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ORIGINAL CONTRIBUTION

S. Nagadome
H. Oda
Y. Hirata
H. Igimi
A. Yamauchi
Y. Sasaki
G. Sugihara

Transport of monooleoylglycerol mediated by bile salt micelles across porous membranes

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S. Nagadome · H. Oda
G. Sugihara (✉)
Department of Chemistry
Faculty of Science
Fukuoka University
Nanakuma, Jonan-ku
Fukuoka 814-80, Japan
(Tel) 81+92-871-6631,
(Fax) 81+92-865-6030

Y. Hirata
Department of Industrial Chemistry
Sasebo College of Technology
Okishincho
Sasebo 857-11, Japan

H. Igimi
Shionogi & Co. Ltd.
2-5-1 Mishima, Settsu
Osaka 566, Japan

A. Yamauchi
Department of Chemistry
Faculty of Science
Kyushu University
Higashi-ku
Fukuoka 812, Japan

Y. Sasaki
San-Ei Gen F.F.I. Inc.
1-1-11 Sanwacho, Toyonaka
Osaka 561, Japan

Abstract In order to study how the bile salts and lipids behave in the vicinity of microvillus, the transport properties of a sodium salt of deoxycholic acid (NaDC) and its mixture with monooleoylglycerol (MO) through artificial membranes were investigated in 0.15 M NaCl saline solution at 37 °C.

The hydrodynamic radius of MO-solubilized micelles was estimated to be approximately 17–20 Å from the transport study. The thermodynamically stable MO-NaDC mixed micelles formed above critical micelle concentration in the higher region of mole fraction of NaDC in the mixture (X_{NaDC} > ca. 0.6), can behave as a single species in transport process and freely pass through the porous membranes of both pore sizes, 0.01 μm and 0.1 μm.

The permeabilities of MO-NaDC mixed micelles are large compared with those of pure NaDC micelles. MO molecules solubilized may probably enhance the interaction between MO and NaDC molecules by better contacting with the respective hydrophobic groups in a mixed micelle (the flexible structure of MO molecule enables it), and in this situation, the smaller micelles compared with those of pure NaDC must be more favorable.

Key words Mixed micelle – bile salt – monoacylglycerol – transport – porous membrane

Introduction

It is well-known that bile salts play an important role when some lipids such as acylglycerols migrate in living tissue and are absorbed through intestinal wall [1–4]. In more details, the bile salts form a mixed micelle with phospholipids and solubilize the ingested fat, cholesterol, and fat-soluble compounds. A comprehensive understanding of transport mechanism requires some knowledge of the physicochemical properties of lipids and bile salts regarding their behavior in an aqueous environment [5–8]. Many studies to clarify the biological activities of the bile salts have been presented from pharmacological and physicochemical points of view up to date [9–14]. Nevertheless, the physicochemical mechanism in vivo has not yet

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been clarified; one of the main reasons may be the complexity of the living system. According to the electron microscopic observation, the surface of epithelial cell of the intestine was visualized to be covered by villus on which many microvillus grow thickly and enhance absorption of the digested materials. The spaces of 30–150 μm with 1–1.5 mm in depth for villi and of 0.01–0.1 μm with approximate 1 μm in depth for microvilli respectively, have been reported [15, 16]. Furthermore, the diffusion process around the absorptive villi or microvilli would be a primary process of absorptive mechanisms. It will be required to elucidate how the bile salts behave in the vicinity of this microvillus [16].

In order to extend the understanding for the behavior of bile salts, we, in the present work, examined the transport properties of a typical bile salt and its mixture with monoacylglycerol through artificial membranes. In this model system, sodium salt of deoxycholic acid (NaDC) was used as the bile salt and monooleoylglycerol (MO), one of acylglycerols, was used as a lipid. The artificial porous membranes with 0.01 μm and 0.1 μm in pore size which correspond to the space between microvillus, were chosen because the digestive absorption has been known to take place predominantly in the vicinity of the microvilli. From the present study, the diffusion coefficient or the size of bile salt micelles with or without solubilizing MO in addition to the composition of the mixed micelles will be estimated.

Experimental

Materials

Monooleoylglycerol (MO) was kindly donated by Kao Co. (Tokyo, Japan) and was used as received. Sodium salt of deoxycholic acid (NaDC) was purchased from Calbiochem. Co. (Sandiego, CA, USA) and recrystallized from ethanol. *p*-Aminobenzoic acid (PABA) and other inorganic salts were reagent grades and used as received from Wako Pure Chem. Ind. (Osaka, Japan). PABA was used as a reference for estimating diffusivity of examined substances through the porous membrane. All the used solvents were purified by distillation at least twice or more. In most cases two kinds of porous nitrocellulose membranes (purchased from Sartorius Co.) were employed and their pore size (and thickness) were 0.01 μm (90 μm), and 0.1 μm (100 μm), respectively.

Diffusion cell

The diffusion apparatus was a conventional glass cell which consists of two compartments, each of which has the volume 10 cm^3. The membrane was fixed between the circular face of two chambers. The effective surface area of the membrane was just π cm^2. A silicon O ring at one side of membrane was placed and supported to avoid the leak of solution. The two chambers were double-jacketed for temperature control at 37 °C and the solutions in compartments were stirred at a speed of 400 rpm by magnetic stirrers. This apparatus has been described elsewhere [17].

Preparation of mixed solutions

Respectively known amounts of MO and NaDC were mixed in 0.15 M NaCl saline solution in test tubes with a glass stopper and the mixed solutions were shaken and incubated in a thermostated water bath at 37 °C for 24 h. During the preparation, air in the mixed solution was completely replaced with dry N_2 gas to avoid oxidation of MO. The saline solutions involving only NaDC were prepared with shaking at 37 °C for 2 h at least.

Measurement

In all experiments, the sample solution and saline solution were placed in the left-(Phase I) and right-(Phase II) hand sides of the membrane, respectively. The aliquot with each time interval was withdrawn from the sample solution in the Phase II using a microsyringe and the increase of NaDC and MO concentration with time was determined by means of a spectrophotometer (JASCO UVIDEC-320). Prior to the measurement, a diffusion study across the membrane was carried out using PABA as a standard diffusate [16]. The concentration of PABA was determined by ultraviolet (UV) assay (277 nm). In the case of PABA, the concentration change as a function of time was analyzed by taking 10 μl of the sample solution for each measurement. The concentration of MO was determined by applying the acetylacetone method (Triglyceride-Test, Wako Pure Chem. Ind. Itd.) to each 100 μl of the sample solution [18, 19]. For each 10 μl of the sample solution taken out, NaDC concentration change with time was measured according to the enzymatic assay (ENZABILE-2, Daiichi Pure Chem, Co., Itd.) [20]. The percent transmittance was measured by means of the spectrophotometer at 500 nm.

Results and discussion

First, we will address the mixing ratio of NaDC to MO in the mixture employed for the present study. Figure 1 shows the plot of transmittance of the NaDC-MO mixture

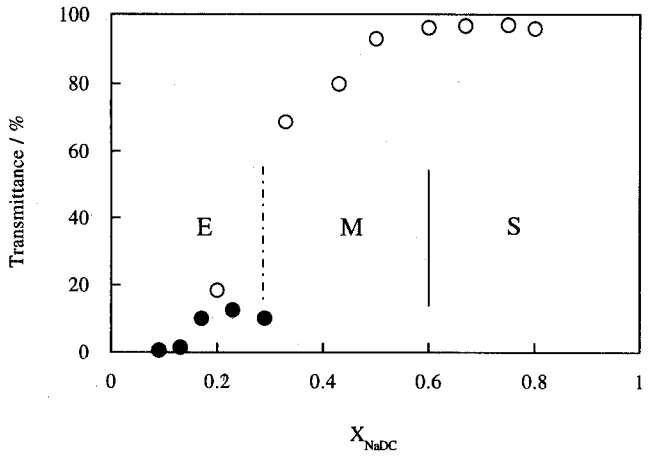


Fig. 1 The plots of transmittance of MO-NaDC mixture in 0.15 M NaCl solution against mole fraction of NaDC in MO-NaDC mixture at 37 °C. NaDC concentration: (●) below 5 mM (CMC), (○) above 5 mM. E,M and S denote regions of emulsion, intermediate state and solubilizing micellar solution, respectively

in saline solution against mole fraction of NaDC, $X_{NaDC} = [NaDC]/\{[NaDC] + [MO]\}$. The solid circles are obtained for the mixed systems containing NaDC the concentration of which ranges below the critical micelle concentration, (CMC ca. 5 mM), indicating that NaDC can help appreciably for MO to disperse, even at the concentration range below CMC. Open circles are for the mixtures of MO and NaDC whose concentration is above CMC. Obviously, the NaDC-MO mixtures are divided into three at X_{NaDC} = ca. 0.3 and ca. 0.6; the mixtures below $X_{NaDC} = 0.6$ were turbid while those above 0.6 were transparent. The regions partitioned by broken and solid lines in terms of transmittance are denoted by E, M, and S as shown in Fig. 1. The region E may be assigned to an emulsion and the region S, micellar solution solubilizing MO. Thus, Fig. 1 indicates that in the mole fraction region above $X_{NaDC} > 0.6$, MO is completely solubilized by NaDC and at $X_{NaDC} = 0.6$, the mixing ratio of MO vs. NaDC is equal to 2:3, meaning that at least three NaDC molecules can solubilize two MO molecules, in other words, thermodynamically stable mixed micelles of NaDC with MO are formed if only there exists 1.5 times more NaDC than MO in molecular number. It is interesting to compare with cholesterol (Ch) solubilization by NaDC; although NaDC as well as sodium salt of chenodeoxycholic acid (NaCDC) is the best solubilizer among all kinds of bile salts, 15 NaDC molecules are needed to solubilize one Ch molecule [6, 12, 21].

It is noteworthy that the region M between about $X_{NaDC} = 0.3$ and 0.6 may be assigned to an intermediate state between emulsion and micellar solution. The phenomenon that the solution properties are different depending on the mole fraction is interesting from the view point of phase equilium or solution state of the mixed system and, indeed, it has attracted some researches to stimulate the investigation in detail [22, 23]. However, further mention about this will not be done hereafter, because the analysis is beyond the scope of the present object. In the present study, we are going to deal with the mixed systems at micellar solution range.

NaDC system

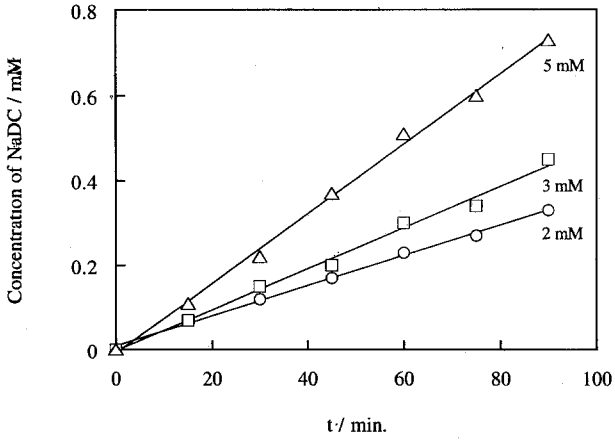
At first, the diffusivity of NaDC across the porous membranes was examined for the NaDC alone dissolved in saline solution at 37 °C. Figure 2 shows the typical results about the concentration changes in Phase II against the lapsed time. At each concentration a good linearity holds over the NaDC concentration range examined. This suggests that the transport of the system is at a steady state. The flux of NaDC, J_{NaDC}, can be obtained by dividing the slope by the effective membrane area [24], and the results obtained about two different membranes are given as a function of NaDC concentration in Fig. 3.

Figure 3 demonstrates that there appears a sharp kink point at a certain NaDC concentration. Irrespective of difference in pore size of the membranes, the break took place at the same NaDC concentration. This suggests that the flux change was caused by micelle formation of NaDC, and the concentration giving the break point corresponds to the CMC's value in literature [6, 25].

Generally, the flux, J_M of a solute M across the membrane, is described simply as a function of solute concentration, C_M,

$$J_M = P_M \cdot \Delta C_M \quad (1)$$

Fig. 2 Concentration change with time in Phase II as a function of initial NaDC concentration in Phase I. Numerical values indicate the concentrations of NaDC. Flux (J) is determined from the slope (in 0.15 M NaCl solution at 37 °C)



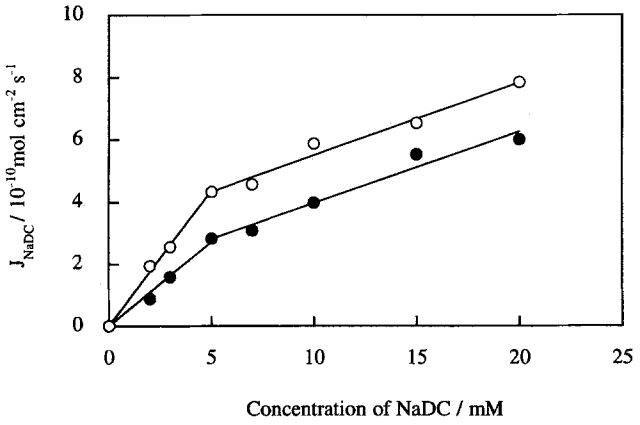


Fig. 3 Flux (J) changes of NaDC alone systems with concentration through the membranes of pore sizes 0.01 μm (●) and 0.1 μm (○) (in 0.15 M NaCl solution at 37 °C)

where P is the permeability coefficient having the dimension of velocity, ΔC, the concentration difference between Phases I and II, and the subscript M, the solute species, respectively. Thus, each slope below and above the intersection concentration (CMC) turns out to be the permeability coefficients concerning the monomeric and micellar states, respectively, and Eq. (1) tells us that the permeability for each state can be obtained from the slope in Fig. 3. Since a PABA molecule is sufficiently smaller than pore sizes in this study, the diffusion coefficient of PABA in aqueous solution can be used as a reference value even in porous membrane. Thus, the following relation may be applied to estimate the diffusion coefficients at below and above CMC,

$$P_{PABA}/D_{PABA} = P_{NaDC}/D_{NaDC}\,, \tag{2}$$

where D denotes the diffusion coefficient. This equation is given on the basis of an assumption that in the expression of the permeability, $P_i = f\beta_i D_i$, the product $f\beta_i$ was supposed to be common to PABA and NaDC, where f is the membrane coefficient and β_i is the partition coefficient between bulk solution and membrane phase for a solute i. This means that these two solutes have a nearly same apparent membrane coefficient $f_i^*(=f\beta_i)$ [26]. The relation of Eq. (2) is further extended to the following Eq. (2') by assuming that the temperature dependence of f_i^* is negligible.

$$\frac{P_{PABA}^{25°C}}{D_{PABA}^{25°C}} = \frac{P_{PABA}^{37°C}}{D_{PABA}^{37°C}} = \frac{P_{NaDC}^{37°C}}{D_{NaDC}^{37°C}} \tag{2'}$$

Here, P_i values are all measurable and the literature value of D_{PABA} at 25 °C is available ($D_{PABA} = 8.4 \times 10^{-6}$ cm^2 s^{-1} at 25 °C [16, 27]) so that the unknown diffusion coefficients of NaDC at monomeric and micellar states are assumed to be determinable. The P values of PABA were preliminarily determined at 25 °C (and 37 °C) for 0.01 μm and 0.1 μm of membranes as 1.1×10^{-4} cm·s^{-1} (25 °C) and 1.2×10^{-4} cm·s^{-1} (25 °C), respectively, including that the apparent membrane coefficients themselves differ depending on the pore size. However, as long as the membranes of the same pore size are used, the apparent membrane coefficient can be canceled out in Eq. (2) and (2') so that PABA can be used as a reference to estimate the D values of NaDC. According to the treatment described above, P_{NaDC} and D_{NaDC} values for two membranes different in pore size were obtained and given in Table 1. As expected, the diffusion coefficients through different membranes indicated almost the same values for the respective membranes, but different values above and below CMC of NaDC. The monomeric NaDC molecules can migrate through the membrane faster than the aggregates.

It is of interest to estimate the size of migrating molecules within the porous membrane using the Stokes–Einstein relation, $D = kT/6\pi\eta r$, where k is the Boltzmann constant, T is the Kelvin temperature, η is the viscosity of the continuous phase, and r is the hydrodynamic radius. The estimated radii are also included in Table 1, giving us the information with respect to micellar size. Judging from radius value of micelle (17–20 Å), it can be easily understood that even micelles can go through porous membrane having 0.01 μm in pore size. In the 0.15 M NaCl saline solution at 37 °C, a NaDC micelle is estimated to be composed of ca. 40–60 NaDC molecules from such a simple calculation: $4\pi r^3/3$ was divided by 530 Å^3 (The molecular volume of a sodium salt of bile acid is ca. 530 Å^3 in dilute aqueous solution [10, 28]). The calculated aggregation numbers coincide well with the data determined by means of light-scattering measurement [29].

NaDC-MO mixed system

In the case of the mixed solution of NaDC with MO, the situation is not so simple as that of the NaDC alone

Table 1 Permiability (P), diffusion coefficient (D) and hydrodynamic radius (r) of monomer and micelle of NaDC

	Monomer		Micelle	
	0.01 μm	0.1 μm	0.01 μm	0.1 μm
P (10^{-5} cm·s^{-1})	5.4	8.7	2.2	2.3
D (10^{-6} cm^2·s^{-1})	4.8	6.4	1.9	1.7
r(Å)	6.8	5.1	17	20

solution system. As seen in Fig. 1, depending on the mixing ratio either solubilization or emulsification takes place. Figure 4 indicates NaDC and MO fluxes across the membrane of 0.01 μm as a function of NaDC concentration under a fixed MO concentration, 10 mM. These results are shown in Fig. 4 by open circles for NaDC and by closed circles for MO, respectively. In this figure, the fluxes increased linearly with increase in NaDC concentration, but deviated from the linearity at a concentration lower than 20 mM of NaDC. The NaDC concentration giving the linearity is within the range above $X_{NaDC} = 0.6$ (see Fig. 1). The measured points at the lowest concentration (10 mM of NaDC) are for the mixed system of $X_{NaDC} = 0.5$, and the deviation from the linearity is interpreted by the fact that the measured point is assigned to the intermediate region, *M*, between emulsion and solubilization, as is seen in Fig. 1. In this region somewhat larger particles (like micelles swelled with supersaturated MO) in between emulsion and micelle may be allowed to pass through the pore.

In Fig. 5 the flux of MO-NaDC micellar solution in which MO concentration is fixed at 10 mM, is plotted against NaDC concentration for the membranes of 0.01 μm (a) and 0.1 μm (b). Although the MO concentration is fixed, a slight increase in amount of transported MO was observed with increase in NaDC concentration. Since all the mixtures given in Fig. 5 are ranged between $X_{NaDC} = 0.67$ and 0.80, the samples examined are all micellar solution and the slightly lower value of flux at lower NaDC concentration is attributed to the smaller concentration of micellar species because the concentration is closer to CMC and singly dispersed species of NaDC can scarcely carry MO molecule.

Contrary to Fig. 5, Fig. 6 shows that the flux was measured as a function of solubilizate concentration in the micellar solution systems, the NaDC concentration of which is kept at 40 mM for both membranes. The flux (open squares) increases very slowly with increase in MO concentration. Comparing the slopes of the flux vs. concentration curves of the micellar solutions (open squares) given in Figs. 5 and 6, the permeability of MO-solubilized micelles is more effectively enhanced by increasing NaDC concentration. Interestingly, the curve of NaDC (open circles) has a negative slope, as shown in Fig. 6. This negative slope does not mean that the permeability of NaDC is negative. This apparent decrease corresponds to the relative decrease in NaDC content in the MO-NaDC mixture.

If the micellar components, MO and NaDC, were the same in Phases I and II, i.e., before and after transport through the membrane, the flux fraction of MO and NaDC to the total, Y_m, should have a linear relation of a) $Y_m(NaDC) = X_{NaDC}$ or $Y_m(MO) = 1 - X_{NaDC}$ when MO concentration is fixed, and b) $Y_m(MO) = X_{MO}$ or $Y_m(NaDC) = 1 - X_{MO}$ when NaDC concentration is fixed. These relations are given by solid lines in Fig. 7(a) and (b), respectively. From the data in Figs. 5 and 6, the

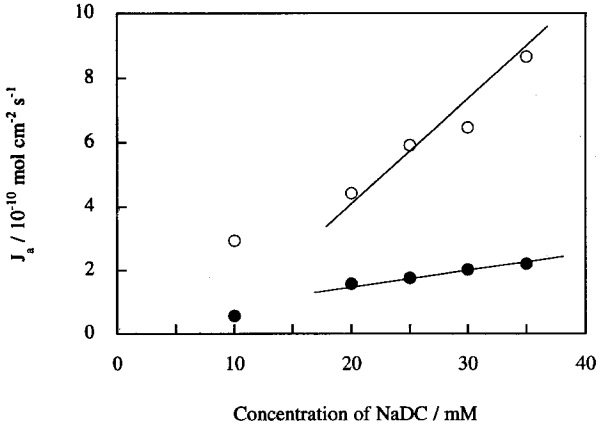


Fig. 4 The fluxes (J_a) of MO (●) and NaDC (○) as a function of concentration of NaDC in mM (in 0.15 M NaDC solution at 37 °C)

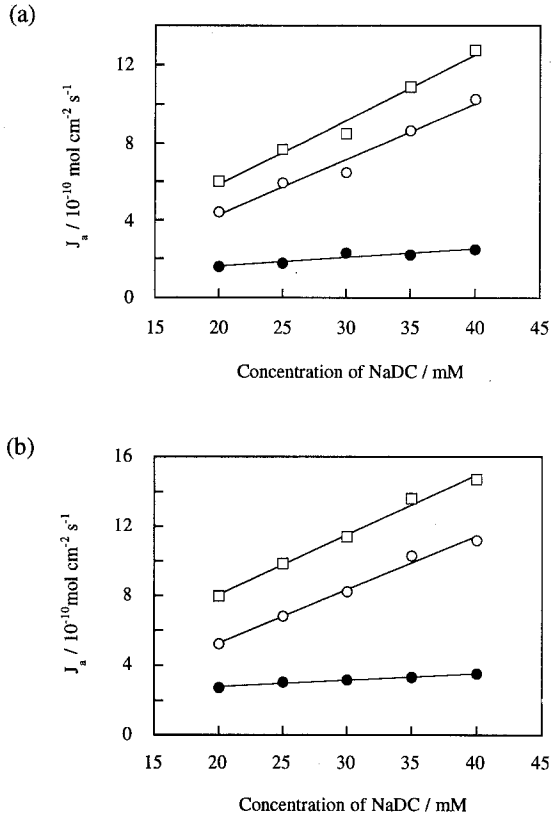


Fig. 5 The plots of fluxes (J_a) of MO (●), NaDC (○) and the total (□) against NaDC concentration for the systems in which MO concentration is fixed at 10 mM (in 0.15 M NaCl solution at 37 °C). Membrane pore size: (a) 0.01 μm, (b) 0.1 μm

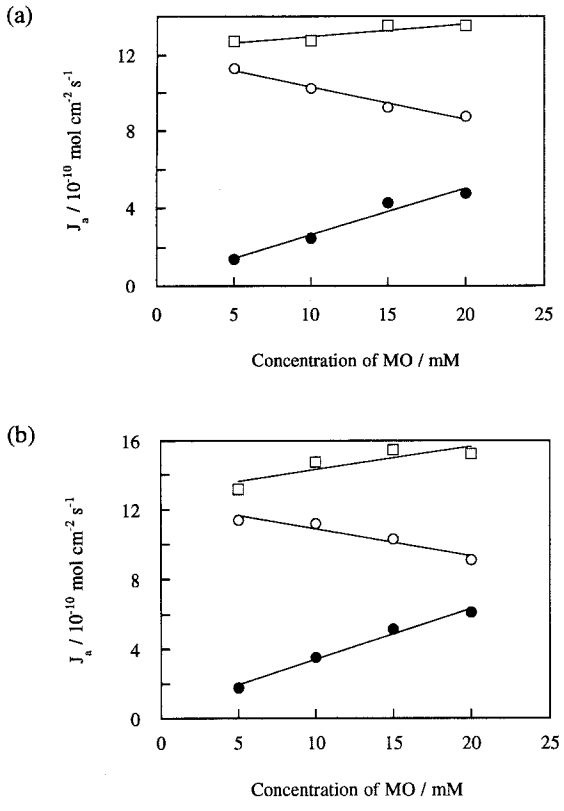


Fig. 6 The plots of fluxes (J_a) of MO (●), NaDC (○) and the total (□) against MO concentration for the systems in which NaDC concentration is fixed at 40 mM (in 0.15 M NaCl solution at 37 °C). Membrane pore size: (a) 0.01 μm, (b) 0.1 μm

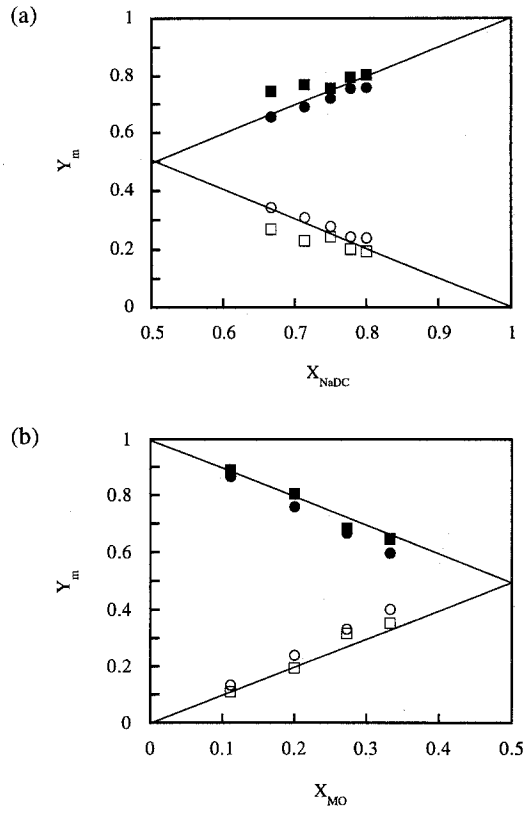


Fig. 7 The relations of mole fraction of MO-NaDC mixture, (a); X_{NaDC} and (b); X_{MO} with flux fraction, Y_m(NaDC) and Y_m(MO), where $Y_m(\text{NaDC}) = J_{(\text{NaDC})}/\{J_{(\text{NaDC})} + J_{(\text{MO})}\}$ and $Y_m(\text{MO}) = J_{(\text{MO})}/\{J_{(\text{NaDC})} + J_{(\text{MO})}\}$. Squares: pore size = 0.01 μm; □ = MO and ■ = NaDC. Circles: pore size = 0.1 μm; ○ = MO and ● = NaDC

flux fraction was calculated for each measured point and plotted against the mole fraction in Phase I, i.e., before transport through the membrane. Figure 7 shows that the measured points are generally in good agreement with the theoretical (hypothetical) lines; this strongly suggests that the MO solubilized micelles can pass through the membrane as just formed in Phase I. In other words, the MO-NaDC mixed micelles formed with given mixing ratio are acting as a single species. Looking at measured points in more details, the higher mole fraction of NaDC results in better coincidence, and the lower mole fraction shows larger deviation from the curve; this has been caused by the lower concentration of micellar species due to the closer to the CMC.

In this experiment, transport of MO-solubilized micelles has been investigated for two kinds of series, i.e., one concentration was fixed and the other concentration was changed between MO and NaDC. In order to obtain the information on component dependence in micellar size, shape, and so on, we should have measured the flux as a function of mole fraction in the mixture for solutions in which mole fractions are discretely fixed by changing the concentration. However, we can derive another important information from the results shown in Figs. 5 and 6, as follows.

Let us consider the curves of $J_{(\text{MO})}$ in Fig. 6. Taking account into the fact that the MO-NaDC mixed micelle (MO-solubilized micelle) behaves as a single species, $J_{(\text{MO})}$ corresponds just to the flux of micelles themselves; the fluxes can be described by the expression, $J_{(\text{MO})} = P_{(\text{MO})} \Delta C_{\text{MO}}$ respectively and the permeability, $P_{(\text{MO})}$ should be equal to that of the micelle at the given X_{NaDC}. The linearity of $J_{(\text{MO})}$ against MO concentration change suggests that $P_{(\text{MO})}$ is independent of MO concentration change and thus the diffusion coefficient as well as the hydrodynamic radius is constant over the concentration range studied. As mentioned above, $P_{(\text{MO})}$ represents the permeability of the MO-NaDC micelles so that the diffusion coefficient D and the hydrodynamic radius r estimated from $P_{(\text{MO})}$ value can be regarded as D and r of the micelle itself. A similar consideration applies to the curve of $J_{(\text{NaDC})}$ in Fig. 5; the curve linking the experimental points is also likely to be linear and its slope is similar to that of $J_{(\text{MO})}$ in Fig. 6. Each $J_{(\text{NaDC})}$ value may be regarded

as being equal to $J_{(\text{mixed micelle})}$, but this $J_{(\text{NaDC})}$ curve involves the contribution from the singly dispersed NaDC species. Therefore, $J_{(\text{NaDC})}$ in Fig. 5 cannot be just equal to the $J_{(\text{MO})}$ in Fig. 6.

The permeabilities of MO-NaDC mixed micelles were estimated from the $J_{(\text{MO})}$ in Fig. 6 as 2.4×10^{-5} cm·s^{-1} for pore size 0.01 μm and 3.0×10^{-5} cm·s^{-1} for pore size 0.1 μm, respectively. These values are large compared with those of pure NaDC micelles, implying that the solubilization of MO by bile salts leads to a reduction of micellar size. MO molecules solubilized may probably enhance the interaction between MO and NaDC molecules by better contacting with the respective hydrophobic groups in a mixed micelle (the flexible structure of MO molecule enables it), and in this situation, the smaller micelles compared with those of pure NaDC must be more favorable.

Next, a thermodynamic consideration about the prominent negative slope of $J_{(\text{NaDC})}$ in Fig. 6 shall interpret the cause in terms of chemical potential of micelle. If the chemical potential of a micelle is expressed as $\mu = \mu^\circ + kT \ln X_{\text{NaDC}}$ where k is the Boltzmann constant and μ° is the standard chemical potential at temperature T, μ is lowered with lowered X_{NaDC} accompanied by increase in MO concentration. The decrease of μ with C_{MO} predominantly results in the lowering of $J_{(\text{NaDC})}$. The good linearity seems to mean that the mixing of MO and NaDC is nearly ideal or energetically favorable. On the other hand, as for the positive slope of $J_{(\text{NaDC})}$ in Fig. 5 it may be considered that the increase in micelle number with increase in NaDC concentration primarily contributes to the increase of $J_{(\text{NaDC})}$. In this case the chemical potential as a function of micellar concentration is raised with NaDC concentration, so that it leads to the positive slope. In conclusion, as compared with micelles consisting of pure NaDC molecules, smaller but well mixed micelles are formed with MO; in other words, NaDC can well solubilize MO by enhancing the hydrophobic interaction with each other. The thermodynamically stable MO-NaDC mixed micelles which are formed above CMC in the region of NaDC content, ($X_{\text{NaDC}} >$ ca. 0.6) can behave as a single species in transport process and freely transport through the porous membranes of both pore sizes, 0.01 μm and 0.1 μm.

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