Comparison of Hydrogen Bonding for Chiral and Racemic 1-Monostearoylglycerols in Solvent

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Abstract: Comparison of the hydrogen bonding of racemic RS-1-monostearoylglycerol and chiral S-1-monostearoylglycerol in benzene was carried out through the NMR measurement. In addition, the concentration dependence of a chirality of S-1-monostearoylglycerol in hexane was studied through UV and circular dichroism (CD) measurements. The chemical shifts of OH protons of the S- and RS-1-monostearoylglycerols indicated that the hydrogen bonding between the R- and S-form molecules of RS-1-monostearoylglycerol is stronger than that between the S- and S-form molecules of S-1-monostearoylglycerol in the low concentration region and that the difference in the strength of hydrogen-bonding between them becomes small in the high concentration region. The chirality of the S-1-monostearoylglycerol in hexane decreased with increasing its concentration. This suggests that the association of chiral S-1-monostearoylglycerol arising from its increasing concentration reduces the chirality of S-1-monostearoylglycerol.

Key words: hydrogen bonding, chiral 1-monostearoyl glycerol, racemic 1-monostearoyl glycerol, NMR chemical shift, circular dichroism

1 INTRODUCTION
Triacylglycerols composed of three acyl groups and one glycerol are the main component of natural oils and fats. In nature there also exist monoacylglycerols in which only one acyl group combines with one glycerol.

The monoacylglycerols, having one hydrophobic moiety and two hydrophilic OH moieties, show strong surface-active ability. Hence, they are used as superior surfactants being safe for living organisms, e.g., for toiletries, medicine and pharmaceuticals and food supplies.

Two types of monoacylglycerols exist depending on the acyl group position combining to glycerol, i.e., 1-monoacylglycerol and 2-monoacylglycerol. The former has an asymmetric carbon atom, whereas the latter does not. Thus the former should have two chiral structures, i.e., R- and S-forms. In general, however, only S-form and racemic RS-form are commercially available as the 1-monoacylglycerol having hydrocarbon chains with the length from C12 to C18.

Properties of the 1-monoacylglycerol especially for its RS-form have been studied by many investigators1-6). However, little has yet been reported on the physicochemical difference between optically active S-form and inactive RS-form.

The racemic RS-form in the crystalline state can be classified, in terms of the interaction of R- and S-molecules in the crystal, into three types of modification: a racemic compound, a solid solution and a simple mixture of R- and S-form molecules7,8). RS-1-monoacylglycerols containing carbon-chains from C12 to C18 are thought to be racemic compounds. This is because the RS-1-monoacylglycerols have higher melting points than their optically active S-forms9). Thus the various crystalline properties of the racemic compounds are expected to differ from those of the optically active species. The thermodynamic and structural properties of the crystals of RS-1-monoacylglycerol were compared with those of S-1-monoacylglycerol5,9). Through the measurements of DSC, solubility in benzene, IR spectra and X-ray diffraction for S- and RS-forms of 1-monostearoylglycerol and 1-monopalmitoylglycerol it was assumed that their RS-forms are the racemic compounds in their melts, in which the R- and S-form molecules are strongly combined with each other, and that in a solvent the interaction between the molecules of R- and S-forms is not so strong that the molecules are free from each other5,9). Recently, however, the near infrared spectroscopic mea-
measurements for alcohols in solvents indicated that the alcohol molecules associate to form their dimers, trimers and oligomers even at relatively low concentration such as 0.1 mol dm\(^{-3}\). Furthermore the fatty acid in CCl\(_4\) associates to form dimer even at extremely low concentration such as \(10^{-2} \text{ to } 10^{-3}\) mol dm\(^{-3}\). Thus the 1-monocacylglycerol having two OH groups would more easily combine with each other by their strong hydrogen bonding in the solvents.

In the present study, the difference in the hydrogen bonding between the \(S\)- and \(RS\)-1-monostearoylglycerols in benzene was studied through the measurement of \(^1\)H NMR. The concentration dependence of chirality of \(S\)-1-monostearoylglycerols in hexane was also studied through the measurements of UV and circular dichroism (CD).

2 EXPERIMENTAL

2.1 Materials

Samples (\(\gg 99\%\) pure) of optically active \(S\)- and racemic \(RS\)-1-monostearoylglycerols obtained from Fluka AG (Switzerland) were purified by repeated recrystallization from fleshly distillated hexane. Hexane (Junsei Kagaku Co., ultra-analytical grade) used for the recrystallization and the UV and CD measurements were prepared by distillation after overnight drying with CaCl\(_2\) and four hours refluxing with metallic sodium. Benzene (Junsei Kagaku Co., spectroscopic grade) for DSC measurements was prepared by distillation after overnight drying with CaCl\(_2\) and four hours refluxing with CaH\(_2\). Deuterated benzene (atomic purity 99.8 \%, Merck (Tokyo)) was used without further purification.

The heat of fusion and the melting temperature were determined on a differential scanning calorimeter (Rigaku Denki, model 8230B) in the temperature range of 300-373 K at a constant rate of 5 K min\(^{-1}\) to rise the temperature. In order to obtain melting temperature-composition diagrams for the mixtures of \(S\)- and \(RS\)-1-monostearoylglycerols, they were mixed in benzene and allowed to stand overnight under a reduced pressure to evaporate the benzene.

The UV-absorption and CD spectra were recorded on a Hitachi U-3210 spectrophotometer and a JASCO J-500 spectropolarimeter, respectively, both of which were equipped with a temperature-control unit.

The \(^1\)H NMR spectra were recorded on a JEOL Model EX 400 NMR spectrometer with a sample tube of 5.0 mm diameter at 25, 35, 50, 60 and 75 ± 0.5°C. The hydroxyl proton chemical shifts, relative to the proton of benzene existing in the deuterated benzene that is used for locking purposes, were measured as a function of the concentration of 1-monostearoylglycerol. The deuterated benzene was contained in a coaxial tube.

3 RESULTS AND DISCUSSION

3.1 Phase diagram

DSC thermograms were obtained by heating scan for pure \(S\)-form, pure \(RS\)-form and \(S\/-RS\)-form mixtures with several compositions. The temperatures of the endothermic peaks of DSC thermograms define the phase boundaries. Thus, the phase diagram of the binary mixture can be constructed by plotting the DSC peak temperatures against the composition. The phase diagram thus obtained for \(S\/-RS\)-form mixture of 1-monostearoylglycerol is shown in Fig. 1, where the composition is expressed in terms of the mole fraction of \(RS\)-form, \(x_{RS}\). This phase diagram exhibits a typical eutectic pattern: \(RS\)-1-monostearoylglycerol is the racemic compound. Thus, the horizontal solidus line extending over the whole composition range suggests that \(S\)- and \(RS\)-forms are completely immiscible and exist as separate phases in a solid phase.

Melting temperatures of \(S\)-form and \(RS\)-form were 349.3 and 355.6 K, respectively, which are in good agreement with the corresponding values available from literature, 349.2 K for \(S\)- and 355.2 K for \(RS\)-forms (\(\beta\)-form crystals)\(^{9,10}\). The obtained heat of fusion for \(S\)-form and \(RS\)-form were 82.4 kJmol\(^{-1}\) and 75.4 kJmol\(^{-1}\), respectively, which are in agreement with the values in literature\(^{10}\). Thus, we confirmed that \(RS\)-form has a higher melting temperature and lower heat of fusion than the \(S\)-form. This anomaly in the order of their values between melting temperatures and heat of fusion is explainable by assuming that the interaction between \(R\)- and \(S\)-form molecules is stronger than that between \(S\)- and \(S\)-form molecules and that \(RS\)-form
crystal does not sufficiently dissociate into completely free $R$- and $S$-form molecules in a liquid just above the melting temperature. Namely, there would remain the more amount of $R$-$S$ compound than the $S$-$S$ compound in their liquid states.

3.2 Comparison of hydrogen bondings of $RS$- and $S$-1-monostearoylglycerols in benzene by their $^1$H NMR chemical shifts

In order to compare the strength of the interaction between $R$- and $S$-form molecules with that between $S$- and $S$-form molecules in solvent, we carried out the $^1$H NMR measurement for $RS$- and $S$-1-monostearoylglycerols in benzene; in this $^1$H NMR experiment we used benzene-$d_6$. This is because the solubility of $RS$- and $S$-1-monostearoylglycerols in benzene is relatively high at room temperature, whereas hexane is a poor solvent for them. The chemical shift assignments were carried out using the proton signal (7.20 ppm) of benzene as a reference, which exists slightly in benzene-$d_6$. Figure 2 shows a typical NMR spectrum for $RS$-1-monostearoylglycerol (4.46 x 10$^{-3}$ mol dm$^{-3}$) in benzene-$d_6$ at 25°C. There exists a proton signal of OH moiety at 1.950 ppm. The chemical shift of each signal except the proton signal of OH moiety was held constant regardless of the temperature and the concentration of $RS$- or $S$-1-monostearoylglycerol. $RS$- and $S$-1-monostearoylglycerol gave almost same signal pattern except for the OH proton signal.

Figure 3 shows the temperature and concentration dependence of the chemical shifts of OH proton for $RS$-1-monostearoylglycerol (○) and $S$-1-monostearoylglycerol (▲) in benzene-$d_6$. At each constant temperature the both chemical shifts, $\delta_{OH}$, of protons in OH moieties of $RS$-1- and $S$-1-monostearoylglycerols increase with increasing their concentrations; the $\delta_{OH}$ values of $RS$-1-monostearoylglycerol are, as a whole, larger than those of $S$-1-monostearoylglycerol. On the other hand, at each constant concentration of the $S$- or $RS$-1-monostearoylglycerol, the $\delta_{OH}$ of $S$- or $RS$-1-monostearoylglycerol decreases with increasing temperature. The proton-signal shift to higher magnetic field with temperature would be attributable to the dissociation of the hydrogen-bonding aggregate, whereas the shift to lower magnetic field with concentration, to the forma-
tion of the hydrogen-bonding aggregate. The $\delta_{\text{OH}}$ of S-form and that of RS-form are in agreement with each other at low concentration ($2.79 \times 10^{-4}$ mol dm$^{-3}$) and a high temperature ($70^\circ$C). Probably, under the condition such as low concentration and high temperature, R- and S-form molecules would exist as free monomers even in the RS-1-monostearoylglycerol solution. Thus, from the results of NMR, it is concluded that the hydrogen-bonding interaction between R- and S-molecules of 1-monostearoylglycerol is slightly stronger than that between S- and S-molecules in benzene.

### 3.3 Effect of concentration of S-1-monostearoylglycerol in hexane on its chirality

In order to know the effect of the concentration of S-1-monostearoylglycerol in a solvent on its chirality, we carried out the UV and CD measurements. In this experiment we used hexane at high temperature ($63.1^\circ$C). This is because benzene has a strong absorption band which interferes the observation of C=O band of S-1-monostearoylglycerol in its UV spectrum, whereas hexane has no such interference.

Figure 4 shows the concentration dependence of UV-absorption and CD spectra for S-1-monostearoylglycerol in hexane at $63.1^\circ$C. The UV-absorption and CD spectra have a band having a peak around 214 nm. The intensity of the UV-absorption and CD band-peaks increases with increasing concentration. The bands in both UV-absorption and CD spectra are due to the ($\pi^*, \pi$) transition of C=O bond$^{14,15}$. The RS-form gave almost the same UV spectra as S-form; the CD spectra for RS-1-monostearoylglycerol were, of course, not observed.

Figure 5 shows the absorbance versus concentration relationships at 214 nm for RS- and S-forms. The two relationships are in fair agreement with each other and indicate a good linearity. Thus Lambert-Beer low is applicable to the RS- and S-forms in this concentration range. From the slope of the line, we obtained the molar absorption coefficient, $e$ ($= 60$ mol$^{-1}$ dm$^3$ cm$^{-1}$), for the absorption band at 214 nm for S-1-monostearoylglycerol.

Figure 6 shows the concentration dependence of the ellipticity at 214 nm, $\theta_{214\text{nm}}$, of S-1-monostearoylglycerol.
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in hexane at 63.1°C. The value of 214 does with increasing concentration but its increment gradually decreases. Linearity is not applicable between the 214 and the concentration. Thus from the 214 value, we evaluated the difference in the molar absorptivity for left and right circularly polarized light,  , using Equation 1.

\[ \Delta e = \frac{33 \theta_{214}}{C \cdot d} \]  

(1)

where \( C \) denotes the concentration (mol dm\(^{-3}\)) and \( d \), the light-path length.

CD spectra are generally influenced by the intensity change of the absorption band itself. Thus, for strict comparison of \( \Delta e \) at a band peak, normalization is necessary; we divided \( \Delta e \) by the molar absorption coefficient, \( \epsilon \), at the same wavelength. The parameter \( \Delta e/\epsilon \) is called as anisotropy factor, \( g \).

Figure 7 shows the variation of \( g \) against the concentration of S-1-monostearoylglycerol at 63.1°C. The \( g \) value decreases with an increase in the concentration of S-1-monostearoylglycerol. It is clear that the reduction in \( g \) factor should be attributable to the decrease in \( \Delta e \) values. Why does the \( g \) value decrease with an increase in the concentration?

Goto et al. reported the X-ray crystal structure of a single crystal of the \( \beta' \) form of S-1-monostearoylglycerol. They indicated that in the crystal of S-1-monostearoylglycerol, the asymmetric unit contains two crystallographically independent molecules, which differ in the conformation of their glycerol parts. This is considered to be the influence of the hydrogen-bond network structures in the crystal. Consequently, S-1-monostearoylglycerol, although it exists as free monomers in its low concentrated solution, is thought to produce most probably two kinds of 1-monostearoylglycerol having two distinct conformations in its high concentrated solution and combine with each other to form associated pairs, which have a less chirality (decreasing \( \Delta e \)). Namely, the decrease in \( g \) value (i.e., the decrease in chirality) for S-1-monostearoylglycerol with a rise of the concentration results from the association of chiral molecules by the hydrogen bonding; the association of chiral molecules is thought to reduce the overall chirality. More specifically, it is thought that a changing of magnetic dipole moment of \( (\alpha, \pi^* \) transition of carbonyl group in glycerol, which affects CD intensity, is caused by the formation of hydrogen-bonding aggregate.

4 CONCLUSION

The hydrogen bonding of racemic 1-monostearoylglycerol in benzene was studied by comparison with that of chiral one through the \( ^1H \) NMR measurement. UV and circular dichroism (CD) measurements were carried out to know the effect of the concentration of S-1-monostearoylglycerol on its chirality. The chemical shift of OH proton indicated that the hydrogen bonding between \( R \)- and \( S \)-form molecules in \( R S \)-1-monocyglycerol is stronger than that between \( S \)- and \( S \)-form molecules in S-1-monocyglycerol and that the difference in the strength of hydrogen-bonding between \( R S \)- and \( SS \)-form become to be small at high concentration. The chirality of S-1-monostearoylglycerol in hexane decreased with an increase in its concentration. This suggests that the two kinds of \( S \)-form molecules having different structures associate with each other in the concentrated solution. This association would decrease the overall chirality of S-1-monostearoylglycerol.

References