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PHYSICAL AND MOLECULAR PROPERTIES OF LIPID POLYMORPHS
– A REVIEW –

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Abstract

The physical and molecular properties of the polymorphism of stearic acid, oleic acid and SOS (1,3-distearoyl-2-oleoyl glycerol) are comparatively discussed. Temperature dependence of Gibbs energy (G-T relation) of three polymorphs of stearic acid; A, B and C, revealed close relationships to each other. The molecular structures subtly differed in these polymorphs. In contrast, three polymorphs of oleic acid, α, β and γ, exhibited remarkably different characteristics. G-T relation showed more diversified features: in particular, the melting points of α and β differ by 3°C. An order-disorder transformation occurred between α and γ, as a result of conformational disordering in the portion of the oleic acid molecule from the double bond to the terminal methyl group in α. Finally, five polymorphs of SOS were newly presented, α, γ, pseudo-β', β_2 and β_1. X-ray spectra and thermal behaviors proved that the above five forms are the independent polymorphs. The author discussed the multiple polymorphism of SOS, taking into account the lamellar sorting of stearic/oleic acids chains accompanied with the change in the chain length structure. In relation to the polymorphism of SOS and other 1,3-disaturated–2-oleoyl glycerides, the author emphasizes the possibility that the conversion from Form V to VI in cocoa butter might be caused principally through the polymorphic transformation from β_2 to β_1 of the higher-melting fat fractions of cocoa butter.

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Introduction

The physical and molecular properties of lipid polymorphs have drawn the attention of many investigators in the fields of biological sciences and oil chemical technology. This is due to the fact that the polymorphism of lipids is highly relevant in biological systems, and also decisive to the physical properties of foods, cosmetics, etc., which comprise lipids as the main compounds of solid fats.

The polymorphism may be discussed in terms of thermodynamic stability, crystal packing and molecular conformation as far as the physical aspects are concerned. The fundamentals of the macroscopic features of polymorphism, such as morphology, solidification kinetics and so on, may be explained in terms of these physical aspects. Each of the above factors is highly dependent on the molecular species which constitute the lipid, under a given set of external conditions. This is easily seen if one compares the melting points of stearic acid (69.6°C) with oleic acid (16.2°C of the high-melting polymorph). Obviously, this difference is the result of a drastic reduction in the chain-packing energy by the introduction of one *cis*-double bond at the central position of the polymethylene chain.

Very recently, many investigators have tried to elucidate the physical and molecular properties of some principal fatty acids and triglycerides. Particular effort has been devoted to the lipids containing unsaturated fatty acids as the main and functionally active constituents. Accordingly, this paper gives a brief review of the polymorphism of stearic acid and oleic acid, being representative of the saturated and unsaturated fatty acids, respectively. Furthermore, new findings are presented on the polymorphism of SOS, 1,3-distearoyl-2-oleoyl triglyceride, as representative of the symmetric S_tOS_t (S_t: saturated acid, O: oleic acid) triglycerides. First, we briefly discuss some conceptual and methodological backgrounds.

Polymorphism: Thermodynamic Stability and Crystallography

In order to discuss the physical properties of different polymorphic forms of certain fatty acids and triglycerides, a precise knowledge of thermodynamic stability is prerequisite. For this purpose, measurements on the solubilities, melting points and transformation pathways of all the polymorphs are most characteristic. The less stable polymorphs melt at lower temperatures, are more soluble in solvent, and transform to more stable ones either via solid-state (Verma and Krishna, 1966) or via solution-mediated (Cardew and Davey, 1985) or via melt-mediated transitions (Sato and Kuroda, 1987). The latter two transformations may actually occur if the solid-state transformation is kinetically hindered. Many long-chain compounds reveal these transformations. Knowing the thermal data, one may draw the relationship between the thermodynamic stability using a crystal Gibbs energy (G) and temperature (T) diagram.

The crystallographic aspects of the polymorphism of fatty acids and triglycerides are reflected in the lateral packing and lamella stacking of the hydrocarbon chains, which are most easily measured by X-ray diffractometry or by Infrared (IR) and Raman spectroscopy. Indicative of the lateral packing, characterized by the subcell structure, are the X-ray short spacings. These have so far exhibited three specific subcells; orthorhombic perpendicular ($O_\perp$), triclinic parallel ($T_{\parallel}$) and pseudo-orthorhombic parallel ($O'_{\parallel}$) as shown in Fig. 1 (Abrahamsson et al., 1978). In addition, a hexagonal subcell is reported to occur in highly metastable states (Abrahamsson et al., 1978). The saturated aliphatic chains are principally packed in $O_\perp$ and $T_{\parallel}$ according to the mode of crystallization and thermodynamic stability, whereas $O'_{\parallel}$ is reported for the low-temperature polymorph of oleic acid (Abrahamsson et al., 1962). Hence this may be one of the subcells characteristic to the *cis*-unsaturated acyl chain.

The lamellar stacking is indicated by the X-ray long spacing spectrum which equals the inter-lamellar distance between the terminal CH_3 groups of the lipid lamellae. The long spacing also can be a measure of the chain length structure, in particular, of triglycerides (Small, 1986). Normal triglycerides, such as monosaturated acids, reveal a double chain length structure where the long spacing equals the length of two fatty acids and one glycerol group. A change from the double to triple chain length structures occurs when the fatty acid moieties become mixed: i.e., large differences in the numbers of carbon atoms, (Kodali et al., 1984), saturated/unsaturated mixed acids, (Lutton, 1972) etc. The triple chain length structure is caused by a sorting of one chain acid from the other two chain acids (Fig. 2). Thus, the long spacing equals the sum of the lengths of three fatty acids and two glycerol groups. Even a six chain length structure is proposed (Fahey et al., 1985), consisting of two triple chain length lamellae in a polytypic relation (Verma and Krishna, 1966).

Last, molecular information about the hydrocarbon portions of the molecules can be elucidated with IR, Raman, high-resolution NMR, etc. The data on chain packing within the lamellar plane, inter-lamellar end packing, configuration and conformation of the carbon chains and hydrogen bonding, etc., obtained with these spectroscopic methods are not only complimentary to those from X-ray diffraction but also diagnostic of subtle structural properties of the polymorphism on a molecular level.

Stearic Acid

Three typical polymorphs of stearic acid, A, B and C, have been known for 30 years (von Sydow, 1956). The fourth form, E, was found later by the spectroscopic methods (Holland and Nielsen, 1962). Form A is triclinic, and B, C and E are monoclinic. The subcell structures of B and C are reported to be $O_\perp$.

As for the thermodynamic stability, the solubilities of A, B and C were measured independently (Table 1) (Beckmann et al., 1984). B has the lowest solubility below 32°C (Sato et al., 1985), whereas C is the least soluble above that temperature. Form A has a higher solubility than the lowest value at all temperatures. Accordingly, the thermodynamic stability of the A, B and C polymorphs of stearic acid may be depicted by the G-T diagram shown in Fig. 3. The G values of B and C are the same around 32°C. This is apparently contradictory to the features of the solid-state transformation. Stenhagen and von Sydow (1953) reported that the transformation temperatures on heating from A to C, and from B to C are 54°C and 46°C, respectively. Another report (Garti et al., 1980) says that B transforms to C at 54°C. All these values are higher than the actual crossing points of the Gibbs energies of A, B and C. This is attributed to a kinetic hindrance of the transformation in the solid-state. So, the actual polymorphic transformation in the crystal may depend on the heating rate, which differs in the above two papers.

Structural analyses using single crystals of B and C showed that the hydrocarbon chains of C are in the all-*trans* conformation (Malta et al., 1971), whereas the C_1–C_3 carbons closest to the carboxyl group of B are in *gauche* conformation (Goto and Asada, 1978).

Oleic Acid

Three polymorphs of oleic acid, α, β and γ, were recently confirmed by means of DSC, X-ray diffraction (Suzuki et al., 1985), IR and Raman spectroscopy (Kobayashi et al., 1986). The transformation circuit of the three polymorphs is depicted in Fig. 4 (Sato and Suzuki, 1986). α is crystallized by chilling the melt, although it is thermodynamically metastable. The preferred crystallization and metastability for α resemble those of α of glycerides. α and γ undergo a reversible transformation in the solid-state at -2.2°C on heating. γ is the form on which the structural determination was done (Abrahamsson et al., 1962). β, the most stable polymorph, crystallizes with very slow rates, both from solution and the melt. There is no solid-state transformation from α (or γ) to β in the melt-grown crystal due to a steric hindrance. Instead, the solution mediates the conversion. Lutton's high-melting form (Lutton, 1946) is equivalent to β in both the melting point and X-ray diffraction spectra. α, however, is contradictory to his X-ray data on the low-melting form, although the melting point is the same.

Table 2 summarizes the melting points and the enthalpy and entropy of fusion, dissolution and transformation. Solubility measurement (Sato and Suzuki, 1986) made it possible to depict the G-T relationship as shown in Fig. 5. The G values of γ and β are parallel to each other, whereas those of β and α come close together with increasing temperature. Far below their crossing point, they melt. This multiple melting is characteristic of oleic acid, and is not observed in saturated fatty acids.

The morphology and X-ray diffraction patterns of the three forms are shown in Figs. 6 and 7. All the crystals were of a tabular shape with a well-developed basal surface in a slightly supersaturated solution. α reveals a slender hexagonal shape, while β shows a truncated lozenge shape. The truncation occurs normal to the bisectrix of 55°. γ reveals a rectangular shape which is consistent with the subcell structure of $O'_{\parallel}$ (Abrahamsson et al., 1962). The morphology of the three forms changes

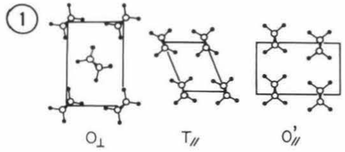

Fig. 1. Subcell structures of $O_\perp$, $T_{//}$ and $O'_{//}$ of aliphatic chains.

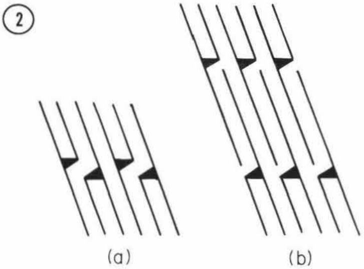

Fig. 2. Double, (a), and triple, (b), chain length structures in triglycerides.

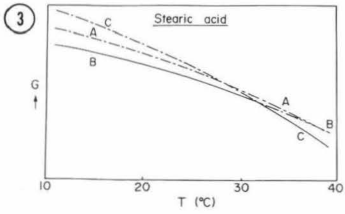

Fig. 3. Relationships between Gibss energy (G) and temperature (T) of A, B and C polymorphs of stearic acid, from solubility data (Beckmann et al., 1984).

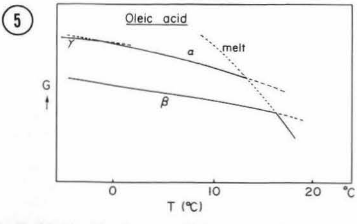

Fig. 5. Relationships between Gibbs energy (G) and temperature (T) of α, β and γ polymorphs of oleic acid, from solubility data (Sato and Suzuki, 1986).

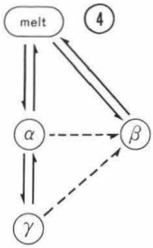

Fig. 4. A transition circuit among the polymorphs of α, β and γ, and melt of oleic acid (Sato and Suzuki, 1986).

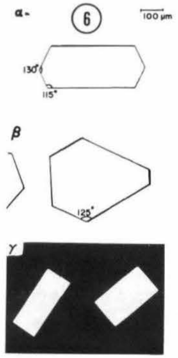

Fig. 6. Crystal shapes of α, β and γ polymorphs of oleic acid (Sato and Suzuki, 1986).

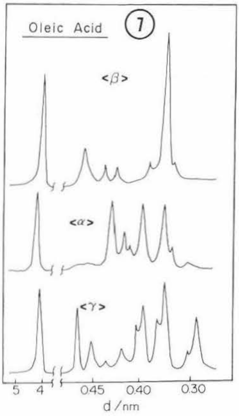

Fig. 7. X-ray diffraction spectra of α, β and γ polymorphs of oleic acid (Suzuki et al., 1985).

Table 1. Enthalpy (ΔH) and entropy (ΔS) of dissolution of A, B and C polymorphs of stearic acid in decane (Beckmann et al., 1984).

ΔH (kJ/mol)			ΔS (J/mol/K)		
A	B	C	A	B	C
65.7	69.0	64.4	193.4	205.0	189.9

Table 2. Enthalpy and entropy of fusion, dissolution and solid-state transformation of α, β and γ polmorphs of oleic acid (Suzuki et al.: 1985, Sato and Suzuki: 1986).

	fusion		dissolution				transitio
polymorph	α	β	γ^a	β^a	α^b	β^b	γ→α
T (°C)	13.3	16.2	–	–	–	–	−2.2
ΔH (kJ/mol)	39.6	51.9	100	100	59.4	76.0	8.7
ΔS (J/mol/K)	138.4	179.3	360	352	222.9	279.7	32.

a. decane, b. acetonitrile.

drastically to needle shape when the supersaturation of solution or supercooling is increased. The short spacing spectra of γ corresponds to the subcell of $O'_{\parallel}$. The other two forms reveal remarkably different patterns, implying different subcell structures. The long spacings are 4.34 nm (α), 4.12 nm (β) and 4.19 nm (γ).

Vibrational spectroscopic studies on α, β and γ forms of oleic acid have resulted in the following (Kobayashi et al., 1986): (a) the γ–α transformation is of an order (γ)-disorder (α) type which is accompanied by a conformational disordering in the portion of aliphatic chain between the double bond and the terminal methyl group (methyl-sided chain). The most conspicuous spectral change is seen in the low-frequency Raman spectrum (Fig. 8a). All the sharp bands of γ collapse to a broad band in α due to a loss of translational symmetry. This originates from a disordered structure. Additionally, a peculiarity, indicating the same conformational disordering, appeared in two strong bands due to a C–C stretching mode. This mode is reflected in a single band for saturated acids. After the γ–α transition, the 1125 cm^{-1} band of methyl-sided chain drastically decreased in intensity, whereas the 1095 cm^{-1} band due to the carboxyl-sided chain remained unchanged. (Fig. 8b). Thus, introduction of one *cis*-double bond at the central position of the alkyl chain induces an increase in the chain mobility, resulting in a new type of transformation of an interfacial melting. Conformational disordering of this kind was not detectable in β. (b) The conformation of the polymethylene chains of γ and β is all-*trans*. It is likely that *gauche* conformations occur in the disordered methyl-sided chain of α. (c) β and γ differ most in the characteristic bands of the olefin groups; skew-*cis*-skew' for γ, whereas β may take skew-*cis*-skew type conformation. (d) As for the subcell structure, γ shows typical spectral bands characteristic of $T_{\parallel}$. This supports the $O'_{\parallel}$ subcell, because $O'_{\parallel}$ may be included in the category of $T_{\parallel}$. The β form assumes a specific subcell structure differing from $O'_{\parallel}$ and $T_{\parallel}$ according to C–C progressive bands reflected in IR spectra. The inferred subcell of β suggests that the C–C zigzag planes of neighboring chains are not parallel to each other but, instead, somewhat inclined.

It is worth noting that the transformation of interfacial melting of γ–α of oleic acid was also observed in γ–α of palmitoleic acid and in γ–α of erucic acid (Suzuki et al., to be submitted). This indicates a characteristic kind of chain disordering in unsaturated fatty acids having one *cis*-double bond.

Comparing the polymorphism of stearic and oleic acids, differences are seen both in the crystal structures and the thermal behaviors as described above. In addition, the kinetic behaviors of crystallization are different. The polymorphs of stearic acid crystallize in a different manner, depending on sovent, supersaturation and temperature. The quantitative differences in the nucleation rate (Sato and Boistelle, 1984) and the crystal growth (Beckmann and Boistelle, 1985) are up to approximately 50% under normal conditions of crystallization. In the case of oleic acid, however, the rate of crystallization of α and β are very different. For example, α exclusively solidifies from the melt with moderate cooling rates, e.g., 2°C/min as examined by DSC. On the contrary, only β occurs when the cooling rate is lower than 0.05°C/min (Suzuki et al., 1985). These differences demonstrate more diversified characteristics of both the thermodynamics and kinetics of the polymorphism of oleic acid. From this, one may conclude that the complexity can exist in the polymorphism of mixed saturated/unsaturated triglycerides. Independent or cooperative changes in the chain packing and molecular motions of the saturated and unsaturated alkyl groups are likely to occur while sharing the common glycerol backbone. This is discussed in the next section.

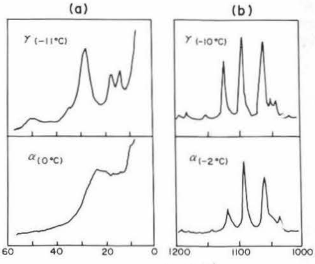

Fig. 8. Raman spectra of α and γ polymorphs of oleic acid, (a) low-frequency bands, (b) C-C stretching bands (Kobayashi et al., 1986).

SOS

The polymorphism of triglycerides has been studied extenstively because of their importance in lipid chemstry (Small, 1986). The polymorphism of monosaturated acid triglycerides

Table 3. Long spacing (LS, nm), melting point (T_m, °C) and enthalpy of fusion (ΔH_f: kJ/mol) of five polymorphs of SOS obtained in the present study and corresponding polymorphs in the literature.

present study					literature					
form	purity	LS	T_m	ΔH_f	(a)	(b)	(c)	(d)	(e)	(f)
α	91%	5.05	28*	–	IV	–	α	α	α	IV
	99%	5.05	23.5	–						
γ	91%	7.37	37*	–	II	β'	subβ	β''	subβ	II
	99%	7.35	35.4	98.5						
pseudo-β'	91%	7.06	38*	–	II	–	β'	β'	–	II
	99%	7.00	36.5	104.8						
β_2	91%	6.50	42*	–	I	β	β	β	β	I
	99%	6.50	41.0	143.0						
β_1	91%	6.50	43*	–	I	β	–	β	–	I
	99%	6.50	43.0	151.0						

(a) Daubert and Clarke, 1944), (b) Filer et al., (1946), (c) Lutton and Jackson (1950), (d) Malkin and Wilson, 1949), (e) Lavery (1958), (f) Landmann et al., (1960).

(*) Examined by viewing transparency temperature of sample with eyes.

has been well established, as far as the thermodynamic properties are concerned. In the literature, however, results are rather contradictory for mixed saturated/unsaturated acid triglycerides, despite the importance of these compounds in confectionery fats. For the same compound, inconsistent results are reported for the number and nomenclature of polymorphs, structures of the subcell and chain length, melting points and thermal behaviors, etc. This is shown in Table 3 which summarizes the nomenclature and the long spacing data of the polymorphs of SOS. Although not presented here, there is a wide variation in the melting point. This confusion might be attributed to the purity of sample employed, or experimental methods and instruments.

The authors have recently studied the polymorphism of a series of S_tOS_t triglycerides (S_t: C_{16}, POP; C_{18}, SOS; C_{20}, AOA, and C_{22}, BOB). Particular concern was given to purity of the samples and techniques for identification of individual polymorphs. Two samples, lower-purity (91%) and higher-purity (99.0%), were examined for each compound using the same thermal treatments to reduce the effect of purity. Polymorphs were identified by their characteristic X-ray diffraction pattern after obtaining a single melting or solidification peak. In taking these data, two thermal treatments were used; transformation from the polymorphs which were directly solidified from the melt at various temperatures and the melt-mediated transformation. The latter transformation is a re-solidification from the melt which was formed by rapidly raising the temperature to just above the melting point of a less stable form (Sato and Kuroda, 1987). The X-ray and DSC measurements were carried out simultaneously for each sample during the above thermal treatments. Polymorphs were named by taking into account the characteristic X-ray diffraction patterns, DSC data, and related previous work. This paper presents a summary of new results of SOS and POP (Sato et al., submitted to J. Am. Oil Chem. Soc.) and other S_tOS_t compounds (Wang et al., 1987).

Five polymorphs of SOS were obtained at ambient temperatures (above 15°C); α, γ, pseudo-β', β_2 and β_1, all of which occurred both in higher- and lower-purity samples. Additionally, the lower-purity samples contain another intermediate polymorph having X-ray short spacing spectra, e.g., three peaks of 0.435 nm, 0.419 nm, and 0.393 nm, similar to β_1' of tristearin (Simpson and Hagemann, 1982). This form, however, did not appear in the higher-purity samples and should be disregarded as a polymorph of pure SOS.

Each polymorph has the following characteristics; (a) DSC (2°C/min) reveals a single melting peak except for α whose melting peak (around 23°C) was followed by solidification of γ. (b) Thermal treatments exhibited successive irreversible transformations in the solid-state. (c) The X-ray short spacings reveal unique patterns for all polymorphs as shown in Fig. 9. Higher- and lower-purity samples gave identical short spacing spectra and there was no doubt in discriminating between specific spectra of α, γ, pseudo-β'. This is also true for long spacings, melting points and enthalpies of fusion, ΔH_f (Table 3). The distinction between β_2 and β_1 is subtle, yet it has an important relevancy. The short spacings, melting points and ΔH_f are distinctly different but the long spacings have the same value. For short spacings, a strong peak for 0.458 nm is common, but the intensity ratios of other peaks show a clear contrast. Furthermore, the two peaks in β_2 denoted by arrows (0.400 nm and 0.390 nm) are split into two in β_1 and a peak in β_2 denoted by a filled triangle (0.375 nm) disappeared in β_1. The difference in β_2 and β_1 is also manifest in the melting curves examined by DSC. Figure 10 depicts DSC curves of the five polymorphs of SOS recorded while heating (2°C/min). When a sample revealing the superimposed X-ray short spacing spectra of β_2 and β_1 was heated, double melting peaks were detected. Subsequent results confirmed that the higher-melting peak increased in size as the duration of tempering increased.

Typical thermal treatments for obtaining the five polymorphs of SOS are as follows. α is formed by chilling the melt below 23°C. γ is formed either by transformation from α (e.g., over 12 h at 17°C) or by the solidification from the melt in a temperature range of 24–28°C. The melt-mediated transformation from α also yielded γ in the same temperature range. γ transformed to pseudo-β' by tempering over 1 h at 30°C. The melt also solidified into pseudo-β' around 29–36°C, although very slowly. The transformations of pseudo-β' to β_2 and β_2 to β_1 occurred over 10 h at 35°C and 11 days at 40°C, respectively. These data confirm the five individual polymorphs of SOS.

The chain length structure of α is double, Fig. 2, but in contrast triple chain length structures are revealed in the other four forms. This means that a conversion in the chain length structure occurs during the transformation in the solid-state.

Analysis of other S_tOS_t compounds show that AOA and BOB possess the same five polymorphs as SOS with regard to X-ray short and long spacings (Wang et al., 1987). POP, however, displayed unusual behavior. α, γ, β_2 and β_1 are found as in all the S_tOS_t compounds, but three more intermediate forms were also obtained. They were named δ, pseudo-β_2' and pseudo-β_1'. The latter two forms show the X-ray short spacing spectra similar to pseudo-β' of SOS, but subtle differences were also observed. Furthermore, their chain length structure was double chain-length. Pseudo-β_2' and pseudo-β_1' were formed either via the transformation from γ or via the melt solidification and differed in melting point by about 2.5°C. Pure δ occurred during the melt solidification in a narrow range of the solidification temperature. Consequently a total of seven polymorphs were obtained in POP.

The description of the polymorphism of the S_tOS_t compounds is concluded with justifications for the nomenclature of the different polymorphs. α corresponds to what is commonly seen in glycerides, being characterized by a single short spacing around 0.42 nm. γ has a strong short spacing line at 0.472 nm, which is not seen in any polymorphs of saturated fatty acids and glycerides. Therefore names of β' (Filer et al., 1946), β'' (Malkin and Wilson, 1949), and sub-β (Lutton and Jackson, 1950, Lavery, 1958) may be inadequate. This peak was observed only in γ of oleic acid (Fig. 7). The name of pseudo-β' is used because the short spacing spectra are similar to patterns of β' for $S_tS_tS_t$, and because Gibon et al. (1986) gave the same name to the polymorph of POP having features equivalent in the present study. β_1 and β_2 have the X-ray short spacings similar to β of $S_tS_tS_t$ with increasing subscripts denoting decreasing melting point. Use of Roman numerals (I, II, etc.,) (Daubert and Clarke, 1944; Landmann et al., 1960), or of A, B, etc., for POP (Lovegren et al., 1971) is disregarded since they are far from the nomenclature traditionally employed in glycerides.

At present, the structures of the subcell and unit cell, and the molecular conformations of the S_tOS_t polymorphs are unknown. Due to subtle differences in X-ray diffraction patterns, slight variations in molecular structure are possible. In this regard, a possible variation may be a change within the saturated or unsaturated lamellae which are separated into the different layers in the triple chain length structure. Since the polymorphic behaviors of the unsaturated and saturated acids are quite different, it is highly feasible that multiple polymorphic forms of S_tOS_t may occur due to independent or cooperative molecular changes in the two different lamellae. Vibrational spectroscopic measurements and X-ray structure analyses using single crystals should give decisive data. Work is in progress.

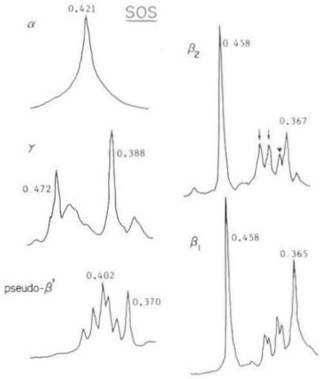

Fig. 9. X-ray short spacing spectra of α, γ, pseudo-β', β_2 and β_1 of SOS. After the transformation, two peaks of β_2 denoted by arrows split into two, and a peak denoted by a filled triangle disappeared. (unit = nm)

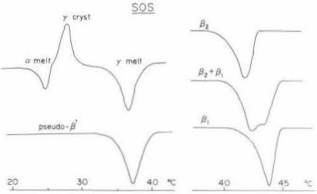

Fig. 10. DSC curves (on heating: 2°C/min) of α-melting/γ-solidification, melting of pseudo-β', β_2, β_1 and the sample which was formed during the transformation from β_2 to β_1.

Cocoa Butter Polymorphs

The peaks of medium strength in the X-ray patterns of β_2 and β_1 are very similar to those of Forms V and VI, respectively, of cocoa butter (Fig. 11) (Wille and Lutton: 1966, Garti et al., 1986). Furthermore, the difference in the melting point is of the same order of magnitude; 2.5°C between Forms V and VI of cocoa butter (Wille and Lutton, 1966) and 2.2°C between β_2 and β_1 (Table 3).

To verify these similarities, the polymorphism of a mixture of POP/POS/SOS (wt% ratio, 18.2/47.8/34.0; % purity: POP, 99.2: SOS, 99.0; POS, 98.3) was examined using the thermal treatments previously described (Sato et al., unpublished). The same DSC and X-ray short spacing patterns were obtained as those of β_2 and β_1 of SOS and POP. The melting points of β_2 and β_1 of the mixture were 33.2°C and 35.3°C, respectively. In addition, mixtures of POS/SOS with a lower content of POS, or without POS, also revealed essentially the same results. From this it is concluded that the β_2 and β_1 polymorphs are characteristic forms in pure S_tOS_t triglycerides and their mixtures, and that the transformation of Forms V → VI in cocoa butter is the result of a polymorphic transformation of the S_tOS_t fractions from β_2 to β_1. Wille and Lutotn (1966) compared the X-ray data of a mixture of SOS (25%), POS (50%) and SOO (25%), and reported the similarity of its pattern to the β polymorph of SOS and POP. No distinction was made, however, between "two β" forms of the two triglycerides.

As for the mechanism of the V → VI transition in cocoa butter, the present consideration agrees better with ideas based on the solid-state transformation (Garti et al., 1986), yet contradicts those which assume that the V → VI change is caused by the separation of a portion rich in high-melting fat from a lower-melting portion of cocoa butter (Manning and Dimick, 1985). Obviously, the actual process of the V → VI transformation in cocoa butter is rather complicated. At elevated temperatures (around 30°C), the higher-melting fractions of cocoa butter, mainly POP/POS/SOS, coexist with liquid oil which consists of the lower-melting fractions. Therefore, transformations mediated in liquid oil may also take place: dissolution of β_2 (Form V) in oil, and re-crystallization of β_1 (Form VI). The transformations mediated via oil (solution)-mediated and via solid-state may concurrently take place. In both processes, however, the basic physical features may be regulated by two different polymorphs of the S_tOS_t triglycerides.

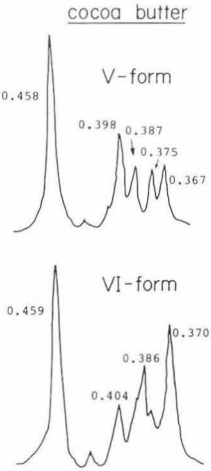

Fig. 11. X-ray short spacing spectra of Forms V and VI of cocoa butter (redrawn from data of Wille and Lutton: 1966). (unit = nm).

Conclusion

The polymorphism of stearic acid, oleic acid and SOS has been discussed. It was shown that, physical and molecular characteristics in the polymorphism is quite different between stearic and oleic acids. This difference is caused by introduction of one *cis*-double bond at the center of the aliphatic chain in oleic acid. Therefore, one may expect that the molecular natures of the polymorphs of various unsaturated fatty acids may be more complicated, depending on the number, position and configuration of the double bond. Extensive work should be devoted to the study of these unsaturated fatty acids, since data are quite lacking.

Knowledge about the polymorphism in mixed saturated/unsaturated acid triglycerides is rather scarce or contradictory. This should be overcome, since many naturally-important triglycerides consist of saturated and unsaturated acyl chains, e.g., cocoa butter. Examples presented in this review, POP and SOS, indicate that the numbers of individual polymorphs are increased and their molecular natures become more complicated in comparison to monoacid triglycerides. More detailed analysis for the crystal and molecular structures of all polymorphs of S_tOS_t or other mixed triglycerides must be necessary.

In these researches, one may have to employ pure sample to get essential features of the polymorphism of each compound to reduce the effects of impurities. Then, the complicated polymorphism of lipids in real systems which consist of varying fats and fatty acids can be analyzed using the data of pure substances. Furthermore, multiple techniques for identification of the individual polymorphs must be applied to the polymorphism of complicated triglycerides, since the differences in thermal and structural behaviors between the polymorphs may be rather subtle. In this regard, to get the molecular properties, spectroscopic methods are very convincing.

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Discussion with Reviewers

J.W. Hagemann: Saturated monoacid triglycerides of even chainlength less than 16 carbons exhibit a third intermediate melting polymorphic form. Using this analogy, do you perceive that the three new intermediate forms of POP reflect an effect due to the shorter chainlength acids? Since the new forms are double chainlength structures, is it possible that chain reordering is occurring between the carboxyl group and double bond?
Author: Complex intermediate polymorphs were found only in POP. Therefore, we think that this complexity is caused by certain interaction between shorter length of saturated acyl chain with oleic acid. Among three intermediate forms of POP, pseudo-β_1' and pseudo-β_2' are double chainlength but δ is of triple chainlength, although all of their X-ray short spacing spectra are similar to that of pseudo-β' of SOS. There are two transition circuits in the crystal after melt crystallization: $\alpha \rightarrow \gamma \rightarrow$ pseudo-$\beta_2' \rightarrow$ pseudo-$\beta_1' \rightarrow \beta_2 \rightarrow \beta_1$, and $\delta \rightarrow$ pseudo-$\beta_1' \rightarrow \beta_2' \rightarrow \beta_1$. Both undergo conversions in the chainlength structure; (double →) triple → double → triple. Presumably these conversions may be accompanied with reordering of oleic and palmitic acyl chains. Thus, it is possible that, in forms of POP, specific lateral packings consisting of oleic and palmitic acyl chains may exist in the same lamella.

K. Larsson: The name α-form in paraffins, simple esters and glycerides corresponds to crystals with a hexagonal (or pseudohexagonal) subcell. The α-form of oleic acid seems to have the triclinic chain packing. Thus the type of disorder is different from that of triglycerides, and might motivate a different name not to cause confusion. What is your view?
Author: We intend to give a proper nomenclature for the polymorphism of unsaturated fatty acids using Greek characters, knowing that the similar nomenclature for glycerides (α, β' and β) has been established in lipid chemistry. Primary concern was paid to discriminate between saturated (even-numbered, A, B etc.; odd-numbered, A′, B′, etc.) and unsaturated fatty acids. In doing so, Greek character was chosen because of its convenience more than Roman numerals, etc. First, we actually feared

that some confusion might arise, e.g., between α of glycerides and α of oleic acid as you pointed out. No similarity is seen both in molecular conformation and subcell structure between these two α-forms, although solidification behaviors look alike. I hope, however, that this confusion will be solved in accordance with progress in research for varying unsaturated fatty acids. I would note that the α-form was observed in a few unsaturated fatty acids, being characterized by an interfacial melting as briefly mentioned in the text. In addition, it was found that the γ-form also exists in erucic and palmitoleic acids, exhibiting the same molecular conformation and subcell structure as γ of oleic acid.

K. Larsson: Your proposal of independent molecular change in the two different lamellae of $\mathrm{S^tOS^t}$ is interesting. Do you think it would be possible that the unsaturated chain layer even could "melt" below the saturated chain layer, corresponding to a liquid-crystal formation?

Author: In each polymorph of SOS, no significant change was detected in X-ray short spacing spectra taken at 5°C and just below its melting point. This means that the lateral packings of stearic and oleic lamellae are uniquely fixed in each polymorph. However, we have no convincing information on oleic acyl chains in any forms, e.g., whether in crystalline state or in liquid-crystalline state, since discrimination in X-ray short spacing patterns between the two lamellae is very difficult. Yet, preliminary studies with Raman technique indicate that the oleic chains are rather disordered in γ and pseudo-β', but more ordered in β_2 and β_1. Further systematic study is needed to solve this important problem.