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Solubilization of *dl*-α-Tocopherol by Bile Salts, Polysorbate 80 and Egg Lecithin

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The solubilization of *dl*-α-tocopherol (VE) into micellar solutions of bile components and non-ionic polysorbate 80 (PS-80) was studied. Dihydroxy conjugated bile salt showed a higher saturation ratio than trihydroxy conjugated bile salt. The mixed micelles of bile salt with egg lecithin (PC) solubilized more VE than the bile salt alone. PS-80 micelles showed a saturation ratio approximately 20 times higher than those of bile salts. When PC was included in PS-80 micellar solution, the solubility of VE decreased with increasing PC content. The order of micellar size of bile salts and PS-80 solubilizing VE, as measured by gel filtration, was sodium taurocholate, sodium taurodeoxycholate and PS-80.

Keywords——*dl*-α-tocopherol; solubilization; micellar solution; bile salt component; polysorbate 80; egg lecithin; micellar size; gel filtration

It is well known that the intestinal absorption of lipophilic substances and insoluble drugs is dependent on the type and the concentration of bile salts and surfactants present.[2] Bile salts and surfactants are considered to enhance the intestinal absorption of drugs by modification of the physico-chemical properties of the drug in solution as a result of micelle formation, and also by altering the permeability of the biological membrane.[2]

dl-α-Tocopherol (VE) has a terpene-like molecular structure and is an insoluble nonswelling amphiphilic lipid. Some studies[3] showing that the uptake of VE by intestinal mucosa depends on the presence of sufficient bile salts to solubilize VE into a micellar phase in the intestinal lumen have been reported. The solubilization of VE by bile salts, however, has not been studied in detail. Thus, in this paper the solubilization of VE and the properties of micellar solutions of bile salts, egg lecithin and polysorbate 80 were studied in order to clarify the mechanism of intestinal absorption of VE.

Experimental

Materials——Sodium deoxycholate (SDC), sodium taurodeoxycholate (STDC), sodium glycochenodeoxycholate (SGCDC), sodium taurocholate (STC), and sodium taurochenodeoxycholate (STCDC) were purchased from Sigma Chemical Co., Ltd., U.S.A. Sodium glycocholate (SGC) and sodium glycodeoxycholate (SGDC) were obtained from Calbiochem Co., Ltd., U.S.A. These materials were used without further purification. These reagents each showed a single spot on a silica gel plate (Merck, Darmstadt, West Germany) when developed with a solvent system[4] composed of propionic acid–isoamyl acetate–*n*-propanol–water (3 : 4 : 2 : 1, v/v/v/v). L-α-Lecithin from egg yolk (PC) was obtained from Calibiochem Co., Ltd. The stability was examined on a silica gel plate developed with mixed solvent (chloroform–methanol–water, 65 : 25 : 4, by volume).[5] Polysorbate 80 (PS-80) was supplied by Kao-Atlas Co., Ltd., Japan. *dl*-α-Tocopherol (VE), supplied by Wako Pure Chem. Ind., Ltd., Japan, was purified by alumina column chromatography[6] with benzene–ethanol (2 : 1, v/v) in the dark, to remove oxidized materials. Sephadex G-75 was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. The proteins used for determining the micellar size were bovine serum albumin (fraction V), cytochrome c and chymotrypsinogen A from Sigma Co., Ltd., U.S.A. All other reagents were of analytical grade.

Measurement of VE Solubility——The method of Hofmann[7] was slightly modified as follows; 1 ml of Krebs

bicarbonate buffer (pH 7.0, 5 mM KCl, 1 mM KH_2PO_4, 8 mM $NaHCO_3$, 136 mM NaCl) containing a bile salt or PS-80 with or without PC and a few drops of VE were put in a glass ampoule, which was filled with nitrogen gas. After being sealed, each ampoule was shaken at 37±0.1 °C for 48 h. The opaque solution was centrifuged (20000 *g*) at 37 °C for 60 min. Finally, the oily upper layer was removed by aspiration. The remaining layer was gently filtered through a 0.22 μm membrane filter (GSWP01300, Millipore Ltd.) under atmospheric pressure in order to remove the insoluble VE. The initial few drops were discarded, and the following filtrate was collected.

The solubilized VE was assayed by the modified Emery–Engel procedure;[8] 0.5 ml of 0.4% bathophenanthroline (Wako Pure Chem. Ind., Japan) in absolute ethanol and 0.5 ml of 0.2% ferric chloride (commercial product) in absolute ethanol were added to an aliquot of the test solution in a 10 ml glass-stoppered amber flask. After mixing, absolute ethanol was added and the mixture was shaken well again. The flask was allowed to stand for 10 min at room temperature, then the absorbance at 530 nm against absolute ethanol as the reference was measured. A standard calibration curve for VE was prepared with ethanol solutions containing 0—100 μg of VE. A straight line passing through the origin was obtained up to 100 μg of VE.

Determination of Micellar Size——An experiment was performed by gel permeation chromatography on a Sephadex G-75 column (2 cm i.d. × 50 cm) preequilibrated and eluted with the micellar solution used to solubilize VE. The flow rate of the column was kept at 12 ml/h and the eluate was collected in fractions of 3 ml each. The elution was done at room temperature. The concentrations of protein and VE were measured spectrophotometrically at 280 and 293 nm, respectively. The distribution coefficient (K_d) was calculated from the following equation:[9]

$$K_d=(V_e-V_o)/(V_t-V_g-V_o)$$

where V_o, the void volume of the column, was obtained by using blue dextran 2000, on the assumption that the blue dextran was completely excluded from the gel. The concentration of blue dextran was measured spectrophotometrically at 600 nm. V_e, the elution volume, was regarded as the volume at which the maximal concentration of the eluted component appeared. V_g and V_t are the gel volume and total volume of the gel bet, respectively, and (V_t-V_g) was estimated by assuming water regain[9] of the gel.

Results and Discussion

Effects of Bile Salt Type and Concentration on Solubilization of VE

In Fig. 1, the time course of VE solubility in STDC micellar solution is shown. The solubilization reached equilibrium within 24 h, and therefore the incubation time was set at 48 h. Solubilization of VE in various bile salt solutions is shown in Fig. 2. The critical micelle concentration (cmc) was determined by extrapolating the linear part of the solubilization curve and obtaining its intercept with the horizontal axis. The saturation ratio (SR) was obtained from the slope of the linear portion of the solubilization curve above the cmc.[7] The cmc and SR values are given in Table I. As shown in Fig. 2, all conjugated bile salts and SDC almost linearly increased the VE solubility with increase of their concentration. The solubilization in SGC showed some tendency to saturate, but SGC was fully dissolved in the buffer. The SR values decreased in order of STDC, STCDC, SGDC, SGCDC, SDC, SGC and STC. The conjugated bile salts (STDC, SGDC) show higher SR values than the unconjugated bile salt (SDC). Dihydroxyl bile salts (STDC, SGCDC) have higher SR and lower cmc values than trihydroxy ones (SGC, STC). This result indicates that the less polarity bile salts have, the easier the formation of micelles with VE is. These effects were similar to the effects of bile salts reported by Hofmann[7] on the solubilities of azobenzene and mono-olein.

Effect of PC on the Solubilization of VE

It is well known that PC forms mixed micelles with bile salts.[10] Figure 3 shows the solubility of VE in mixed micellar solution when the total concentration of PC and bile salt is 10 mM at various mixing ratios. PC itself has no ability to solubilize VE but can solubilize it by forming mixed micelles with bile salt. A micellar solution of 8.3 mM STC solubilized only 0.7 mM VE, while a mixed micellar solution with 1.7 mM PC solubilized approximately 2.2 mM VE. A comparison of the solubilities with and without PC indicates that 7.5 mM SGC mixed micellar solution solubilized VE 3.4 times more effectively. The mixed micelles of 4 mM PC with 6 mM STDC or SGCDC produced solubility enhancements of 3.6 and 7.0 times, respectively. The result that different maximum values of solubilization were obtained at

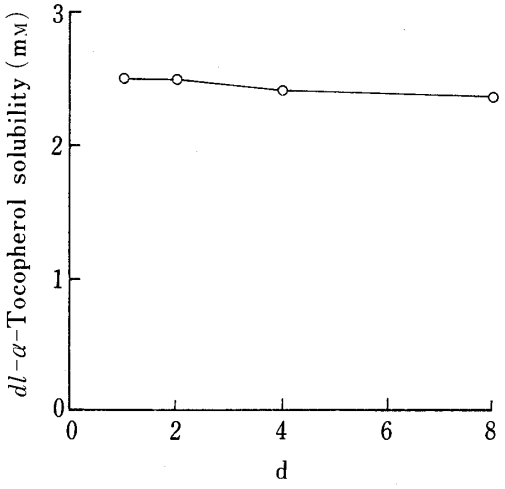

Fig. 1. Solubilization Behavior *dl*-α-Tocopherol in 10 mM STDC Solution at 37±0.1 °C

Each point represents the mean of three experiments.

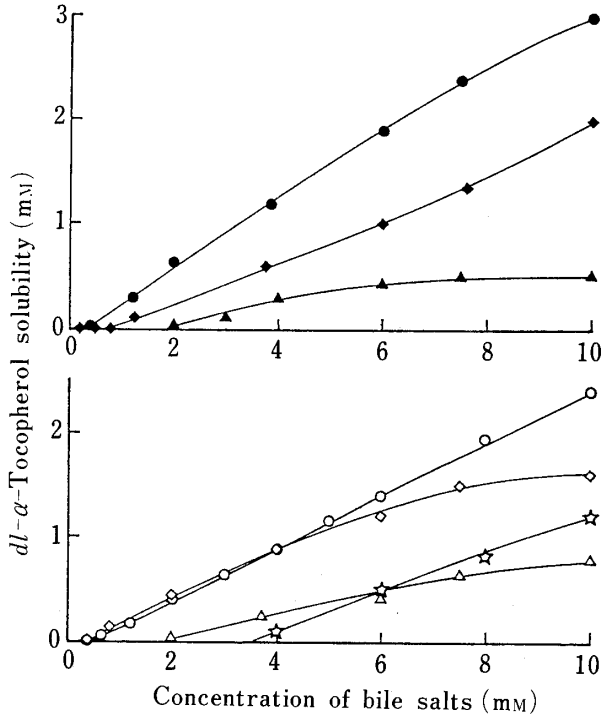

Fig. 2. Effect of Bile Salt Concentration on Solubilization of *dl*-α-Tocopherol at 37±0.1 °C

Each point represents the mean of three experiments.

◆, SGCDC; ●, SGDC; ▲, SGC; ◇, STCDC; ○, STDC; △, STC; ☆, SDC.

TABLE I. Values of Critical Micelle Concentration and Saturation Ratio for Bile Salts and Surfactant with *dl*-α-Tocopherol

	Critical micelle concentration (mM)	Saturation ratio[a)]
Sodium glycocholate (SGC)	2.00	0.15
Sodium taurocholate (STC)	1.90	0.12
Sodium glycochenodeoxycholate (SGCDC)	0.50	0.20
Sodium taurochenodeoxycholate (STCDC)	0.52	0.24
Sodium glycodeoxycholate (SGDC)	0.35	0.22
Sodium taurodeoxycholate (STDC)	0.48	0.25
Sodium deoxycholate (SDC)	3.30	0.17
Polysorbate 80 (PS-80)	0.40	4.13
Egg lecithin (PC) : STDC (1 : 10)	0.38	0.37
PC : STDC (1 : 5)	0.22	0.41
PC : STDC (1 : 1.5)	0.19	0.55

a) Slope of the linear portion of the solubilization curve determined by the least-squares method.

different molar ratios can be considered to be due to the differences of the chemical structure of bile salts and/or phase change of the micelles induced by the changes in the composition ratio of the bile salts and PC.[11)]

As shown in Fig. 4, at all mixing ratios of PC, the solubility of VE increased linearly with the total concentration of micellar component. The cmc and SR values obtained from these

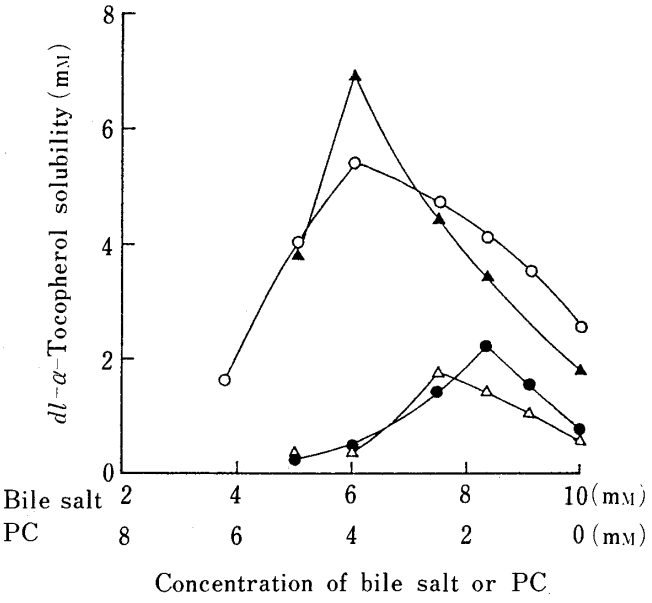

Fig. 3. Solubilization of *dl*-α-Tocopherol in Mixed Micellar Solutions of Bile Salt and PC at 37±0.1 °C

Each point represents the mean of three experiments.
○, STDC–PC; ▲, SGCDC–PC; ●, STC–PC; △, SGC–PC.

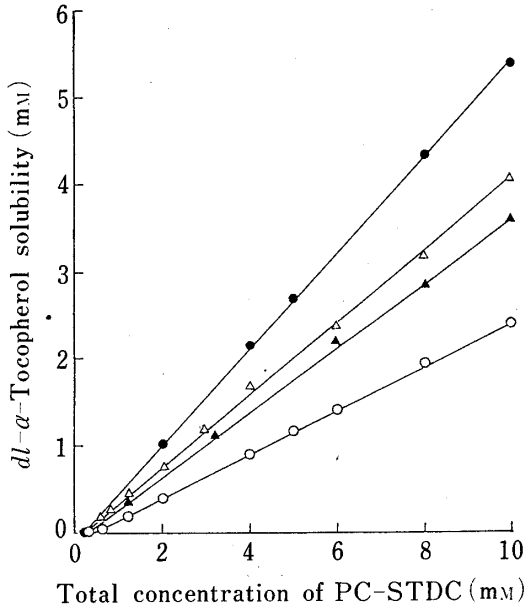

Fig. 4. Solubilization of *dl*-α-Tocopherol in Mixed Micellar Solutions with Various Ratios of PC to STDC at 37±0.1 °C

Each point represents the mean of three experiments.
○, PC–STDC (0:1); ▲, PC–STDC (1:10); △, PC–STDC (1:5); ●, PC–STDC (1:1.5).

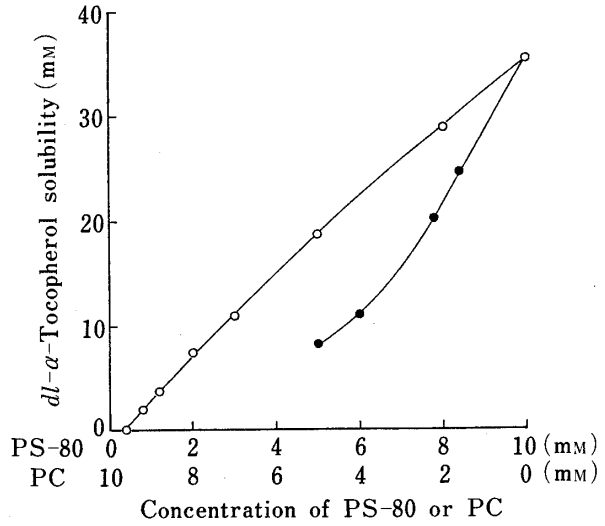

Fig. 5. Solubilization of *dl*-α-Tocopherol in PS-80 Solution and Mixed Micellar Solutions of PS-80 and PC at 37±0.1 °C

Each point represents the mean of three experiments.
○, PS-80; ●, PS-80 and PC.

straight lines are shown in Table I. The increasing solubility in the STDC and PC system is considered to be the effect of increasing micellar size, as shown by Carey and Small,[12] who observed an improved solubilization of cholesterol by bile salt and PC mixed micelles.

The solubility of VE in PS-80 micellar solution was also studied; it increased linearly with increasing concentration of PS-80 (Fig. 5) PS-80 showed higher solubilizing ability than the bile salts, and the SR value was 4.1, which was approximately twenty times larger than those of the bile salts, though the cmc values were similar (Table I). In Fig. 5 the solubilities of VE in mixed micellar solutions of PS-80 with PC are also depicted. In contrast to the case of bile salts, the solubility of VE in the mixed micellar solution decreased as the mixing ratio of PC increased. At 1:1, the solubility of VE was only about half that in PS-80 alone. Such a difference of solubility of VE might be due to differences in the arrangement of PS-80 molecules in the micelles of the PS-80 alone and in the mixed micelles.[12b]

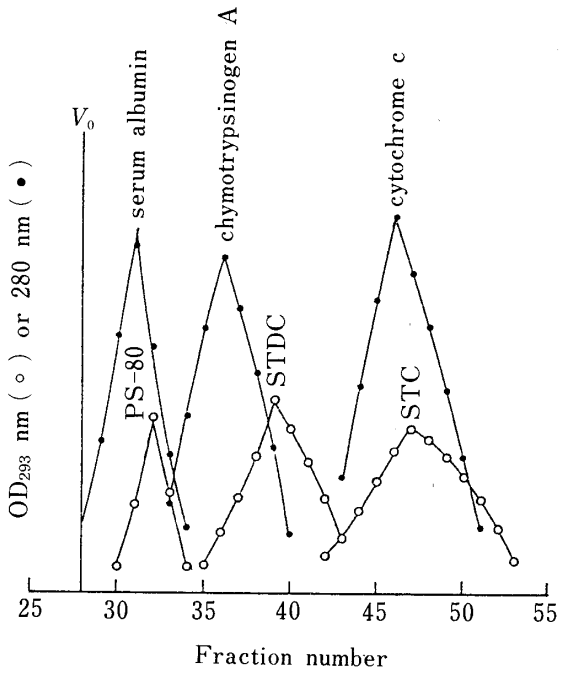

Fig. 6. Elution Curves of Three Standard Proteins and Micelles of STC, STDC and PS-80 Solubilizing *dl*-α-Tocopherol from a Sephadex G-75 Column

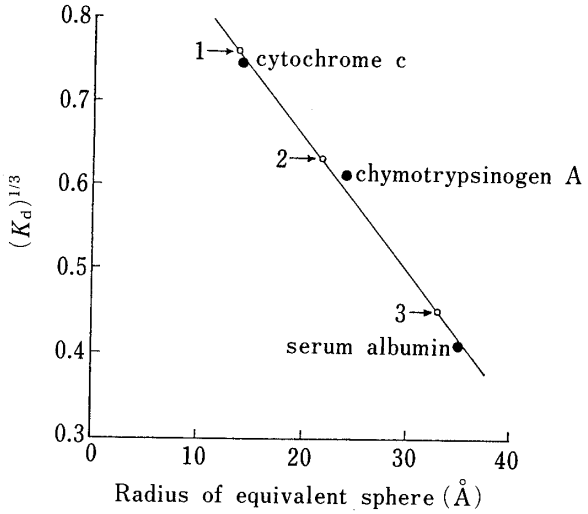

Fig. 7. Relationship between the $(K_d)^{1/3}$ Values for Three Standard Proteins and the Radii of Their Equivalent Spheres

The $(K_d)^{1/3}$ values obtained for the micelles are also shown: 1, STC; 2, STDC; 3, PS-80 micelles.

Micellar Size in the Solubilized Solution

Figure 6 shows the elution pattern in gel filtration of standard proteins and bile salt micelles solubilizing VE. Assuming the micelles to be spherical,[12] Fig. 7 shows the relationship between $(K_d)^{1/3}$ values and the sizes of the proteins and micelles (the sizes of the proteins are given as the radii of the equivalent spheres).[13] The micellar radii of STD, STDC and PS-80 obtained by interpolation on the plot for the standard proteins in Fig. 7 were 15, 22, and 33 Å, respectively.

The micellar size, shape and aggregation number of bile salts in micelles depend markedly on such variables as the species of bile salts, the concentration of bile salts, NaCl concentration in the solution and the temperature. Our results for micellar radius, 10 Å for STDC and 22 Å for STDC, are similar to these found by Mazer *et al.*[14] using quasielastic light scattering. Comparing the sizes of these micelles of surfactants with the saturation ratios, that is, 0.12, 0.25, 4.13 in STC, STDC and PS-80 (Table I), respectively, it appears that the larger the micellar size, the larger the solubilization of VE is.

In conclusion, the solubilized amount of VE in PS-80 micellar solution was larger than that in bile salt micellar solution. However, although the solubilizing ability of mixed micelles with PC increased in the case of bile salts, that in the case of PS-80 decreased. There was a relationship between the micellar size and solubilization of VE. Based on these characteristic effects of various surfactants on the solubilization of VE, the intestinal absorption of VE from micellar solutions will be dealt with in the following paper.

References and Notes

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