tially higher activation barrier compared to that for the proton transfer to the N-protonated form in methyl carbamate. Therefore we conclude that the methoxy O-protonated tautomer(III) is not only unstable thermodynamically (Table 1) but also is difficult to form kinetically so that the abundance of (III) in the acid solution will be negligible. Thus the protonation behavior of methyl carbamate will be similar to that of acetamide. This conclusion is fully consistent with experimental results of protonation equilibria of amides and carbamates.[9]

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Studies on the Formation and Stability of Colloids (I): Perturbation of Micelle Formation of Sodium Deoxycholate by Amides

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The critical micelle concentration (CMC) of sodium deoxycholate (NaDC) and the effects of amides on the micellization processes have been studied by fluorometric technique using pyrene as a probe. The addition of amides as cosolvent destabilized the NaDC micelle and increased the CMC. The order of effectiveness for the perturbation of NaDC micelle was N-methylacetamide≥DMF>acetamide>formamide, which is the order of hydrophobicity of the amides. This indicated that the effect of amides on the micellization processes of NaDC arises from diminution of the hydrophobic effect. The electrostatic repulsion between ionic head groups in the NaDC micelle appeared to be much less than that in aliphatic ionic micelle. This was also revealed in the weaker dependence of the CMC on ionic strength. The premicellar association of NaDC was not significantly involved in the micellization processes of the bile salt.

Introduction

Bile salts are well known as biological surfactants and for their solubilizing action for lecithin, cholesterol and many other hydrophobic dietary lipids. The physiological role of bile salts depends mainly on their self-associating, *i.e.* micelle forming properties. Early works on the micellization and related physicochemical properties of bile salts were extensively surveyed by Small.[1]

The bile salt micelles are quite different from ordinary micelles in aggregative number and shape of the micelles. The exact nature of the driving forces for micellization of bile salts is not unequivocally explained.[2] But, several evidences suggest the presence of primary (aggregation of about 10 monomers to small micelles) and secondary (aggregation of primary micelles to large micelles) micellization phenomena for dihydroxy bile salts such as sodium deoxycholate (NaDC).[1,10,11] Generally, it is believed that the major driving force for micellization of a surfactant is hydrophobic effect, which arises from local ordering of water molecules into ice-like structure at hydrocarbon-water interface. The aggregation of hydrophobic part of surfactants results in decrease of the interfacial area, and thus, the micellization process is entropically favored.

Amides are known as a water-breaking chaotropic solutes, and they could reduce the hydrophobic interaction between water and surfactants molecules, and thus destabilize the micelles.[10b,12-14] This paper reports the effect of added amides on the critical micelle concentration(CMC) of NaDC. This is of particular interest because various compounds bearing

amide bond are coexistent with the bile slat in biological fluids. It was also hoped that the difference in the micelle forming properties between ordinary surfactants and bile salts could also be revealed in the effects of amides on the micellization processes. The determination of CMC was made by fluorometric technique using pyrene as a probe at low concentration of NaDC, where primary micelle forms.

Experimental

Materials. Sodium deoxycholate(NaDC) was obtained from DIFCO and recrystallized from water-acetone(1:9). N,N-Dimethylformamide(DMF) and formamide were purchased from Junsei and distilled under reduced pressure. Acetamide and N-methylacetamide were from Junsei and Tokyo Kasei, respectively, and used as received. Demineralized distilled water was used for preparation of solutions.

Preparation of Solutions. An excess amount of pyrene (Kanto Chemicals) was stirred overnight in aqueous 10 mM NaDC solution. The saturated solution was filtered, and concentration of pyrene was calculated from absorbance value at 334 nm after diluting the solution 10 times with methanol using $\varepsilon = 50000\ \ell\ mol^{-1}\ cm^{-1}$ at the wavelength. The pyrene stock solution was mixed with 20 mM NaDC stock solution and proper amide-water solvents to obtain series of NaDC solutions containing the same amount of pyrene (3×10^{-7} M) in desired solvent compositions. The pH of solutions were maintained at 8.1±0.2 with 0.01 M tris buffer.

Fluorescence Spectra were recorded from a Hitachi 650-10S Spectrofluorometer equipped with a thermostatically controlled cell holder at 25°C. Excitation wavelength was 333.3 nm. The CMC values were determined from the plots of ratio of emission intensities of the first and the third vibronic bands of pyrene (I_3/I_1) against concentration of NaDC.

Results and Discussion

Figure 1 shows the fluorescence emission spectra of pyrene in various concentration of NaDC. The emission spectra exhibit four vibronic bands of monomeric pyrene at 372, 379, 383 and 392 nm. It is well known that the ratio of intensities of pyrene emission of the first (372 nm) and the third (383 nm) bands (I_3/I_1) varies sensitively with polarity of medium where the fluorophor resides.[7,15] This values is about 0.59 in water, and 1.85 in cyclohexane.[7]

The variations of I_3/I_1 values of pyrene fluorescence spectra with concentration of NaDC are plotted in Figure 2. The interpretation of the data in Fig. 2 is straightforward. At low concentration of NaDC, pyrene dissolves in the bulk aqueous phase and thus, I_3/I_1 value of its fluorescence is close to the value in water. Near the CMC, NaDC monomers begin to aggregate forming micelles, and the hydrophobic pyrene dissolves into the micelles. As a result, the I_3/I_1 value of pyrene fluorescene increase at or above the cmc. We took the concentration of NaDC at which I_3/I_1 starts to increase abruptly as the CMC of the surfactant. The value was 4.7 mM at 25°C in aqueous solution of ionic strength of 0.01 M without amide.

The CMC's of NaDC and other bile salts in aqueous solutions without amide were measured by many investigators using a variety of techniques and experimental conditions.[1,10,16-19] The reported CMC of NaDC varies as much as from 1 to 70 mM. This is primarily due to the sensitivity of the CMC on

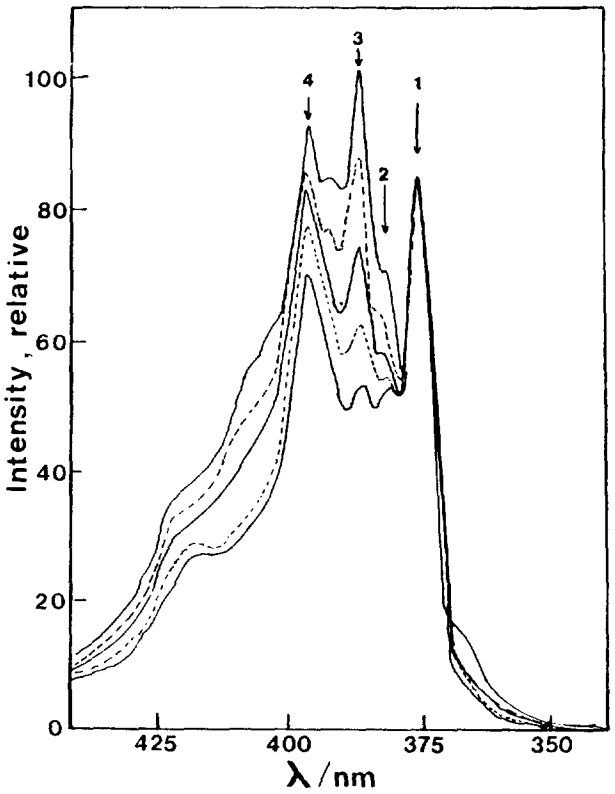

Figure 1. Fluorescence spectra of pyrene in aqueous NaDC solutions. From bottom to top, NaDC concentrations are 0.1, 5.1, 5.6, 7.1 and 10.1 mM.

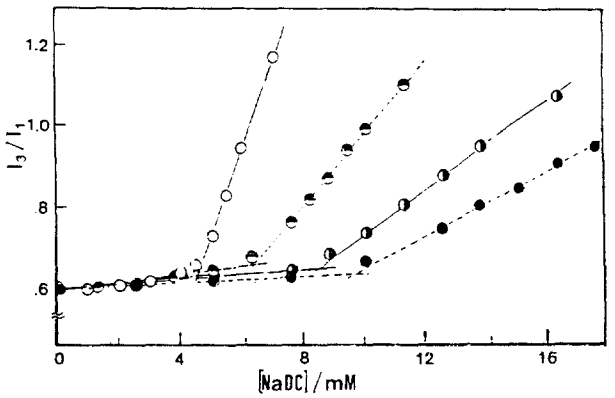

Figure 2. The variations of I_3/I_1 of pyrene fluorescence as functions of NaDC concentration. The proportions of DMF in solvent were 0(○), 1(◓), 2(◑) and 2.5(●) mole %.

the purity of the sample, and on the pH, ionic strength and temperature of medium. The measured CMC of NaDC, 4.7 mM, is close to the literature values of 4.7 mM in 0.02 M NaCl at pH 8.3 and 30°C determined by conductometric method,[16] and 6.4 mM in 0.01 M NaOH at 25°C by surface tension measurement.[17] This indicates that pyrene can be used as an useful probe for micellization of NaDC. It would be worthwhile to mention that, as the concept of CMC is convenient but inexact, the CMC values of a surfactant under given condition determined by different methods rarely agree exactly.

In Figure 2, we also presented the variations of I_3/I_1 of pyrene fluorescence with concentration of NaDC in the

presence of DMF as cosolvent. The Figure clearly shows that the presence of DMF in medium destabilizes the NaDC micelle and increases the CMC. The destabilizing effect of amide on NaDC micelle was also observed for other amides employed in this study, and agrees with the observation in aliphatic surfactant systems.[12-14] The dependences of CMC on the mole fraction of amides were graphed in Figure 3. The nearly linear relationship between log CMC and mole fraction of amide in amide-water cosolvent was same as the trend observed in n-dodecyltrimethylammonium bromide(DTAB)[13] and sodium dodecylsufate(SDS)[14], when the fraction of amide is small. The decreasing order of effectiveness for destabilizing the NaDC micelle among amides was N-methylacetamide≥N,N-dimethylformamide>acetamide>formamide. This is the decreasing order of hydrophobicity of the amides. The parallelism between hydrophobicity and micelle destabilizing ability was also noticed in the effect of substituted ureas on SDS[13] and other amides on cetyltrimethylammonium bromide (CTAB).[12]

The thermodynamic consideration of micelle formation predicts that the addition of micelle-penetrating solute to the solvent *decreases* the CMC.[20] Thus, the observation of the increase in the CMC of NaDC by amides suggests that the amides behave as non-penetrating solute and do not incorporate into the micelle. The change in dielectric constant of medium by the addition of amides can affect the electrostatic repulsion between ionic head groups and the CMC of the micelle. It was shown that acetamide ($\varepsilon = 59$ for 83°C liquid) lowers the dielectric constant slightly(about 3% for 3 M solution).[13] Probably, DMF ($\varepsilon = 37$) would also lower it. On the other hand, N-methylacetamide($\varepsilon = 180$) and formamide($\varepsilon = 110$) might raise it. Considering only electrostatic effect, it is expected that acetamide and DMF stabilize the ionic micelle and decrease the CMC, wherease N-methylacetamide and formamide cause the opposite effect. This expectation is quite different from our observation, which shows little correlation between dielectric constant and NaDC micelle destabilizing ability of the amides. Therefore the observed destabilizing effect of amides on the NaDC micelle formation must be explained by the diminution of the hydrophobic effect, which arises from the destruction of the ordered water at hydrocarbon-water interface of the surfactant. It seems that as the hydrophobicity of an amide increases, the amide is more effective for breaking the water structure and thus raising CMC.

The increase of the CMC of NaDC by the addition of formamide is a quite contrast to the effect of the amide on the aliphatic ionic micelles. It was reported that formamide up to 70 wt.% lowers the CMC of SDS.[13] We also showed that the CMC of CTAB as a function of the proportion of formamide shows a minimum at about 12 mole % of formamide above 45°C, whereas the CMC increases monotonically with the content of the amide below 35°C. However, we observed only monotonic increase in the CMC of NaDC even at 45°C: 5.3 mole % of formamide increased the CMC from 4.7 to 7.2 mM at 25°C, and from 6.2 to 8.7 mM at 45°C. The stabilizing effect of formamide for SDS micelle can be explained by the greater effect of dielectric constant than the hydrophobic effect. The observation of the minimum in CMC of CTAB can be attributed to the balance between the two opposing effect. This suggests that the electrostatic repulsion between head groups in the micelle is in order of NaDC<CTAB<SDS.

The weaker electrostatic repulsion in NaDC micelle than that in aliphatic micelles was also revealed in the dependence of CMC on the ionic strength. In the agreement with Mass-Action law of ionic micelle formation,[1] the logarithm of CMC of NaDC varied linearly with the logarithm of the ionic strength of the medium with the slope of 0.4 (Figure 4): the slope represents the fraction of the counter ion bound to the micelle when the ionic surfactant forms micelle and is parallel to the strength of the electrostatic field of the micellar surface. The slopes for SDS micelle and DTAB were calculated to be about 0.65 and 0.55, respectively.[21] (The value for CTAB is expected to be the same as that for DTAB as both have

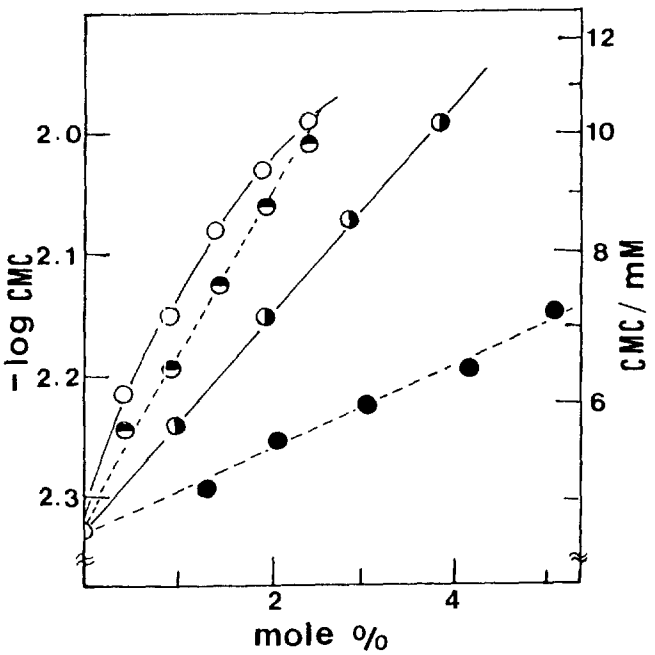

Figure 3. The dependence of the critical micelle concentration of NaDC on the proportion of amides: —O—, N-methylacetamide; --●--;DMF; —◑—, acetamide; --•--, formamide.

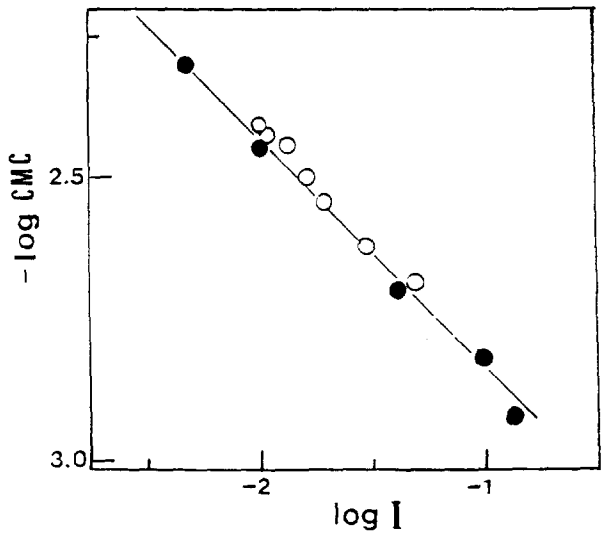

Figure 4. The dependence of the critical micelle concentration of NaDC on the ionic strength of medium. The ionic strength was adujusted with NaCl in 0.001 M tris buffer (O) or with phosphate buffer (●).

the same head group, $-N(CH)_3^+$.)

Another interesting finding in this experiment is that the I_3/I_1 ratio of pyrene probe varies only slightly with the concentration of NaDC below the CMC. This indicates that there is only weak premicellar association, if any, between the bile salts, and contradicts to the claim of dimer formation made by Oakenfull and Fischer,[4,5] but supports the conclusion presented by Zana and others.[6-8]

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