Formation of a Cytotamine Complex with Fatty Acid in Dichloroethane\(^1\)

YASUKO KOMATA,*, SHOHO YOKOYAMA, AKIKO KANEKO, AND TADAO FUJII

Kyoritsu College of Pharmacy, 1–5–30, Shibukoen, Minato-ku, Tokyo 105, Japan. Received August 16, 1989

The formation of complexes between cytotoamine (CCT) and fatty acids (FA) (tetradecanoic acid, hexadecanoic acid, and octadecanoic acid) in 1,2-dichloroethane was studied by phase solubility analysis and ultraviolet absorption spectroscopy. The apparent stability constants of the equimolar complexes between CCT and FA were determined at 288, 298, and 310 K, and thermodynamically discussed. The complex formation ability of FA depended on the number of carbon atoms in FA, but the differences were small. The ability of 1-octadecanol was very small, and octadecanoic acid methyl ester had none. From these results CCT–FA were concluded to be hydrogen bonded complexes in which FA act as hydrogen donors. The complex formation reactions were exothermic and enthalpically controlled.

**Keywords** cytotoamine; fatty acid; complex; 1,2-dichloroethane

We previously reported the mechanism of formation of a complex between thiamine disulfide (TDS) and fatty acids (FA).\(^2\) Complexes of TDS with higher saturated FA have the pharmaceutical advantage of improved hygroscopicity and bitter taste of TDS.\(^3\) For further study of the mode of the formation of the complex, we tested many thiamine derivatives for formation of a complex with FA. Cytotoamine (CCT), a S-acetylated derivative of thiamine, was found to interact with FA.\(^4\)

This paper reports studies on the formation of complexes between CCT and FA (CCT–FA) in 1,2-dichloroethane (dichloroethane) by phase solubility analysis and ultraviolet (UV) spectroscopy. The mode of interaction is discussed.

**Experimental**

**Materials** Cytotoamine, N-[[1-2-oxo-1,3-oxathin-4-ylidenemethyl]N-[[4-amino-2-methyl-5-pyrimidinyl]methyl]formamide, was the gift of Yamanouchi Pharmaceutical Co., Ltd. Tetradecanoic acid (14:0) was purchased from Wako Pure Chemical Industries Co., Ltd., hexadecanoic acid (16:0) and octadecanoic acid (18:0) were purchased from Koso Chemical Co., Ltd. 1,2-Dichloroethane was purchased from Kokusan Chemical Co., Ltd. All other chemicals were of guaranteed reagent grade.

**Phase Solubility Analysis** The phase solubility analysis method was taken from Higuchi and Lach.\(^5\) An excess amount of CCT was added to fatty acid solution (0–1 × 10\(^{-2}\) m) and was shaken for 24 h at a given temperature (288, 298, and 310 K) until the solution attained equilibrium. This solution was filtered, and the filtrate was diluted with dichloroethane.

The amount of CCT in this filtrate was determined spectrophotometrically (273 nm, \(ε = 4.99 × 10^4\)). Fatty acid had no effect on the absorption of CCT.

**UV Spectroscopy** UV spectra were measured with a Hitachi 556 double beam spectrophotometer at ambient temperature.

**Results and Discussion**

**Solubility Studies** Figure 1 shows the dependence of solubility of CCT in dichloroethane on the concentration of FA. Increasing the concentration of FA resulted in increase in CCT solubility, concurrent with the formation of CCT–FA. The effect of 1-octadecanol (18 OH) on the solubility of CCT was, however, very small and almost undetectable at this concentration, and octadecanoic acid methyl ester (18:0 methyl) had no effect. These results show that the ability of 18 OH to form CCT–FA was much smaller than those of FA, and that 18:0 methyl had no ability. Therefore, it was suggested that FA acted as hydrogen donors to form hydrogen-bonded complexes of CCT–FA, and that FA are more capable of acting as hydrogen donors than alcohol.

To compare the differences of the ability of FA to form CCT–FA depending on the number of carbon atoms and the temperature, the apparent stability constant (K value) of an equimolar complex of CCT–FA was determined by the following equation according to Conners and Mollica:\(6\):

\[ K = \text{slope}(S_0(1 - \text{slope})) \]

in which \(S_0\) denotes the solubility of CCT in the absence of FA, and slope denotes the slope of the phase solubility diagram at a given temperature. The determined K values are listed in Table 1. By increasing the temperature the values for slope decreased and the values

![chart](chart1.png)

**Table 1.** K Values for CCT–FA in Dichloroethane

<table>
<thead>
<tr>
<th>System</th>
<th>Temperature (K)</th>
<th>K (M(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>288</td>
<td>298</td>
</tr>
<tr>
<td>CCT-14:0</td>
<td>153</td>
<td>116</td>
</tr>
<tr>
<td>CCT-16:0</td>
<td>144</td>
<td>109</td>
</tr>
<tr>
<td>CCT-18:0</td>
<td>123</td>
<td>103</td>
</tr>
</tbody>
</table>

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for $S_2$ increased, resulting in a decrease in the $K$ values. By increasing the number of carbon atoms in FA the values for slope decreased slightly, resulting in a small decrease in the $K$ values. This result is thought to ascribe to the potency of FA to act as hydrogen donors depending on the number of carbon atoms in their alkyl chains.71

The position acting as a hydrogen acceptor in CCT is unknown, so we are now studying it using nuclear magnetic resonance spectroscopy.

**UV Spectroscopic Studies** Addition of FA to CCT in dichloroethane resulted in the UV spectral changes with an isosbestic point at 277 nm, as shown in Fig. 2. The addition of 18 OH or 18:0 methyl caused no spectral change of CCT. These results support those obtained by solubility studies.

**Thermodynamic Studies** Figure 3 shows the Arrhenius plots of the apparent $K$ values for CCT–FA. As each plot showed linearity, free energy changes ($\Delta G$), enthalpy changes ($\Delta H$), and entropy changes ($\Delta S$) for CCT–FA formation were determined according to the equation as previously reported.83 These determined parameters are listed in Table II. All the values for $\Delta S$ were negative, but the values for $\Delta H$ were even larger negative. So, the reactions are entropically unfavorable but enthalpically favorable resulting in being free energetically favorable; they are therefore exothermic and enthalpically controlled. Decreasing the number of carbon atoms in FA resulted in increase both in the negative values of $\Delta S$ and $\Delta H$, resulting in an increase in the negative values of $\Delta G$. This result shows that decreasing the number of carbon atoms in FA affects entropically unfavorably but enthalpically favorably leading to free energetically favorably CCT–FA. The unfavorable $\Delta S$ was thought to be related to the solvent orientation, which is observed in the case of hydrogen-bonded complexes between $p$-fluorophenol and pyridines or amines in aprotic solvents including dichloroethane.83

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**References**

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