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Atomic resolution structure analysis of β′ polymorph crystal of a triacylglycerol: 1,2-dipalmitoyl-3-myristoyl-*sn*-glycerol

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Abstract The crystal structure of the β′-2 form of a mixed chain triacylglycerol (TAG), 1,2-dipalmitoyl-3-myristoyl-*sn*-glycerol (PPM), was determined to a final reliability factor of 0.074. This work is the first to resolve the atomic-level structure of the β′ polymorph, which is of the highest functionality among multiple polymorphs in asymmetric TAG. In particular, fat crystals present in food emulsions are in β′, whose transformation into β causes deterioration in their physical properties. β′-2, one of the two β′ forms of PPM, forms a monoclinic unit cell with a space group of C2; Z = 8, a = 16.534(5) Å, b = 7.537(2) Å, c = 81.626(9) Å; β = 90.28(2)°, V = 10171(3) Å^3, density = 1.018 g/cm^3, and μ = 4.96 cm^{-1}. The following characteristics were obtained: *1*) two asymmetric units, named A and B, form a hybrid-type orthorhombic perpendicular subcell; *2*) the two asymmetric units reveal different glycerol conformations: *trans* for *sn*-1 palmitic acid and *sn*-2 palmitic acid, but *gauche* for *sn*-3 myristic acid in A; and *trans* for *sn*-2 palmitic acid and *sn*-3 myristic acid, but *gauche* for *sn*-1 palmitic acid in B; *3*) a unit lamellae reveals a four-chain-length structure consisting of two double-layer leaflets; *4*) the two double-layer leaflets are combined end-by-end in a unit lamellae; and *5*) the chain axes are alternatively inclined against the lamellar interface.—Sato, K., M. Goto, J. Yano, K. Honda, D. R. Kodali, and D. M. Small. **Atomic resolution structure analysis of β′ polymorph crystal of a triacylglycerol: 1,2-dipalmitoyl-3-myristoyl-*sn*-glycerol.** *J. Lipid Res.* **2001.** 42: **338–345.**

Supplementary key words polymorphism • crystal structure • molecular packing • X-ray diffraction • molecular conformation

Triacylglycerols (TAG) are the major storage lipids in cells, eggs, and seeds. They are widely used in foods, cosmetics, and pharmaceuticals as nutrients or matrix materials (1, 2). Structural determination of acylglycerol crystals at an atomic level has critical implications in understanding the structure-function relationships of lipids, lipoproteins, fat deposits, and biomembrane lipids whose numerous structural data were reviewed recently (3–9). The molecular structures of TAG are also related to biochemical reactions in absorption, transport, and metabolism of fat (10). In addition, physical properties such as polymorphism, melting and solidification, density, and molecular flexibility are influenced by the crystalline phases of TAG, as indicated almost a half century ago (11, 12). Recent reviews indicate that the structural properties of TAG crystals are sensibly influenced by molecular properties of TAG such as saturation/unsaturation of the fatty acid moieties, glycerol conformations, symmetry/asymmetry of the fatty acid compositions connected to the glycerol groups, and so forth (13, 14).

TAG crystals exhibit multiple polymorphic modifications, specifically named α, β′, and β (15). Among the three forms, the β′ polymorph reveals the most optimal functional physical properties compared with the thermodynamically least stable α forms as well as the more stable β forms, in terms of crystal density, morphology, and network formation of microcrystals, flexibility, and molecular conformations (16). For instance, TAG in food fats such as butter, margarine, and chocolate are crystallized in the β′ polymorph in end products through optimal tempering or other crystallization processes. The same results occur in suppositories. Compared to β′, the most stable β form shows less optimal properties, such as morphology and hardness of crystals. Therefore, the inhibition of the polymorphic transformations from β′ to β is prerequisite in determining the qualities of food fats, and detailed structural information for β′ crystals has been of high interest. Although crystal structures of the most stable β forms have so far been clarified for three TAG (17–20),

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Abbreviations: CCC, tricaproyl-glycerol; CLC, 1,3-dicaproyl-2-lauroyl-*sn*-glycerol; PPM, 1,2-dipalmitoyl-3-myristoyl-*sn*-glycerol; PP2, 1,2-dipalmitoyl-3-acetyl-*sn*-glycerol; TAG, triacylglycerol.

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no structural data of β′ at the atomic level have been reported. This has been attributed to the fact that growth of single crystals of β′ is quite difficult because this form is thermodynamically metastable in most cases. Investigators have tried to figure out the molecular-level features of the β′ forms with X-ray diffraction and calculation (21–25), atomic force microscopy (26), and Fourier transformed infrared (FT-IR)spectroscopy (27) using powder crystals or oriented crystalline films. However, no definitive information has been available for the β′ form in terms of glycerol conformation, chain-chain end stacking, detailed chain structure, definitive subcell packing, chain inclination against lamellar interfaces, molecular motions of the chains closer to the end groups, etc., all of which are major dominating factors involved in the β′ to β transformations.

In the present work, atomic resolution structure analysis was performed on the β′ polymorph crystal of the TAG, 1,2-dipalmitoyl-3-myristoyl-*sn*-glycerol (PPM). PPM was synthesized previously (28). PPM has two stable β′ forms: β′-1 and β′-2 (28, 29). The molecular structural properties of the β′-1 form were recently studied by FT-IR spectroscopy using very tiny single crystals (27). However, these small crystals did not allow structure determination by X-ray. The bulky single crystal of β′-2 of PPM was eventually analyzed by the X-ray diffraction method, providing quite unique molecular features that are far different from those of the β-form crystals of TAG so far reported (17–20).

MATERIALS AND METHODS

PPM was synthesized by the method described elsewhere (28, 29). The single crystal of β′-2 was grown from *n*-hexane solution at 8°C by slow evaporation, (about 100 mg PPM solute materials in 10 ml solvent). All the X-ray data were obtained with a RIGAKU AFC7R four-circle diffractometer (Rigaku, Tokyo, Japan) by using graphite monochromated Cu-K_α radiation at room temperature.

RESULTS

The single crystal exhibited a rhombohedral shape with interplanar angles of 45° and 135°, which are largely different from those of 60° and 120° for β′-1 of PPM (27), 75° and 105° for the B form of stearic acid, and 55° and 125° for the C form of stearic acid (30). All of these crystals are packed in orthorhombic perpendicular subcells, which is also observed for β′-2 of PPM (29). The differential scanning calorimetry melting point and enthalpy of melting of the solution-grown β′-2 crystals were 54.8°C and 115.2 kJ/mol, respectively, and both were in good agreement with those obtained for the melt-grown crystals (28). Crystal data and summary of the experimental details are given in **Table 1**.

The structure was solved by the direct methods (shelxs 97). At the first stage, three continuous carbon chains appeared on an E map. The most difficult part of the analysis appeared in determining glycerol groups and the terminal methyl end of the carbon chains. After determining the right position of the glycerol moiety, the R factor was 0.33, but the end carbon atoms of palmitic acids (P) were not located. The positions of the end carbon atoms were deliberately determined by Fourier and D-Fourier methods. Then, the R factor reduced to be 0.18. Later, all of the nonhydrogen atoms were refined anisotropically, and hydrogen atoms were located at ideal positions and fixed. In the end, the R factor was reduced to 0.074. The atomic coordinates of nonhydrogen atoms are shown in **Table 2**.

TABLE 1. Experimental details

Crystal data	
Chemical formula	$C_{49}O_6H_{94}$
Chemical formula weight	779.28
Crystal system	monoclinic
Space group	C2
Lattice parameters	
a (Å)	16.534(5)
b (Å)	7.537(2)
c (Å)	81.626(9)
β (degree)	90.28(2)
V (Å^3)	10171(3)
Z	8
d (calc)	1.018 g/cm^3
Crystal habit	Rhombohedral plate
Crystal size (mm)	0.3 × 0.5 × 0.03
Data collection	
Diffractometer	Rigaku AFC7R
Radiation type	CuK_α
Wavelengths (Å)	1.5418
Data collection method	ω-scan
No. of measured reflections	15216
No. of unique reflections	8375
Rint	0.035
2θ (degree)	120.2
Intensity decay (%)	5.86
Refinement	F Full-matrix least-squares
Least squares weight	$w = 1/\sigma^2$ (Fo)
No. observations [I > 1.5 σ (I)]	3522
No. of parameters used	991
R	0.074
Rw	0.076
S	1.65
$(\Delta/\sigma)_{max}$	0.11
ρ_{max} (eÅ^{-}3)	0.21
ρ_{min} (eÅ^{-}3)	−0.21

Description of the structure

The asymmetric units (**Fig. 1**) contained two crystallographically independent molecules: A (carbon atoms of C1–C49) and B (C50–C98). The molecular conformations of A and B with respect to the acyl chain moieties are different (**Fig. 2**). In A, *sn*-1 palmitic acid (C1–C16) and *sn*-2 palmitic acid (C17–C32) formed an almost straight zigzag chain (dihedral angle of 178° for O1-C1-C2-C3 and −175° for O2-C17-C18-C19, respectively). The third *sn*-3 myristic acid (C33–C46), which is branched off of the third glycerol C49 atom and O3 atom, was bent and the dihedral angle of O3-C33-C34-C35 was 61°. Remarkably, in

TABLE 2. Positional parameters and B(eq) for nonhydrogen atoms

Atom	x	y	z	B(eq)
O(1)	−0.3486(4)	1.025(2)	0.23530(7)	5.9(2)
O(2)	−0.4507(3)	0.914(2)	0.27268(7)	5.1(2)
O(3)	−0.3255(4)	0.671(2)	0.27312(8)	5.3(2)
O(4)	−0.1623(4)	0.329(2)	0.24094(7)	5.4(2)
O(5)	−0.0351(3)	0.085(2)	0.24179(7)	5.1(2)
O(6)	−0.1391(4)	−0.032(2)	0.27866(7)	5.8(2)
O(7)	−0.4208(6)	1.194(2)	0.21838(9)	9.2(3)
O(8)	−0.3832(4)	1.012(2)	0.29453(8)	6.6(2)
O(9)	−0.3302(5)	0.406(3)	0.26087(10)	7.6(3)
O(10)	−0.1574(4)	0.592(2)	0.25344(8)	6.6(2)
O(11)	−0.1034(3)	−0.016(2)	0.21964(7)	5.5(2)
O(12)	−0.0647(5)	−0.191(2)	0.29566(8)	8.3(3)
C(1)	−0.3612(8)	1.109(3)	0.2210(1)	5.6(4)
C(2)	−0.2901(5)	1.085(3)	0.2100(1)	5.3(3)
C(3)	−0.3021(6)	1.172(3)	0.1933(1)	5.3(3)
C(4)	−0.2350(6)	1.137(3)	0.1817(1)	5.3(3)
C(5)	−0.2410(6)	1.221(3)	0.1653(1)	5.4(3)
C(6)	−0.1738(6)	1.184(3)	0.1538(1)	5.7(3)
C(7)	−0.1795(6)	1.272(3)	0.1370(1)	5.6(3)
C(8)	−0.1116(7)	1.236(3)	0.1253(1)	6.4(3)
C(9)	−0.1190(6)	1.323(3)	0.1086(1)	5.5(3)
C(10)	−0.0530(7)	1.279(3)	0.0970(1)	7.3(4)
C(11)	−0.0597(6)	1.369(3)	0.0807(1)	6.6(3)
C(12)	0.0077(6)	1.331(3)	0.0686(1)	6.7(3)
C(13)	0.0020(6)	1.422(3)	0.0521(1)	6.2(3)
C(14)	0.0667(6)	1.379(3)	0.0402(1)	7.8(4)
C(15)	0.0562(7)	1.468(3)	0.0238(1)	9.7(4)
C(16)	0.1220(7)	1.430(3)	0.0116(1)	12.2(5)
C(17)	−0.4405(7)	0.943(3)	0.2888(1)	5.3(3)
C(18)	−0.5145(6)	0.880(3)	0.2979(1)	6.0(3)
C(19)	−0.5097(5)	0.920(3)	0.3162(1)	5.0(3)
C(20)	−0.5746(6)	0.827(3)	0.3260(1)	5.7(3)
C(21)	−0.5733(6)	0.870(3)	0.3440(1)	6.6(4)
C(22)	−0.6355(7)	0.774(3)	0.3542(1)	7.1(4)
C(23)	−0.6317(6)	0.815(3)	0.3722(1)	5.8(3)
C(24)	−0.6938(7)	0.720(3)	0.3827(1)	6.1(4)
C(25)	−0.6893(7)	0.770(3)	0.4008(1)	7.5(4)
C(26)	−0.7503(7)	0.675(3)	0.4112(1)	6.9(4)
C(27)	−0.7467(7)	0.726(3)	0.4291(1)	8.3(4)
C(28)	−0.8070(7)	0.625(3)	0.4401(1)	10.0(5)
C(29)	−0.7980(8)	0.669(3)	0.4584(1)	11.5(5)
C(30)	−0.854(1)	0.565(3)	0.4685(2)	16.3(8)
C(31)	−0.829(2)	0.626(6)	0.4870(2)	36(1)
C(32)	−0.872(2)	0.497(8)	0.4946(3)	54(2)
C(33)	−0.3172(7)	0.495(3)	0.2732(1)	6.7(4)
C(34)	−0.2950(6)	0.424(3)	0.2894(1)	5.7(3)
C(35)	−0.3574(6)	0.462(3)	0.3023(1)	6.2(3)
C(36)	−0.3457(5)	0.377(3)	0.31867(10)	4.9(3)
C(37)	−0.4115(5)	0.414(3)	0.3305(1)	5.5(3)
C(38)	−0.4028(6)	0.316(3)	0.3470(1)	6.0(3)
C(39)	−0.4728(7)	0.363(2)	0.35871(10)	6.0(3)
C(40)	−0.4661(6)	0.261(3)	0.3746(1)	6.9(4)
C(41)	−0.5304(6)	0.312(3)	0.3868(1)	5.3(3)
C(42)	−0.5231(6)	0.213(3)	0.4030(1)	6.5(4)
C(43)	−0.5911(7)	0.257(3)	0.4154(1)	7.5(4)
C(44)	−0.5854(7)	0.163(3)	0.4317(1)	7.5(4)
C(45)	−0.6506(7)	0.217(3)	0.4434(1)	8.7(5)
C(46)	−0.6451(8)	0.122(3)	0.4598(1)	13.8(5)
C(47)	−0.4111(6)	1.039(3)	0.2473(1)	6.0(3)
C(48)	−0.3814(6)	0.939(3)	0.2622(1)	4.7(3)
C(49)	−0.3509(7)	0.758(3)	0.2577(1)	7.0(4)
C(50)	−0.1682(7)	0.505(3)	0.2411(1)	5.1(3)
C(51)	−0.1921(6)	0.573(3)	0.2245(1)	5.7(3)
C(52)	−0.1267(5)	0.531(3)	0.2118(1)	5.1(3)
C(53)	−0.1415(6)	0.620(3)	0.19568(10)	5.8(3)
C(54)	−0.0729(6)	0.579(3)	0.18383(9)	5.6(3)
C(55)	−0.0802(6)	0.678(3)	0.1676(1)	5.9(3)
C(56)	−0.0158(5)	0.633(3)	0.15569(10)	5.6(3)
C(57)	−0.0204(7)	0.732(3)	0.1394(1)	5.9(3)
C(58)	0.0472(6)	0.688(3)	0.1273(1)	6.7(4)
C(59)	0.0438(7)	0.790(3)	0.1115(1)	7.1(4)
C(60)	0.1086(6)	0.737(3)	0.0995(1)	5.8(3)
C(61)	0.1009(6)	0.833(3)	0.0831(1)	6.6(3)
C(62)	0.1662(6)	0.781(3)	0.07133(10)	7.2(3)
C(63)	0.1630(6)	0.879(3)	0.0547(1)	6.5(4)
C(64)	0.2285(6)	0.823(3)	0.0428(1)	8.7(4)
C(65)	0.2228(8)	0.923(3)	0.0264(1)	12.0(4)
C(66)	−0.0436(5)	0.049(3)	0.2255(1)	4.9(3)
C(67)	0.0279(6)	0.112(3)	0.2166(1)	5.6(3)
C(68)	0.0247(6)	0.077(3)	0.1982(1)	5.7(3)
C(69)	0.0898(6)	0.165(3)	0.1883(1)	5.4(3)
C(70)	0.0844(6)	0.122(3)	0.1704(1)	6.2(3)
C(71)	0.1483(6)	0.218(3)	0.1601(1)	5.7(3)
C(72)	0.1457(6)	0.171(3)	0.1421(1)	6.2(3)
C(73)	0.2087(6)	0.271(3)	0.1321(1)	5.9(3)
C(74)	0.2062(6)	0.220(3)	0.1139(1)	6.3(3)
C(75)	0.2690(6)	0.318(3)	0.1036(1)	5.7(3)
C(76)	0.2657(6)	0.275(3)	0.0856(1)	6.5(3)
C(77)	0.3277(6)	0.368(3)	0.0753(1)	5.4(3)
C(78)	0.3246(6)	0.324(3)	0.0575(1)	7.1(4)
C(79)	0.3850(6)	0.417(3)	0.0468(1)	7.4(3)
C(80)	0.3735(8)	0.370(3)	0.0288(1)	10.1(5)
C(81)	0.4324(8)	0.471(3)	0.0182(1)	14.3(5)
C(82)	−0.1246(6)	−0.113(3)	0.2929(1)	4.9(3)
C(83)	−0.1932(6)	−0.087(3)	0.3043(1)	6.0(3)
C(84)	−0.1837(6)	−0.179(3)	0.3207(1)	6.3(3)
C(85)	−0.2530(6)	−0.140(3)	0.3326(1)	6.4(4)
C(86)	−0.2444(6)	−0.231(3)	0.3491(1)	6.0(3)
C(87)	−0.3126(6)	−0.187(3)	0.3608(1)	6.0(3)
C(88)	−0.3028(6)	−0.279(3)	0.3775(1)	6.5(4)
C(89)	−0.3698(6)	−0.234(3)	0.3892(1)	5.9(3)
C(90)	−0.3633(7)	−0.330(3)	0.4056(1)	8.2(4)
C(91)	−0.4310(6)	−0.288(3)	0.4180(1)	6.2(3)
C(92)	−0.4223(7)	−0.383(3)	0.4341(1)	8.6(4)
C(93)	−0.4844(7)	−0.338(3)	0.4466(1)	10.3(5)
C(94)	−0.4715(8)	−0.434(3)	0.4627(1)	12.9(5)
C(95)	−0.5389(10)	−0.386(4)	0.4748(1)	18.4(7)
C(96)	−0.1368(6)	0.246(3)	0.2559(1)	4.6(3)
C(97)	−0.1061(6)	0.062(3)	0.2522(1)	5.9(3)
C(98)	−0.0748(5)	−0.033(3)	0.26701(10)	4.6(3)

B, the *sn*-3 myristic acid (C95–C82) and the *sn*-2 palmitic acid (C66–C81) formed the same zigzag straight chain involving the glycerol group (the dihedral angles were 179° for O5-C66-C67-C68 and 178° for O6-C82-C83-C84, respectively). Whereas, the *sn*-1 palmitic acid (C50–C65) was bent and the dihedral angle of O4-C50-C51-C52 was −63°. The selective bond distances, bond angles, and torsion angles are shown in **Table 3**.

The molecular arrangements expressed in the (a,c) and (b,c) projections of the unit cell are shown in **Fig. 3**. The four-chain-length structure is formed, involving two double-layer leaflets that are related by two rotational axes and tilted to each other by 17°. Each leaflet is terminated by two types of the methyl end packing: -PPP-PPP-PPP- and -PMM-PMM-PMM- (P, palmitic chain; M, myristic chain). In the latter type, the temperature factors of the carbon atoms (especially the *sn*-2 palmitic acid C31 and C32 in the A molecule) were very large, as shown in Fig. 1. This is probably due to enhanced atomic mobility of this chain, which is lying in a gap between the opposing PPM end planes.

The polymethylene chains are packed in hybrid subcell

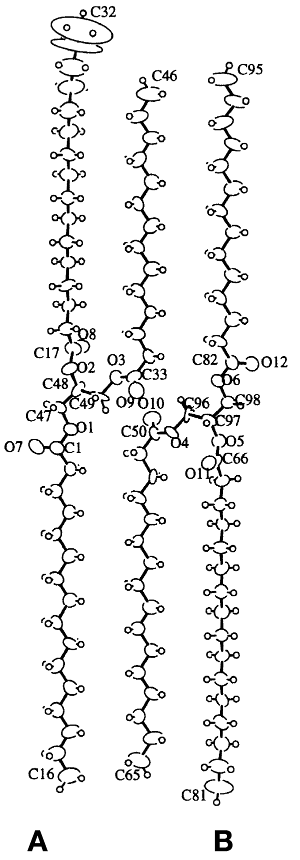

Fig. 1. Two asymmetric units, A and B, in a unit cell of β′-2 of PPM.

(HS) arrangements of a disordered orthorhombic perpendicular type. The precise dihedral angles between the zigzag polymethylene planes are summarized in **Table 4**. **Figure 4** shows the subcell arrangements of the polymethylene planes expressed for adjacent two carbon atoms at the layer composed of PPP in leaflet I (as noted by arrow X in Fig. 3). The fatty acid chains of 1, 4, and 5 (defined in Table 4), which are all palmitic acid chains, form the subcell arrangements of Fig. 4. The same subcell structure was observed for the layer composed of the 2, 3, and 6 chains (Table 4) for the layers denoted by an arrow Y in Fig. 3. The orthorhombic subcell parameters are a_s = 15.0 Å, b_s = 7.5 Å, c_s = 2.55 Å.

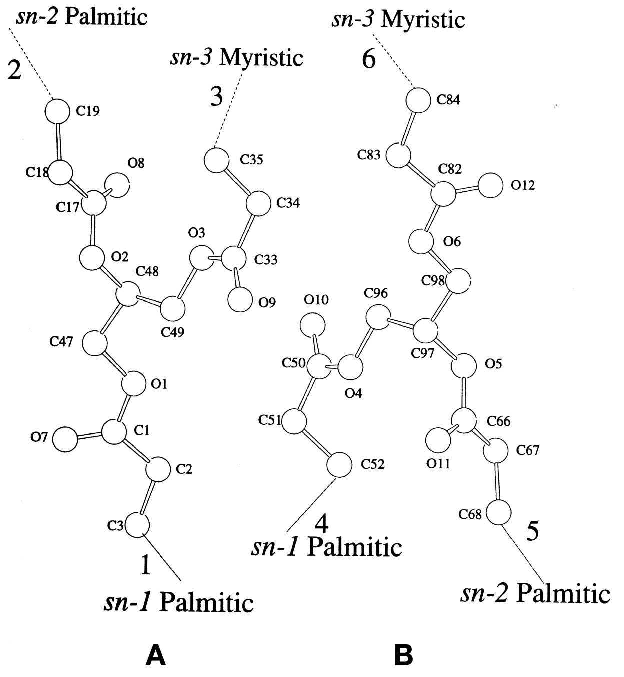

Fig. 2. Atomic structures close to glycerol groups of two asymmetric units, A and B, of β′-2 of PPM. Hydrogen atoms are not shown.

DISCUSSION

The crystal structure of β′-2 of PPM is unique in relation to those of the other acylglycerols in terms of chain end-stacking mode, subcell packing, and glycerol conformation.

Chain end-stacking mode

In lamella-type crystals formed in the long-chain aliphatic compounds, the chain end-stacking mode is a major factor for the structural stabilization for chains packed in specific subcells such as orthorhombic or triclinic types. The β′-2 form of PPM showed two types of the methyl end-stacking modes (Fig. 3), in which zigzag-type stacking is mutually revealed with the arrangement of terraces, and every terrace is composed of three fatty acid chains. The degree of inclination of the terrace plane, however, is quite different between those composed of the PPP layer (the outer layer in Fig. 3) and the PMM layer (the inner layer). The inclination angles with respect to the lamella plane defined in Fig. 3 are θ_1 = 9.6° for PPP and θ_2 = 38.0° for PMM.

By contrast, many TAG crystals reveal much simpler methyl end-stacking modes. For instance, the β form of tricaproyl-glycerol (CCC) shows a single type of the methyl end-stacking mode having the θ value of 12° (18). In the β form of 1,2-dipalmitoyl-3-acetyl-*sn*-glycerol (PP2), a zigzag-type terrace is formed at the end stacking of the PPP layer having the θ value of 11° (20). Complexity in the methyl end-stacking mode of β′-2 of PPM is related to the formation of the two types of the end-stacking mode of the four-chain-length structure, the outer interface composed of PPP and the inner interfaces composed of PMM. It seems that the instability of the methyl end stacking of the methyl end terrace planes having the large θ value may be compensated by arranging them face-to face at the interior interfaces of the four-chain-length structure.

Subcell packing mode

In the lipid crystals, the polymethylene chains can adopt a variety of different modes of lateral packing, which are described by subcells. The subcells with triclinic, orthorhombic, monoclinic, and hexagonal symmetry are defined, and parallel or perpendicular arrangements are defined in such a manner that the polymethylene planes can be mutually parallel or perpendicular with re-

TABLE 3. Seleced bond distances, bond angles, and torsion angles

A Molecule		B Molecule	
Selected bond distances (Å)		Selected bond distances (Å)	
C3–C2	1.52(1)	C52–C51	1.53(1)
C2–C1	1.49(2)	C51–C50	1.50(2)
C1–O7	1.19(2)	C50–O10	1.21(1)
C1–O1	1.34(2)	C50–O4	1.33(2)
O1–C47	1.43(1)	O4–C96	1.44(1)
C47–C48	1.51(2)	C96–C97	1.51(2)
C48–C49	1.51(2)	C97–C98	1.49(2)
O2–C48	1.45(1)	O5–C97	1.46(1)
O8–C17	1.18(1)	O11–C66	1.20(1)
C17–C18	1.51(2)	C66–C67	1.47(2)
C18–C19	1.52(1)	C67–C68	1.52(1)
C49–O3	1.48(1)	C98–O6	1.43(1)
O3–C33	1.34(2)	O6–C82	1.34(1)
O9–C33	1.22(2)	O12–C82	1.17(1)
C33–C34	1.47(2)	C82–C83	1.48(1)
C34–C35	1.51(1)	C83–C84	1.52(1)
Selected bond angles (°)		Selected bond angles (°)	
O1-C1-C2	110(1)	O4-C50-C51	110(1)
C1-O1-C47	116(1)	C50-O4-C96	116(1)
O1-C47-C48	106(1)	O4-C96-C97	109(1)
O2-C48-C49	106(1)	O5-C97-C98	104(1)
C17-O2-C48	117(1)	C66-O6-C97	118(1)
O2-C17-O8	123(1)	O5-C66-O11	123(1)
O3-C49-C48	106(1)	O6-C98-C97	106(1)
C33-O3-C49	117(1)	C82-O6-C98	116(1)
O3-C33-C34	112(1)	O6-C82-C83	110(1)
Selected torsion angles (°)		Selected torsion angles (°)	
C2-C1-O1-C47	178(1)	C51-C50-O4-C96	178(1)
O7-C1-O1-C47	1(2)	O10-C50-O4-C96	−4(2)
C1-O1-C47-C48	−178(1)	C50-O4-C96-C97	−160(1)
C18-C17-O2-C48	−170(1)	C67-C66-O5-C97	168(1)
O8-C17-O2-C48	11(2)	O11-C66-O5-C97	−7(2)
C17-O2-C48-C47	−136(1)	C66-O5-C97-C96	−104(1)
C34-C33-O3-C49	−177(1)	C83-C82-O6-C98	174(1)
O9-C33-O3-C49	0(2)	O12-C82-O6-C98	−3(2)
C33-O3-C49-C48	161(1)	C82-O6-C98-C97	178(1)
C17-O2-C48-C49	104(1)	C66-O5-C97-C98	135(1)
O1-C47-C48-O2	−166(1)	O4-C96-C97-O5	63(1)
O1-C47-C48-C49	−49(1)	O4-C96-C97-C98	178(1)
O2-C48-C49-O3	−62(1)	O5-C97-C98-O6	170(1)

spect to their neighbors. The subcell structures are further differentiated into simple and hybrid (8, 31), of which the latter (HS) is defined to be those involving more than two different asymmetric units in a subcell. The subcell structures of the β forms of CCC and PP2 are both triclinic parallel type, which may be categorized as the simple subcell. Two types of the HS, HS1, and HS2 (**Fig. 5**), have so far been observed in biomembrane lipids: an orthorhombic HS1 has been observed in a phospholipid, and 1,2-dilauroyl-DL-phosphatidylethanolamine (32, 33) and a monoclinic HS2 observed in a cerebroside [β-D-galactosyl-N-(2-D-hydroxy-octadecanoyl)-D-dihydrosphingosine] (34). The subcell lattice parameters are a_s = 10.3 Å, b_s = 7.5 Å, and c_s = 2.54 Å for HS1, and a_s = 9.2 Å, b_s = 9.8 Å, c_s = 2.55 Å, and γ = 98° for HS2. Actually, HS2 is a hybridization of orthorhombic, triclinic, and monoclinic properties.

The subcell structure present in β′-2 of PPM is a completely new type of the HS that has never been observed in TAG (31), phospholipid, and glycolipid crystals (8).

The specificity of the HS of β′-2 of PPM stands for superimposing periodicity along the a_s direction as shown in Fig. 5, which gives rise to triple of the usual simple orthorhombic perpendicular subcells. Therefore, this new type of the HS is named HS3. The formation of HS3 may be attributed to the presence of two asymmetric units, as well as to the glycerol conformations discussed below. The same situation also occurred for HS2 in the cerebroside crystal, in which there were two independent molecules even in the asymmetric unit, partly related by a noncrystallographic symmetry (34).

Glycerol conformation

Figure 6 compares the glycerol conformations of three TAG crystals of CCC β (17, 18), PP2 β (20), 1,3-dicaproyl-2-lauroyl-*sn*-glycerol (CLC) β′ (26), and PPM β′-2. The structure model of CLC β′ was made by computer simulation based on powder crystal X-ray diffraction data (26). In PPM β′-2, the glycerol conformations of the two asymmetric units, A and B, are different in terms of relative orientation of the three glycerol carbon atoms with respect to the lamella plane (dashed lines in Fig. 6) and the fatty acid moieties connected to each glycerol carbon. How-

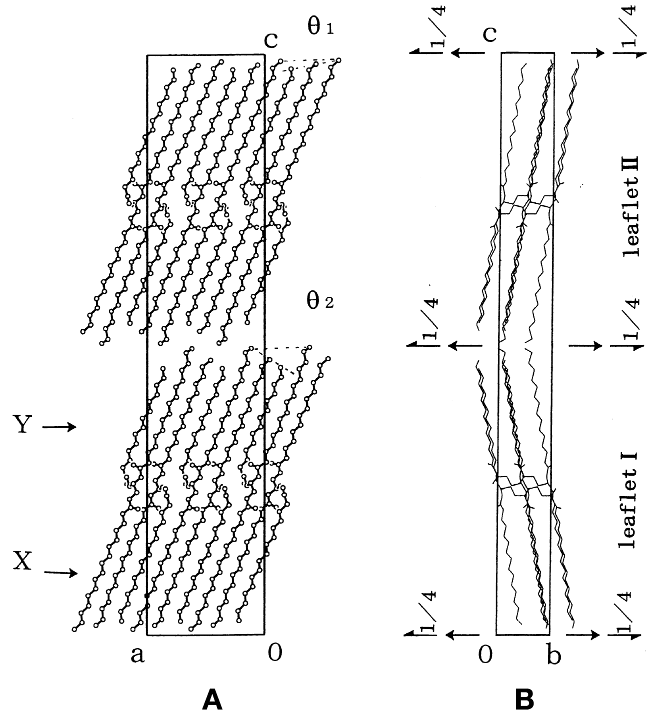

Fig. 3. Molecular packing of β′-2 of PPM projected along the b axis (A), and along the a axis (B).

ever, the symmetry operation, (x, y, z) to (−x, −y, −z), for B produces B′, which gives rise to basically the same glycerol conformation as that in A, as shown in Fig. 6. The difference between A and B′ singly appears in relative positions of adjacent atoms with respect to the molecular plane (sheet of printed page). This means that the two asymmetric units of A and B actually have the same glycerol structure, except for the fatty acid moieties connected to the glycerol carbons. In other words, diversity in the glycerol conformation in the two asymmetric units of PPM β′-2 is minimized so that the methyl end-stacking mode is most optimally stabilized, as discussed above.

As for the difference between PPM β′-2 and other TAG, CCC β reveals the glycerol conformation quite similar to that of B molecule of PPM β′-2, except for the configuration of the *sn*-1 chains; for example, the *sn*-3 (or *sn*-1) chain in CCC β extends to the opposite direction to the *sn*-2 chain, whereas the *sn*-1 and *sn*-2 chains are directed toward the same direction in the B molecule. The glycerol conformations of PP2 β and CLC β′ are quite different from both of the two asymmetric units of PPM β′-2.

TABLE 4. Dihedral angles between polymethylene zigzag planes (°)

Plane[a]	1	2	3	4	5
2	111.00	—	—	—	—
3	1.78	109.22	—	—	—
4	2.45	108.54	0.68	—	—
5	109.10	1.92	107.33	106.65	—
6	1.48	109.52	0.31	0.98	107.63

[a] Planes: 1 = C1–C16; 2 = C17–C30 (except C31, C32); 3 = C33–C46; 4 = C50–C65; 5 = C66–C81; and 6 = C82–C95.

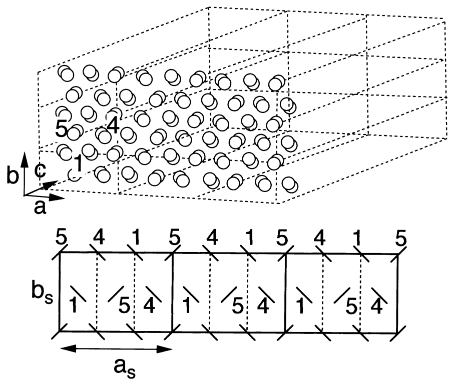

Fig. 4. Subcell arrangements of β′-2 of PPM.

Chain inclination

One of the striking features in the molecular structures of PPM β′-2 is alternative chain inclination of every double-layer leaflet against the lamella interface, as shown in Fig. 3. The chain inclination of this type looks similar to a polytype structure, in which the lamella-lamella stacking is primarily determined by the packing of the methyl end groups that are usually localized close to the lamella interface (30). The chain inclination, however, exhibited in the PPM β′-2 is not exactly of the polytypic nature because the two-dimensional arrangements within the individual lamella are not the same.

Within each double-layer leaflet, the TAG molecules form a straight-chain configuration involving the glycerol carbon and oxygen atoms. This contrasts to the glycerol configurations and the chain inclination properties of β′ form calculated for C_n-C_{n+2}-C_n TAG, which were assessed by computer calculation based on powder X-ray diffraction spectra (25, 26). For instance, van de Streek et al. (25) claimed that the fatty acid chains are bent at the glycerol position with respect to the methyl end-group plane. Although PPM is asymmetric and C_n-C_{n+2}-C_n is symmetric in terms of the fatty acid compositions in the mixed-acid TAG, the chain inclination is related to the stabilization of the methyl end stacking of both types of TAG. The origin of the chain inclination concerning whether it is coming from the glycerol position or from the methyl end stacking is critically important. In the case of PPM β′-2, it was clearly verified that the chain inclination originates from the methyl end stacking.

To summarize, the structural properties of β′-2 of PPM are quite unique compared with those observed in the

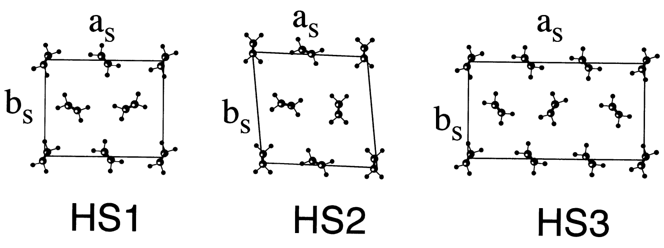

Fig. 5. Hybrid subcell arrangements.

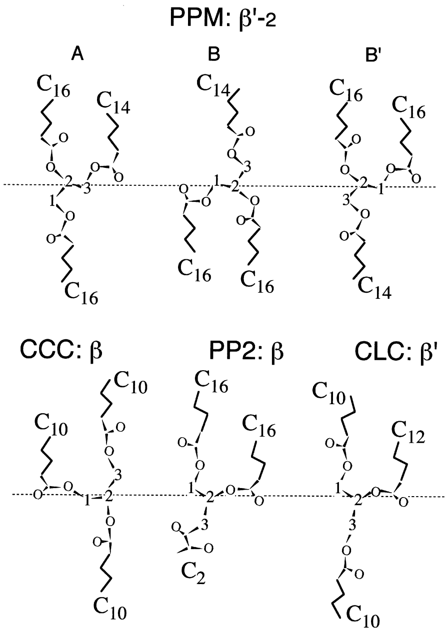

Fig. 6. Stereochemical conformation of the chains closest to the glycerol groups of four TAG crystals. Two asymmetric units, A, B, and B′ of B′-2 of PPM, which is formed by symmetry operation of B. The molecules have been arranged with the glycerol C-2 arranged along the dashed line, the oxygen on C-2 coming out of the plane of the page, and C-2 acyl chain perpendicular to the dashed line. (Acyl chains have been truncated.) The carbons of the glycerol backbone have been numbered and the oxygen atoms are identified. (H atoms are not indicated.) Relative positions of adjacent atoms from the head group through the carbonyl groups are indicated by the width of the line connecting the atoms. The narrow portion of the line goes behind the page and the wide portion comes out of the plane of the page. Structures were drawn according to CCC β (18), PP2 β (20), and CLC β′ (26). In CCC, the numbering of glycerol carbons 1 and 3 is arbitrary and could be revised.

most stable β forms of TAG so far reported. The uniqueness is revealed in the presence of two asymmetric units in the unit cell, the chain end-stacking mode, the lateral chain packing expressed in the HS3 subcell, the chain inclination against the lamella interface, and the glycerol conformation. One may assume that based on the present work, the stabilization of the β′-form structure may be realized through combined interactions of the methyl end stacking, the lateral chain packing, and the glycerol conformation between the PPM molecules. For the moment, it is rather difficult to assess how these interactions cooperate, and which interaction plays the dominant role. It has been widely observed (35) that the β′ form is stabilized when a TAG contains different types of fatty acid moieties that are connected to the glycerol carbons in an asymmetric manner such as in PPM. This indicates relative importance of the interactions operating at the methyl end stacking for the stabilization of the β′ form. This indication may be rationalized by the fact that *rac*-PPM (PPM and MPP) showed β′ to β transformation within 3 days in a solid state at 50°C, which is 2°C below the melting point of β′. We have confirmed this result, although not shown here.

As to the importance of the glycerol conformation, FT-IR study of another β′ form of PPM, β′-1, suggested the possible structure in which the chain inclination may occur at the glycerol group, which is quite different from the present β′-2 form having the all-extend glycerol conformation (27). It is highly interesting, therefore, to compare the crystal structures of the two β′ forms of PPM at the atomic level to examine structural diversity in the polymorphic modifications of β′.

NOTE ADDED IN PROOF

The atomic structure of β′ of symmetric TAG was reported recently by Van Lagevelde et al.(2000. *Acta Cryst.* **B56**: 1103–1111).

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