Communications to the Editor

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INTERACTION OF β-CYCLODEXTRIN WITH BILE SALTS IN AQUEOUS SOLUTIONS

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β-Cyclodextrin (β-CD) forms inclusion complexes with bile salts (sodium cholate, sodium deoxycholate, sodium glycocholate and sodium taurocholate) in aqueous solutions. In the presence of bile salts, the guest molecules of β-CD complexes are excluded from the cavity of β-CD and the free molecules increase with the concentration of bile salt up to cmc. Above cmc they are partitioned between the aqueous and micellar phases. Below cmc the exchange reaction proceeds depending on the formation constants of the guest molecule with β-CD and the concentration of bile salt. Above cmc, the free molecules in aqueous phase decrease with increasing concentration of bile salt because of the partitioning to the micellar phase. These results may be related to the absorption of β-CD complexes administered orally and also the metabolism of cholesterol when the complexes are administered orally for a long period of time.

KEYWORDS ————formation constant; bile salt - β-CD complex; partition coefficient; ANS; TNS; cmc; host-guest exchange reaction

The inclusion complex of β-CD administered orally may be absorbed from the small intestine in a free form.[1] The liberation mechanism of the guest molecule from the complex is very complicated. Beside the simple dissociation mechanism, many kinds of hydrophobic compounds in the meal and secretions are concerned with the exchange of the guest molecule. Particularly the interaction of bile salts with the β-CD complex seems to be important, because the guest molecule and bile salts have hydrophobic moieties and the exchange of the guest molecule may possibly take place in the duodenum, where large amounts of bile salts are excreted from the gallbladder.

On the other hand, the total amount of liver lipids and triglycerides of rat decreased by food containing large amounts of CD mixture eaten over long period of time.[2]

From these viewpoints, we investigated the complex formations of TNS (6-toluidinylnaphthalene-2-sulfonate) and ANS (8-anilinonaphthalene-1-sulfonate) with β-CD in aqueous bile salts solutions.

ANS and TNS form inclusion complexes with β-CD[3,4] in aqueous solutions and

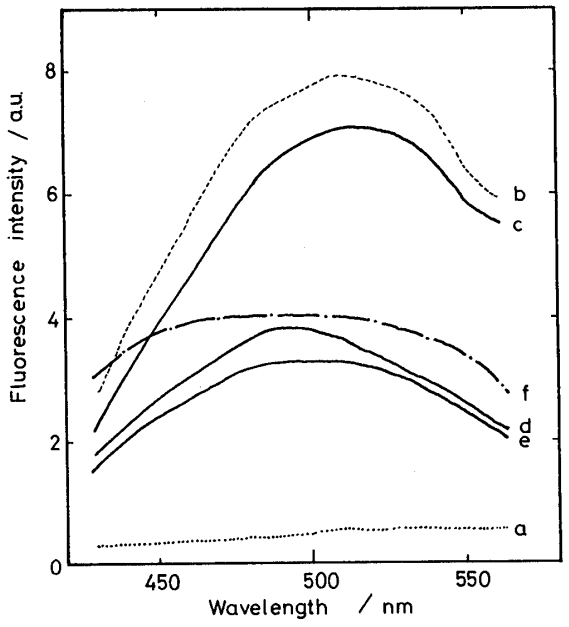

Fig. 1. Effect of β-CD and Sodium Cholate on the Fluorescence Spectrum of TNS in Aqueous Phosphate Buffer Solution (pH: 7.2 Ionic Strength: 0.024)
a) 1 x 10^{-4} M TNS, b) (a) + 2 x 10^{-3}M β-CD, c) (b) + 1 x 10^{-3}M sodium cholate, d) (b) + 5 x 10^{-3}M sodium cholate, e) (b) + 10 x 10^{-3}M sodium cholate, f) (b) + 12 x 10^{-3}M sodium cholate.

the fluorescence intensities at 510 and 480 nm increase with the concentration of β-CD. However, as seen in Fig. 1, the fluorescence intensities are decreased by the addition of sodium cholate up to the cmc and then increase with the concentration of sodium cholate. This indicates that the exchange of guest mole takes place up to the cmc and above that the free TNS molecules are partitioned between the aqueous and micellar phases. By modifying the method of Matsui,[5] who determined the formation constants of various alcohol-CD complexes with Methyl Orange, we determined the formation constants of bile salts - β-CD complexes with TNS, instead of Methyl Orange, in aqueous phosphate buffer solution. The fluorescence intensities of TNS in aqueous bile salt solutions increase gradually with the concentration of bile salt, even below the cmc. The increment of fluorescence intensity based on the interaction of TNS with bile salt below cmc corresponds to 10-15% of the total intensity of TNS in a mixed solution of TNS, β-CD and bile salt, depending on the kinds and concentration of bile salt. Taking this effect into account, the formation constants of bile salt - β-CD complexes determined by this method are shown in Table I, on the assumption that each bile salt forms a 1-1 complex with β-CD. The partition coefficients of ANS and TNS were

Table I. Characteristics of Bile Salts in Aqueous Buffer Solution at 25 C°

Bile salt	cmc /mM	Formation constant /M^{-1}	Partition coefficient P	
			ANS	TNS
Sodium cholate	9.0	1100	44	350
Sodium deoxycholate	4.2	2670	30	110
Sodium glycocholate	7.0	410	26	220
Sodium taurocholate	6.0	406	50	160

$P = (x/V_m) / \{(a - x)/V_w\}$, (a: total mole of dye, X: mole of dye in micelle, V_m: volume of micellar phase, V_w: volume of aqueous phase)

determined from the slope and the intercept in the reciprocal of the fluorescence intensity vs. volume fraction of the micellar phase curve (above cmc), assuming that the density of micelle is unity.[6] The cmc of bile salt in aqueous buffer solution was determined by fluorometry with TNS.[7] Using these values and the formation constants of ANS- and TNS - β-CD complexes (ANS: 72, TNS: 1000 M^{-1}), the concentration of the guest molecules of each species is calculated as a function of the concentration of bile salts. This assumes that the cmc's of bile salts in buffer solutions containing 2 mM β-CD are the concentrations of free bile salts corresponding to the cmc in buffer solutions and the CD complexes are not partitioned into the micellar phase. The exchange profiles thus obtained are shown in Fig. 2. The concentration of free ANS and TNS increases with the concentration

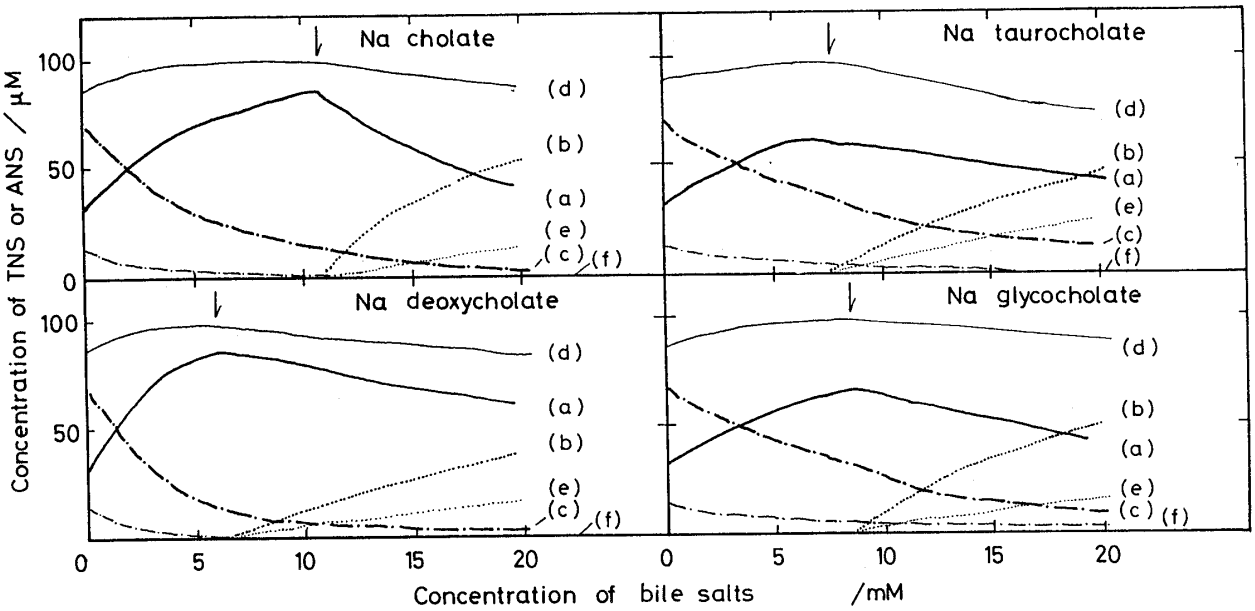

Fig. 2. The Concentrations of Free Dyes in Aqueous and Micellar Phases and the Concentration of Dye - β-CD Complexes in Aqueous Phase as a Function of Bile Salts Concentration in the Presence of 2 mM β-CD (Total concentration of dye : 4×10^{-4}M)
a) Free TNS in aqueous phase, b) TNS in micellar phase, c) TNS - β-CD in aqueous phase, d) Free ANS in aqueous phase, e) ANS in micellar phase, f) ANS - β-CD in aqueous phase.

of bile salt up to the cmc in buffer solution containing 2 mM β-CD and decreases beyond that. This is because both dyes are partitioned into the micellar phase of bile salt above cmc. The maximum concentrations of free dyes indicated by the arrow signs correspond to the cmc's in buffer solution containing 2 mM β-CD. They are larger than the cmc's in Table I, because the concentration of free bile salt decreases in the presence of β-CD by complexing with the β-CD and the complexed bile salt molecules can not participate in micelle formation. The concentration of

free guest molecules depends on the magnitude of the formation constant of guest molecules relative to that of bile salts. Above cmc, both the partition coefficient and the formation constant of dye are the key factors determining the free concentration. Compared with TNS, the concentration of free ANS is always larger than that of TNS at constant bile salt concentration. This is because of the small formation constant and partition coefficient of ANS. The amount of TNS solubilized in micelle is appreciable, especially in high concentrations of bile salt. This implies that the absorption of hydrophobic guest molecules may take place via solubilized micelle in the small intestine, as in the case of oily compounds.[8] In general, the larger the formation constant and the partition coefficient, the smaller the number of free guest molecules in the aqueous phase.

The more effective absorption of drugs with CD-complex is considered to be attributable to the constant supply of free guest molecules regulated by the dissociation of the CD-complex in the gastro-intestinal tract. However, our results indicate that the interaction of CD complex with bile salt plays an important role in the absorption of orally administered CD complex.

As shown in Fig. 2, we can calculate the concentration of free guest molecules as a function of bile salt concentration if we know the formation constant and the partition coefficient of the guest molecule. This may be useful for analyzing absorption of CD complex in vivo. Bile salts are produced by the catabolism of cholesterol in human liver. Judging from the fact that the CD complex is not absorbed from the small intestine, the oral administration of CD complex for a long period of time may possibly reduce the cholesterol in the human body through the consumption of bile salts by the above mentioned mechanism.

The natural bile excreted from the gallbladder contains not only bile salts but also lecithin and cholesterol, that is, bile salts are excreted as a mixed micelle. The cmc of these mixed micelles is far lower than that of bile salt alone. So our research is directed to the interaction of mixed micelle with CD complex.

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