On the Sub-α-Form and the α-Form in Monoacylglycerols

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ABSTRACT: The concentration of 2-monostearoyl-glycerol (2-MS) in 1-MS kept at 100°C reaches a plateau concentration of about 12% (w/w) according to ^{1}H nuclear magnetic resonance. The phase diagram of these two components, made by differential scanning calorimetry (DSC), showed that the temperature of the transition from the sub-α_1-form to the α-form and the melting point of the α-form decreased when the content of 2-MS increased above 9%. The temperature of transition from the sub-α_2-form to the sub-α_1-form, however, decreased at even lower contents of 2-MS. The reversible phase transition of mixtures of 2-MS and 1-MS from the sub-α-form to the α-form has been followed by DSC and X-ray diffraction. Concentrations of 2-MS above 9% change the tilt of the molecules in the mixture abruptly during the transition from the sub-α-form to the α-form. At higher temperature, the conformational disorder in the α-form of the mixture changes continuously. This conformational disorder is, however, unstable enough to change gradually into a more stable one. Even for pure 1-*rac*-MS, small changes were observed. In addition, monobehenoyl-glycerol and monopalmitoyl-glycerol, which also contained 2-monoacyl-glycerol, were also investigated. The longer fatty acid chains tend to give a higher degree of conformational disorder. The transition behavior has been analyzed on the basis of the crystal structures. Water swelling behavior has also been studied and compared for the sub-α-form and the α-form. The α-form can incorporate more water, probably because it has more disordered polar groups.

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KEY WORDS: Conformational disorder, DSC, α-form, isomerization, long spacing, phase transition, sub-α-form, water

Monoacyl-glycerols (MG) are important amphiphilic substances that are frequently used in foods as well as in drugs and cosmetics.

In previous studies (1–4), the thermal behavior and polymorphism in pure 1-*rac*-MG of saturated fatty acids have been discussed. Malkin and Shurbagy (1) found three different crystal forms, the α-form with vertical chains and the β′-form and the β-form, both of which have tilted fatty acid chains with the same angle of tilt. Lutton and Jackson (2), however, claimed that not only the β′-form and the β-form but also the α-form are tilted at almost the same angle and, furthermore, that 1-MG has one more polymorphic form, which they called the sub-α-form because it is transformed into the α-form reversibly. The latter description was confirmed by Chapman with infrared absorption data (3), and by Larsson from X-ray diffraction pattern vs. temperature (DPT) diagrams (4).

Larsson's studies (4) on molecular arrangements of pure 1-racemic monosaturated acyl-glycerol indicated that the sub-α-form shows the common orthorhombic chain packing (O⊥), and that the chains are tilted about 55° toward the end-group planes. This indicates that the end-group planes have subcell indices (021). Recently, Goto *et al.* (5,6) have determined the crystal structure from single-crystal data of the stable forms of pure 1-*rac*-monolaurin and optically active 1-monostearin. Stereospecific 3-monoacyl-*sn*-glycerols with saturated fatty acids were synthesized, and their structures were investigated by Small's group (7).

Pure 2-monoacyl-glycerols (2-MG) are easily transformed into 1-*rac*-MG (8). The thermal behavior was studied by Malkin (9). Larsson determined the crystal structure of the stable β-form of 2-monolaurin (4). The chains are packed according to the triclinic chain packing (T//) and tilted 44° toward the end-group planes.

MG used in technical applications, such as food systems, however, are equilibrium mixtures of 1-MG and 2-MG. In an earlier study of the mixture of 1-MG and 2-MG by Filer *et al.* (10), no polymorphism was observed.

The α-form is a useful polymorphic form in food systems, because this phase can swell with water. It can therefore contribute to emulsion stability (11–13).

In this article, we describe the phase diagram between 1-monostearoyl-glycerol (1-MS) and 2-MS, obtained by heating 1-*rac*-MS for different periods. Recently, Lutton (14) reported that there are two sub-α-forms, related by a reversible sub-$\alpha_2 \rightarrow$sub-α_1 transition, and that 10% and more of 2-MG makes this transition disappear. We will also discuss this phenomenon. Our work was focused on the transition from the sub-α-form to the α-form at different 2-MS contents.

MATERIALS AND METHODS

Materials. The 1-*rac*-monostearin and 1-*rac*-monopalmitin were purchased from Sigma Chemical Co. (St. Louis, MO). The purity was checked and found to be over 99%. The 1-monobehenoyl-glycerol sample was kindly supplied by Mo-

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gens Nielsen and Niels Krog (Danisco, Brabrand, Denmark). It was prepared by molecular distillation in an industrial pilot plant and consisted of about 90% 1-*rac*-monobehenoyl-glycerol and 10% 2-monobehenoyl-glycerol. The concentration of 2-MG was determined by 300 MHz ^{1}H nuclear magnetic resonance (NMR) (15).

Sample preparation. Two pure components are usually mixed to make a binary phase diagram, but 2-MG is so unstable that it is difficult to get well-defined mixtures. Therefore, heating of pure 1-*rac*-MG to give different levels of 2-MG has been used. The heated samples were subjected to ^{1}H NMR to determine the concentration of 2-MG. Mixtures with varying concentrations of 2-MS were prepared by heating pure 1-*rac*-MS at 100°C between 0 and 5000 min. The samples were rapidly cooled to 0°C in an ice bath.

X-ray diffractometry (XRD). For XRD measurements, a Guinier camera was used (CuKα; λ = 0.1542 nm). To check the dependence of crystal spacing on temperature and time, diffraction patterns vs. temperature or vs. time were recorded.

Differential scanning calorimetry (DSC). Transition and melting temperatures were measured with a DSC-2C (Perkin-Elmer, Norwalk, CT). The samples were heated from 0 to 100°C at 5°C/min.

Water-swelling behavior. Water (20% w/w) was added to the MG, and the samples were then subjected to XRD. The water-layer thickness was calculated as the difference between the samples with water and without water in the long-spacing region.

RESULTS AND DISCUSSION

Phase diagram. When 1-*rac*-MS is kept at 100°C for a long time, the concentration of 2-MS increases rapidly to about 8% and after that gradually reaches a plateau at about 12%. The observed reaction curve fits the equation:

$$y = 7.50 + 1.102 \log (x);\ R^2 = 0.962 \qquad [1]$$

where y = the concentration of 2-MS (%), and x = time (min). Our phase diagram is limited to 12% content of 2-MS.

Figure 1 shows that the temperature of the transition from the sub-α_1-form to the α-form and the melting point of the α-form decrease when the content of 2-MS increases above 9%. It suggests that the occurrence of the phase transition needs the space made by contamination created by >9% 2-MS. On the other hand, the transition temperature from the sub-α_2-form to the sub-α_1-form decreases even at a lower content of 2-MS. The general similarities in the XRD data between the sub-α_2-form and the sub-α_1-form of pure 1-MS show that their crystal structures are closely related (Table 1). Both forms are tilted, with orthorhombic chain packing. The short spacings of the sub-α_2-form are, however, shorter than those of the sub-α_1-form, and the long spacing of the sub-α_2-form is also a little shorter than that of the sub-α_1-form. A decrease in transition enthalpy was observed as well. These results indicate that the sub-α_2-form has a closer-packed crystal structure and that, with a certain amount of 2-MS contamination, there is enough disorder (thus extra space) to allow a change from the sub-α_2-form to the sub-α_1-form.

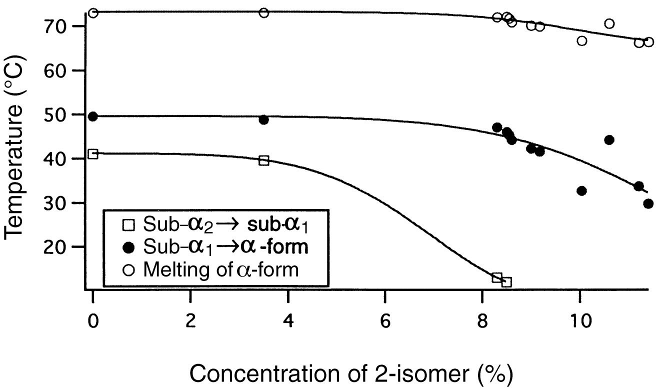

FIG. 1. Phase diagram as a function of 2-monostearoyl-glycerol concentration.

Effect of concentration of 2-MS. The α-forms and the sub-α-forms of samples heated for different periods were analyzed by XRD, and the difference in the long spacings between the sub-α-form, measured at 20°C, and the α-form measured at 60°C, are shown in Figure 2. The concentration of 2-MS was calculated from Equation 1. A previous study (1) reported that the sub-α-form and the α-form have almost the same long spacing, but Figure 2 indicates that the long spacing of the α-form is 0.5 Å longer than that of the sub-α-form at a 2-MS concentration of 8.5%. The difference in the long spacing between these two phases becomes almost 4 Å at 10% 2-MS, and this difference seems to reach a plateau value above 10%. Therefore, the α-form has a somewhat longer long spacing than the sub-α-form in 1-MS, and with increasing concentration of 2-MS this difference is successively increased. The strongest short spacing line near 4.2 Å also shows a similar increase in difference between the sub-α-form and the α-form with the amount of 2-MS.

Influence of temperature. The influence of temperature on the samples heated to 90°C for 90 min was followed by XRD measurement at room temperature and from 35 to 70°C at 5°C intervals. In each measurement, a new sample was used immediately after cooling to 0°C.

Figure 3 shows that the long spacing of the sub-α-form is

TABLE 1
Transition of Monostearin[a]

Form	Sub-α_2		Sub-α_1		α
Short (Å)	4.45 (vw)	→	4.54 (vw)		4.65 (w)
	4.29 (m)	→	4.32 (w)		
	4.13 (vs)	→	4.21 (vs)	→	4.18 (s)
		↘	4.10 (m)	↗	
	3.88 (m)	→	3.97 (m)		
	3.73 (w+)	→	3.81 (w)		
	3.57 (m)	→	3.65 (m)		
Long (Å)	49.7	→	50.0		50.5
Temperature (°C)	23.4		48.4		59.7

[a] Arrows indicate continuous change. Abbreviations: vs, very sharp; s, sharp; m, medium; w, weak; vw, very weak.

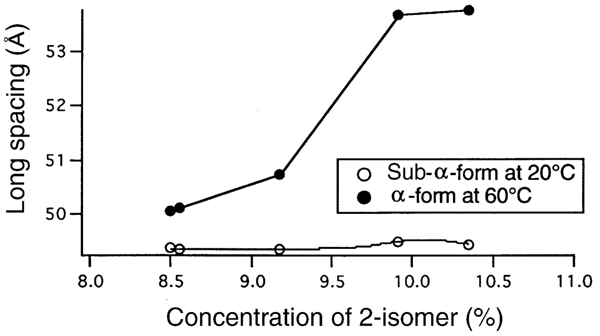

FIG. 2. Long spacing of the sub-α-form and the α-form vs. the 2-monostearoyl-glycerol.

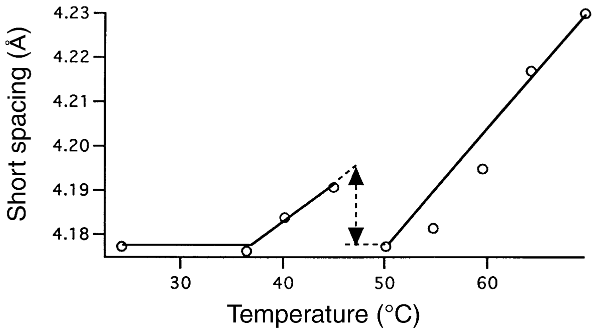

FIG. 4. The dominant short-spacing line vs. temperature. Arrow indicates a discontinuous change.

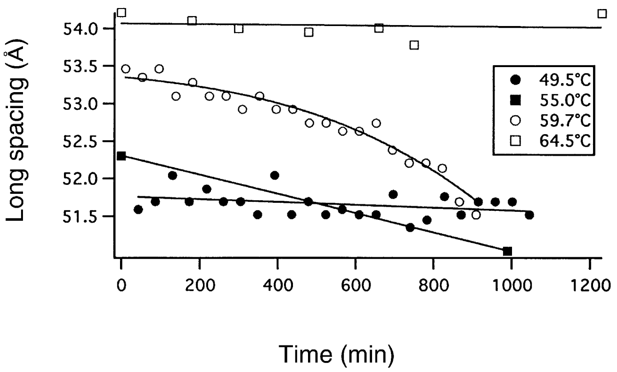

FIG. 5. The long spacing of the α-form diffraction data vs. time.

independent of temperature. At the transition from the sub-α-form to the α-form, however, a discontinuous increase was observed. Even in samples with small amounts of 2-MS, there is still a discontinuous increase (0.5 Å). In the α-form, the long spacing increases continuously with temperature. The strongest short-spacing line near 4.2 Å was also followed because it reflects the close packing of the chains (Fig. 4). In both the sub-α-form and the α-form, higher values were observed with increasing temperature. It is important in this connection to point out that the 4.2 Å line represents the distance between the chain planes in the hexagonal packing of the α-form, whereas in the orthorhombic packing of the sub-α-form it is one interchain distance. The other is about 3.8 Å. At the transition, the short spacing shows a discontinuity as well.

To see whether this phenomenon represents equilibrium, the long spacings were recorded for samples kept for 1200 min at specific temperatures (49.5, 55.0, 59.7, and 64.5°C). When the long spacing is below 53.5 Å, it has a tendency to decrease to about 51 Å (the long spacing at 49.5°C), whereas samples with long-spacing values over 54 Å do not decrease (Fig. 5).

1-MG with different saturated fatty acids. The long spacings of the sub-α-form and the α-form of 1-MG with different saturated fatty acids were also investigated, and the results are shown in Table 2. To obtain calculated tilt angles in the sub-α-forms of MG with different fatty acid chains, the long spacings that corresponded to the vertical structure of MG were calculated with Goto's results (5,6) on the single-crystal data that indicated a gauche conformation of the glycerol skeleton on the β′-form of 1-MG. If these data are applied to the crystal structure of the sub-α-form, because both the sub-α-form and the β′-form have the orthorhombic chain packing, the 1-MS molecule is estimated to have a length of 30.15 Å. The tilt angle in the sub-α-form can then be calculated, and a value of 55.2° toward the end plane is obtained. In 1-monopalmitoyl-glycerol and 1-monobehenoyl-glycerol, the corresponding tilt angles are 55.1 and 55.7°, respectively. These results show that even MG with different fatty acid chains have almost the same calculated tilt angle in the sub-α-form, but that the α-form of the MG with a longer fatty acid chain (1-monobehenoyl-glycerol) has a larger calculated tilt angle.

Conformational changes of formation of the α-form. The

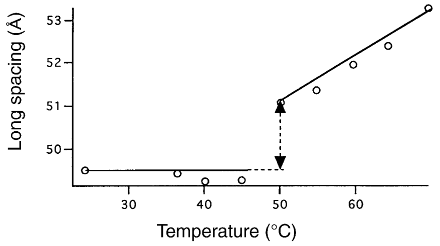

FIG. 3. Long spacing vs. temperature. Arrow indicates a discontinuous change.

TABLE 2
The Difference in Long Spacing Between the α-Form and the Sub-α-Form

	Long spacing	Calculated tilt angle	Temperature (°C)	Polymorphic form
Monopalmitoyl-glycerol	45.3 (Å)	55.2°	25.3	Sub-α
(55.2 Å)[a]	46.8 (Å)	58.0°	40.0	α
Monostearoyl-glycerol	49.5 (Å)	55.1°	21.9	Sub-α
(60.3 Å)[a]	54.0 (Å)	63.6°	69.2	α
Monobehenoyl-glycerol	58.2 (Å)	55.7°	22.0	Sub-α
(70.5 Å)[a]	63.5 (Å)	64.3°	55.0	α

[a] Length of vertical molecule.

long spacing and the strongest short spacing of 1-MS with 2-MS above 10% concentration vary continuously with temperature. It is proposed here that fatty acid chains with increasing disorder bend like a bow. This conformational disorder phenomenon has been observed by Kaneko *et al.* (16). They report that disordering in the α-form can be regarded as partial melting. Successive reduction in the tilt (as in a bow shape) toward the methyl end groups will give more space to each methylene group and thus allow a higher disorder. This feature can also explain the continuous change in the long spacing that we observed and the phenomenon indicated by the α-form of MG with varying fatty acid.

Transition from the sub-α-form to the α-form. This discontinuous increase in the long spacing at the transition from the sub-α-form to the α-form might be due to a change in the alkyl chain tilt or in the glycerol region. The change in the alkyl region includes a transition from orthorhombic to hexagonal chain packing and a change in tilt angle of the chains. In the paraffin crystal form of $C_{33}H_{68}$, the chainlength has been reported to increase about 0.6 Å per molecule at the transition from the orthorhombic phase to the hexagonal phase, mainly because the thickness of the plane layers of voids, which separate adjacent lamellae, becomes larger owing to thermal pressure arising from longitudinal motions of the chains (17).

This figure (0.6 Å) is almost the same as the difference between the sub-α-form and the α-form of the sample with a small amount of 2-MG. The discontinuous increase in the long spacing between the sub-α-form and the α-form is, however, larger for 1-MS that includes more 2-MS. This may indicate that a rearrangement of the polar group region takes place (2-MS has larger effective polar heads).

A similar discontinuous increase in the long spacing in 1-*O*-alkylglycerols has been reported by Persson (18). It was interpreted as a change from crystallinity to noncrystallinity in polar groups at the transition from the sub-α-form to the α-form.

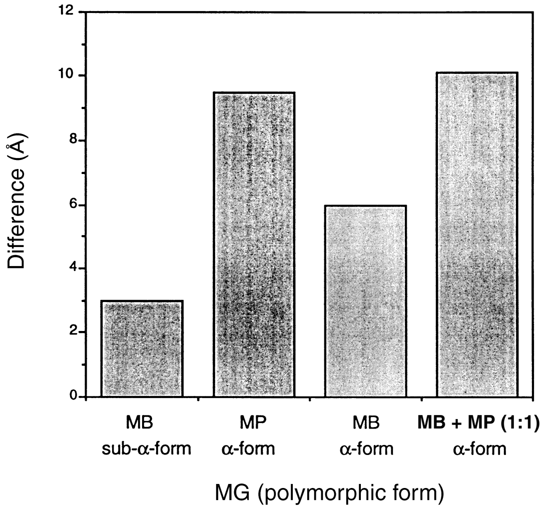

FIG. 6. Differences in long spacing after water swelling. Abbreviations: MG, monoacyl-glycerol; MB, monobehenoyl-glycerol; MP, monopalmitoyl-glycerol.

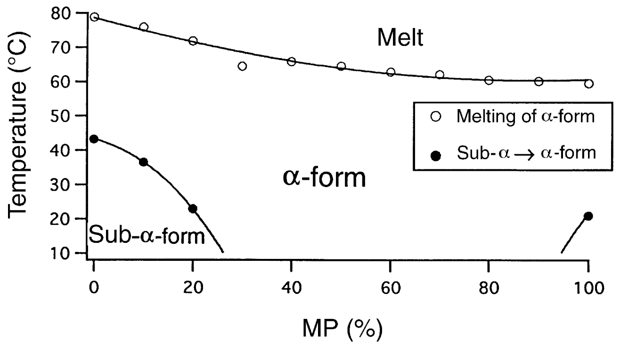

FIG. 7. Phase diagram between MP and MB. See Figure 6 for abbreviations.

Water-swelling behavior of the sub-α-form and the α-form. Water-swelling effects on the long spacing of the α-form and the sub-α-form are shown in Figure 6. The α-form can obviously take up more water and form a gel phase (19); Figure 6 indicates that all α-forms of MG contain more water than the sub-α-forms. In addition, the mixture of monobehenoyl-glycerol (MB) and monopalmitoyl-glycerol (MP) shows a stable α-form at room temperature (Fig. 7). Any MG with a chainlength above C_{16} cannot form a stable gel phase at room temperature in pure form. Therefore, the mixture of MB and MP (1:1w/w) can take up more water (Fig. 6) and form a well-defined gel phase. Even in the α-forms, there are differences in water-swelling behavior. The difference between MB and the mixture of MB and MP may be attributed to the disorder of the molecular structure.

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