_{375} on Pyrene Concentration

○, I_E/I_M; ●, I_{392}/I_{375}. The values are expressed as relative to those in 10^{-6} M pyrene. See the legend to Fig. 2 for other comments.

The monomer fluorescence intensity and I_{392}/I_{375} ratio sigmoidally increased with increasing SDS concentration, reaching their maxima at about 8 mM SDS in common (Fig. 4A). This value was in good agreement with the critical micelle concentration (CMC) value of SDS obtained by other methods,[9] indicating that these fluorescence parameters are good indicator of SDS micelle formation.

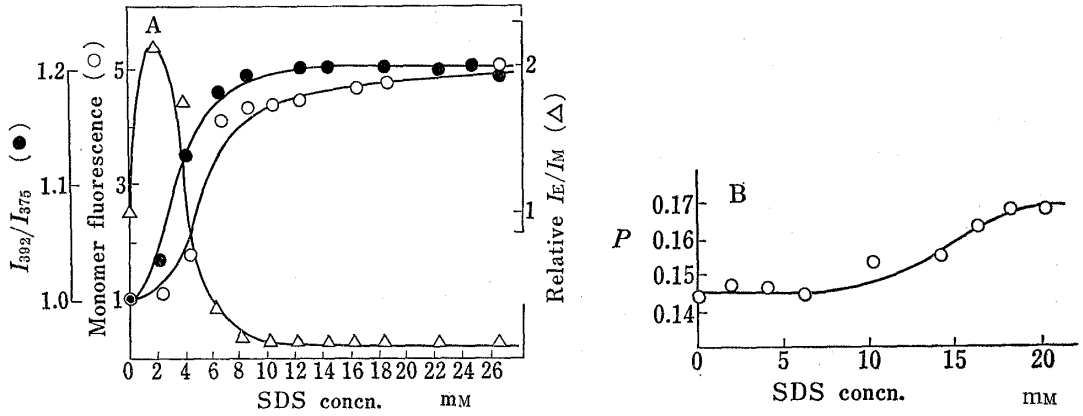

Fig. 4. Dependence of the Fluorescence Parameters on SDS Concentration

A: Changes of the monomer fluorescence intensity (○), I_{392}/I_{375} (●) and I_E/I_M (△). The values are expressed as relative to those in the absence of SDS. B: Change of fluorescence polarization. Emission was read at 392 nm. See the legend to Fig. 2 for other comments.

In contrast, the I_E/I_M ratio showed a very distinguished increase at about 2 mM of SDS and a steep decrease in higher SDS concentrations down to a level even lower than that of pyrene alone in the concentrations above 6 mM (Fig. 4A). This phenomenon would suggest that a specific enhancement in excimer fluorescence of pyrene occurs around 2 mM of SDS as an indication of some oriented arrangement of SDS molecules.

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On the other hand, the fluorescence polarization of pyrene did not change in SDS concentration below 8 mM, the CMC of SDS, showing a rise in higher concentrations than that (Fig. 4B).

Effect of $MgCl_2$ on SDS Micelle Formation

The effect of 1 mM $MgCl_2$ on the micelle formation is shown in Fig. 5.

The values of I_{392}/I_{375} were larger in the presence of $MgCl_2$ than those in the absence in SDS concentrations below 6 mM, indicating that the CMC is lowered by $MgCl_2$ (Fig. 5A).

The SDS concentration showing the peak of I_E/I_M was slightly shifted to the lower concentration of it by the addition of $MgCl_2$ as shown in Fig. 5B.

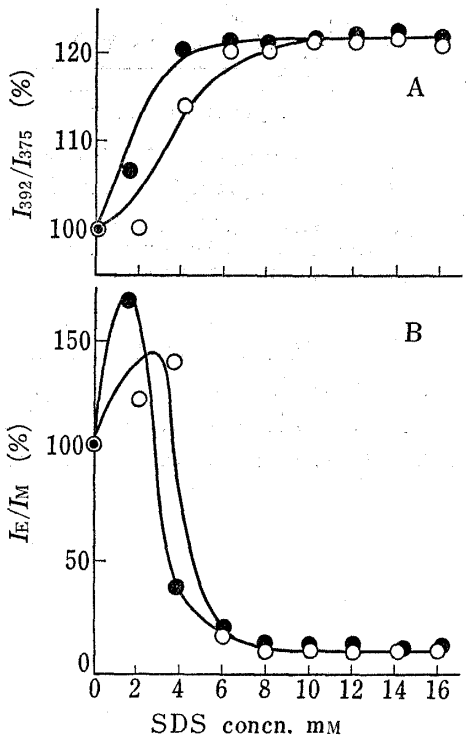

Fig. 5. Effect of $MgCl_2$ on I_{392}/I_{375} (A) and I_E/I_M (B) in Varied SDS Concentrations

○, control; ●, 1 mM $MgCl_2$. See the legend to Fig. 4 for other comments.

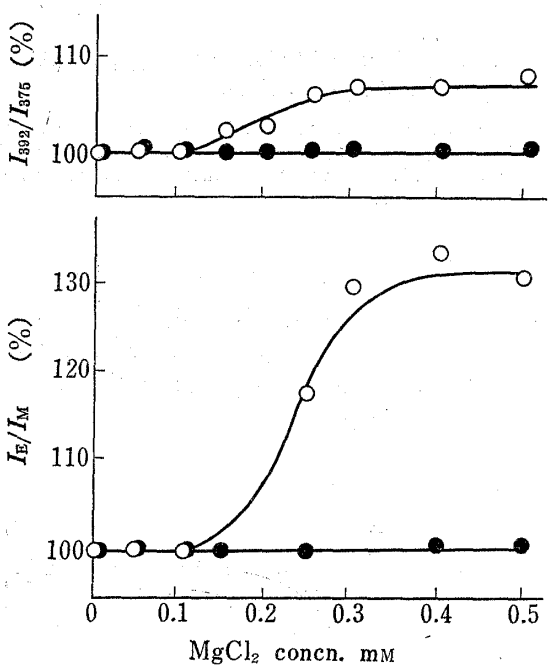

Fig. 6. Dependence of I_{392}/I_{375} and I_E/I_M on $MgCl_2$ Concentration in 2 and 16 mM SDS

○, 2 mM SDS; ●, 16 mM SDS. The values are expressed as relative to those without $MgCl_2$. See the legend to Fig. 2 for other comments.

Figure 6 shows the dependence of the fluorescence parameters of pyrene on $MgCl_2$ concentration in the presence of 2 and 16 mM SDS.

The values of I_E/I_M and I_{392}/I_{375} of pyrene increased as $MgCl_2$ concentration was increased above 0.1 mM, attaining their maxima at about 0.3 mM of $MgCl_2$ in the presence of 2 mM SDS. In 16 mM SDS–pyrene system, these parameters showed no change over the $MgCl_2$ concentration tested.

Responses to Temperature and NaI

In order to obtain further information about the arrangement of the hydrocarbon chains of SDS molecules in both low and high concentrations of SDS, the temperature dependence of the efficiency of pyrene excimer formation and the effect of a quencher, NaI, on monomer fluorescence of pyrene were measured.

As seen in Fig. 7, I_E/I_M of the 2 mM SDS-pyrene system was significantly decreased by increasing temperature of medium in the same way as that of pyrene alone.

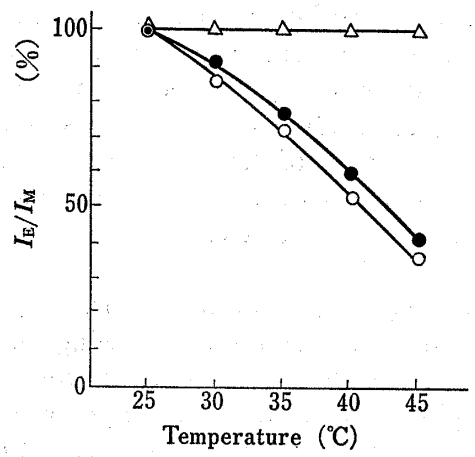

Fig. 7. Effect of Temperature on I_E/I_M in 2 and 20 mM SDS

○, pyrene alone; ●, 2 mM SDS; △, 20 mM SDS. The values are expressed as relative to those at 25°. See the legend to Fig. 2 for other comments.

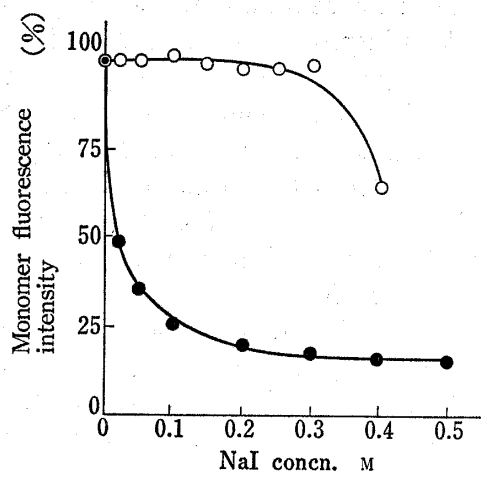

Fig. 8. Effect of NaI on the Monomer Fluorescence Intensity of Pyrene in the Presence and Absence of 16 mM SDS

●, no SDS; ○, 16 mM SDS. The values are expressed as relative to those in the absence of NaI.

In contrast, in 20 mM SDS–pyrene system, no change in I_E/I_M was observed in the temperature range investigated.

Figure 8 shows the effect of concentrations of NaI on the fluorescence intensity in 16 mM SDS–pyrene system.

The monomer fluorescence of pyrene alone was remarkably quenched by addition of NaI, but that of the 16 mM SDS–pyrene system did not show any change up to 0.3 M NaI, indicating that pyrene molecules are located deep in the hydrocarbon cores of the micelles. On the other hand, in the 2 mM SDS–pyrene system, a steady increment of the monomer fluorescence of pyrene was observed up to 0.15 M of NaI (data not shown). This phenomenon can be reasonably considered as a result of salt-induced acceleration of SDS micelle formation.[10]

Discussion

We suggested in this paper the small aggregates of SDS molecules at an early stage of micellar formation with increasing SDS concentration on the basis of the analyses summarized below using some of fluorescence parameters of pyrene used as a probe.

The monomer fluorescence intensity, I_{392}/I_{375} ratio, polarization and excimer formation efficiency of pyrene were very sensitive to SDS concentration (Fig. 1 and 4). The CMC value, 8 mM, of SDS was determined from the changes of these parameters, especially the former three, in good agreement with that obtained by other methods.[9]

An important finding in the present study is the biphasic behavior of excimer formation efficiency of pyrene in the low SDS concentrations around 2 mM (Fig. 4A). The other fluorescence parameters gave no indication of such a behavior in this range of SDS concentration. Thus it is unlikely that the observed phenomenon in the I_E/I_M is a consequence of artifact due to light scattering. As shown in Fig. 2 and 3, the excimer formation of pyrene strongly depends on the dye concentration and/or the freedom of movement of dye molecules during the excited state lifetime. Therefore the discontinuous enhancement of the I_E/I_M value in the low SDS concentrations is plausibly explained by the formation of the small aggregates of SDS molecules to make the arrangement of concentrated pyrene molecules

10) M.F. Emerson and A. Holtzer, *J. Phys. Chem.*, 71, 1898 (1967).

favorable to excimer formation. We have termed temporarily this status "premicellar state". An anomalous increment of I_E/I_M was observed in the very low concentrations of bile salt below its CMC (data not shown) in agreement with the results obtained by means of conductimetry[11] and nuclear magnetic resonance,[12] possibly reflecting detection of formation of premicellar aggregates. It would show the reliance and usefulness of this fluorescence parameter in detection of the formation of premicellar aggregates of detergent molecules. The relation of dimer formation of SDS molecules in their low concentrations reported by Mukerjee *et al.*[13] to our concept of the premicellar state mentioned above is a very interesting problem to be studied hereafter.

On the other hand, the anomalous decrease of the I_E/I_M value by further addition of SDS may be attributed to the loss of mobility of the dye molecules, showing decreased fluidity of the hydrocarbon chains of SDS along with growing to complete micelles.

The difference in the nature of hydrocarbon regions of SDS between the premicellar and micellar states was clearly demonstrated by the different fluorescence responses to $MgCl_2$ (Fig. 6) and temperature (Fig. 7). Judging from the results of measuring polarization (Fig. 4B) and temperature dependence of excimer formation efficiency (Fig. 7) with the 2 mM SDS–pyrene system, SDS molecules in the premicellar state are still in random arrangement like those in the monomeric state in which pyrene molecules possess a high degree of freedom of translocational motion.

In contrast, in the micellar state, fluorescence properties of incorporated pyrene are all insensitive to ion binding (Fig. 6), quencher (Fig. 8) and temperature (Fig. 7), indicating that pyrene molecules are deeply located in the highly ordered hydrocarbon cores of the micelles. An increased polarization in the high concentrations above CMC (Fig. 4B) would support it.

11) D.G. Oakenfull and L.R. Fisher, *J. Phys. Chem.*, **81**, 1838 (1977).
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