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Studies on the Formation and Stability of Colloids(II): pH and Temperature Effects on the Secondary Micelle Formation of Sodium Deoxycholate

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The micelle formation of NaDC was studied by fluorometric and viscometric measurements. The thermodynamic parameters of the primary and secondary micellization of the bile salt were evaluated. The primary micelle formation was appeared to be an entropy driven process due to hydrophobic effect, while the major driving force for secondary micelle formation of the bile salt is the large negative enthalpy. The secondary micelle provides less hydrophobic environment to pyrene than the primary micelle does. The cooperative aggregation of primary micelles via hydrogen bond formation was proposed for the secondary micelle formation.

Introduction

The micellization of bile salts has been a subject of extensive investigation for a long period of time due to their unique characteristics which are closely related to the physiological and biochemical roles of bile salts[1]. The micellizing properties of bile salts depend greatly on the number of hydroxy groups attached to the parent cholanic acid. At low ionic strength, typically adjusted with NaCl, the bile salts form micelles of aggregation number of about or less than 10. Unlike trihydroxy series, the dihydroxy bile salts form large micelles at moderately increased ionic strength and bile salts concentration. D. M. Small termed the large micelle as secondary micelle to differentiate this from small (primary) micelle[1].

The formation of secondary micelles, which is maifested by increased viscosity and ultimate gelation on standing of the bile salt solutions was first reported for sodium deoxycholate(NaDC) by two independent groups in 1958[2,3]. Sodium salts of ursodeoxycholate(NaUDC) and chenodeoxycholate (NaCDC), and mixtures of these with conjugated dihydroxy bile salts also form secondary micelles, but require lower pH, and much higher bile salts and NaCl concentrations than NaDC[4-6]. The secondary micellization(gelation) of NaDC solutions is favored by low pH and high ionic strength[7]. The micelle is also stabilized as temperature is lowered[2,8], and high pressure is applied[8]. Most of investigators agreed that the secondary micelles, gel, of the bile salts are formed by aggregation of primary micelles. However, details on the mechanism of the aggregation is not known unequivocally. Sugihara et al.[8] measured the sol-gel transition of NaDC by conductometry and reported thermodynamic parameters of gelation. The critical concentration of secondary micelle formation of NaDC was also measured by Sesta et al. by densitometry and viscometry[9]. These studies were performed in the absence of NaCl. On the other hand, the light scattering[5,6] and ultrasound[10] studies on the conjugated dihydroxy bile salt did not reveal the critical concentration for the secondary micellization, but indicated that the primary micelles grow gradually above the CMC of primary micelle formation.

In this study, we examined the critical concentration of NaDC, for both primary and secondary micellization. We also investigated the effects of pH and temperature on the processes to obtain information on the mechanism of the micellization. These experiments were performed near physiological pH and ionic strength, because of the interest of the phenomena in biological fluids. The formation of primary micelle was followed by fluorometric method using pyrene as a probe[11], and the secondary micellization(gelation) was studied by viscometric measurements.

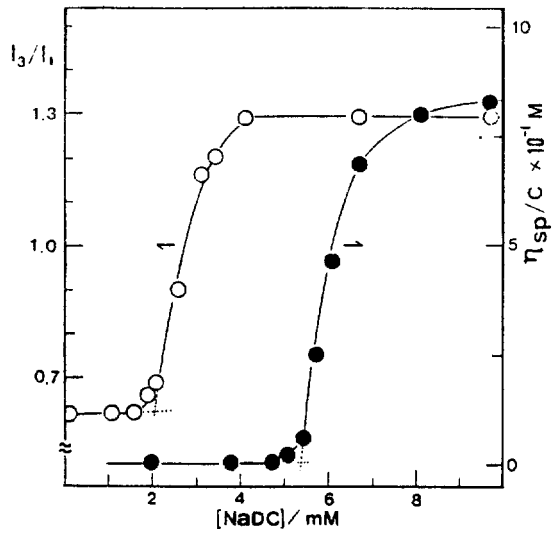

Figure 1. The changes of fluorescene intensity ratio(I_3/I_1) of pyrene probe(○) and reduced viscosity(●) as functions of NaDC concentration at 40°C, pH 7.15 and NaCl concentration of 0.2 M.

Experimental

Sodium deoxycholate(NaDC) was obtained from DIFCO and purified by recrystallization from water-acetone(1:9) mixture. Pyrene was purchased from KANTO and used as received. NaDC solutions were prepared with deionized distilled water and ionic strength of solutions was adjusted to 0.2 M with NaCl. The pH of the solutions was adjusted to 7.15 with 0.01 M phosphate buffer, unless otherwise specified. Details on the preparation of solutions and fluorescence measurement for determination of the critical micelle concentration(CMC) of primary micelle formation were described elsewhere[11]. Viscosity of solutions was measured by using an Ostwald type viscometer of which flow-time of water was 9.5 sec at 25°C. The NaDC solutions for viscosity measurements were stored in a refrigerator overnight, and the temperature of the solutions was not raised above the temperature at which the viscosity is measured.

Results and Discussion

Temperature Dependencies of Critical Micelle Concentrations and Thermodynamic Parameters of Micellizations. The emission spectra of pyrene in NaDC solutions excited at 333.3 nm show four vibronic bands at 372, 379, 383 and 392 nm[11]. Figure 1 shows the variation of the ratio of emission intensities of the 383 nm(I_3) and the 372 nm(I_1) bands on the concentration of NaDC at 40°C, pH 7.15. The CMC value was obtained from the plot by intersecting the slope of the I_3/I_1 curve at inflection point with the base line. The value was 2.0 mM. We also measured CMC of NaDC at other temperature by the same method and the results are listed in Table 1.

The phase separation model of micellization relates the Gibbs free energy of micellization to CMC by $\Delta G_m^\circ = RT\ln CMC$[12]. The enthalpy and entropy of micellization can be evaluated from the basic relationships, $\Delta H = d(\Delta G/T)/d(1/T)$ and $\Delta S = (\Delta G - \Delta H)/T$. The average ΔH_m° for the primary micellization was about −5 KJ/mole, and ΔS_m° was estimated to be +35 J/K/mole under our experimental condition[13]. The thermodynamic parameters are included in Table 1.

Table 1. Parameters of NaDC Micellization in 0.2 M NaCl at pH 7.15

Temerature, °C		25	35	40	45
Primary Micelle	CMC, mM	1.9	1.9_5	2.0	2.2
	ΔG, KJ/mole	−15.5	−16.0	−16.2	−16.2
	ΔH, KJ/mole		ca. −5		
	ΔS, J/K/mole		ca. +35		
Secondary Micelle	$(CMC)_2$, mM	2.4	3.4	5.3	>20
	Ag#/micelle	16	14	12	
	$C_M \times 10^5$, M	3.1[a]	10[a]	28[a]	
per micelle[b]	ΔG, KJ/mole	−25.8	−23.6	−21.3	
	ΔH, KJ/mole	−92	−130	−160	
	ΔS, J/K/mole	−220	−345	−440	
per monomer[c]	ΔG, KJ/mole	−1.6	−1.7	−1.8	
	ΔH, KJ/mole	−5.8	−9.3	−13	
	ΔS, J/K/mole	−14	−25	−37	

[a] M denotes the critical concentration of primary micelle for secondary micellization. [b] These values are the estimated thermodynamic parameters for secondary micellization per mole of primary micelle. [c] These values are the same per mole of monomer in primary micelle.

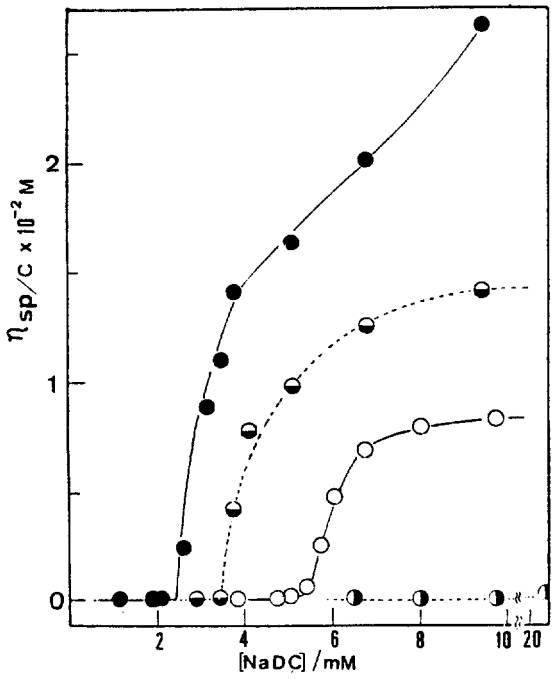

Figure 2. Variations of reduced viscosity with concentration of NaDC at 25(●), 35(◒), 40(○) and 45°C(◑). The other conditions are the same as those in Figure 1.

In Figure 1, we also present the variation of reduced viscosity, $\eta_{sp}/C = (\eta_{soln}/\eta_{solv} - 1)/C$, of NaDC solutions as a function of NaDC concentration(C). The η_{sp}/C value was virtually zero up to certain critical concentration, but it increased sharply beyond the concentration and ultimately the solution became gel. The critical concentration of the viscosity increase was always higher than the CMC of the primary micelle formation, which was manifested by increased I_3/I_1 ratio of pyrene probe.

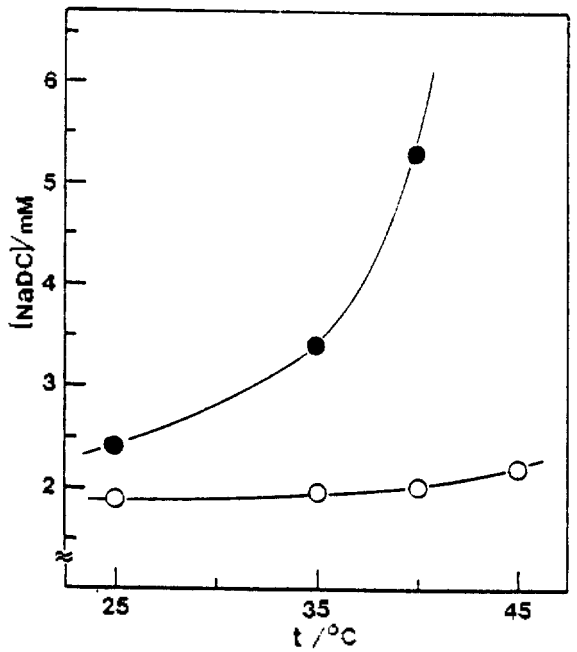

Figure 3. Variations of critical concentration of the primary(O) and secondary(●) micelle formation of NaDC with temperature at pH 7.15 and 0.2 M NaCl.

The sharp increase in viscosity of NaDC solutions at critical concentration reflects the formation of secondary NaDC micelle by cooperative aggregation of primary micelle. The observation of critical concentration for secondary micellization of NaDC is in agreement with the results of Sesta et al.[9], but in contrast to the reports[5,6,10] of gradual growing of primary micelle into secondary micelle which was observed in conjugated dihydroxy bile salts.

Unlike the primary micelle formation, the secondary micellization of NaDC is greatly temperature dependent as demonstrated in Figure 2. The critical concentration of secondary micelle formation, $(CMC)_2$, was determined from η_{sp}/C vs. NaDC plots in Figure 2 by the same method applied for the determination of CMC of primary micelle formation from I_3/I_1 curves. The results are summarized in Table 1. The temperature dependencies of the critical concentration of the primary and secondary micellization were compared in Figure 3.

The thermodynamic parameters of secondary micellization can be estimated by the same method used for primary micelle formation described in a previous section. For this, however, we have to use the critical concentration of primary *micelle*, rather than *monomer*. Based on the tabulated data by Small[14], the aggregation numbers(Ag#) of NaDC primary micelles in our systems are assumed to be 16 at 25°C, 14 at 35°C and 12 at 40°C. The critical concentration of primary micelle(M) in terms of mole of micelle per dm^3 was calculated by $C_M = [(CMC)_2 - CMC]/Ag\#$. The values were used to estimate the thermodynamic parameters of secondary micellization of NaDC per mole of primary micelle. For comparison of the data with those of primary micellization, which are expressed in terms of per mole of monomer, we also calculated the thermodynamic parameters of secondary micellization in terms of per mole of monomer by dividing the per mole of micelle data by Ag#. These results are included in Table 1.

PH Effects on the Secondary Micelle Formation. The

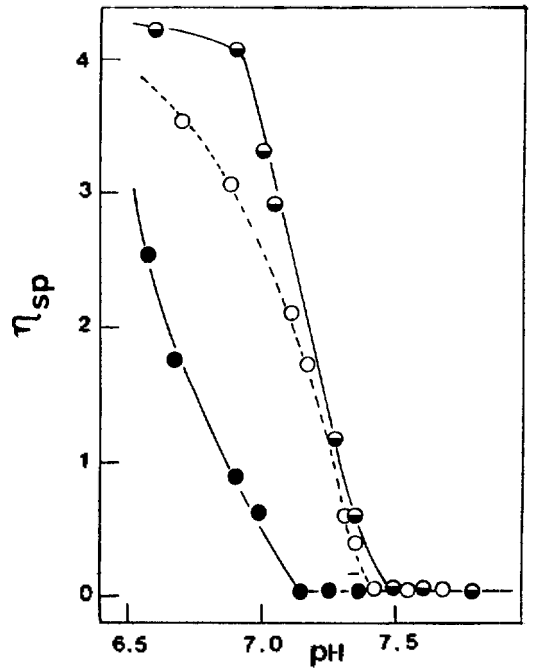

Figure 4. Specific viscosity of 10 mM NaDC solutions as functions of pH at 15(◒), 25(O) and 35°C(●).

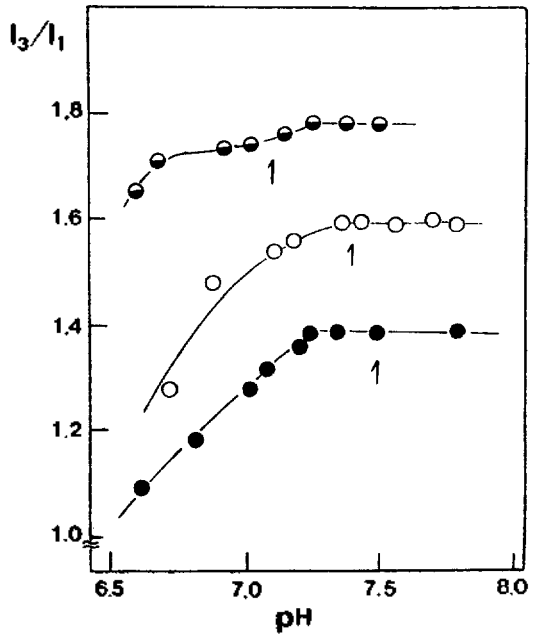

Figure 5. The change of fluorescence intensity ratio of pyrene probe with pH in 10 mM NaDC solutions at 15(●), 25(O) and 35°C(◒). Arrows indicate the critical pH of secondary micellization from viscosity measurements. The data of 25 and 35°C are shifted by +0.2 and +0.4, respectively.

secondary micelle of NaDC is induced by lowering the pH of NaDC solutions. Figure 4 shows the variations of specific viscosity of 10 mM NaDC solutions with pH. The critical pH's for the viscosity rise, i.e., secondary micellization were 7.5 at 15°C, 7.4 at 25°C and 7.1 at 35°C. The lowering of pH results in protonation of carboxylate groups. Thus, the observation of critical pH indicates that critical fraction of COOH groups is required to form secondary micelle. This fraction

is greater as the temperature of solutions is raised and the concentration of NaDC is lowered. The protonation of COO^- groups by lowering pH reduces electrostatic repulsion between charged NaDC primary micelles when they are brought into proximity to form the secondary micelle. In this aspect, the lowering of pH might affect the secondary micellization in similar fashion as the increased ionic strength does.

We also followed I_3/I_1 ratios of pyrene probe with pH and the results are graphed in Figure 5. At high pH, the ratio was about 1.4 indicating that pyrene is dissolved in hydrophobic environment of NaDC micelle(the concentration of NaDC, 10 mM, is higher than the CMC of primary micelle formation). Interestingly, the I_3/I_1 ratio decreases as the pH is lowered below the critical pH of secondary micellization which is reflected by the sharp increase in viscosity(Figure 4). This suggests that the pyrene probe resides, on the average, in less hydrophobic environment in secondary micelle than in primary micelle. The interfacial area between primary micelles formed when the micelles are brought into contact to aggregate could also be the solubilizing site of pyrene. The newly formed site would provide less hydrophobic environment to pyrene than the primary micelle does as hydrophilic hydroxy groups of the bile salts are faced into the interface.

Thermodynamics and Model of NaDC Micellization. The thermodynamic parameters of micellization can be presented by summation of various factors involved in the process(Eq.1).

$$\Delta X = \Delta X_{EI} + \Delta X_{HE} + \Delta X_{H\text{-}bond} + \Delta X_{solvation} \quad (1)$$

X can be Gibbs free energy(G), enthalpy(H) and entropy(S) change of micellization, and other thermodynamic quantities. ΔX_{EI} is the thermodynamic quantity arised from electrostatic interaction for ionic micelle. This interaction contributes positive quantities to free energy and enthalpy of micellization. ΔX_{HE} is due to hydrophobic effect. The water around hydrophobic part of a surfactant molecule is believed to be highly ordered. The formation of micelle reduces the hydrocarbon–water interface and results in decrease in free energy and enthalpy, but increase in entropy. This is the major driving force for micellization of ordinary surfactants. As temperature is raised, the contribution of the hydrophobic interaction is less important, and thus usually the ΔH becomes more negative and ΔS becomes less positive. Hydrogen bond formation, $\Delta X_{H\text{-}bond}$, generally contributes negative quantities to free energy and enthalpy as well as to entropy. Finally, the solvation term, $\Delta X_{solvation}$, is due to changes in solvation to hydrophilic groups of a surfactant molecule when these groups are involved in micellization process, for example, by hydrogen bonding. The contribution of this to the overall thermodynamic quantities is in opposite direction to the hydrogen bond formation, but in less degree for most cases.

The thermodynamic parameters of NaDC primary micellization (Table 1) are typical of the corresponding values observed in the ordinary aliphatic surfactant systems. The major driving force for micelle formation is positive entropy change. This is manifestation of the hydrophobic effect and agrees with the conclusions made by Zana and others[15], and with our previous report[11]. The entropy change cannot account for the hydrogen bond formation suggested by others[16,17]. This point becomes clearer when one compares the thermodynamic data with those of secondary micellization(see Table 1 and following arguments).

In contrast to the primary micelle formation, the secondary micellization of NaDC accompanies large negative entropy change and the process is enthalpy driven. One of the possible explanation for this is that the secondary micelle is formed by intermicellar hydrogen bond formation between hydroxyl groups suggested by Small[8,18]. The hydroxyl groups in primary micelle lie in hydrophilic surface and are in contact with water. The association of the micelles by hydrogen bonding might require preferable orientation of the hydroxyl groups, and should overcome the increased electrostatic repulsion between the carboxylate groups. The former requirement can explain why NaCDC forms secondary micelle in much less degree than NaDC does[4-6] though both NaDC and NaCDC exhibit similar CMC values for the primary micelle. The critical dependence of CMC of secondary micellization points the latter requirement. The hydrogen bond formation results in desolvation of the polar hydroxyl groups. The hydrophilic surface of primary micelle also contains large hydrophobic region. The association of the micelle reduces the hydrocarbon-water interfacial area contributing hydrophobic effect to the process. The overall positive quantity of entropy of secondary micellization indicates that the effect of hydrogen bond formation overwhelms the effects of desolvation and hydrophobic interaction. However, the latter two endothermic factors can explain the much weaker exothermic nature of the secondary micellization ($\Delta H = -6 \sim -13$KJ/mol) than that expected for the normal hydrogen bond formation ($-15 \sim -40$KJ/mol).

It seems necessary to differentiate the usual growth of primary micelle [14,19,20] and secondary micellization of NaDC discussed in this study. The aggregation number(Ag#) of most ionic surfactants including bile salts increases with increasing concentration of surfactant and ionic strength. And the number decreases with temperature. The growth is gradual and does not require critical condition. On the other hand, the secondary micellization of NaDC is cooperative and requires critical conditions. Also the usual growth of micelle is kinetically rapid, but the latter process is slow and takes sometimes several hours[7,21]. These criteria can resolve ambiguity casted on the mechanism of secondary micelle formation of bile salts[6,8,9,15,19,22].

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13. The plots of $\Delta G/T$ vs. $1/T$ for micellization processes are rarely linear. ΔH is temperature dependent and sometimes they exhibit minima. Generally, ΔH becomes more negative as temperature is raised above room temperature[12]. This trend can also be noticed in our data of the dependence of CMC on temperature. Because of low sensitivity of the CMC on temperature, we failed to calculate the temperature dependencies of ΔH and ΔS of the primary micellization of NaDC. Thus, we estimated the average values of those. But these data can be used for comparison with those of secondary micellization.
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Relativistic Effects on Orbital Energies in AgH and AuH; A Clue to the Origin of Relativistic Correlation Effects

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Oribtal energies for AuH and AgH are calculated by an all-electron relativistic self-consistent-field method using Slater type basis functions. Major relativistic effects for AgH are spin-orbit splittings and those for AuH are large shifts in orbital energies in addition to spin-orbit splittings. Relativistic effects on orbital energies in AgH and AuH imply that changes in correlation energies for relativistic calculations of AuH will be significantly larger than those of AgH, providing partial explanation for the large discrepencies in equilibrium bond length and the dissociation energy between experiments and theoretical estimates for AuH. Large relativistic effects on orbital energies indicate that relativistic contributions should be included for the correct interpretation of ionization potentials for these molecules. Relativistic effects are also evident in dipole moments for these molecules.

Introduction

Diatomic molecules AgH and AuH are quite interesting molecules from the computational point of view, exhibiting very large relativistic and correlation effects on bond lengths and dissociation energies as shown by the previous studies[1,2]. When the relativistic effects estimated from Dirac-Hartree-Fock (DHF) calculations using analytic expansions, which is refered as the all-electron relativistic self-consitent-field (RSCF) calculations, and the correlation effects from non-relativistic calculations are combined[3], the equilibrium bond length and the dissociation energy for AgH from the calculations are in good agreement with experimental values whereas those for AuH are not. It is evident that the correlation effects caused by the shift of energy levels and the change of orbital shapes due to the relativistic effects play an important role for the correct description of the electronic structure of AuH.

In order to understand the synergistic effect between correlation and relativity, both of them should be treated simultaneously in one calculation using a correlated method based upon a relativistic formalism. Although this procedure for all-electron calculations is not difficult to conceptualize and a formalism has been reported[4], the actual calculations even for small molecules are not available. The majority of calculations which include both the relativistic and correlation effects are based upon effective core potentials with only the minimum amount of valence correlations required for the qualitatively correct description of several valence state potential curves[5,6].

Since all-electron calculations including both the relativistic and the correlation effects are not readily available at present, we try to find a clue to the source of relativistic correlation effects, the extra correlation effects caused by the introduction of relativistic effects, for AuH from the result of previous RSCF calculations for AgH and AuH.