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Fluorometric Analysis of the Micelle Formation Process of Surfactants in Aqueous Solution. I. Utility of Pyrene in Determination of the Critical Micelle Concentration

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Pyrene, a fluorescent hydrocarbon, is a useful, sensitive probe for the determination of the critical micelle concentration of all types of detergents, non-ionic, anionic and cationic. In addition, this reagent has proved useful in research on the micelle formation process in aqueous solution and on differences in the rearrangement of the hydrocarbon chains of surfactants during micelle formation, based on measurements of the excimer formation efficiency.

Keywords——surfactant micelle; pyrene; fluorometric analysis; critical micelle concentration

The structures of micelles and the process of their formation afford not only physicochemical problems on the solubilization of lipophilic compounds, but also useful model systems of biological membrane structures and organization. It is, therefore, of interest to explore the changes of aggregation of surfactant monomers with increasing concentration to achieve an understanding of the nature of the organization of their hydrocarbon chains in association with micelle formation. One approach to this problem would be to find a sensitive probe or means of detecting possible changes in the arrangement of hydrocarbon chains in surfactant molecules associated with their micelle formation. However, various physical techniques used for determination of the critical micelle concentration (cmc) of surfactants, such as conductimetry,[1,2] nuclear magnetic resonance (NMR),[3,4] electron spin resonance[5-7] and Raman spectroscopy,[8,9] have limited applicability to studies of minute changes in the physical properties of the hydrocarbon domain of clustering surfactant molecules at very low concentrations.

Recently, fluorometry has been introduced as a sensitive method to elucidate micellar properties,[10-15] but few reports have dealt with its application for cmc determination.[10,14] Indeed no fluorescence probe that can be utilized for the cmc determination of all types of detergent has yet been reported.

Experimental results in the present report show that pyrene is useful probe for the c.m.c. determination of many types of detergents, including non-ionic, anionic and cationic ones, and suggest the occurrence of a transient state at the early stage of aggregation of hydrocarbon chains in the process of micelle formation of anionic detergent molecules.

Experimental

Chemicals——Pyrene was purchased from Wako Pure Chemical Co. and recrystallized from ethanol and water before use. 1-Laurylpyridinium chloride, cetylpyridinium chloride, cetyltrimethylammonium bromide (CTAB), Triton X-100 and sodium dodecyl sulfate were obtained from Wako Pure Chemical Co. Sodium cholate, sodium deoxycholate and sodium taurocholate were purchased from Nakarai Chemicals Co. These reagents were used without further purification.

Fluorometry——Steady-state fluorescence measurements were performed at 90° to the exciting beam, using a Hitachi MPF-4 fluorescence spectrophotometer with a rhodamine B quantum counter. The sample compartment was maintained at a constant temperature (25°C) by circulating water through the cell holder. The fluorescence intensity of pyrene was followed at 375, 392 and 470 nm with excitation at 340 nm. Pyrene was used at a final concentration of 33 μM unless otherwise specified. Excimer formation efficiency of pyrene was expressed as the ratio of fluorescence intensity of the dimer to that of monomer, I_E/I_M, or the ratio of the fluorescence intensity at 470 nm to that at 392 nm, with excitation at 340 nm.

Results and Discussion

Typical changes in the fluorescence spectrum of pyrene upon addition of surfactant are illustrated in Fig. 1. The emission spectrum of pyrene alone exhibits a superposition of the emission peaks of monomer at 375 and 392 nm and structureless broad excimer emission with a peak at about 470 nm. Addition of sodium deoxycholate (10 mM) to that system resulted in a large enhancement of the intensity of the monomer fluorescence and disappearance of the excimer fluorescence. Since the solubility of pyrene is very high in lipid, it is expected that pyrene will be distributed mainly in thehydrocarbon region of soap micelles.[11-13] An increased monomer fluorescence of pyrene reflects a solubilization of the probe in the micelle interior composed of the aggregated hydrocarbon chains of detergent molecules.

Another noticeable change in the fluorescence spectrum of the probe on addition of sodium deoxycholate was the increase of the ratio of intensities at 375 and 392 nm, I_{392}/I_{375}. Since the ratio, I_{392}/I_{375}, is dependent on the solvent polarity[16] rather than solvent viscosity, this parameter reflects the polarity of the microenvironmentin which the probe is solubilized.

As can be seen in Table I, the ratio, I_{392}/I_{375}, of pyrene fluorescence in the presence of anionic detergents such as sodium cholate and sodium deoxycholate was generally larger than that of pyrene alone. As described in our previous paper,[16] the I_{394}/I_{375} ratio of pyrene is enhanced with decreasing solvent polarity. Therefore, this result suggests that the environment surrounding the absorbed chromophore is hydrophobic. On the other hand, the ratios in the presence of non-ionic (Triton X-100) and cationic (CTAB) surfactants were almost the same as that with pyrene alone, suggesting that the interior of these surfactant micelles is

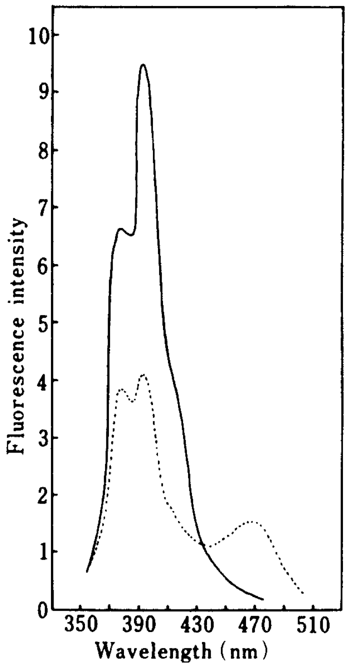

Fig. 1. Emission Spectra of Pyrene in the Presence and Absence of Surfactant (10 mM)

Symbols: ……, pyrene alone; ——, with sodium deoxycholate.

Fluorescence intensities are expressed in arbitrary units. Pyrene concentration is 0.5 μM.

TABLE I. Comparison of I_{392}/I_{375} Ratio of Pyrene in the Presence of Various Types of Surfactant Micelles

System	Ratio (I_{392}/I_{375})
Pyrene alone	1.09
CTAB	1.09
Triton X-100	1.12
Sodium cholate	1.32
Sodium deoxycholate	1.43

Conditions were the same as in the legend to Fig. 1.
Peak ratio was calculated from the emission spectrum.

rather polar. One possible explanation for these results is the difference in location of the probe in various micelles due to the difference in the arrangement of their hydrocarbon chains.

Figures 2A—D show typical concentration-dependent effects of surfactants on the fluorescence parameters of pyrene. The responses of the monomer fluorescence of pyrene to addition of various detergents were all biphasic within the concentrations tested, each showing a concentration-dependent slight or marked decrease at lower concentrations followed by a marked increase at higher ones, except for pyridinium-type detergent (Fig. 2D). These results are assumed to indicate that qualitative change occurs in the arrangement of molecules

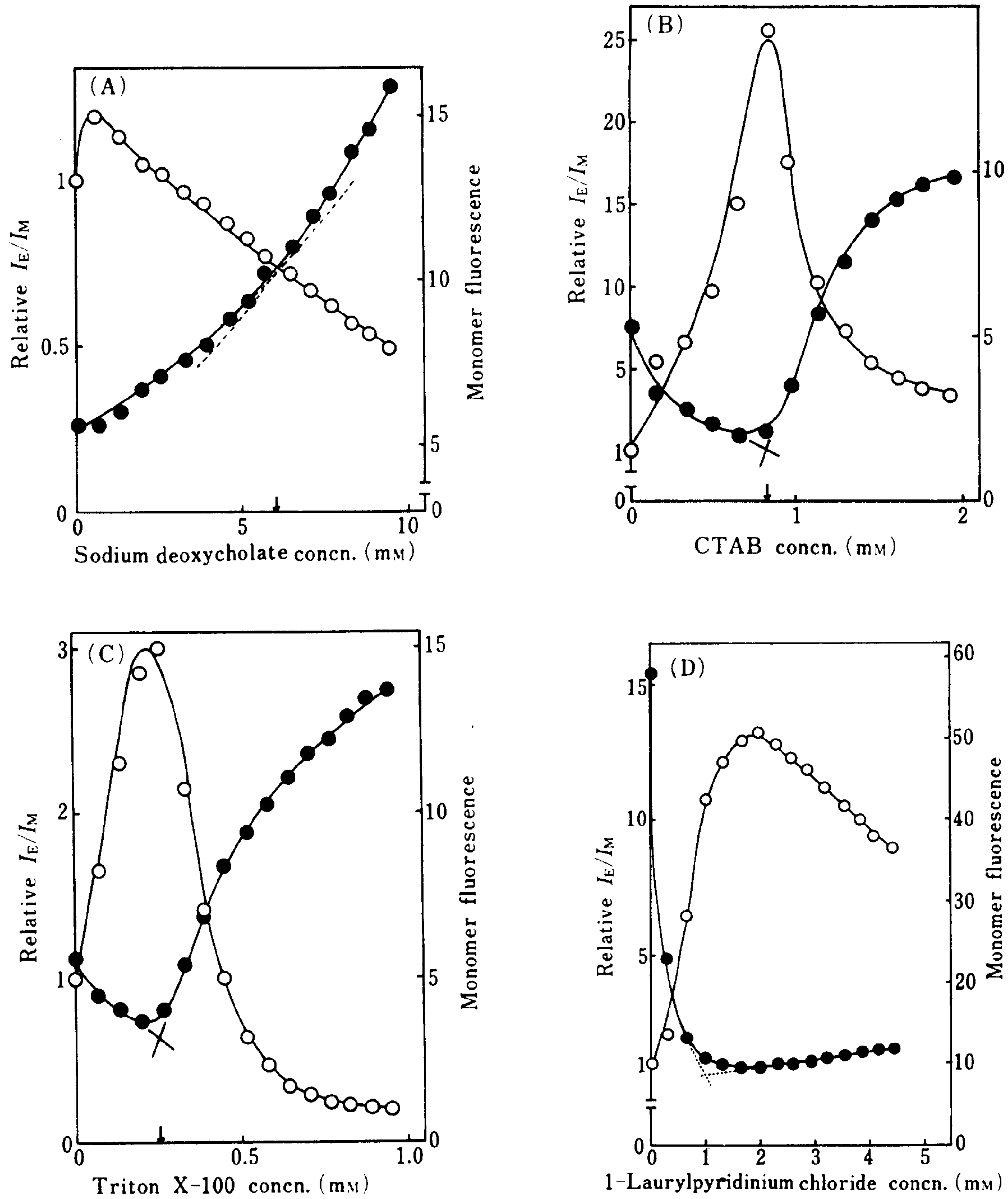

Fig. 2. Surfactant Concentration Dependence Profiles of the Fluorescence of Pyrene in Water

●, monomer fluorescence intensity; ○, I_E/I_M. (A) sodium deoxycholate; (B) cetyltrimethylammonium bromide; (C) Triton X-100; (D) 1-laurylpyridinium chloride. Monomer fluorescence intensity is given in arbitrary units and I_E/I_M ratio is expressed as the value relative to that in the absence of surfactant. Consult Experimental for the calculation of I_E/I_M. Other conditions were the same as in the legend to Fig. 1.

at particular concentrations of the detergents below 5 mM. In the cases of cationic (Fig. 2B), except for pyridinium-type (Fig. 2D) detergents, and non-ionic (Fig. 2C) detergents, the cmc is easily determined from the concentration of detergent at which a marked increase of the monomer fluorescence of pyrene begins. The dose-response curve of the pyrene monomer fluorescence with sodium deoxycholate (Fig. 2A) was somewhat different from those with cationic and non-ionic detergents. In this case, an increase in the pyrene monomer fluorescence begins at a detergent concentration far below the cmc[17,18] and then a further increase in the fluorescence intensity is observed near the range of detergent concentrations corresponding to the cmc determined by other methods.[17,18] A similar phenomenon was also observed with sodium cholate or sodium taurocholate (data not shown). In these cases, the cmc values were determined from the intersection point of the two curves calculated by the non-linear least-squares method, because the degree of the monomer fluorescence change around the cmc is not large. In contrast, the monomer fluorescence of pyrene in sodium dodecyl sulfate solution monotonically increased with increasing detergent concentration and reached a constant level at the concentration corresponding to the cmc value.[16] It seems likely that the discrepancy of the fluorescence response of pyrene between sodium dodecyl sulfate and bile salts may be due to a difference in the mode of rearrangement of the hydrocarbon chains in the detergent molecules during their micelle formation, but this requires confirmation.

On the other hand, in the case of pyridinium-type detergents such as 1-laurylpyridinium chloride and cetylpyridinium chloride, the monomer fluorescence of pyrene decreased sharply with increasing detergent concentration and reached an almost constant level above a certain concentration of detergents. Thus, in these cases, the cmc values were estimated by extrapolation of the two portions of the plots, as shown in Fig. 2D.

The cmc values determined by this method for various surfactants are summarized in Table II. On the basis of these results, it is concluded that the cmc of detergents can be rapidly and simply estimated by measurement of the change of pyrene monomer fluorescence with detergent concentration. These results also suggest that pyrene can be used as an useful probe for analysis of the aggregation process of detergent molecules.

TABLE II. cmc Values determined by the Fluorometric Method using Pyrene

	Surfactants	cmc (mM)	
		Present study[a]	Other methods
A	Sodium dodecyl sulfate	8	8—8.2[17,19]
	Sodium cholate	13.6	13—15[17,18]
	Sodium deoxycholate	6	4—6[17,18]
	Sodium taurocholate	9.5	10—15[17]
C	Cetyltrimethylammonium bromide (CTAB)	0.91	0.9[15,17]
	1-Laurylpyridinium chloride	0.9—1.3	—
	Cetylpyridinium chloride	0.6—0.9	0.9[17]
N	Triton X-100	0.25	0.24[17,20]

A, Anionic detergents; C, Cationic detergents; N, Non-ionic detergents

a) Determined from monomer fluorescence change of pyrene.

On the other hand, the change of the excimer formation efficiency of pyrene, expressed as the value of I_E/I_M ratio, gave a characteristic pattern with increasing concentration of each detergent; a sharp increase in the lower range of concentration of detergent followed by a sharp decrease in the higher range. For both non-ionic and cationic types of detergent the maximum of the ratio I_E/I_M was in good coincidence with the minimum of monomer fluorescence. However, for anionic-type detergent, the maximum of the excimer formation efficiency of pyrene was in the concentration range far below the cmc.

It is well known[12,21] that the excimer formation of pyrene is a diffusion-controlled process and therefore its efficiency is dependent on the local concentration of the probe. Thus, changes in the pyrene excimer fluorescence with increasing surfactant concentration may reflect rearrangement of the hydrocarbon chains of the surfactant molecules during their micelle formation process, *i.e.*, an increased I_E/I_M ratio may be attributed to an enhanced solubilization of pyrene molecules and/or alignment of the pyrene molecules in favor of excimer formation. On the other hand, a sharp reduction of I_E/I_M value may reflect the restriction of the rotational movement of the probe molecules due to a decrease in the fluidity of the hydrocarbon regions of the micelle interior in the process of completion of micelles.

The particular pattern of the change of excimer formation efficiency with increasing concentration of anionic detergents is very interesting (Fig. 2A). In the previous paper16) we described a similar phenomenon for sodium dodecyl sulfate. The phenomenon was then assumed to be evidence of the production of small aggregates arbitrarily named the premicellar state at the early stage of the micelle formation process. However, the possibility remained that it might be an artifact induced by interaction between pyrene and surfactant molecules with no relation to the change of molecular arrangement of surfactants. This possibility has now been almost excluded because such a phenomenon was not observed in cationic or non-ionic detergent systems.

Small *et al.*22,23) have inferred the formation of small aggregates designated as primary micelles of bile salts in the concentration range lower than the cmc on the basis of the results of conductimetry and NMR spectroscopy.

Experiments are in progress in our laboratory with sodium cholate to characterize the premicellar state of anionic surfactants by means of fluorometry.

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