Remarkable functions of sn-3 hydroxy and phosphocholine groups in 1,2-diacyl-sn-glycerolipids to induce clockwise (+)-helicity around the 1,2-diacyl moiety: Evidence from conformation analysis by $^1$H NMR spectroscopy

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**Abstract**

Cell-membrane glycerolipids exhibit a common structural backbone of asymmetric 1,2-diacyl-sn-glycerol bearing polar head groups in the sn-3 position. In this study, the possible effects of sn-3 head groups on the helical conformational property around the 1,2-diacyl moiety in the solution state were examined. $^1$H NMR Karplus relation studies were carried out using a series of 1,2-dipalmitoyl-sn-glycerols bearing different sn-3 substituents (namely palmitoyl, benzyl, hydrogen, and phosphates). The $^1$H NMR analysis indicated that the helical property around the 1,2-diacyl moiety is considerably affected by these sn-3 substituents. The sn-3 hydroxy group induced a unique helical property, which was considerably dependent on the solvents used. In CDCl$_3$ solution, three staggered conformers, namely gt(+), gg(−) and tg, were randomized, while in more polar solvents, the gt(+) conformer with (+)-helicity was amplified at the expense of gg(−) and tg conformers. The sn-3 phosphocholine in phosphatidylcholine exhibited a greater effect on the gt(+) conformer, which was independent of the solvents used. From the $^1$H NMR analysis, the helical conformational properties around the 1,2-diacyl moiety conformed to a simple empirical rule, which permitted the proposal of a conformational diagram for 1,2-dipalmitoyl-sn-glycerols in the solution states.

**Introduction**

Glycerophospholipids, constituting the basic elements of cytoplasm bilayer membranes, are responsible for several cell functions [1-3]. These chiral biomolecules have an asymmetric sn-glycerol backbone. Although sn-glycerol is symmetric, an sn-3 phosphate group makes it chiral with an (R)-configuration at the sn-2 position [4]. Such molecular chirality is crucial to
not only their biological activities but also for their metaphysical properties, as glycerophospholipids comprise elements of fluid membrane [5] and nanoscale vesicles called liposomes [6].

In addition, the chiral \( sn \)-glycerol backbone is composed of acyclic polyols that produce several conformers through the free rotation about each of the C–C single bonds. For example, the free rotation about the \( sn \)-1,2 and \( sn \)-2,3 C–C bonds furnishes nine conformers by the combination of three staggered rotamers, namely gt (gauche–trans), gg (gauche–gauche) and tg (trans–gauche, Figure 1). Conformational flexibility often leads to the ambiguous characterization of acyclic molecules, thereby making it difficult to precisely examine their biological activities. This observation is applicable for cell-membrane glycerophospholipids that have been targets in numerous conformational studies [7-15].

Cell-membrane glycerophospholipids are known to adopt the gt(+) and gg(−) conformations around the 1,2-diacyl moiety (Figure 1). From X-ray crystallography data, a common structure in which the 1,2-diacyl chains are aligned in parallel is observed, which adopts either the gt(+) or gg(−) conformer [7,10,12]. An analogous conformation has been reportedly observed among \( \alpha \)-glycosyl 1,2-diacyl-\( sn \)-glycerols in the solution state [16]. Probably, the two gauche conformers, namely gt(+) and gg(−), are stabilized in a manner so as to permit stacking interactions between the 1,2-diacyl chains.

In our previously reported circular dichroism (CD) studies [17,18], helical conformational properties of a series of 1,2-dibenzoyl-\( sn \)-glycerols bearing different \( sn \)-3 substituting groups were examined. As shown in Figure 1, gt(+) is one of the gauche conformers with a right-handed (+)-helicity around 1,2-diol, while gg(−) is another gauche conformer with an antipodal left-handed (−)-helicity. Harada and Nakanishi [19] reported the dibenzoate chirality CD methodology, which helps in the analysis of the chirality originating from the disparity between these two helical conformers. We have found thereby that the 1,2-dibenzoyl moiety favors the right-hand screwed gt(+) conformer over the left-handed one [17]. The gt(+)-preference was kept irrespective of the \( sn \)-3 substituting groups and the solvents used. Moreover, a relation in the order as gt(+) > gg(−) > tg was maintained. On the other hand, the intensity of exciton couplet CD bands changed remarkably among the 1,2-dibenzoyl-\( sn \)-glycerols [18], indicating that the disparity between gt(+) and gg(−) conformers varies widely by influences from \( sn \)-3 groups.

Helical properties constitute one of the major factors in determining the molecular chirality [20] of not only proteins and nucleic acids but also simpler biomolecules [17-19] such as acyclic \( sn \)-glycerols and glycerophospholipids [8,21]. In this study, the helical properties of four 1,2-dipalmitoyl-\( sn \)-glycerols 1–4 (Scheme 1) are examined; these 1,2-dipalmitoyl-\( sn \)-glycerols are composed of different substituents (X) at the \( sn \)-3 position, and each of them serves as a representative model for the 1,2-diacyl-\( sn \)-glycerols, as categorized in Scheme 1. Although the exciton chirality CD methodology is not applicable for these 1,2-diacyl-\( sn \)-glycerolipids without an appropriate UV/CD chromophore, \(^1\)H NMR spectroscopy will permit the precise determination of their helical conformational properties.

![Figure 1](image-url): (a) Structures of cell-membrane glycerophospholipids with a common asymmetric 1,2-diacyl-\( sn \)-glycerol-3-phosphate structure and (b) the conformational equilibrium among three staggered conformers, namely gt(+), gg(−) and tg around the 1,2-diacyl moiety.
Results and Discussion

1. Helical conformational properties of tripalmitin 1 and 3-O-benzyl 1,2-dipalmitoylsn-glycerol (2) in CDCl₃ solutions

First, the helical property of tripalmitin 1 (entry 1, Table 1) is examined according to a previously reported method [18]. Briefly, fractional populations (%) of the three staggered conformers [gt(+), gg(−) and tg] are calculated using two Karplus equations, Equation 1 [22] and Equation 2 [18]. From the conformer populations (%), the “helicity index” is determined according to the method previously reported by our group [18].

\[
\begin{align*}
3J_{H_1prOR,H_2}(\text{Hz}) & = 3.1gt(+)+2.8gg(−)+10.7tg \\
3J_{H_1prOR,H_2}(\text{Hz}) & = 10.7gt(+)+0.9gg(−)+5.0tg \\
3J_{H_1prOR,H_2}(\text{Hz}) & = 2.5gt(+)+2.3gg(−)+10.6tg \\
3J_{H_1prOR,H_2}(\text{Hz}) & = 10.2gt(+)+1.3gg(−)+5.8tg
\end{align*}
\]

The result in entry 1 (Table 1) indicates that tripalmitin 1 favors gt(+) with right-handed (+)-helicity compared to gg(−) with left-handed helicity (helical disparity = +6%−7%). According to our previously reported study [18], the disparity, as estimated from Equation 2, is linear with respect to the magnitude and intensity of exciton coupling CD bands, indicating that the 1,2-diacyl moiety in 1 exhibits (+)-chirality corresponding to the equilibrium imbalance between gt(+) and gg(−) conformers as indicated by the helicity index (entry 1 in Table 1). The helical volume of 1 (76% by Equation 2 and 81% by Equation 1) indicates that this glycerolipid favors the two helical conformers in addition to the antiperiplanar tg conformer (ca. 25% by Equation 2) at equilibrium.

Next, the helical property of chiral 3-O-benzyl derivative 2 is examined. In our previously reported CD study [17], the intensity of the exciton couplet CD bands for 3-O-benzyl-1,2-dibenzoyl-sn-glycerol is greater than those of 3-palmitoyl-1,2-dibenzoyl-sn-glycerol. From the preceding result, the replacement of the sn-3 palmitoyl group in 1 with a benzyl ether is expected to enhance the helical property. As can be seen from the result of 2

\[
\begin{align*}
3J_{H_1prOR,H_2}(\text{Hz}) & = 3.1gt(+)+2.8gg(−)+10.7tg \\
3J_{H_1prOR,H_2}(\text{Hz}) & = 10.7gt(+)+0.9gg(−)+5.0tg \\
3J_{H_1prOR,H_2}(\text{Hz}) & = 2.5gt(+)+2.3gg(−)+10.6tg \\
3J_{H_1prOR,H_2}(\text{Hz}) & = 10.2gt(+)+1.3gg(−)+5.8tg
\end{align*}
\]

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(Table 1, entries 2 and 3), the helical disparity (+15%, Equation 1 and Equation 2) increases with the introduction of a benzyl group. This result is in good agreement with our expectation. In addition, the helical volume (%) was increased by 7−8% as compared with that of 1. The 3-O-benzyl group apparently enhances the (+)-chirality around the 1,2-diacyl moiety.

To examine the possible effects of solvents, the helical property of 2 is also examined in a mixed solvent containing ca. 10% methanol-d₄ in CDCl₃ (C/M 10:1, v/v). The result in entry 3 (Table 1) indicates that the helical property of 2 is marginally affected by protic solvents.

2. Helical conformational property of chiral 1,2-dipalmitoyl-sn-glycerol (3) using different solvents

Next, the helical property of 1,2-dipalmitin 3 with a hydroxy (OH) group in the sn-3 position is examined. This compound is selected as a representative model of 1,2-diacyl-sn-glycerols, which play essential roles in the metabolism and anabolism of sn-glycerols [25-28]. Compound 3 is prepared by the catalytic hydrogenolysis of benzyl ether (for the synthetic details, see Supporting Information File 1).

The ¹H NMR spectrum of 3 in a CDCl₃ solution (Figure 2a) shows a pair of double doublet signals of H₁proS (δ 4.32 ppm) and H₁proR (δ 4.23 ppm), which exhibit a spectral feature similar to that of 1 [23]. On the other hand, the signals of H₃proR and H₃proS in 3 collapse in a narrow region around δ 3.73 ppm.

These observations are in good agreement with the ¹H NMR data of 3 reported by Vilceze and Bittman [29].

From the analysis of the ¹H NMR data using Equations 1 and 2, 1,2-dipalmitin 3 in CDCl₃ exhibits a very unique helical conformational property. That is, the populations of the gt(+) and gg(−) conformers are almost equal to give a helical disparity of around 0% (Table 2, entries 1 and 2). A helical volume of around 75% (Equation 2) is analogous to that observed in 1. In contrast to the ¹H NMR data of 2, those of 3 showed remark-

![Figure 2: ¹H NMR spectra of 1,2-dipalmitin (3) in CDCl₃ after partial isomerization into the 1,3-isomer. (a) The expanded spectrum of 3 in CDCl₃. (b) 3 in a mixed solvent with ca. 10% methanol-d₄ in CDCl₃ (C/M ca 10:1, v/v). The signal marked with an asterisk * corresponds to a 1,3-diacyl isomer, which is derived from 3 during storage in a CDCl₃ solution.](image)

| Table 2: ¹H NMR data and helical conformational properties of 1,2-dipalmitin 3 using different solvents. |

| Entry | Compound (head X =) | Solventa | ¹H NMR data δ (ppm) | Populations (%) of staggered conformers in sn-1,2 position | Helicity index in sn-1,2 position |
|-------|--------------------|----------|----------------------|----------------------------------------------------------|----------------------------------|
|       |                    |          | H₁proR | H₁proS | g(+) | g(−) | t(g) | f(+) | g(−) | tg | Sign (+/−) | Disparity [gt−gg]% | Volume [gt+gg]% |
| 1     | 3 (-H)             | CDCl₃    | 4.23b  | 4.33b  | 40   | 40   | 20   | 35   | 39   | 26 | −          | −4 (0)          | 74 (80)        |
| 2     |                   | CDCl₃    | 4.23   | 4.32   | 41   | 40   | 19   | 37   | 39   | 24 | −/+        | −2 (1)          | 76 (81)        |
| 3     |                   | C/M (10:1)| 4.20   | 4.33   | 48   | 38   | 13   | 45   | 35   | 20 | +          | 10 (10)         | 80 (86)        |
| 4     |                   | C/M (5:1)| 4.19   | 4.34   | 52   | 38   | 9    | 49   | 35   | 16 | +          | 14 (14)         | 84 (90)        |
| 5     |                   | C/M (2:1)| 4.19   | 4.37   | 53   | 37   | 10   | 50   | 34   | 16 | +          | 16 (16)         | 84 (90)        |
| 6     |                   | C/M (2:1)+D₂O| 4.18 | 4.37   | 55   | 38   | 7    | 53   | 34   | 13 | +          | 19 (17)         | 87 (93)        |

aC/M (v/v) represents the ratios of the mixed solvents CDCl₃ (C) and methanol-d₄ (M). b¹H NMR data from the study reported by Vilceze and Bittman [29].
able changes in the “mixed solvents” containing methanol-$d_4$ in CDCl$_3$. With the addition of methanol-$d_4$, the H1proR and H1proS signals shift to high and low fields, respectively (Figure 2b). Simultaneously, the H3 signals shift upfield by 0.04 ppm. The shift of these H1 signals increases with an increase in the content of methanol-$d_4$ in the mixed solvents, while the H3 signals are marginally changed; thereafter, their positions are maintained at $\delta$ 3.69 ppm (Figure 2b). As shown in Table 2, entries 1–6, the change in the chemical shifts is related to that in the vicinal coupling constants, indicative of a change in the dynamic conformations occurring around the 1,2-diacyl moiety in 3.

From the analysis of the $^1$H NMR data using the Karplus equations (Equation 1 and 2), an equilibrium shift mainly occurs between the gt(+) and tg conformers. In the mixed solvents with high methanol-$d_4$ contents, the population of the gt(+) conformer seemingly increases at the expense of the tg conformer. The population of the gg(−) conformer decreases by several percent after the addition of ca. 10% of methanol-$d_4$ (Table 2, entry 3). Thereafter, the gg(−) population remains constant at around 35% irrespective of the solvents.

Because of the shift in the equilibrium from tg to gt(+) in the mixed solvents with high methanol contents, the helical disparity (%) and helical volume (%) increase. With an increase in the methanol-$d_4$ content to 17% (C/M 5:1), the helical property of 3 becomes similar to that of 2 (Figure 3). Although this change seems to be saturated in the mixed solvent containing 33% methanol-$d_4$ (C/M 2:1, v/v), the addition of one aliquot of D$_2$O to this solution further changes the gt(+) and tg populations by a few percent (Table 2, entry 6). Moreover, the H2 signal of 3 shifts downfield by 0.03 ppm in the presence of D$_2$O, although this signal marginally changes in the mixed solvents without D$_2$O.

From the $^1$H NMR spectra in Figure 2, a part of 3 is isomerized to 1,3-isomer during storage in solutions. To examine the possible effects from this isomer, the isomerization is promoted up to 50%, and the $^1$H NMR spectrum of the isomeric mixture is analyzed. This experiment indicates that the presence of the 1,3-isomer marginally affects the $^1$H NMR signals of 3.

As shown in Table 1, entries 2 and 3, the solvents marginally affect the $^1$H NMR signals of 2. Clearly, sn-3 OH plays an essential role in the conformational dynamics, as shown above. The dynamic change is probably caused by solvation by methanol-$d_4$ and/or D$_2$O around the 3-OH group as well as the increasing polarity of the mixed solvent. As judged from the chemical shift change in the H3 signals, the solvation is possibly saturated in the mixed solvent with 10% methanol-$d_4$ (C/M = 10:1). In the solvent containing more than 33% methanol-$d_4$ (C/M = 2:1), the solvation by methanol-$d_4$ might be partly replaced with D$_2$O.

Hamilton et al. [30] employed $^{13}$C NMR spectroscopy to examine the dynamic molecular behavior of 1,2-dilauroyl-sn-glycerol located in liposomes mixed with glycerophospholipids. Their
$^{13}$C NMR analysis revealed that the hydration occurring around the carbonyl groups in the 1,2-diacyl moiety triggers the dynamics of the molecular alignments in liposomes. Probably, an analogous phenomenon related to the solvation around sn-3 OH was observed. Thus, solvation is thought to play a key role in the dynamic conformation change around the 1,2-diacyl moiety.

3. Helical conformational properties of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (4, DPPC) and other glycerophospholipids in the solution state

The current $^1$H NMR analysis is extended to four 1,2-dipalmitoyl-sn-glycerophospholipids (Scheme 2) bearing different terminal groups (Y). Large portions of their $^1$H NMR data were collated by Hauser et al. [10]. In our experiment, the $^1$H NMR data of phosphatidylcholine 4 are obtained using the mixed solvent C/M = 10:1.

![Scheme 2: Structures of glycerophospholipids with a common structural skeleton of 1,2-dipalmitoyl-sn-glycerol 3-phosphate. Abbreviations: DPPC = 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, DPPE = 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine, DPPS = 1,2-dipalmitoyl-sn-glycero-3-phospho-L-serine, DPPA = 1,2-dipalmitoyl-sn-glycerol 3-phosphate.](image)

As shown in Figure 4, the $^1$H NMR spectrum of 4 shows a pair of well-separated double doublet signals of H1proR ($\delta$ 4.14 ppm) and H1proS ($\delta$ 4.40 ppm). Compared to the other 1,2-diacyl-sn-glycerols 1–3, this phospholipid exhibits a higher vicinal coupling constant to H1proR ($^3J_{H1R,H2} = 7.2$ Hz) and a lower one to H1proS ($^3J_{H1S,H2} = 3.5$ Hz). In addition, the difference in the chemical shift ($\Delta\delta = 0.26$ ppm) between the H1proR and H1proS signals increases in 4. These observations predict that the 1,2-diacyl moiety in 4 exhibits an extremely unique conformational property.

![Figure 4: Partial $^1$H NMR spectrum of 4 in a mixture of CDCl$_3$ and methanol-d$_4$ (C/M = 10:1, v/v).](image)

In fact, the $^1$H NMR Karplus analysis indicates that the helical disparity of 4 increases above 30% (Table 3, entries 1 and 2); the disparity is greater than that observed thus far in previously reported studies [16-18]. When previously reported $^1$H NMR data for 4 are examined [8,10,31], the strong (+)-chirality is independent of the solvents used (Table 3, entries 1–4). Moreover, the data in entries 5–7 (Table 3) indicate that this property is commonly observed in the glycerophospholipids listed in Scheme 2, indicating that an sn-3 phosphate group plays a key role. From Table 3, the sn-3 phosphate group can also simultaneously increase the helical volume (%). The helical volumes (%) of 4 using Equation 1 nearly reach the theoretical limit (100%). This result is in good agreement with the conformational properties of cell-membrane glycerophospholipids reported previously [10-15]. On the other hand, in our calculations using Equation 2 as the advanced Karplus equation [18], the helical volumes of these glycerophospholipids are around 90%, which permits the presence of the tg conformer by ca. 10%. Note, that the tg conformer is crucial [32,33] because the antiperiplanar relation is thought to deform lamellar phases and trigger membrane fusion.

With respect to the antiperiplanar tg conformer, Hauser et al. [10] examined the effect of self-assembly using 1,2-dihexanoyl (C6) homologs of glycerophospholipids. They added these acyl homologs into D$_2$O at concentrations less than or greater than the critical micellar concentration. In their $^1$H NMR spectroscopy analysis, the tg conformer is almost absent under the self-assembled conditions [10]. In addition, in our calculation by Equation 2, the helical volume (%) reaches the theoretical limit (100%), and the helical disparity (%) is greater 40% [18]. Probably, cell-membrane glycerophospholipid 4 can adopt the unusual rotational mode, where the 1,2-diacyl chains swing between gt(+) and gg(−) conformers. However, such extraordinary rotation would be possible only when molecules are located under self-assembled conditions.
4. General trend in the helical conformational properties of 1,2-dipalmitoyl-sn-glycerols 1–4 in the solution state

By plotting the helical disparity (%) obtained by Equation 2 against the population (%) of the gt(+) conformers for glycerolipids 1–4 examined herein, a linear relation is obtained (Figure 5). From the linearity, we obtain Equation 3 and Equation 4:

\[
\text{Helical disparity} \% = \left[ \text{gt(+)} - \text{gg(-)} \right] \% \\
= 1.34[\text{gt(+)} \% - 37.9] \\
\text{(3)}
\]

\[
\text{Population} \% \text{ of gt(+) = 2.94}[50.8 - \text{gg(-)}] \% \\
\text{(4)}
\]

Equation 3 indicates that the helical disparity (%) increases as a function of gt(+) population (%). Equation 4 indicates that the population (%) of the gt(+) conformer increases at the expense of the gg(-) conformer. When the rule of 100 > gt(+) > 0 (%) is applied to Equation 4, the gg(-) population can assume values in a narrow range between 25% and 51%. At a gg(-) population of 25%, the gt(+) population and helical volume (%) reach their theoretical limits (75% and 100%, respectively). At a gg(-) population of 51%, the gt(+) population reaches 0% (tg = 49%).

When the gt(+) population is arbitrarily changed between 30% (B1 section) and 75% (C2 section) in these empirical formulae, a diagram shown in Figure 6 is obtained. The derived diagram is apparently useful for summarizing the overall helical conformational properties of the four 1,2-dipalmitoyl-sn-glycerols 1–4.

In this diagram, an intersection, denoted by B2, is observed, indicating that the helical disparity becomes 0% when both gt(+) and gg(-) populations are 38%. At this point, the helical volume is 76%, and the tg population is 24%. 1,2-Dipalmitin 3 exhibits a similar behavior when dissolved in CDCl3 (Table 2, entry 2). When methanol-d4 is added to the CDCl3 solution of 3, the gt(+) population increases from 37% up to 50% at the expense of the gg(-) and tg conformers. The observed change is well reproduced in this diagram. Glycerophospholipid 4 shows the largest gt(+) population (64%) in the CDCl3 solution (Table 3, entry 1). A similar situation is denoted by a section C1, where the populations of gt(+), gg(-) and tg are 64%, 29% and 7%, respectively. These values are in good agreement with the experimental results (Table 3, entry 1).

In Table 4, the applicability of Equation 3 and Equation 4 is evaluated using α-D- and α-L-glucopyranosyl 1,2-dipalmitoyl-sn-glycerols (Table 4, entries 1–4). The helical conformational...
properties of these α-glycolipids are determined by Equation 2 applying the $^1$H NMR data reported in a preceding paper [16]. The results of the $^1$H NMR analyses are compared with those calculated by Equation 4. Entries 1–4 (Table 4) indicate that Equation 4 can reproduce also the helical conformational properties of these α-glycolipids.

**Conclusion**

In this study, a $^1$H NMR spectroscopy analysis of 1,2-dipalmitoyl-sn-glycerols 1–4 in the solution state was carried out to elucidate their helical conformational properties around the 1,2-diacyl moiety. In addition, the possible effects from the substituents at the sn-3 position were evaluated. In the current analysis, the chiral $^2$H-labeled triacylglycerols [23,24] provided a key basis to discriminate between the H1proR and H1proS signals (Materials and methods). Throughout this study, each of the 1,2-dipalmitoyl-sn-glycerols 1–4 exhibited a unique helical property, indicating that not only sn-configurations but also sn-3 substituents govern the helical conformational property around the 1,2-diacyl moiety. The biological systems in nature effectively utilize the sn-3 substituents. For example, the sn-3 OH group in 1,2-diacyl-sn-glycerols is essential for the dynamic conformational behavior, which possibly plays major roles in their biological functions as transmembrane second messengers [25-30,34]. The sn-3 phosphocholine in phosphatidylcholine induced strong (+)-chirality regardless of the solvents used, which should considerably contribute to their functions as activators of membrane-bound glycoproteins [35-37].

The helical conformational properties observed in the four 1,2-dipalmitoyl-sn-glycerols (Scheme 1) conformed to an empirical rule, as shown in Equation 3 and in the diagram shown in Figure 6. This rule revealed that the helical disparity (%)
linearly changes by the function of $gt(\%)$ populations, albeit in an allowed range. Probably, the range between B2 and C1 sections in the diagram covers the conformational properties of most 1,2-diacyl-$sn$-glycerols in the solution state. The conformational properties in this region can be characterized by the relation of $gt(\%) > gg(\%) > tg(\%)$, which has been commonly observed in our preceding studies [16-18].

The $^1H$ NMR spectroscopy analysis was carried out in organic solvents. It is possible that the conclusions obtained herein deviate from those examined under physiological conditions. For example, glycerophospholipids are located in self-assembled lamellar structures that show liquid crystalline properties. Plasma membranes comprise glycerophospholipids which interact with other membrane components such as glycoproteins and sterols [38,39]. Moreover, natural glycerolipids are composed of heterogeneous acyl chains with different alkyl lengths and alkenyl –C=C– bonds. Thus, it will be of high significance in extensional studies to evaluate the helical conformational properties of 1,2-diacyl-$sn$-glycerols assuming these heterogeneous situations which may occur in nature.

Materials and Methods

Model compounds

Tripalmitin 1 was prepared together with chirally deuterated $sn$-glycerols and identified in our former studies [22,23]. 1,2-Dipalmitoyl-$sn$-glycerol (3) and its 3-$O$-benzyl derivative 2 were prepared in a reported manner [8,29] (for details, see Supporting Information File 1). 1,2-Dipalmitoyl-$sn$-glycerol-3-phosphocholine (4 DPPC) was purchased from Tokyo Kasei Co. Ltd. and used without purification. All the compounds studied here have chemical purities over 95% ($^1H$ NMR) except for 3 which isomerizes into the 1,3-diacyl isomer during storage in CDCl$_3$ solution.

Table 4: Helical conformational properties of $\alpha$-$D$- and $\alpha$-$L$-glucopyranosyl 1,2-dipalmitoyl-$sn$-glycerols in the solvent mixture of CDCl$_3$ and methanol-$d_4$ (C/M = 10:1).

| Entry | Compound$^a$ (head groups at sn-3) | Results$^b$ (%) from $^1H$ NMR spectroscopic analyses by Equation 2 | Calculated values$^c$ (%) with Equation 4 |
|-------|----------------------------------|-------------------------------------------------|------------------------------------------|
| 1     | $\alpha$-$D$-Glc                 | $gt$: 53, $gg$: 36, $tg$: 11, $disparity$: 17 | $volume$: 89, $gt$: 53, $gg$: 33, $tg$: 14, $disparity$: 20, $volume$: 86 |
| 2     | 6-phosphocholine $\alpha$-$D$-Glc| $gt$: 53, $gg$: 36, $tg$: 11, $disparity$: 17 | $volume$: 89, $gt$: 53, $gg$: 33, $tg$: 14, $disparity$: 20, $volume$: 86 |
| 3     | 6-palmitoyl $\alpha$-$D$-Glc     | $gt$: 49, $gg$: 37, $tg$: 14, $disparity$: 12 | $volume$: 86, $gt$: 49, $gg$: 34, $tg$: 17, $disparity$: 15, $volume$: 83 |
| 4     | 6-phosphocholine $\alpha$-$L$-Glc| $gt$: 55, $gg$: 33, $tg$: 12, $disparity$: 22 | $volume$: 88, $gt$: 55, $gg$: 32, $tg$: 13, $disparity$: 23, $volume$: 87 |

$^a$Abbreviations: $\alpha$-$D$- or $\alpha$-$L$-Glc = $\alpha$-$D$- or $\alpha$-$L$-glucopyranoside. $^b$H NMR data in our preceding study [16] are analyzed with Equation 2; $^c$calculated values (%) from Equation 4 by adapting the $gt$ population (%) in the $^1H$ NMR spectroscopy analysis.

Acquisition of the $^1H$ NMR spectral data of $H1proR$ and $H1proS$ signals

Each of the four glycerolipids 1–4 is dissolved in either CDCl$_3$ or the mixed solvents containing methanol-$d_4$ in CDCl$_3$ (deuterium content > 99.5%) at ca. 10 mM concentrations. $^1H$ NMR spectroscopy is measured on a JEOL 400 MHz or 500 MHz instruments at temperatures between 22–25 °C. Chemical shifts ($\delta$, ppm) and coupling constants ($J$, Hz) of $H1proR$ and $H1proS$ signals are obtained manually with $^1H$ NMR spectra expanded in the region between $\delta$ 4.0 ppm and $\delta$ 4.5 ppm. The manual process is of high significance for the current $^1H$ NMR analysis since a peak top by computer system does not always point at a weighted center correctly.

The discrimination between $H1proR$ and $H1proS$ signals is another crucial process. In our former studies [22,23], chiral $^2$H-labelled triacylglycerols were prepared (Scheme 3) and applied for the assignment of these diastereomeric protons, namely $H1proR$ and $H1proS$. The results have shown an empirical relation between the two $H1$ signals; the $H1proS$ signals appear downfield from the $H1proR$ signals ($\delta$ $H1proS > \delta$ $H1proR$ ppm) and have lower smaller coupling constants ($J_{H1proR,H2} > J_{H1proS,H2}$ Hz). This rule is maintained among 1,2-diacyl, 1,2-dipalmitoyl-, and 1,2-dibenzyol-$sn$-glycerols and substituents at the $sn$-3 position. The validity of this rule is confirmed in a comparative analysis using circular dichroism (CD) spectroscopy [17,18]. The current study applies these relations established in our preceding $^1H$ NMR and CD studies.

Calculation of fractional populations (%) of three staggered conformers around the 1,2-diacyl group with a Karplus relation

A general Karplus equation of Haasnoot et al. [40] is extended into the simultaneous linear equations Equation 1 [22] and Equation 2 [18].
From the vicinal coupling constants ($^3J$ Hz) of H1proR and H1proS signals, the fractional populations (%) of the three staggered conformers are calculated. Equation 1 is a standard equation, in which the three staggered conformers have the dihedral angles of ±60° or 180° around 1,2-diols.

Equation 2 is an advanced equation [18], which is optimized for the analysis of 1,2-diacyl-sn-glycerols in the solution state. The results by Equation 1 and Equation 2 produce some deviations each other. In general, Equation 1 tends to overestimate the population (%) of $gt(+)$ and $gg(−)$ conformers by 3–5% compared to those by Equation 2. The current study applies both Equation 1 and Equation 2 in parallel while the main discussion utilizes the results by Equation 2 as the advanced equation.

Definition of ‘helicity index’, ‘helical disparity (%)’ and ‘helical volume (%)’

The ‘helicity index’ [18] comprises three items, namely ‘(+)-sign’, ‘helical disparity (%)’ and ‘helical volume (%)’. The helical disparity (%) is the difference in populations (%) between $gt(+) \text{ and } gg(−)$ conformers. The disparity has either a ‘(+)- or ‘(−)-sign’, which corresponds to the sign of exciton couplet CD bands. When the $gt(\text{-})$ conformer is preferred over the $gg(\text{-})$ conformer, the sign is positive. The absolute value in the helical disparity (%) corresponds to the magnitude of the exciton couplet CD bands.

The helical volume (%) is the summation of $gt(\text{+})$ and $gg(\text{-})$ conformers. The volume expresses to what extent a given glycerolipid can adopt the two helical conformers around the 1,2-diacyl moiety. The helical volume (%) may reach the theoretical limit (100%) under self-assembled conditions [18].

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