Solubilization of Thiamine Disulfide by Fatty Acid or Its Analog in 1,2-Dichloroethane[1)]

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The solubility change of thiamine disulfide (TDS) in 1,2-dichloroethane by the addition of fatty acid (FA), fatty alcohol or fatty acid methyl ester was determined by phase solubility analysis at 25 °C. The solubility of TDS increased linearly with added concentrations of stearic acid, palmitic acid or myristic acid, but the diagram did not exhibit a plateau due to the appearance of a solid complex. The dependency of the FA slope values on the number of carbon atoms in FA was very little. The solubility of TDS also increased linearly with added concentrations of stearyl alcohol, while the value for the slope was smaller than FA. On the other hand, the solubility of TDS decreased by the addition of stearic acid methyl ester. The results agreed well with those for the solubilization of cycotiamine, a thiamine derivative, by FA and FA analogs in 1,2-dichloroethane.

Keywords thiamine disulfide; fatty acid; fatty alcohol; fatty acid methyl ester; 1,2-dichloroethane; solubilization; complex; interaction

The crystalline complex between thiamine disulfide (TDS), an oxidized dimer of thiamine, and saturated fatty acid (FA) is obtained in 1,2-dichloroethane solution.[2)] The stoichiometric ratio of the crystalline complex is $(FA)_6$ (TDS). The advantages of $(FA)_6$ (TDS) to the pharmaceutical field, namely, creating a milder taste and smell of thiamine and exhibiting a possibility of controlled release, have been reported.[3)] It has been reported that the solubility of TDS increases with the addition of stearic acid in chloroform, acetone, benzene, and ethanol, although $(FA)_6$ (TDS) cannot be crystallized in any of these solvents.[2)] A solubility change of TDS by the addition of stearic acid or another FA in 1,2-dichloroethane from which $(FA)_6$ (TDS) is obtained has not yet been determined.

We have tested the interaction between TDS or other thiamine derivatives with FA or FA analogs in a liquid phase, and found that the solubility of cycotiamine, *N*-[1-(2-oxo-1,3-oxathin-4-ylidene)ethyl]-*N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-formamide, is increased by the addition of FA in 1,2-dichloroethane.[4)]

In this paper, we determined the effect of FA with various carbon numbers (C_n, 14, 16 and 18) and FA analogs (fatty alcohol and fatty acid methyl ester) on the solubility of TDS in 1,2-dichloroethane at 25 °C by phase solubility analysis. The results were compared with those obtained for cycotiamine (CCT), and the mode of the interaction between TDS and FA in 1,2-dichloroethane is discussed.

Experimental

Materials TDS (hydrated), extra pure grade, was purchased from Tokyo Kasei Industries Co., Ltd. Myristic acid (14:0), guaranteed reagent grade, and stearic acid methyl ester, extra pure grade (18:0 methyl), were purchased from Wako Pure Chemical Industries Co., Ltd. Palmitic acid, extra pure grade (16:0), and stearic acid, guaranteed reagent grade (18:0), were purchased from Koso Chemical Co., Ltd. Stearyl alcohol, extra pure grade (18 OH), and 1,2-dichloroethane, guaranteed reagent grade, were purchased from Yoneyama Yakuhin Industries Co., Ltd.

Phase Solubility Analysis The phase solubility method was carried out as described in the previous report.[4b)] An excess amount of TDS was added to 0—1×10^{-1} M of FA or FA analog solution in 1,2-dichloroethane. 18:0 and 18 OH 1,2-dichloroethane solutions were prepared as 0—1×10^{-2} and 0—8×10^{-2} M, respectively. The solution was shaken for 24 h at 25 °C until the solution attained equilibrium. This solution was filtered quickly, and an aliquot of the filtrate was diluted with 1,2-dichloroethane kept at 25 °C. The amount of TDS in the filtrate was determined as unhydrate spectrophotometrically at 277 nm at room temperature using $\varepsilon = 9.45 \times 10^3$ $M^{-1} cm^{-1}$. All experiments were carried out at least three times and the results were highly reproducible.

Results and Discussion

Figure 1 shows the effect of FA analogs on the solubility of TDS in 1,2-dichloroethane depending on their concentration. The solubility change of TDS *vs.* concentrations above 1×10^{-2} and 8×10^{-2} M for 18:0 and 18 OH, respectively, cannot be measured due to their solubility in 1,2-dichloroethane at 25 °C. The solubility of TDS was increased by the addition of 18:0. A plot of the solubility of TDS against the concentration of 18:0 yielded a single straight line. The solubility of TDS was also increased linearly with added concentrations of 18 OH, but the increment was much smaller than 18:0. Conversely, the solubility of TDS was decreased slightly by the addition of 18:0 methyl. Figure 2 shows the change in solubility of TDS depending on the concentration of FA with various C_n. The solubility of TDS was increased by the addition of 14:0 or 16:0 in the same manner as 18:0. The linear increases in the solubility of TDS by the addition of FA or fatty alcohol were not followed by a plateau or a decrease in the solubility; therefore, those phase diagrams can be classified as A_L type.[5)] The phase diagram obtained for 18:0 methyl does not fit either type A or type B. The values for slope determined from Figs. 1 and 2 are listed in Table I. All the values for slope of these phase diagrams are less

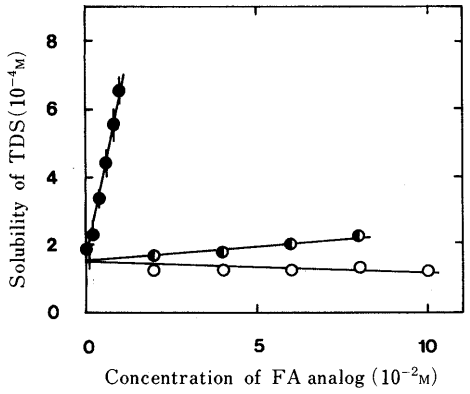

Fig. 1. Effect of FA Analogs on the Solubility of TDS in 1,2-Dichloroethane at 25 °C

FA analogs: ●, 18:0; ◐, 18 OH; ○, 18:0 methyl. Points and vertical bars represent means and S.D. ($n=3$), respectively.

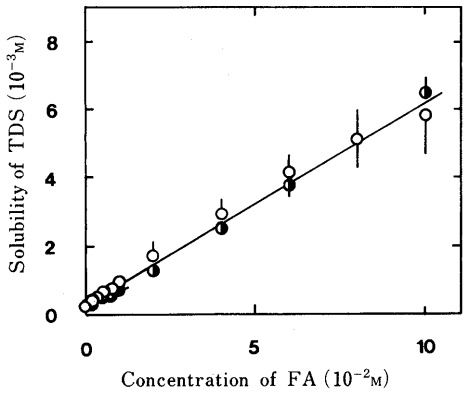

Fig. 2. Effect of FA on the Solubility of TDS in 1,2-Dichloroethane at 25 °C

FA: ○, 12:0; ◑, 14:0; ●, 18:0. Points and vertical bars represent means and S.D. ($n=3$), respectively.

TABLE I. The Values for Slope from Phase Solubility Diagram of TDS at 25 °C in 1,2-Dichloroethane

Additive	Slope
14:0	$(6.5\pm0.5)\times10^{-2}$
16:0	$(6.4\pm0.2)\times10^{-2}$
18:0	$(6.2\pm0.1)\times10^{-2}$
18 OH	$(8.1\pm1.5)\times10^{-4}$
18:0 methyl	$-(1.5\pm1.1)\times10^{-4}$

than unity. The dependency of the values for slope on C_n in FA is very little.

It has been reported that 18:0 exists as a monomer at concentrations below 1×10^{-2} M, and begins to form a hexamer in the structure of a reversed micelle at a concentration of $1-2\times10^{-2}$ M at 40 °C in 1,2-dichloroethane.[6] It has also been reported that monomers and dimers are the only important species of carboxylic acids in aprotic solvents, and that the dimer is the predominant molecular form except in very dilute solutions (0.2 v/v% or less).[7] According to these reports, FA, especially 14:0 and 16:0, may exist not only as monomers but also in other species under our experimental conditions. From the results obtained by phase solubility analysis, however, one cannot determine whether an increase in solubility of TDS by the addition of FA resulted from the solubilization of TDS in a reversed micelle of FA[6] or from the formation of a soluble complex between TDS and certain species of FA. Thus, for the purpose of evaluating the extent of interaction between TDS and FA with various C_n, we supposed that a single complex is responsible for the increase in solubility of TDS, and estimated the apparent stability constant of the complex whose stoichiometric ratio is 1:1 ($K_{1:1}$). The values for $K_{1:1}$ were calculated according to the equation[5]: $K_{1:1}=$ slope/(S_0(1 − slope)), in which S_0 denotes the solubility of TDS in the absence of FA, and slope denotes the slope of the phase solubility diagram. The values for $K_{1:1}$ of the complex between TDS and 14:0, 16:0 or 18:0 are calculated to be 331.0, 325.6 and 314.8 M^{-1}, respectively. The values for $K_{1:1}$ depend only slightly on C_n in FA. This suggests that the extent of the interaction between TDS and FA depends very little on C_n in FA, if the stoichiometric ratios of complexes formed between TDS and FAs in 1,2-dichloroethane are identical like those of the crystalline complexes of $(FA)_6$ (TDS) are.[2]

Kertes *et al.*[8] have reported that alcohols predominantly exist as dimers, tetramers, and higher oligomers, depending on the solvent, except for in a very dilute solution (10^{-3} mol/kg range) in inert alkanes. According to this report, the molecular species of fatty alcohol with which TDS interacted may also not be a monomer. But, the smaller slope of 18 OH than 18:0, as shown in Table I, may be related to the fact that monomer alcohol is less interactive than monomeric acid.[7] The very small negative value for the slope of 18:0 methyl suggests that FA ester does not form a soluble complex with TDS like FA or fatty alcohol do in 1,2-dichloroethane.

We have previously reported the solubilization of CCT, a thiamine derivative, by FA or FA analog in 1,2-dichloroethane.[4b] The results obtained for CCT at 25 °C agree well with that for TDS: 1) the solubility of CCT is increased linearly with added concentrations ($0-1\times10^{-2}$ M) of FA; the dependency of the values for slope on C_n in FA is very small (0.719, 0.730 and 0.743 for 18:0, 16:0 and 14:0, respectively); 2) the solubility of CCT is increased linearly with added concentrations of 18 OH ($0-1\times10^{-1}$ M); the value for the slope of 18 OH (0.016) is smaller than 18:0; 3) the solubility of CCT is decreased linearly with the added concentration of 18:0 methyl (0—1×10^{-1} M); the value for slope is −0.03. This agreement suggests a similarity in the mode of interaction between CCT–FA and TDS–FA in 1,2-dichloroethane. The N-1 nitrogen atom and the NH_2 in the pyrimidine ring of CCT are involved in the interaction with FA in 1,2-dichloroethane.[9] As the aminopyrimidine ring is common to CCT and TDS, the N-1 nitrogen atom and the NH_2 in the pyrimidine ring of TDS may be needed for the interaction with FA in 1,2-dichloroethane.

The diagram obtained for FA did not exhibit a plateau or any decrease in the solubility of TDS, as shown in Fig. 2. This suggests that a solid complex, $(FA)_6$(TDS) did not appear. $(FA)_6$(TDS) is crystalized by keepting a solution of TDS and FA in a molar ratio of 1:8 in 1,2-dichloroethane at 20—30 °C[2]; the concentrations of FA for obtaining each $(FA)_6$ (TDS) are calculated to be approximately 0.09, 0.1 and 2 M for 18:0, 16:0 and 14:0, respectively; the solution is heated once to dissolve TDS and FA thoroughly. Namely, the concentration of FA and the temperature are both higher than under our experimental conditions.

A complex between CCT and FA connot be crystallized even in the same manner as for $(FA)_6$ (TDS). CCT is more soluble in 1,2-dichloroethane (2.48×10^{-2} M at 25 °C) than TDS (2.10×10^{-4} M at 25 °C). Furthermore, the values for slope of CCT (both positive and negative values) are larger than TDS. These differences may explain the solubility of each complex in the solvent.

Conclusion

The solubility of TDS was increased by the addition of 14:0 or 16:0 in the concentration range of $0-1\times10^{-1}$ M, or 18:0 in the concentration range of $0-1\times10^{-2}$ M in 1,2-dichloroethane at 25 °C. But $(FA)_6$(TDS) did not precipitate. It is assumed that there is some similarity in the mode of interaction between TDS-FA and CCT-FA, although a complex between CCT and FA cannot be crystallized.

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