Effects of Sodium Glycocholate and Sodium Taurocholate on the Mixed Micelles of Bile Salts and Nonionic Surfactant

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Effects of sodium glycocholate (NaGC) and sodium taurocholate (NaTC) on the mixed micelles for two systems consisting of NaGC-octaoxyethylene glycol mono n-decyl ether $(C_{10}E_8)$ and NaTC-$C_{10}E_8$ have been studied as a function of the mixed micelles' compositions, polarities of the micelles' interior and mean aggregation numbers. The compositions of the mixed micelles are calculated from critical micelle concentration (CMC) data by using excess thermodynamic quantities. The polarities and mean aggregation numbers are determined from pyrene fluorescence in the mixed micelles. Both mixed systems were nonideal, and the mole fraction of NaGC or NaTC in a mixed micelle near the CMC was less than that in the aqueous mixed solution. However, the mixed micelle of the NaTC-$C_{10}E_8$ system contained more bile salt molecules than that of the NaGC-$C_{10}E_8$ system because of a good miscibility of NaTC and $C_{10}E_8$ molecules. The pyrene fluorescence results suggested that the mixed micelles changed from $C_{10}E_8$-rich micelles to NaGC- or NaTC-rich micelles, and mean aggregation numbers of the mixed micelles decreased abruptly with increasing mole fraction of bile salts. In the low mole fraction range of bile salts, however, both polarities and the mean aggregation numbers for the NaTC-$C_{10}E_8$ system are lower than those for the NaGC-$C_{10}E_8$ system because of the high mole fraction of NaTC in a mixed micelle, and also because of the different effect of the conjugated group between NaTC and NaGC molecules in the mixed micelles.

KEY WORDS: Excess thermodynamic quantities, pyrene fluorescence, sodium glycocholate, sodium taurocholate.

Bile salts possess bulky hydrophobic parts in their molecules. These hydrophobic parts have a strong affinity between the steroid rings so that they form rigid, back-to-back micelles (1–5). On the other hand, a nonionic surfactant with a linear hydrocarbon chain has typical colloidal properties in water and forms loosely packed micelles. Therefore, we have studied some extraordinary properties of bile salts by adding a nonionic surfactant to bile–salt micelles; bile salt properties can be derived from the information obtained; for example, bile–salt micelles are loosely packed by nonionic surfactant. In previous papers (6–9) the mixed micelles of bile salts and nonionic surfactants have been described as nonideal mixtures, and the extent of nonideal mixing for these mixed systems was found to depend on the conjugation of glycine to sodium cholate (NaC) and the deoxydation at the 7-position of NaC.

In this work, effects of conjugated glycine and taurine to the NaC molecule on the mixed micelles consisting of bile salts and nonionic surfactants will be discussed based on the data obtained from surface tension measurements and fluorescence techniques (10–14).

EXPERIMENTAL PROCEDURES

Materials. Sodium taurocholate (NaTC; Sigma Chemical Co., St. Louis, MO) and sodium glycocholate (NaGC; Akabane, Tokyo, Japan) were purified several times by recrystallization from the mixed solvent of ethanol and methanol, and then a Soxhlet extraction was performed with acetone for 72 h.

Octaoxyethylene glycol mono n-decyl ether $(C_{10}E_8;$ Nikko Chemicals, Tokyo, Japan) was used as a nonionic surfactant, pyrene (Tokyo Kasei Kogyo, Tokyo, Japan) was used as a probe and dodecylpyridinium chloride (DPCl; Tokyo Kasei Kogyo) was used as a quencher; and all were purified by methods reported in a previous paper (6–9).

Aqueous solutions of surfactants were prepared with tris/HCl buffer solution adjusted to pH 9.00 ± 0.05 with an ionic strength of 0.026.

Methods. Before all measurements, aqueous solutions of NaTC, NaGC and $C_{10}E_8$ by themselves were prepared as well as binary mixtures of each bile salt and $C_{10}E_8$ at mole fractions of 0.25, 0.50, 0.75 and 0.90, respectively.

Surface tensions were measured by the Wilhelmy vertical plate method with a glass plate in a Shimadzu surface tensiometer ST-1. Critical micelle concentrations (CMC) for each bile salt and $C_{10}E_8$ alone and for the binary mixtures at various mole fractions were obtained from curves of the surface tension *vs.* the logarithm of the apparent surfactant concentration. These CMCs were used for the theoretical calculations of the micellar compositions by the excess thermodynamic quantities according to Motomura (15).

Pyrene fluorescence emission spectra were measured by monitoring the spectra from 350 to 500 nm upon exciting the pyrene molecules near 340 nm with a Shimadzu fluorescence spectrophotometer RF-540. For measurements of the ratio of the first and third vibronic peak, I_1/I_3, in a monomeric fluorescence emission spectrum of pyrene (10–12), pyrene concentrations in the micellar solutions were prepared in the range from 0.1 × 10⁻⁵ to 1.0 × 10⁻⁵ mol/L, depending on the total surfactant concentration. The mean aggregation numbers of micelles were obtained from luminescence quenching measurements (13,14), and the concentrations of pyrene were prepared in the range from 0.2 × 10⁻⁶ to 1.0 × 10⁻⁶ mol/L. The concentrations of DPCl as a quencher, [DPCl], were prepared in the range of the ratio of [DPCl] to the micelle concentration, which was confined within narrow limits from 0.5 to 1.5. Throughout this work the temperature was maintained at 25.00 ± 0.05 °C.

RESULTS AND DISCUSSION

Composition in the mixed micelles. Figure 1 shows the surface tension *vs.* logarithm of total surfactant concentration for aqueous solutions of the NaTC-$C_{10}E_8$ system (a) and the NaGC-$C_{10}E_8$ system (b), respectively. Mole fractions of bile salts for each solution were 0.00, 0.25, 0.50, 0.75, 0.90 and 1.00. Each curve for the NaTC-$C_{10}E_8$
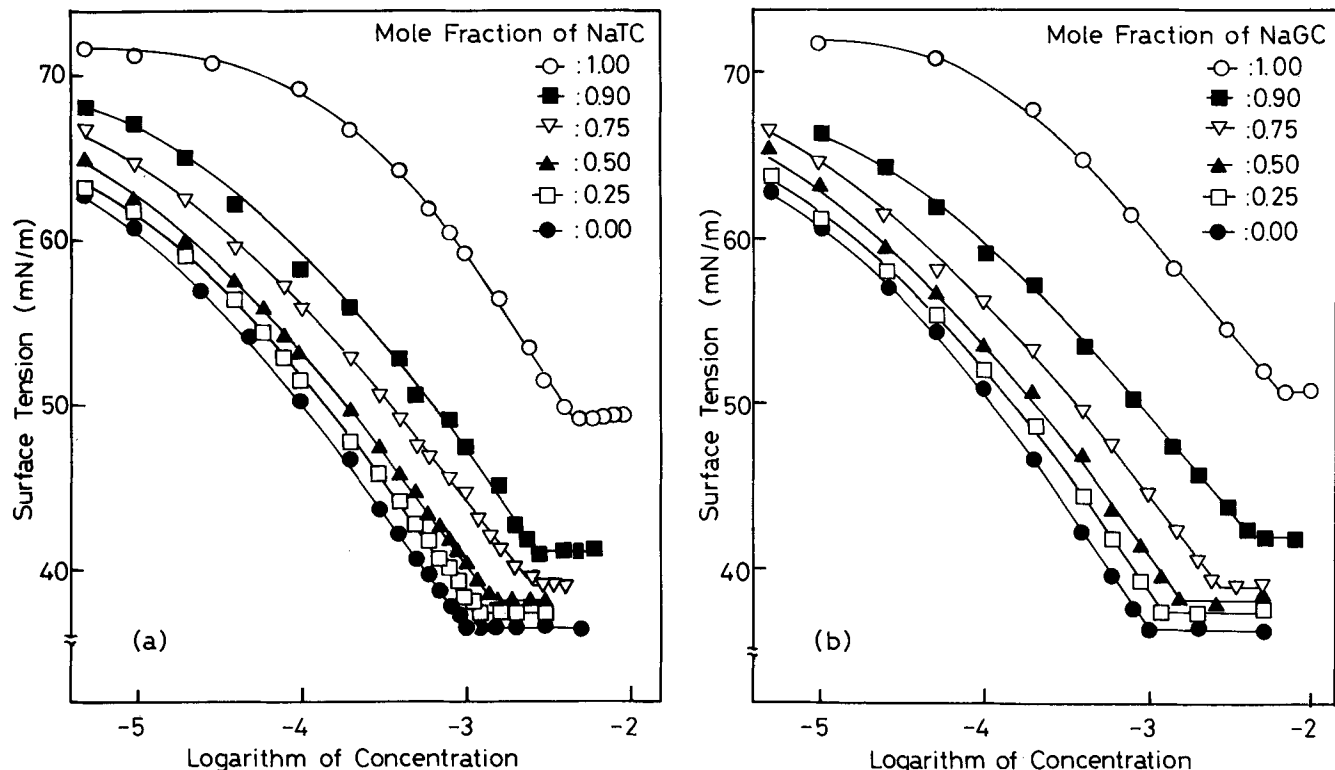
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FIG. 1. Curves of the surface tension for the NaTC-C$_{10}$E$_8$ system (a) and for the NaGC-C$_{10}$E$_8$ system (b) *vs.* total surfactant concentration (logarithmic scale) with various mole fractions of bile salts. Abbreviations as in Table 1.

system for the NaGC-C$_{10}$E$_8$ system showed only one break point, corresponding to a CMC, and these CMC values are listed in Table 1. The mole fractions of bile salt in a mixed micelle were theoretically calculated from these CMC values. If the mixed state of bile salt and C$_{10}$E$_8$ were ideal, the mole fraction of bile salt in a mixed micelle (X$_{ideal}^M$) can be calculated from the apparent mole fraction of bile salt in the solution, X$_{bile}$, and the CMC values for bile salt and C$_{10}$E$_8$ alone (9,16). On the other hand, in the case of nonideal mixture of these two surfactants, the mole fraction of bile salt in a mixed micelle (X$_{excess}^M$) can be determined by a theoretical treatment of the excess thermodynamic quantities of Motomura (8,9,15) from the values of X$_{bile}$ and CMCs for the mixtures. These values (X$_{ideal}^M$ and X$_{excess}^M$) are listed in Table 1.

Figure 2 shows each plot of X$_{excess}^M$ as a function of X$_{bile}$ for the NaTC-C$_{10}$E$_8$ system (a) and the NaGC-C$_{10}$E$_8$ system (b), respectively, and each dotted curve shows X$_{ideal}^M$ values for each system. The X$_{excess}^M$ values for both systems deviated negatively from the dotted X$_{ideal}^M$ curves. These negative deviations suggest that nonionic-rich micelles are formed in these binary mixed solutions and that both systems are nonideal mixtures. However, the extent of deviation from the X$_{excess}^M$ curve for the NaTC-C$_{10}$E$_8$ system is smaller than that for the NaGC-C$_{10}$E$_8$ system, and the former X$_{excess}^M$ values are higher than the latter ones, as shown in Table 1. Small (1) reported that pK$_a$ values for the acid of NaTC were much lower than those of NaGC, and that NaTC has higher solubility in the aqueous solutions than NaGC because

TABLE 1

Critical Micelle Concentration (CMC) for Each Solution of Bile Salts, C$_{10}$E$_8$ and Their Mixtures at 25°C

Mole fraction of bile salts in the solution	CMC (mmol/L)		Mole fraction of bile salt in a mixed micelle X$_{excess}^M$	
x$_{bile}$	NaTC-C$_{10}$E$_8$[a]	NaGC-C$_{10}$E$_8$[a]	NaTC-C$_{10}$E$_8$	NaGC-C$_{10}$E$_8$
0.00	1.00	1.00	0.00	0.00
0.25	1.17	1.16	0.06	0.03
0.50	1.53	1.65	0.17	0.06
0.75	2.04	2.20	0.37	0.12
0.90	2.72	4.04	0.66	0.38
1.00	4.33	6.34	1.00	1.00

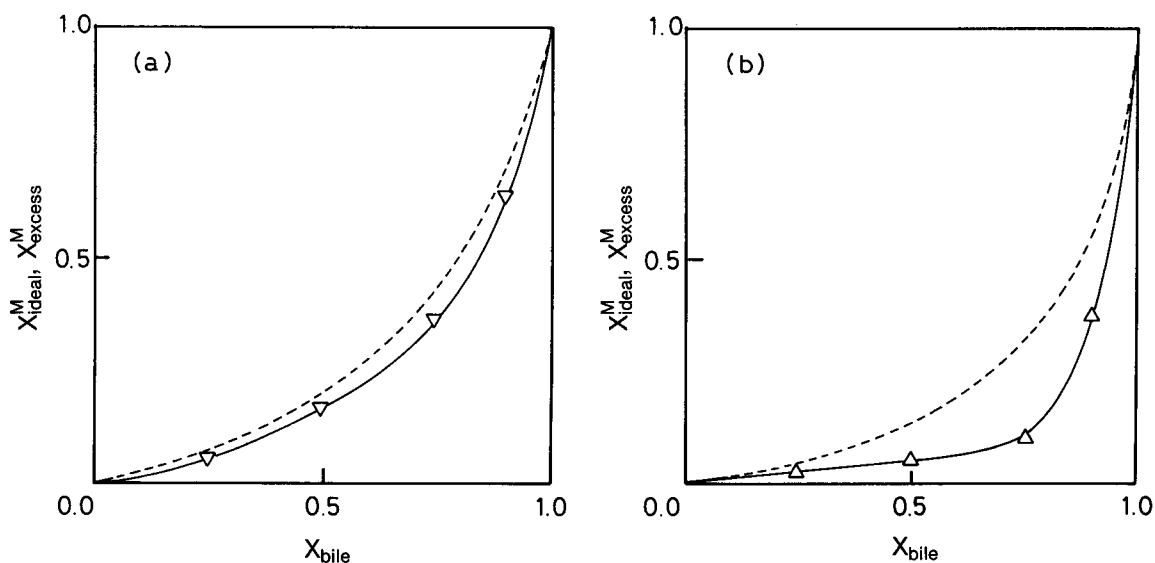[a]NaTC, sodium taurocholate; C$_{10}$,E$_8$, octaoxyethylene glycol mono *n*-decyl ether; NaGC, sodium glycocholate.

FIG. 2. Curves of the mole fraction of bile salts in the mixed micelle, X_{ideal}^M (•••) for an ideal mixture and X_{excess}^M (∇, $\triangle$) calculated from the theory of Motomura (Ref. 15), respectively, vs. the mole fraction of bile salts in the solution, X_{bile}. (a) NaTC-$C_{10}E_8$ system, (b) NaGC-$C_{10}E_8$ system. Abbreviations as in Table 1.

of hydration water around the taurine group. Also, $C_{10}E_8$ is hydrated around the polyoxyethylene chain. Therefore, because the miscibility between NaTC and $C_{10}E_8$ is superior to that between NaGC and $C_{10}E_8$, the deviation from the X_{excess}^M curve for NaTC-$C_{10}E_8$ is expected to become smaller.

Polarities of the mixed micelle. The polarities of in-tramicelles can be estimated by measuring the ratio of the first and third vibronic peaks, I_1/I_3, in a monomeric fluorescence emission spectrum of pyrene (10-12), assum-

ing that the microenvironment of the solubilized pyrene is polar or hydrophilic for high values of I_1/I_3 and non-polar or hydrophobic for low values.

Figure 3 shows the dependency of I_1/I_3 values on total surfactant concentration above the CMCs for the NaTC-$C_{10}E_8$ system (a) and the NaGC-$C_{10}E_8$ system in (b), respectively. In analogy with the results in a previous paper (6-9), the I_1/I_3 values for the mixtures show tend-encies approaching those of $C_{10}E_8$ at low concentration near the CMC. This means that the microenvironments
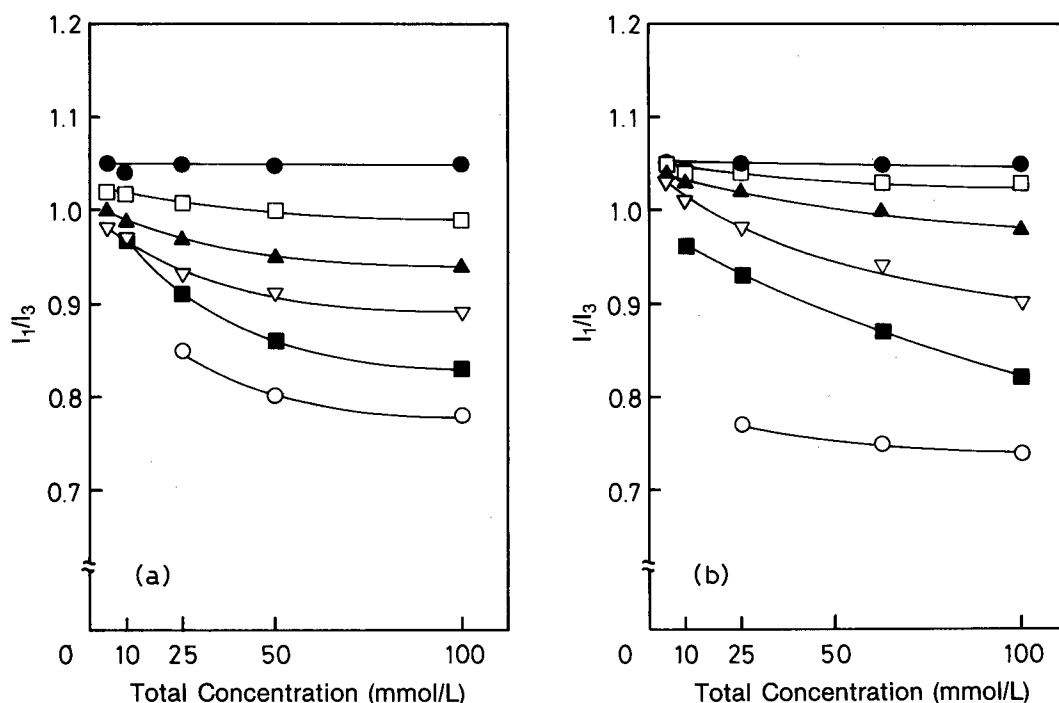

FIG. 3. Curves of I_1/I_3 for the NaTC-$C_{10}E_8$ system (a) and for the NaGC-$C_{10}E_8$ system (b) vs. total surfac-tant concentration with various mole fractions of bile salts; mole fraction of bile salt = 0.00 (●), 0.25 (□), 0.50 (▲), 0.75 (▽), 0.90 (■) and 1.00 (○). Abbreviations as in Table 2.
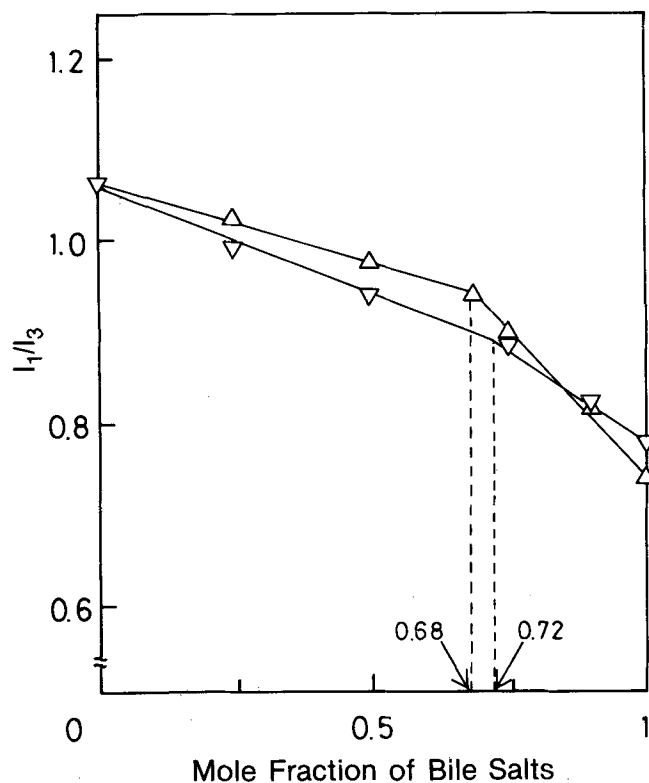
FIG. 4. Plots of I_1/I_3 values for the NaTC-$C_{10}E_8$ system (▽) and for the NaGC-$C_{10}E_8$ system (△) vs. mole fraction of bile salts. Abbreviations as in Table 2.

papers (8,9) a value of 0.69 was given for NaC. In general, these low values for micellar solutions of bile salts suggest that the solubilized pyrene is located in bile-salt micelles with a microenvironment that is nearly as nonpolar as in hydrocarbon solvents, and that bile-salt molecules form more hydrophobic micelles than ordinary surfactants. However, there are differences in the I_1/I_3 values of these bile salts, and the order of the I_1/I_3 values is NaC < NaGC < NaTC. The reason is that NaTC and NaGC molecules differ from the NaC molecule by the taurine or glycine group, and the NaTC molecule has a hydrated taurine group, which is more polar than the glycine group. Accordingly, the solubilized pyrene in NaTC micelles is located in the most polar microenvironment when compared to the other bile salts.

For the mixed NaTC-$C_{10}E_8$ or NaGC-$C_{10}E_8$ systems, the I_1/I_3 values decreased slightly with increasing mole fractions of bile salts and showed break points at the mole fraction of 0.72 for the NaTC-$C_{10}E_8$ system and 0.68 for the NaGC-$C_{10}E_8$ system, respectively. Thereafter, the I_1/I_3 values decreased more steeply above these mole fractions. In general, nonionic surfactants form spherical micelles in dilute solution, whereas bile-salt molecules aggregate because of the hydrocarbon character of the steroid nucleus and form lamellar micelles, so-called back-to-back micelles as shown by Small (1). Therefore, the break points in each curve suggest that these mixed micelles change from $C_{10}E_8$-rich to bile salt-rich, and that below and above each break point micellar shapes change from spherical to lamellar.

In the mole fraction range from 0 to about 0.8 for bile salt, the I_1/I_3 values for the NaTC-$C_{10}E_8$ system were lower than those for the NaGC-$C_{10}E_8$ system, but the I_1/I_3 values were reversed above mole fraction 0.8. The reasons are that the mixed micelles of the NaTC-$C_{10}E_8$ system tend to contain more bile-salt molecules than those of the NaGC-$C_{10}E_8$ system (Fig. 2 and Table 1), and that the miscibility between NaTC and $C_{10}E_8$ molecules in the mixed micelle is better than that between NaGC and $C_{10}E_8$ molecules, due to hydration around the conjugated taurine group of NaTC molecules.

Mean aggregation number. Figure 5 shows plots of mean aggregation numbers, $\bar{n}$, for the NaTC-$C_{10}E_8$ and NaGC-$C_{10}E_8$ systems as a function of the mole fraction of bile salts, and the $\bar{n}$ values are listed in Table 2. Similar to previous results (7–9) of $\bar{n}$ values in the mixed systems

of the intramicelles are nearly the same as those of $C_{10}E_8$ micelles near the CMC. This is in agreement with the results of the theoretical analysis of the mixed micelle composition and suggests that the mole fraction of bile salt in a micelle for each system is much lower than the apparent mole fraction of bile salt.

Figure 4 shows plots of I_1/I_3 values for the NaTC-$C_{10}E_8$ and NaGC-$C_{10}E_8$ systems at a constant total surfactant concentration of 100 mmol/L as a function of the mole fraction of bile salt. These values are listed in Table 2.

The I_1/I_3 values for the single systems of bile salt were 0.78 for NaTC and 0.74 for NaGC, respectively. In previous

TABLE 2

Values of I_1/I_3 and Mean Aggregation Numbers for Various Mole Fractions at 25°C

Mole fraction of bile salts in the solution	I_1/I_3[a]		Mean aggregation number[b] ($\bar{n}$)	
x_{bile}	NaTC-$C_{10}E_8$[c]	NaGC-$C_{10}E_8$[c]	NaTC-$C_{10}E_8$	NaGC-$C_{10}E_8$
0.00	1.05	1.05	69	69
0.25	0.99	1.03	33	36
0.50	0.94	0.98	27	32
0.75	0.89	0.90	22	23
0.90	0.83	0.82	18	18
1.00	0.78	0.74	15	14

[a]Total surfactant concentration is 100/mmol/L. I_1/I_3, ratio of the first and third vibronic peak.
[b]Total surfactant concentration is 50/mmol/L.
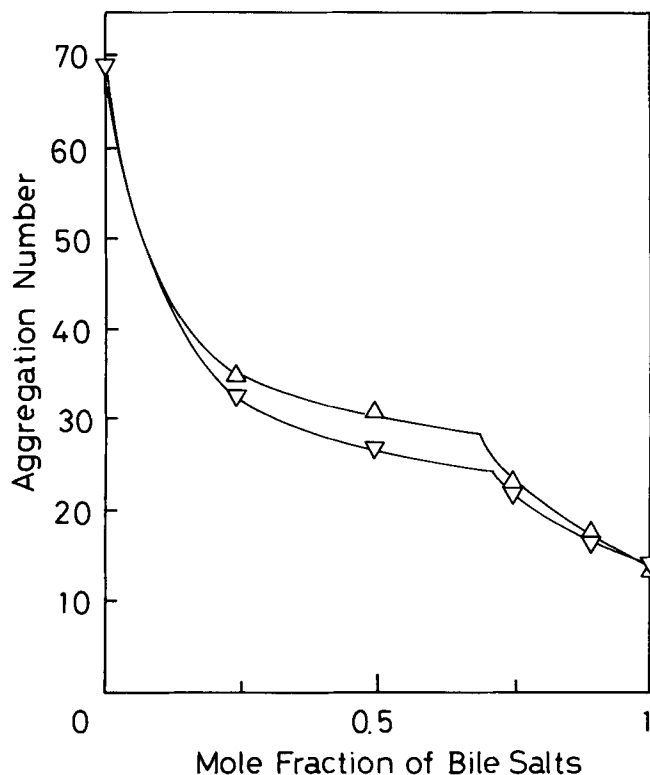[c]Abbreviations as in Table 1.

FIG. 5. Plots of the mean aggregation number for the NaTC-$C_{10}E_8$ system (∇) and for the NaGC-$C_{10}E_8$ system ($\triangle$) vs. mole fraction of bile salts. Abbreviations as in Table 1.

of NaC-$C_{10}E_8$ and sodium deoxycholate-$C_{10}E_8$ (8,9), abrupt decreases of $\bar{n}$ values were observed at low mole fractions of bile salt. Such decreases in $\bar{n}$ values may be attributed to the difference in molecular cohesion between bile salt and $C_{10}E_8$, as well as to the bulky bile–salt molecules.

The $\bar{n}$ values for each single system of bile salt were 15 for NaTC, 14 for NaGC and 15 for NaC, respectively. This suggests that the $\bar{n}$ values are not appreciably influenced by the difference of the conjugation to the terminal carboxyl group.

However, the $\bar{n}$ values for mixtures of the NaTC-$C_{10}E_8$ system were smaller than those of the NaGC-$C_{10}E_8$ system around the mole fractions of 0.25 and 0.50 of bile salts. At these low mole fractions of bile salts, when the X_{excess}^M values for the NaTC-$C_{10}E_8$ system (i.e., the mole fraction of NaTC in a mixed micelle) are larger than those for the NaGC-$C_{10}E_8$ system, the $\bar{n}$ values of the mixed micelles for the NaTC-$C_{10}E_8$ system become small (be-

cause of the lower $\bar{n}$ values for bile salt alone and because of the bulky structure of the bile salt) compared with those for the NaGC-$C_{10}E_8$ system with the same X_{bile}.

From these results, the effects of NaTC and NaGC on mixed micelles in aqueous binary mixtures consisting of bile salt and $C_{10}E_8$ can be described as follows.

Both NaTC-$C_{10}E_8$ and NaGC-$C_{10}E_8$ systems tend to form nonionic-rich micelles in binary mixed solutions. However, the extent of the deviation of the X_{excess}^M curve from the X_{ideal}^M curve for the NaTC-$C_{10}E_8$ system is smaller than that for the NaGC-$C_{10}E_8$ system. The I_1/I_3 values for NaTC-$C_{10}E_8$ mixtures are lower than those for NaGC-$C_{10}E_8$ at low mole fractions of bile salt. This may be due to differences in the hydration around the terminal conjugated group and to the incorporation of pyrene into the mixed micelles. At low mole fractions of bile salt, the $\bar{n}$ values for the NaTC-$C_{10}E_8$ system are lower than those for the NaGC-$C_{10}E_8$ system because of the larger X_{excess}^M values of the NaTC-$C_{10}E_8$ system, the lower $\bar{n}$ values for bile salt alone and the bulky structure of the bile–salt molecules.

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