Interaction between Thiaminetetrahydrofurfuryl Disulfide with Fatty Acid in 1,2-Dichloroethane

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The interaction between thiamine tetrahydrofurfuryl disulfide (TTFD) and saturated fatty acid (FA) in 1,2-dichloroethane at 25 °C was studied by phase solubility analysis using FA with various carbon numbers (14, 16, and 18), stearyl alcohol, and stearic acid methyl ester. The solubility of TTFD increased with the addition of FA. The solubility of TTFD also increased with the addition of stearyl alcohol, while its solubility decreased when stearic acid methyl ester was added.

It was found that the solubility of stearic acid also increased with the addition of TTFD, and that TTFD cannot form a crystalline complex with stearic acid.

These results are compared with those obtained for thiamine disulfide (TDS) and cycotiamine. The mode of the solubilization of TDS by FA in 1,2-dichloroethane, and its relation to the crystallization of the complex between TDS and FA was discussed.

Keywords thiaminetetrahydrofurfuryl disulfide; fatty acid; interaction; 1,2-dichloroethane; complex; thiamine derivative

Thiamine disulfide (TDS), an oxidized dimer of thiamine, forms a crystalline complex with fatty acid (FA).[1] Several advantages of the complex to the pharmaceutical field have been reported, namely, the creation of a milder taste and smell for TDS, and demonstration of the possibility of controlled release.[2] The complex is a clathrate or an inclusion compound,[3] whose stoichiometry is expressed as $(FA)_6(TDS)$.[1] $(FA)_6(TDS)$ is obtained in 1,2-dichloroethane.[1]

We have studied the interaction between TDS and FA in 1,2-dichloroethane by the solubility method at 25 °C,[4] and found that the solubility of TDS in 1,2-dichloroethane increases with the addition of myristic acid or palmitic acid in a concentration range below 1×10^{-1} M, or with stearic acid in a concentration range below 1×10^{-2} M. However, $(FA)_6$ (TDS) does not precipitate under these experimental conditions. It has been suggested that this solubilizing mode of TDS by FA is similar to that of cycotiamine (CCT) by FA in 1,2-dichloroethane.[5] CCT shares a common structure with TDS (Chart 1); however, it does not form a crystalline complex with FA. The mode of solubilization of TDS and CCT by FA, and the relationship between the solubilization of TDS and the crystallization of $(FA)_6$ (TDS) is unknown.

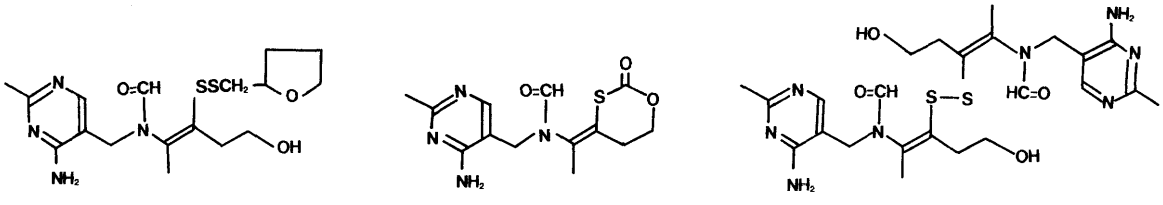
In this paper, we tested the interaction of thiaminetetrahydrofurfuryl disulfide (TTFD) (Chart 1) with FA. The effect of FA with various carbon numbers (C_n) and FA analogs on the solubility of TTFD in 1,2-dichloroethane at 25 °C was determined by phase solubility analysis. The results were compared with those obtained for TDS[4] and CCT,[5] and the mode of the solubilization of TDS by FA in 1,2-dichloroethane and its relation to the formation of $(FA)_6$ (TDS) is discussed.

Experimental

Materials TTF D, *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-*N*-[4-hydroxy-1-methyl-2-[(tetrahydrofurfuryl)dithio]-1-butenyl]formamide, was the gift of Takeda Pharmaceutical Co., Ltd. Myristic acid, guaranteed reagent grade (14 : 0), 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 previous reports.[4,5] An excess amount of TTFD was added to various concentrations of FA or FA analog solution in 1,2-dichloroethane. The solution was shaken for 48 h at 25 °C until it 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 TTFD in the filtrate was determined spectrophotometrically at 272.4 nm at room temperature using $\varepsilon = 5.75 \times 10^3$ M^{-1} cm^{-1}. Fatty acid had no effect on the absorption of TTFD. All experiments were carried out at least three times and the results were highly reproducible.

In the method described above, the solubility of FA in 1,2-dichloroethane restricted the concentration of FA added. And, $(FA)_6$(TDS) is prepared at a higher concentration of FA.[1] Therefore, the solubility change of both TTFD and FA in a higher concentration range was also tested. 18 : 0 was used, and the condition was taken from the method of Ueda *et al.* for $(18:0)_6$(TDS).[1] TTFD and 18 : 0 were added to 1,2-dichloroethane in various molar ratios, then heated until both TTFD and 18 : 0 were completely dissolved. The solution was kept at room temperature (20—30 °C) for 1 d. This solution was filtered, and the residue was dried



TTFD CCT TDS

Chart 1

in a dessicator at room temperature. The residue was identified by its melting point (mp).

Results

Figure 1 shows the change in solubility of TTFD in 1,2-dichloroethane depending on the added concentration of FA with various C_n at 25 °C. The solubility of TTFD was increased by the addition of FA. All these plots of the solubility of TTFD against the concentration of FA yielded single straight lines. The values for the slope determined from Fig. 1 are 1.02 ± 0.06, 0.95 ± 0.04, and 1.03 ± 0.07 for 14 : 0, 16 : 0, and 18 : 0, respectively. The values were almost equal to or greater than unity. The dependency of the values for slope on the C_n of FA is very slight. From these results, it was suggested that TTFD interacts with FA in 1,2-dichloroethane, and that the degree of the interaction depends on the C_n of FA only slightly.

Figure 2 shows the change in solubility of TTFD in 1,2-dichloroethane, depending on the added concentration of FA analogs. The solubility of TTFD also increased linearly with the added concentration of 18 OH, although the increment was smaller than 18 : 0. On the other hand, the solubility of TTFD decreased slightly by the addition of 18 : 0 methyl. The values for slope determined from Fig. 2 are listed in Table I. These results show that 18 : 0 and 18 OH, which have an OH moiety, can solubilize TTFD, but 18 : 0 methyl, which does not have an OH moiety, cannot. Thus, it was suggested that the OH moiety of FA is necessary for interaction with TTFD, which leads to the solubilization of TTFD.

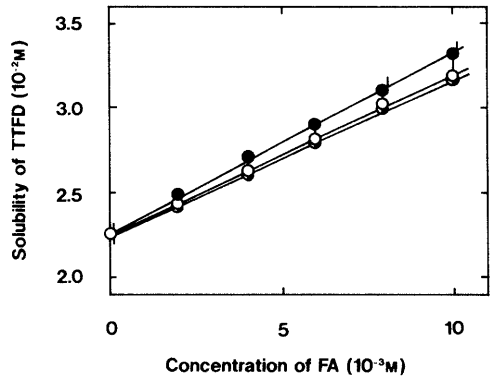


Fig. 1. Effect of FA on the Solubility of TTFD in 1,2-Dichloroethane at 25 °C

FA: ○, 14 : 0; ◑, 16 : 0; ●, 18 : 0. Points and vertical bars represent the mean and S.D. ($n = 3$), respectively.

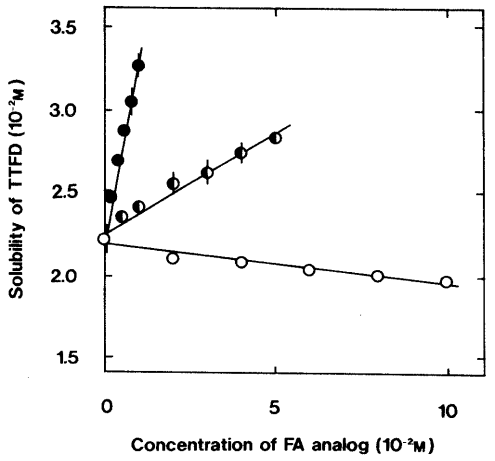


Fig. 2. Effect of FA Analogs on the Solubility of TTFD in 1,2-Dichloroethane at 25 °C

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

The solubility change at a higher concentration region was tested at the same concentration of 18 : 0 as for obtaining $(18:0)_6$ (TDS)[1] as listed in Table II (No. 1—3). The residue obtained from No. 1 solution was only 18 : 0. As listed in Table I, the solubility of TTFD at 25 °C was 2.26×10^{-2} M (0.27 g/30 ml), which demonstrated that TTFD was solubilized by 18 : 0. When the molar ratio of TTFD was increased (No. 2), both TTFD and 18 : 0 were solubilized and nothing was left. When the molar ratio of TTFD was further increased, only TTFD was left (No. 3). Next, both TTFD and 18 : 0 were increased at the molar ratio of 1.1, the same molar ratio as No. 2 (No. 4, 5). But, under the condition of No. 4, nothing precipitated. From the No. 5 solution, finally, TTFD and 18 : 0 precipitated independently.

These results show that the interaction between TTFD and 18 : 0 in 1,2-dichloroethane eventually leads to the solubilization not only of TTFD but also of 18 : 0. But, despite the interaction, no crystalline complex can be formed between TTFD and 18 : 0.

Discussion

We have previously reported on changes in the solubility of TDS[4] and CCT[5] by the addition of FA and its analogs in the concentration range below 1×10^{-2} M. The results obtained for them agree well with that obtained for TTFD: 1) the solubility of TDS/CCT is increased linearly with an added concentration (below 1×10^{-2} M) of FA; the dependency of the values for slope on C_n of FA (14—18) is very small (0.06—0.07 for TDS, and 0.72—0.74 for CCT); 2) the solubility of TDS/CCT is increased linearly with an

Table I. The Solubility and Slope from Phase Solubility Diagram of TTFD, TDS,[4] and CCT[5] at 25 °C in 1,2-Dichloroethane

	Solubility (M)	Slope		
		18 : 0	18 OH	18 : 0 methyl
TTFD	2.26×10^{-2}	1.03 ± 0.07	0.10 ± 0.01	$-(0.016 \pm 0.002)$
TDS	2.10×10^{-4}	$(6.2 \pm 0.1) \times 10^{-2}$	$(8.1 \pm 1.5) \times 10^{-4}$	$-(1.5 \pm 1.1) \times 10^{-4}$
CCT	2.48×10^{-2}	0.72 ± 0.00	0.02 ± 0.01	$-(0.030 \pm 0.001)$

Table II. Solubilization of TTFD and 18 : 0 at a Higher Concentration

No.	TTFD		18 : 0			Reside
	g	$\times 10^{-3}$ mol	g	$\times 10^{-3}$ mol	Ratio[a]	mp (°C)[b]
1	0.4	1.0	0.8	2.8	0.4	67—69
2	1.2	3.0	0.8	2.8	1.1	—
3	2.4	6.0	0.8	2.8	2.1	132—136
4	1.8	4.5	1.2	4.2	1.1	—
5	2.4	6.0	1.6	5.6	1.1	67—69, 137—142

These materials are added to 30 ml of 1,2-dichloroethane. *a*) Ratio of added moles of TTFD to 18 : 0. *b*) The melting point of the appearing material. The melting point of 18 : 0 and TTFD were 67—69 °C and 142—145 °C, respectively.

added concentration of 18 OH (below 1×10^{-1} M); as listed in Table I, the value for slope of 18 OH is smaller than 18 : 0; 3) the solubility of TDS/CCT is decreased linearly with an added concentration of 18 : 0 methyl. It cannot be concluded from these results that the mechanisms of interaction between FA and these thiamine derivatives, which lead to solubilization, are identical. But, the agreement of the results suggests some similarity in their mode of interaction.

$(FA)_6$(TDS) is a clathrate or an inclusion compound.[3] It has been suggested that the process of the formation of $(FA)_6$ (TDS) includes the solubilization of TDS in the 18 : 0 association, similar to a reversed micelle.[6] However, the solubilization of TDS and other thiamine derivatives by the addition of FA in the concentration ragion below 1×10^{-2} M is not thought to be due to the solubilization of these substances in the FA associations, for the following reasons: 1) $(FA)_6$ (TDS) does not appear in the concentration range of FA below 1×10^{-2} M, where TDS is solubilized. And even though TTFD or CCT are solubilized by FA in 1,2-dichloroethane, they cannot form a crystalline complex like $(FA)_6$ (TDS). 2) When a solubility study is carried out, a break in the solubility curve is indicative of micelle formation to differentiate between complex formation and micellar solubilization.[7] As previously described, every phase solubility diagram of TTFD, TDS, and CCT can be expressed as a single line, namely, no break is observed. In the case of TDS, the solubility change by the addition of 14 : 0/16 : 0 in a concentration range over 1×10^{-2} M (up to 1×10^{-1} M) has been determined, but those phase diagrams are also expressed as single lines, and no break is observed. 3) It has been reported that the 18 : 0 hexamer in the structure of a reversed micelle is formed in the concentration region above 4×10^{-2} M at 40 °C in 1,2-dichloroethane.[6] The concentration range below 1×10^{-2} M may correspond to a range below the critical micelle concentration, though the temperature is different. Therefore, it is suggested that the solubilization by the addition of FA in the concentration region below 1×10^{-2} M, at least, is not that in the reversed micelle of FA.

The results obtained by phase solubility analysis using FA analogs shows the necessity of the OH moieties of FA for the solubilization of TTFD, TDS, and CCT. From these results, it was thought that the OH moiety, probably that of the FA monomer, is involved in the interaction with those thiamine derivatives in 1,2-dichloroethane. The value of slope for 18 OH smaller than 18 : 0 is thought to suggest a hydrogen bonding ability of 18 OH smaller than 18 : 0.

It has already been suggested that NH_2 and N-1 in the pyrimidine ring of CCT are involved in the interaction with FA.[8] As shown in Chart 1, 4-amino-2-methylpyrimidine and adjacent methylene *N*-formamide are common to TTFD, TDS, and CCT. Therefore, NH_2 and N-1 in pyrimidine in TTFD/TDS may be involved in the interaction with the FA monomer.

When $(18 : 0)_6$ (TDS) is formed, 18 : 0 is suggested to form an association similar to a reversed micelle, and TDS is solubilized in the micelle.[6] By contrast, the solubilization of TDS, CCT, and TTFD by FA is thought to be caused by the hydrogen bonding interaction between the FA monomer and these thiamine derivatives. Therefore, some steps in the process of the formation of a crystalline complex, a clathrate compound, may be interfered with the hydrogen bonding interaction in which the FA monomer is involved. In the case of TDS, even under the condition of No. 1 in Table II, $(18 : 0)_6$(TDS) appears. But, as shown in Table II, The solubility of both TTFD (and CCT, data not shown) and FA are increased due to the interaction. It is assumed that TTFD and CCT cannot form a crystalline complex because they interact with FA in the concentration region avove the critical micelle concentration cmc of FA. As shown in Table I, the solubility of TTFD and CCT in 1,2-dichloroethane is higher than TDS, indicating that TTFD and CCT have a higher affinity for 1,2-dichloroethane than TDS. It is assumed that the solubilization of FA by the addition of CCT/TTFD, and the inability of CCT/TTFD to form their crystalline complex, is related to their high solubility in 1,2-dichloroethane.

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