

## A Calorimetric Study of the Thermotropic Behavior of Pure Sphingomyelin Diastereomers<sup>†</sup>

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**ABSTRACT:** The phase-transition properties of sphingomyelins were investigated in detail with totally synthetic, chemically and stereochemically pure (2*S*,3*R*)-(N-stearoylsphingosyl)-1-phosphocholine (D-erythro-C<sub>18</sub>-SPM) (**1**) and the corresponding 2*S*,3*S* isomer (L-threo-C<sub>18</sub>-SPM) (**2**). Heating scans of an unsonicated dispersion of **1** right after hydration showed a main transition (I) at 44.7 °C ( $\Delta H = 6.8$  kcal/mol). Upon incubation at 20–25 °C a second transition (II) appeared at 36.0 °C ( $\Delta H = 5.7$  kcal/mol). The two gel phases were designated as G <sub>$\alpha$</sub>  and G <sub>$\beta$</sub>  phases, respectively. The G <sub>$\beta$</sub>  phase was also metastable and relaxed to a third gel phase (G <sub>$\gamma$</sub> ) upon incubation below 10 °C. Conversion of the G <sub>$\gamma$</sub>  phase to the liquid-crystalline phase occurred via two new endotherms at 33.4 °C (2.6 kcal/mol) (III) and 43.6 °C (8.0 kcal/mol) (IV) as well as a main transition at 44.7 °C (9.5 kcal/mol). Possible interpretations have been proposed to account for the observed phase transitions. The L-threo isomer **2** showed similar thermotropic behavior to dipalmitoylphosphatidylcholine (DPPC): a “main transition” at 44.2 °C (6.0 kcal/mol), a “pretransition” at 43.1 °C (1.8 kcal/mol), and upon incubation at 7 °C for 2 weeks, a very broad “subtransition” at ca. 35 °C. The results are substantially different from previous studies of sphingomyelins using mixtures of stereoisomers. Mixing of **1** with **2**, **1** with DPPC, and **2** with DPPC removed the metastability of the gel phase and resulted in a single transition.

**T**hermal phase transitions of sphingomyelins (SPM)<sup>1</sup> have been a subject of numerous investigations over the last decade, as summarized in two recent reviews (Barenholz & Thompson, 1980; Barenholz & Gatt, 1982). Three different types of sphingomyelins have been used for these studies: (a) natural sphingomyelins isolated from various sources (Calhoun & Shipley, 1979a; Shipley et al., 1974; Barenholz et al., 1976), (b) semisynthetic SPM obtained from natural compounds through the deacylation–reacylation sequence (Calhoun & Shipley, 1979b; Cohen et al., 1984; Maulik et al., 1986), and (c) totally synthetic SPM (Barenholz et al., 1976; Maulik et al., 1986; Estep et al., 1979, 1980, 1981).

The natural sphingomyelins have D-erythro 2*S*,3*R* configuration at the sphingosine moiety, but the fatty acyl residue

is usually a mixture of several species depending on the sources of isolation, and the long-chain base may also be heterogeneous (sphingosine is the major component, but dihydrosphingosine, C<sub>20</sub>-sphingosine, etc., may be present as minor components) (Barenholz & Gatt, 1982). The semisynthetic SPM should be stereochemically pure. However, we have found that the commercially available semisynthetic SPM is indeed a mixture of D-erythro 2*S*,3*R* and L-threo 2*S*,3*S* isomers (Bruzik, 1987).<sup>2</sup> The totally synthetic SPM was obtained as a mixture of en-

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<sup>1</sup> Abbreviations: C<sub>18</sub>-SPM, (N-stearoylsphingosyl)-1-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DSC, differential scanning calorimetry; HPLC, high-pressure liquid chromatography; Mops, 3-(N-morpholino)propanesulfonic acid; SPM, sphingomyelin;  $\Delta T_{1/2}$ , half-width of the transition;  $T_m$ , main transition temperature.

<sup>2</sup> In accordance with the 1976 recommendations by IUPAC–IUB Commission on Biochemical Nomenclature (1978) on the nomenclature of lipids, the sphingomyelin derived from (2*S*,3*R*)-sphingosine is designated as D-erythro-sphingomyelin or simply as sphingomyelin. The 2*S*,3*S* diastereomer of sphingomyelin is defined as L-threo-sphingomyelin.

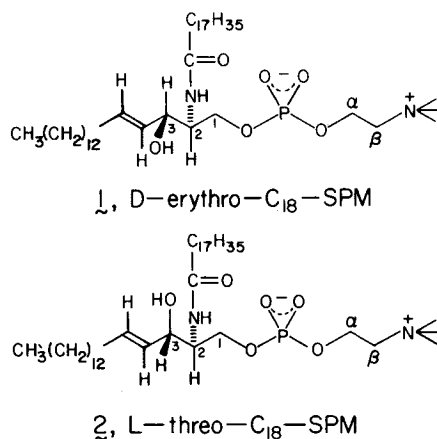


FIGURE 1: Structures of D-erythro-C<sub>18</sub>-SPM (1) and L-threo-C<sub>18</sub>-SPM (2).

antiomers, DL-erythro-SPM. Although synthesis of enantiomerically pure SPM have been reported later [for a summary, see Barenholz and Gatt (1982) and Bruzik (1987)], biophysical studies of these compounds are scarce.

In view of our recent observation of the configurational effect on the thermotropic property of the diastereomers of thiophosphatidylcholines (Wisner et al., 1986), we have investigated the thermotropic properties of sphingomyelins and their mixtures with other lipids using chemically and stereochemically pure sphingomyelins, D-erythro-stearoylsphingomyelin (D-erythro-C<sub>18</sub>-SPM) (1) and L-threo-stearoylsphingomyelin (L-threo-C<sub>18</sub>-SPM) (2). The structures of 1 and 2 are shown in Figure 1. The synthesis of these compounds has been reported in a communication recently (Bruzik, 1986) and will be reported in detail elsewhere (Bruzik, 1987). The results indicate that the thermotropic properties of 1 and 2 are significantly different from each other and from those determined in the previous reports with chemically or stereochemically inhomogeneous sphingomyelins.

#### EXPERIMENTAL PROCEDURES

**Materials.** Diastereomeric D-erythro-C<sub>18</sub>-SPM (1) and L-threo-C<sub>18</sub>-SPM (2) were synthesized according to the recently published procedure (Bruzik, 1986, 1987). After normal chromatographic purification, the D-erythro isomer 1 was further purified by double crystallization from hot acetone, followed by precipitation of sphingomyelin with acetone from chloroform (10:1 v/v; 4 times) and then with acetone from methanol (twice). The purity of samples was judged on the basis of DSC curves, and purification was continued until subsequent precipitation showed no change in  $T_m$  and  $\Delta T_{1/2}$  of the main transition. The L-threo isomer 2 was purified first by preparative HPLC (Bondapak ODS column; methanol/hexane/water = 100/5/5 to methanol/hexane = 100/5, linear gradient) followed with the precipitation procedures as described above. Distilled HPLC-grade chloroform and methanol (Baker) and freshly distilled low-residue acetone (Mallinckrodt) were used in the above purifications.

DPPC was purchased from Avanti Polar Lipids and was used without further purification.

**DSC Experiments.** The samples (multilamellar vesicles) were prepared as follows: a preweighed amount of sphingomyelin (2.5–3.0 mg) was placed in a 1.5-mL Teflon-lined screw-cap vial and was added with 0.625–0.75 mL of Na<sup>+</sup>-Mops buffer (2.5 mM, pH 7.0). After the vial was tightly capped, it was placed in the shaker bath, thermostated at 60 °C, and was shaken for 1 h. A sample of 0.5 mL of the dispersion (equivalent to 2 mg of sphingomyelin) was then

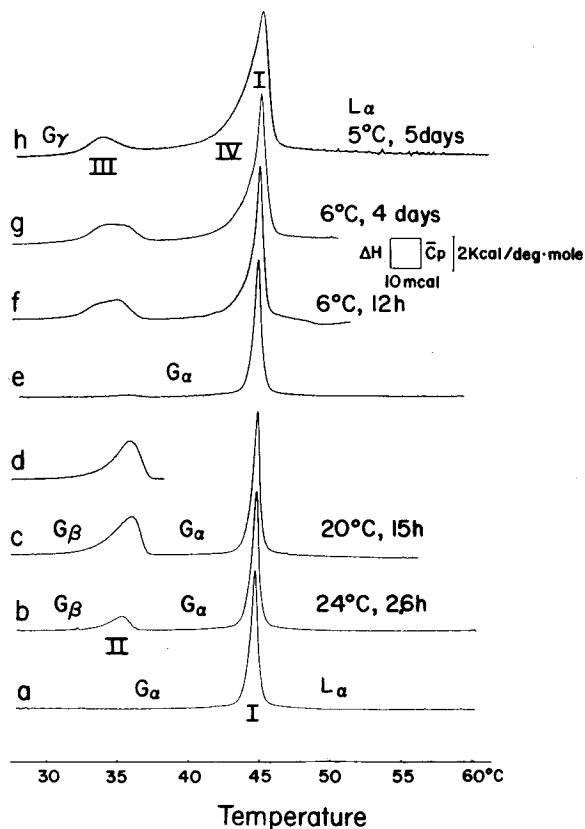


FIGURE 2: DSC profiles of 1: (a) scanned immediately after hydration at 60 °C, (b) after equilibration at 24 °C for 2.6 h, (c) at 20 °C for 15 h, (d) at 20 °C for 7 h, (e) rescanned immediately after run d, (f) at 6 °C for 12 h, (g) at 6 °C for 4 days, and (h) at 5 °C for 5 days.

transferred into the calorimeter cell through a precision syringe fitted with a long needle.

All measurements were performed on a high-sensitivity differential scanning calorimeter, Model MC-2, Microcal Inc. (Amherst, MA), equipped with a circulator bath. All reported DSC profiles were obtained at a scanning rate of 1.1 deg/min. Occasionally, DSC curves at lower scanning rates (20 deg/h) were obtained and compared with those at a higher scanning rate. No measurable differences could be detected. Phase-transition enthalpies were determined by cutting out and weighing the peaks. All transition enthalpies reported for 1 and 2 were accurate within  $\pm 10\%$ . The widths of the main transitions were greatly affected by the presence of impurities, and broader peaks were observed at intermediate stages of sphingomyelin purification.

#### RESULTS

**Thermotropic Properties of D-erythro-C<sub>18</sub>-SPM (1).** Figure 2 shows the DSC profiles of 1 hydrated in excess 2.5 mM Na<sup>+</sup>-Mops buffer, pH 7.0, at different thermal histories. Immediately after hydration at 60 °C, the sample displayed a single sharp endotherm (peak I, trace a) at 44.7 °C, with half-height  $\Delta T_{1/2} = 0.5$  K and a transition enthalpy  $\Delta H = 6.8 \pm 0.2$  kcal/mol. This endotherm is assigned as the "main transition" in accordance with the previous DSC studies of sphingomyelins (Barenholz & Gatt, 1982). The phase above  $T_m$  should be the liquid-crystalline phase, and the phase below  $T_m$  is the gel phase (Shipley et al., 1974). Due to the metastability of the gel phase described below, we designate this phase as gel- $\alpha$  phase, or "G $\alpha$  phase".

When the dispersion, after being heated above  $T_m$ , was incubated at 24 °C for 2.6 h and rescanned, another broad

Table I: Summary of the DSC Data of 1 and 2 and Their Mixtures with DPPC

sample (phase) <sup>a</sup>	stability	main transition (I) <sup>b</sup>			other transitions <sup>c</sup>									Figure		
		$T_m$ (°C)	$\Delta T_{1/2}$ (K)	$\Delta H$ (kcal/mol)	II			III			IV					
					$T$	$\Delta T_{1/2}$	$\Delta H$	$T$	$\Delta T_{1/2}$	$\Delta H$	$T$	$\Delta T_{1/2}$	$\Delta H$			
1 ( $G_\alpha$ )	metastable	44.7	0.5	6.8												2a
1 ( $G_\beta$ )	metastable	44.7	0.5	7.0	36.0	2.2	5.7									2c
1 ( $G_\gamma$ )	stable	44.7	0.5	9.5				33.4	2.4	2.6	43.6	2.8	8.0			2h
2 ( $G_\beta$ )	metastable	44.2	0.4	6.0	43.1	1.5	1.8									4c
2 ( $G_\gamma$ )	stable	44.2	0.5	6.5	43.1	1.5	1.9	35	12	6						4d
1 + 2 (gel)	stable	44.0	0.8	7.5												4b
1 + DPPC (gel)	stable	43.2	0.4	7.0												4e
2 + DPPC (gel)	stable	42.2	1.0	6.7												4f

<sup>a</sup>The phase in parentheses indicates the starting phase of the DSC trace. <sup>b</sup>The estimated error in  $T_m$  and  $\Delta T_{1/2}$  is  $\pm 0.1$  deg, while that in  $\Delta H$  is  $\pm 10\%$ . <sup>c</sup>The errors could be larger for transitions II, III, and IV, depending on the widths of the transitions. The  $\Delta H$  values reported are based on the assumption that the samples were fully relaxed.

endotherm (peak II) appeared at 36 °C (trace b). The enthalpy of this new transition is time and temperature dependent (compare traces b and c) and the rate of its appearance increased as the temperature of incubation decreased (traces not shown). At 24 °C the half-life of the metastable  $G_\alpha$  phase is  $\sim 5$  h. The dispersion which has been heated above 36 °C (trace d) then cooled quickly down to 20 °C displayed only the phase transition at 44.7 °C upon the next heating scan (trace e). When the sample heated above 36 °C was again incubated at 20 °C, a melting profile (not shown) with two endotherms (I and II) was observed again. The enthalpy and the temperature of transition I were not influenced by the appearance of transition II. These experiments clearly establish that the  $G_\alpha$  phase of 1 relaxes to a second gel phase, designated as " $G_\beta$  phase", reversibly, but slowly, upon incubation at 20–25 °C.

Incubation of 1 at lower temperatures (<10 °C) resulted in relaxation to a third gel phase, designated as " $G_\gamma$  phase". The DSC profile of a fully relaxed sample (5 °C, 5 days) is shown in trace h of Figure 2, which shows two new transitions: transition III at 33.4 °C ( $\Delta H = 2.6$  kcal/mol) and transition IV, which is partially overlapped with transition I. At shorter times of incubation at 6 °C, coexistence of  $G_\beta$  and  $G_\gamma$  phases can be observed (traces f and g), as manifested by two overlapping peaks at 33.4 and 36 °C and by the increasing width and enthalpy of the main transition.

The thermotropic property of the  $G_\gamma$  phase of 1 was further characterized by the difference DSC heating profile obtained with two samples of 1 of different thermal history, as shown in Figure 3. A dispersion of 1 was incubated at 5 °C for 5 days, and half of it was loaded into the reference cell of the calorimeter and heated against a buffer solution in a sample cell. Trace a thus obtained is identical with the "mirror image" of trace h of Figure 2. If the scan was to be repeated immediately, the sample in the reference cell should give the mirror image of trace a of Figure 2. Instead of doing this, we obtained a difference trace between traces h and a of Figure 2 as follows. The buffer in the sample cell was emptied, the entire system cooled down to 25 °C, and the sample cell loaded with the remaining half of the dispersion. The heating scan obtained subsequently is shown in trace b. When the next heating cycle was performed with the same dispersions, all transitions were nearly nulled (trace c), which demonstrated the accuracy of matching sample volumes in both cells. From the difference trace b, transition IV can be clearly identified at 43.6 °C associated with  $\Delta H = 8.0$  kcal/mol. In addition, the residual transition at 44.7 °C ( $\Delta H = 2.7$  kcal/mol) in trace b indicates that the  $\Delta H$  of the main transition  $G_\alpha \rightarrow L_\alpha$  should be 9.5 kcal/mol, i.e., the sum of the  $\Delta H$  of the  $G_\alpha \rightarrow L_\alpha$  transition (6.8 kcal/mol) and the residual 2.7 kcal/mol.

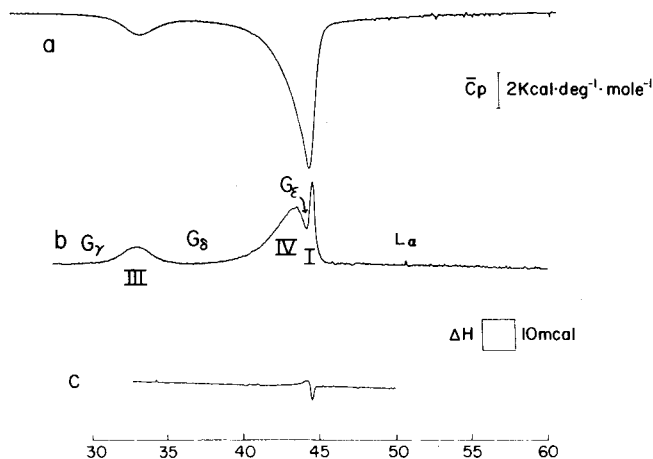


FIGURE 3: Difference DSC scans of 1. (a) Sample cell contained buffer; reference cell contained sample 1 equilibrated at 5 °C for 5 days. (b) After scan a, the sample cell was emptied and filled with another sample of 1 equilibrated at 5 °C for 5 days. (c) Rescan after scans a and b. The exact procedure and meaning of this set of experiments are described in the text.

The results obtained for *D-erythro*- $C_{18}$ -SPM (1) can be summarized as follows: The sharp transition at 44.7 °C converts the metastable gel phase  $G_\alpha$  into a liquid-crystalline state. Incubation of the sample at 20–25 °C causes rearrangement of the metastable gel phase to another metastable gel phase,  $G_\beta$ . Upon heating, the  $G_\beta$  phase is converted to the  $G_\alpha$  phase through the transition at 36 °C. Prolonged cooling of the sample at <10 °C induces rearrangement of the  $G_\beta$  phase into a stable gel phase  $G_\gamma$ . Upon heating, the  $G_\gamma$  phase undergoes three transitions (III, IV, and I) to the liquid-crystalline phase. The two intermediate gel phases in the heating profile of the  $G_\gamma$  phase are designated as  $G_\delta$  and  $G_\epsilon$  phases, as shown in Figure 3b. The transition temperatures and enthalpies are summarized in Table I.

**DSC Properties of 1 + 2.** Because it has been shown that commercially available semisynthetic SPM is a mixture of *L-threo*- and *D-erythro*-sphingomyelin (Bruzik, 1987), we have carried out the DSC scans of the dispersions of a 1:1 mixture of 1 and 2. As is clear from Figure 4b, the mixture shows only one slightly asymmetric peak at 44.0 °C, with  $\Delta H = 7.5$  kcal/mol. The shape of the profile is completely insensitive to incubations at room and refrigerator temperatures for several days. Such results may explain the lack of metastability in previous DSC studies of semisynthetic SPM (Calhoun & Shipley, 1979b; Barenholz & Gatt, 1982).

**Thermotropic Properties of Pure *L-threo*- $C_{18}$ -SPM (2).** The excess heat capacity curve obtained for *L-threo*- $C_{18}$ -SPM (2) is shown in Figure 4c. The behavior of 2 is characterized by

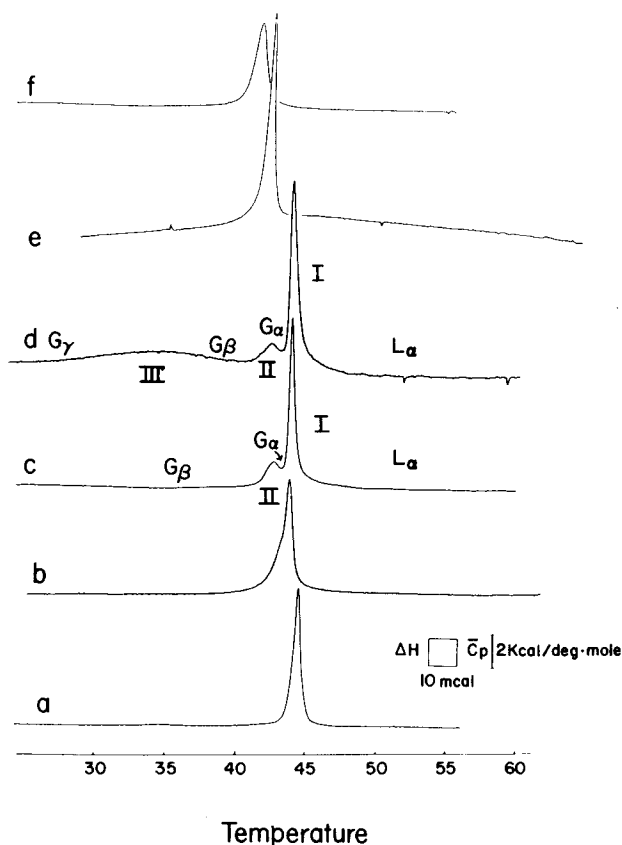


FIGURE 4: DSC profiles of various sphingomyelin-containing dispersions: (a) **1** heated above  $T_m$ ; (b) equimolar mixture of **1** and **2**; (c) **2** heated above  $T_m$ ; (d) **2** incubated at 6 °C for 13 days; (e) equimolar mixture of **1** and DPPC; (f) equimolar mixture of **2** and DPPC.

a sharp endotherm (I) at 44.2 °C ( $\Delta T_{1/2} = 0.4$  K) associated with an enthalpy of 6.0 kcal/mol and a smaller peak (II) at 43.0 °C (1.8 kcal/mol). The two transitions were not affected to a measurable extent by the thermal history of the sample. However, a third, very broad hump extending over the range of 30–40 °C became visible when this sample was held at 6 °C for 2 weeks (peak III of Figure 4d). Thus, the thermotropic property of **2** is quite different from that of pure **1** and the mixture **1** + **2**. The three gel phases of **2** are designated as  $G_\alpha$ ,  $G_\beta$ , and  $G_\gamma$  as shown in traces c and d of Figure 4. The numerical data of the thermal transitions of **2** are also summarized in Table I. It should be noted that we do not know the structures of the various phases at this stage and do not imply any correlation between the  $G_\alpha$ ,  $G_\beta$ , and  $G_\gamma$  phases of **1** and those of **2**. The various possibilities are described under Discussion.

**Mixtures of Sphingomyelins and Dipalmitoylphosphatidylcholine.** The dispersions of the mixture of **1** and DPPC at a molar ratio of 1:1 displayed only one sharp endotherm at 43.2 °C (Figure 4e), while the dispersion of **2** and DPPC showed a main transition at 42.2 °C (Figure 4f). In the latter case the observed transition is slightly broadened. No additional transitions were seen upon storing of **1** + DPPC and **2** + DPPC samples at either 20 or 5 °C for several days. The data are also listed in Table I.

## DISCUSSION

**Similarity between the Thermotropic Properties of **2** and DPPC.** It is interesting to note that the thermotropic behavior of *L-threo*- $C_{18}$ -SPM (**2**) is similar to that of phosphatidylcholines. DPPC, for example, shows a pretransition at 35.1

°C ( $\Delta H = 1.1$  kcal/mol), a main transition at 41.1 °C ( $\Delta H = 6.9$  kcal/mol), and, upon incubation at 0–5 °C for several days, a subtransition at 18 °C ( $\Delta H = 3.2$  kcal/mol) (Chen et al., 1980). Transitions I and II of **2** may correspond to the main transition and the pretransition, respectively, of DPPC, while transition III of long-incubated **2** may correspond to the subtransition of DPPC. Whether the structures of the  $G_\alpha$ ,  $G_\beta$ , and  $G_\gamma$  phases of **2** also resemble those of the  $P_\beta$ ,  $L_\beta$ , and  $L_\epsilon$  phases respectively of DPPC remains to be established by further investigation employing various biophysical techniques.

### Tentative Interpretation on the Thermotropic Property of **1**

1. Understanding of the structural properties of each of the observed phases for *D-erythro*- $C_{18}$ -SPM (**1**) would require extensive studies employing various physical techniques such as X-ray diffraction, Fourier transform infrared spectroscopy, freeze–fracture electron microscopy,  $^{31}\text{P}$  NMR, and  $^2\text{H}$  NMR. As a basis for such future studies, we attempt to compare the thermotropic property of **1** with that of **2** and DPPC.

By comparing traces a–c of Figure 2 with traces c and d of Figure 4, it seems that transitions I and II of **1** correspond to transitions I and III of **2** (main transition and subtransition, respectively). Thus, the differences between **1** and **2** are that **1** does not show a “pretransition” and that the “subtransition” of **2** is much broader than that of **1**.

The  $G_\beta \rightarrow G_\gamma$  relaxation of **1** and trace h of Figure 2, however, are more difficult to interpret. One of the possible explanations is that  $G_\gamma$  is a second subgel phase of **1** and that the  $G_\gamma \rightarrow G_\delta \rightarrow G_\epsilon \rightarrow L_\alpha$  transitions (see trace b in Figure 3) correspond to the  $L_\epsilon \rightarrow L_\beta \rightarrow P_\beta \rightarrow L_\alpha$  transitions of DPPC. A problem with such an interpretation is that the pretransition (transition IV) of **1** has an unusually large  $\Delta H$  (8.0 kcal/mol).

The complex, temperature-dependent relaxation of **1** at different phases has analogy in DPPC. While cooling of DPPC between –8 and 6 °C induces relaxation of the  $L_\beta$  phase to the subgel ( $L_\epsilon$ ) phase (Chen et al., 1980), gradual cooling of DPPC to –30 °C induces transformation from the  $L_\beta$  phase to another gel phase assigned as the “GIII gel phase” (Wong & Mantsch, 1983). In addition, two or more peaks of subtransitions for DPPC have been observed recently (Wu et al., 1985; Silvius et al., 1985; Tristram-Nagle & Nagle, 1986), which implies more than one subgel phase.

The detailed kinetic studies by Nagle and co-workers (Yang et al., 1987; Tristram-Nagle et al., 1987) have concluded that the multiple subtransition peak observed in the DSC of DPPC is simply a kinetic artifact. Such a possibility may also apply to the  $G_\beta$  and  $G_\gamma$  phases of **1**. However, we consider this rather unlikely for three reasons. (a) Heating traces of the two phases are very different. The  $G_\gamma$  phase undergoes three transitions in transforming to the  $L_\alpha$  phase, and the main transition  $G_\epsilon \rightarrow L_\alpha$  has considerably larger  $\Delta H$  (9.5 kcal/mol) than the main transition  $G_\alpha \rightarrow L_\alpha$  (6.8 kcal/mol). (b) In the case of DPPC, incubation at higher temperature (5 °C) gave slower formation of the subgel phase (nucleation is rate limiting) while incubation at lower temperature (0.1 °C) resulted in faster formation of the subgel phase (Yang et al., 1987). The situation with the SPM **1** is just the opposite. It took only 15 h for  $G_\alpha \rightarrow G_\beta$  relaxation at 20 °C (trace c, Figure 2) but a much longer time was required for relaxation to the  $G_\gamma$  phase at 6 or 5 °C (traces f–h, Figure 2). (c) The DSC trace of DPPC equilibrated for a shorter time gave a subtransition at lower temperature due to more crystal defects. The case of **1** is also the opposite. The “subtransition” of the  $G_\gamma$  phase (transition III) actually occurred at a lower temperature (33.4 °C) than the subtransition of the  $G_\beta$  phase (transition II, 36.0 °C).

**Configurational Effect on the Phase Behavior.** We have recently demonstrated significantly different thermotropic properties between diastereomers of chiral thiophospholipids (Wisner et al., 1986). The different behavior between **1** and **2** reported in this paper further suggests the importance of using pure stereoisomers in biophysical studies of membranes. Mixing of **1** and **2**, for example, removed the metastability of the gel phase and resulted in a deceptively simple system. While it is not possible for us to fully explain the differences between our results and every previous report on the DSC studies of sphingomyelins and their interactions with cholesterol [for reviews, see Barenholz and Thompson, (1980) and Barenholz and Gatt (1982)], we believe the lack of metastability in previous reports (except DL-erythro-C<sub>18</sub>-SPM described below) is due to inhomogeneity in their samples, either chemically or stereochemically.

Estep et al. (1980) and Barenholz et al. (1976) indeed observed metastability in synthetic DL-erythro-C<sub>18</sub>-SPM, but in a different sense. The aqueous dispersion of DL-erythro-C<sub>18</sub>-SPM held for several hours at room temperature displayed a main transition at 57 °C associated with a large  $\Delta H$  (20 kcal/mol). If the sample was scanned immediately after heating, an endotherm at 43–47 °C ( $\Delta H < 7$  kcal/mol) was observed. It is unclear whether the difference between their results and ours are due to differences in sample preparation (e.g., purity or buffer), or due to the "enantiomer discrimination" defined by Stewart and Arnett (1982). In any case, it seems difficult to rationalize the large difference between D- and DL-erythro-C<sub>18</sub>-SPM as described above, and the similarity between racemic and enantiomeric N-palmitoyl-sphingomyelins as reported by Neuringer et al. (1979), Calhoun and Shipley (1979b), and Barenholz and Gatt (1982).

**Conclusion.** Detailed thermotropic properties of D-erythro-C<sub>18</sub>-SPM (**1**) and L-threo-C<sub>18</sub>-SPM (**2**) have been reported. The results are quite different from various previous reports which employed chemically or stereochemically inhomogeneous sphingomyelins. The various phases of **1** and **2** have been tentatively assigned in analogy to the thermotropic phases of DPPC. A striking difference between SPM and DPPC is that isomer **1** displayed two subgel phases with distinct kinetic and thermodynamic properties. Such results will serve as the basis for a detailed investigation employing various biophysical techniques. They also suggest that it is important to use pure stereoisomers in studying the biophysical properties of sphingomyelins.

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