Phospholipids Chiral at Phosphorus. 12. Configurational Effect on the Thermotropic Properties of Chiral Dipalmitoylthiophosphatidylcholine[1]

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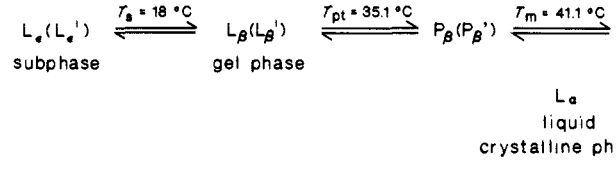
Abstract: Differential scanning calorimetry (DSC) studies of the diastereomers of 1,2-dipalmitoyl-*sn*-glycero-3-thiophosphocholine (DPPsC) have been carried out to determine the effect of phosphate structure and configuration on the phase-transition properties of these analogues of natural 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) in the multilamellar form. Both the S_P and $R_P + S_P$ isomers showed a "pretransition" (T_{pt} = 43.7 and 43.8 °C, respectively) and a "main transition" (T_m = 45.0 and 44.8 °C, respectively). The corresponding values for DPPC were 35.1 and 41.5 °C, respectively. (R_P)-DPPsC, however, showed a single, broad transition at 45.9 °C with significantly higher ΔH. Addition of 15% (S_P)-DPPsC to the R_P isomer converted the broad DSC trace to a "normal" pattern, with T_{pt} and T_m at 42.8 and 44.7 °C, respectively. These suggest that the broad, highly endothermic transition is a unique property of pure (R_P)-DPPsC, which could be a superposition of subtransition, pretransition, and main transition based on the following results. While (S_P)- and ($R_P + S_P$)-DPPsC showed a subtransition near 20 °C following prolonged incubation at 0 °C, a property also possessed by DPPC, (R_P)-DPPsC showed no discernible subtransition following incubation at 0 °C. Furthermore, it was found that (R_P)-DPPsC can indeed exist in the gel phase but relaxes rapidly to a lower energy phase, presumably the subphase. The half-life of the metastable gel phase was found to increase with decreasing diastereomeric purity of (R_P)-DPPsC. These studies suggest that (R_P)-DPPsC is thermodynamically and kinetically more stable at the subphase and demonstrate that the structure and configuration at the phosphate group of phospholipids have a large effect on the thermotropic properties of membranes, particularly in the subtransition temperature. The results are discussed in terms of intermolecular interactions and chiral discrimination in phospholipid membranes.

The effect of chirality on the molecular interactions of membranes has received increasing attention in recent years.[2] One important question is whether there are chiral discrimination factors in membranes, as their main constituents and many of the compounds which must traverse them are chiral. The general approach to determine "enantiomer discrimination" has been to compare the physical properties of isomerically pure phospholipids with those of the mixture of enantiomers. Arnett and co-workers have clearly demonstrated enantiomer discrimination in the monolayers of enantiomeric and racemic *N*-(α-methylbenzyl)-stearamide.[2,3] Chiral discrimination between monolayers of enantiomeric and racemic phospholipids has also been reported in the force–area isotherms of monolayers of 1-stearoyl-2-lauroylphosphatidylcholine[4] and in the differential scanning calorimetry (DSC) studies of multilamellar DPPC.[5-7] However, Arnett and Gold[8] have been unable to detect any significant difference between racemic DPPC and its enantiomers in their meticulous studies using DSC, NMR, and monolayer techniques. Minones et al.[9a] were unable to detect any difference between force–area isotherms of L-DPPC and racemic DPPC on water at 20 °C. The DSC studies by Rainier et al.[9b] also showed no significant difference in the transition temperatures of the *sn*-1,1, *sn*-3,3, and *meso-sn*-1,3 isomers of phosphatidyldiacylglycerol.

The controversy in the DSC studies of DPPC needs further elaboration. The phase behavior of DPPC in excess H_2O can be described as follows[10-13]



where T_s, T_{pt}, and T_m represent transition temperatures of the subtransition, pretransition, and main transition, respectively. Whereas a subtransition around 18 °C was seen in L-DPPC bilayers following incubation near 0 °C for several days,[10] Boyanov et al.[5] reported an absence of the subtransition in DL-DPPC. They also noted that the phase transitions of DL-DPPC are less cooperative than those of L-DPPC. Kodama et al.[6] observed smaller subtransition and pretransition for DL-DPPC relative to L-DPPC. While these results seem interesting and have prompted us to investigate the subtransition of DPPsC as discussed later, the authors did not employ the "absolute method" as suggested by Stewart and Arnett,[2] that is, to first purify D and L enantiomers until they exhibit identical properties and then compare their properties with those of the mixture of the same pure enantiomers. The lack of enantiomer discrimination reported by Arnett and

(1) For paper 11, see ref 20. Abbreviations used: DLPE, 1,2-dilauroyl-*sn*-glycero-3-phosphoethanolamine; DMPE, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPsC, 1,2-dipalmitoyl-*sn*-glycero-3-thiophosphocholine; DSC, differential scanning calorimetry; ΔH_m, enthalpy of main transition; ΔH_{pt}, enthalpy of pretransition; ΔH_s, enthalpy of subtransition; EDTA, ethylenediaminetetraacetate; FT-IR, Fourier transform infrared; Pipes, piperazine-*N*,*N*'-bis(2-ethanesulfonic acid); SUV, small unilamellar vesicles; TLC, thin-layer chromatography; T_m, temperature of main transition; T_{pt}, temperature of pretransition; and T_s, temperature of subtransition.

(2) Stewart, M. V.; Arnett, E. M. In *Topics in Stereochemistry*; Allinger, N. L., Eliel, E. L., Wilen, S. H., Eds.; Wiley: New York, 1982; Vol. 13, pp 195–262 and references therein.

(3) Arnett, E. M.; Chao, J.; Kinzig, B.; Stewart, M.; Thompson, O. *J. Am. Chem. Soc.* **1978**, *100*, 5575–5576.

(4) van Deenen, L. L. M.; Houtsmuller, U. M. T.; de Haas, G. H.; Mulder, E. *J. Pharm. Pharmacol.* **1962**, *14*, 429–444.

(5) Boyanov, A. I.; Tenchov, B. G.; Koynova, R. D.; Koumanov, K. S. *Biochim. Biophys. Acta* **1983**, *732*, 711–713.

(6) Kodama, M.; Hashigami, H.; Seki, S. *Biochim. Biophys. Acta* **1985**, *814*, 300–306.

(7) According to the abbreviations defined in ref 1, DPPC and DPPsC represent 3-*sn* isomers in the conventions of the IUPAC–IUB stereospecific numbering system (which corresponds to the L configuration). While this is the case in all of our work described in this paper, we use DPPC to represent either enantiomer in the introduction when we discuss the literature.

(8) Arnett, E. M.; Gold, J. M. *J. Am. Chem. Soc.* **1982**, *104*, 636–639.

(9) (a) Minones, J.; Sandez Macho, M. I.; Iribarnegaray Jado, E.; Sanz Pedro, P. *Med. Segur. Trab.* **1979**, *27*, 40–47. (b) Rainier, S.; Jain, M. K.; Ramirez, F.; Ioannou, P. V.; Marecek, J. F.; Wagner, R. *Biochem. Biophys. Acta* **1979**, *558*, 187–198.

(10) Chen, S. C.; Sturtevant, J. M.; Gaffney, B. J. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5060–5063.

(11) Lipka, G.; Chowdhry, B. Z.; Sturtevant, J. M. *J. Phys. Chem.* **1984**, *88*, 5401–5406.

(12) Stümpfel, J.; Eibl, H.; Niksch, A. *Biochim. Biophys. Acta* **1983**, *727*, 246–254.

(13) Throughout this paper the primed and unprimed phases are not distinguished.

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Table I. Summary of Pretransition and Main Transition Properties of DPPC and DPPsC[a]

compd	pretransition			main transition			source
	T_{pt}, °C	$\Delta T_{1/2}$, K	ΔH_{pt}, kcal/mol	T_m, °C	$\Delta T_{1/2}$, K	ΔH_m, kcal/mol	
DPPC							
ref 10[b]	35.1	1.8	1.09	41.1	0.18	6.9	
this work	35.1	1.5	1.1	41.5	0.27	7.2	
(R_P + S_P)-DPPsC	43.8	0.75	1.7	44.8	0.26	6.8	Figure 2a
(S_P)-DPPsC	43.7	0.75	1.6	45.0	0.27	7.1	Figure 2b
(R_P)-DPPsC							
>99% purity							
equilibrated				45.9	1.46	13.4	Figure 2c
nonequilibrated	42.7	1.0	1.4	44.9	0.36	7.4	Figure 4a
97% purity							
equilibrated				45.6	1.44	14.7	
nonequilibrated	42.4	0.94	1.6	44.7	0.25	7.5	
85% purity	42.8	0.98	1.8	44.7	0.28	6.6	Figure 2d

[a] The estimated error is ±0.1 °C for transition temperatures and ±10% for ΔH. [b] Obtained in sodium phosphate buffer, pH 7.4

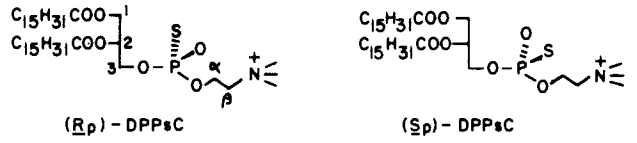

Figure 1. Structures of (R_P)- and (S_P)-DPPsC. (R_P + S_P)-DPPsC is the chemically synthesized 1:1 mixture of the two isomers. In this paper the description "isomers of DPPsC" includes the R_P and the S_P isomers as well as the mixture.

Gold,[8] using the absolute method, seems more convincing, but they apparently did not examine the subtransition.

Our approach to the question of chiral discrimination in membranes has been somewhat different in that we have introduced a second chiral center at the phosphate by virtue of replacing O by S such as to make a pair of unnatural thiophosphatidylcholine diastereomers, (R_P)- and (S_P)-DPPsC[14,15] (Figure 1), which, nonetheless, retain the natural L configuration at carbon-2 of the glycerol moiety. The difference between the physical properties of (R_P)- and (S_P)-DPPsC is analogous to, but not exactly the same as, the "diastereomer discrimination" defined by Stewart and Arnett.[2] The latter term refers to any measurable difference between diastereomeric pairs. For example, the detectable difference between such diastereomeric pairs, L-DPPC/(−)-*N*-(α-methylbenzyl)stearamide and L-DPPC/(±)-*N*-(α-methylbenzyl)stearamide, suggests the possibility of the transmission of chirality in "fluid" membranes.

In the case of DPPsC, we are comparing the difference between membranes of two diastereomeric phospholipids. *The question is not whether chiral discrimination exists but where it exists*; that is, since the configuration at carbon-2 of the glycerol backbone is fixed, a large difference between (R_P)- and (S_P)-DPPsC may suggest a possible stereospecific interaction or a possible conformational change at the phosphate group. The best example to illustrate such applications of DPPsC is the comparison of (R_P)- and (S_P)-DPPsC as substrates of phospholipase A_2 and lecithin–cholesterol acyl transferase (though this is not exactly the chiral discrimination described above). The enzymes catalyze transfer of the 2-acyl group of L-lecithins to H_2O and cholesterol, respectively. In the case of lecithin–cholesterol acyl transferase the K_m and V_{max} were indistinguishable for (R_P)-DPPsC, (S_P)-DPPsC, and DPPC, which suggests that the phosphate group is not involved in enzyme-substrate interactions.[16] On the other hand, phospholipase A_2 showed a high stereospecificity toward (R_P)-DPPsC, suggesting a possible stereospecific interaction between the phosphate group and Ca^{2+}, which was further supported by the metal-ion dependence of stereospecificity.[17]

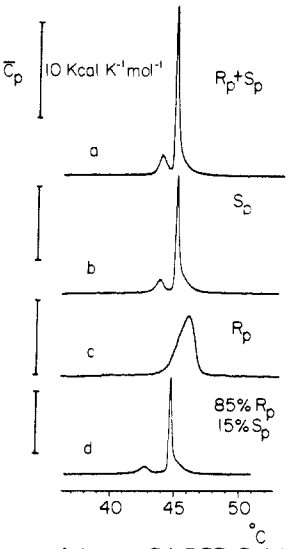

Figure 2. DSC traces of (R_P + S_P)-DPPsC (a), (S_P)-DPPsC (b), (R_P)-DPPsC (c), and (85% R_P + 15% S_P)-DPPsC (d).

Concerning the biophysical properties of (R_P)- and (S_P)-DPPsC, our previous emphasis has been to demonstrate that (R_P)-, (S_P)-, and (R_P + S_P)-DPPsC can form unilamellar[18] and multilamellar vesicles[19] and that they possess different physical properties in the liquid crystalline phase as revealed by ^{14}N NMR,[19] ^{31}P NMR,[18,19] and FT-IR.[20] Such differences prompted us to investigate the thermotropic properties of multilamellar DPPsC in detail[21] by using samples of very high chemical and diastereomeric purity. The results indicate that the configuration at phosphorus has a dramatic effect on the kinetics and thermodynamics of the phase-transition properties of phospholipids, particularly in regard to the subtransition.

Results

Pretransition and Main Transition Properties. Unless otherwise specified, our standard sample condition was 5% (wt/wt) phosphatidylcholine in 20 mM Pipes/NaOH, pH 7.4. As shown in Table I, the values of T_{pt} and T_m and the corresponding ΔH values

(14) Bruzik, K.; Jiang, R.-T.; Tsai, M.-D. *Biochemistry* **1983**, *22*, 2478–2486.
(15) Jiang, R.-T.; Shyy, Y.-J.; Tsai, M.-D. *Biochemistry* **1984**, *23*, 1661–1667.
(16) Pownall, H. J.; Rosario-Jansen, T.; Tsai, M.-D., unpublished results.
(17) Tsai, T.-C.; Hart, J.; Jiang, R.-T.; Bruzik, K.; Tsai, M.-D. *Biochemistry* **1985**, *24*, 3180–3188.
(18) Tsai, T.-C.; Jiang, R.-T.; Tsai, M.-D. *Biochemistry* **1984**, *23*, 5564–5570.
(19) Tsai, M.-D.; Jiang, R.-T.; Bruzik, K. *J. Am. Chem. Soc.* **1983**, *105*, 2478–2480.
(20) Chang, S.-B.; Alben, J. O.; Wisner, D. A.; Tsai, M.-D. *Biochemistry* **1986**, *25*, 3435–3440.
(21) Due to the unavailability of a DSC instrument earlier, we determined the T_m of multilamellar DPPsC preliminarily only to ensure that the NMR spectra were measured above the main transition temperature.[19] The T_m of small unilamellar vesicles (SUV) of DPPsC has been found to be 43.8 ± 0.1 °C for all isomers, with the R_P isomer showing a broader transition.[18] However, the DSC data of SUV cannot be readily interpreted since it is known to be dependent on the size of the vesicles (see ref in ref 18), and the sizes of the SUV of isomers of DPPsC are different.

Table II. Summary of Subtransition Properties of DPPC and DPPsC[a]

compd	T_s, °C	$\Delta T_{1/2}$, K	ΔH_s, kcal/mol	cooling time at 0 °C, days	time for 50% relaxation to subphase[c]
DPPC					
ref 10[b]	18.4	3.0	3.23	3.5	1.2 days
this work	18.3	2.1	2.8	3.6	
	19.0	2.2	2.8	5.1	
(R_P + S_P)-DPPsC	21.7	2.6	2.6	34.0	3 days
(S_P)-DPPsC	22.0	2.8	2.9	34.8	4 days
(R_P)-DPPsC					
>99% purity					0.5 h
97% purity					2.5 h

[a] The estimated error is ±0.1 °C for transition temperatures and ±10% for ΔH. [b] Obtained in sodium phosphate buffer, pH 7.4 [c] Based on ΔH_s. (R_P)-DPPsC was incubated at 25 °C while other samples were incubated at 0 °C.

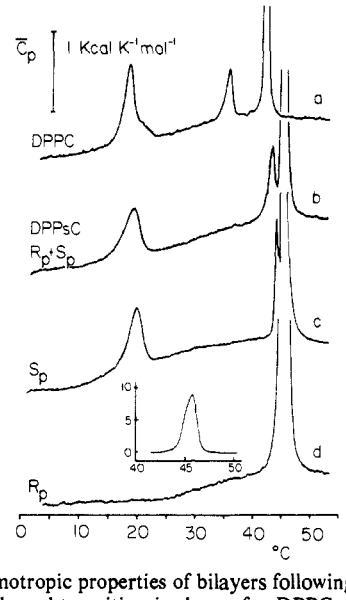

Figure 3. Thermotropic properties of bilayers following incubation at 0 °C (on ice). The subtransition is shown for DPPC after 3.6 days of incubation (a), (R_P + S_P)-DPPsC after 13.8 days (b), and (S_P)-DPPsC after 16.3 days (c). The subtransition is not seen for (R_P)-DPPsC even after 14.1 days (d).

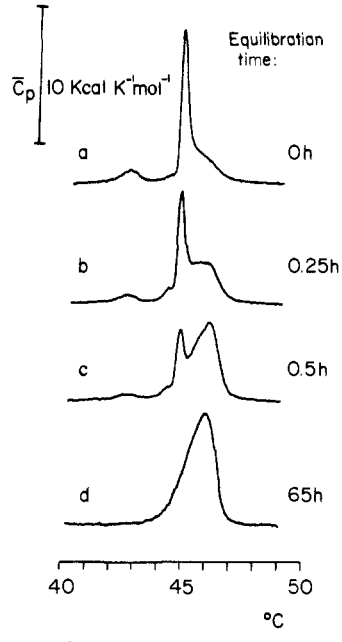

Figure 4. DSC traces of (R_P)-DPPsC of greater isomeric purity (>99%), showing the time dependence of the metastable gel phase. The sample (4.20 mg) was heated to 70 °C for 5 min and then equilibrated at 25 °C for 0 (a), 0.25 (b), 0.5 (c), and 65 h (d). Thermal equilibration of the calorimeter with the sample was conducted at 38 °C for 10–15 min just prior to scanning.

for DPPC are in good agreement with the results of Chen et al.[10] The DSC traces of the isomers of DPPsC are shown in Figure 2, with associated transition parameters also given in Table I. (R_P + S_P)-DPPsC (Figure 2a) and the S_P isomer (Figure 2b) showed very similar phase-transition behavior with pretransitions at 43.8 and 43.7 °C and main transitions at 44.8 and 45.0 °C, respectively. This represents an increase of approximately 9 °C in T_{Pt} and 3.5 °C in T_m relative to DPPC. In addition, the associated pretransition enthalpies of DPPsC are greater than that of DPPC by ca. 50%, and the half-widths ($\Delta T_{1/2}$) of the pretransition of DPPsC were also substantially smaller than that of DPPC.

(R_P)-DPPsC showed anomalous behavior in that only a single, broad transition at 45.9 °C was seen (ΔH = 13.4 kcal/mol) (Figure 2c). This should not be due to impurities since when the sample was subjected to several additional purification steps (precipitation), or when a second independently synthesized sample was employed, the same result was obtained. Further, when 15% (S_P)-DPPsC was added to the R_P isomer, the normal transition behavior was observed with T_{pt} = 42.8 and T_m = 44.7 °C (Figure 2d). The results indicate a chiral discrimination in the phase-transition properties of DPPsC.

The slight asymmetry and the broadness in the main transition of (R_P)-DPPsC suggest that it may be a composite of several transitions. The large ΔH (13.4 kcal/mol) suggests that it could be a superposition of subtransition, pretransition, and main transition. Our results on the subtransition properties of the isomers of DPPsC are consistent with this interpretation.

Subtransition Properties of DPPsC. Figure 3a shows a typical trace of the DPPC subtransition under our experimental conditions. DPPC equilibrated at 0 °C for 3.6 days gave a subtransition centered at T_s = 18.3 °C, consistent with the results of Chen et al.[10] Typical subtransition results of the (R_P + S_P)- and (S_P)-DPPsC isomers are shown in Figure 3b and 3c, after 13.8 and 16.3 days of incubation at 0 °C, respectively. The R_P + S_P and S_p isomers closely resemble naturally occurring DPPC in respect to the subtransition where they have similar enthalpies, transition widths, and only slightly higher subtransition temperatures (Table II). The most significant point of difference is that the (R_P)-DPPsC sample showed no additional peak around 20 °C typical of the subtransition after incubation at 0 °C for 14.1 days. Instead, only the usual broad peak at 45.9 °C was observed. Thus, it is most likely that the R_P isomer is thermodynamically stable in the subphase and does not undergo subtransition until the temperature approaches the main transition. Such a thermodynamic stability can also account for a larger "intrinsic ΔH_s" and thus a larger ΔH_{total} (the sum of the ΔH's of subtransition, pretransition, and main transition) for (R_p)-DPPsC (13.4 kcal/mol), relative to the ΔH_{total} of (S_P)-DPPsC (11.6 kcal/mol) and (R_P + S_P)-DPPsC (11.1 kcal/mol).

The above results and interpretation were further supported by the finding that (R_P)-DPPsC can indeed exist in the gel phase but relaxes rapidly to the subphase. The following section describes the metastability of the gel phase and the kinetics of the gel phase → subphase transition.

Metastability of the Gel (L_β) Phase. When (R_P)-DPPsC was heated at 70 °C for 5 min, cooled to 25 °C, and scanned immediately, a normal pattern as shown in Figure 4a was observed. The pretransition and main transition parameters, as also included

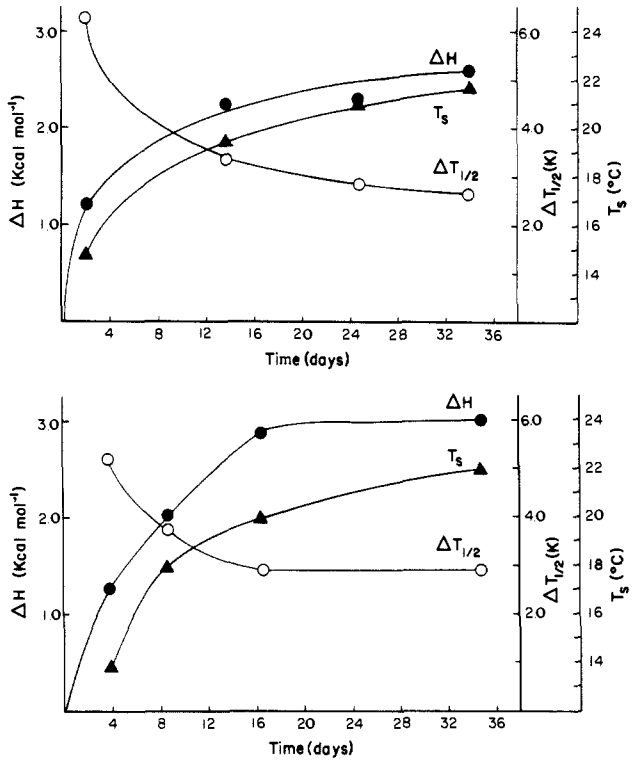

Figure 5. Dependence of subtransition parameters on equilibration time at 0 °C for (R_P + S_P)-DPPsC (a, top) and (S_P)-DPPsC (b, bottom). Shown are the calorimetric enthalpy, ΔH (●); the subtransition temperature, T_s (▲); and the transition width at half maximum height, $\Delta T_{1/2}$ (○).

in Table I, are similar to those of (S_P)- and (R_P + S_P)-DPPsC. As shown in Figure 4b–d, the gel phase of (R_P)-DPPsC relaxed rapidly to a lower energy phase, most likely the subphase on the basis of the above discussion. The time required for 50% relaxation was ca. 0.5 h, although the relaxation was not quite first order. This indicates that (R_P)-DPPsC could also exist in the gel phase but that this gel phase at 25 °C is metastable with respect to the subphase. In contrast, DPPC and other isomers of DPPsC have a stable gel phase at 25 °C that becomes metastable at temperatures approaching 0 °C. The metastable behavior of the gel phase of (R_P)-DPPsC resembles that reported for DLPE[22a,b] and DMPE.[23] It should also be noted that when (R_P)-DPPsC in the L_α phase (after 5 min at 70 °C) was cooled to 0 °C, it relaxed into the subphase immediately or at least within the 10 min required to do the DSC scan; that is, DSC showed a broad endotherm at 45.9 °C.

The metastability of the gel phase appeared to depend on the diastereomeric purity. A separate sample with 97% (R_P)- and 3% (S_P)-DPPsC showed slower relaxation. The time required for 50% relaxation at 25 °C was ca. 2.5 h after this sample was heated to 70 °C for 5 min. The rate of relaxation at 25 °C increased by a factor of ca. 2 when this sample was heated at 50 °C instead of 70 °C for 5 min.

On the other hand, (S_P)- and (R_P + S_P)-DPPsC are kinetically more stable at the gel phase relative to DPPC. The time required for 50% relaxation was ca. 1.2 days for DPPC based on the result of Chen et al.[10] The plots of the temperature T_s, the enthalpy ΔH, and the half-width of the subtransition as a function of incubation time are shown in Figure 5a (for R_P + S_P) and 5b (for S_P). Again the kinetics are not entirely linear, possibly due to complication by the nucleation process,[24] and it is unclear why ΔH and $\Delta T_{1/2}$ have reached a plateau sooner than T_s. The "slow"

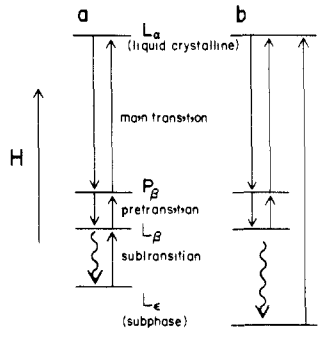

Figure 6. Comparison of thermotropic properties of the diastereomers of DPPsC. The phase-transition properties of DPPC, (R_P + S_P)-DPPsC, and (S_P)-DPPsC are represented in a. Represented in b is our interpretation of the phase-transition behavior of (R_P)-DPPsC.

nature of these processes has hampered more detailed kinetic studies. However, the time required for 50% relaxation can be estimated as ca. 3 and 4 days for (R_P + S_P)- and (S_P)-DPPsC, respectively, and the data are sufficient to conclude that the kinetics of the gel → subphase relaxation at 0 °C follow the order (R_P)-DPPsC > DPPC > (S_P)-DPPsC ≃ (R_P + S_P)-DPPsC.

Discussion

Thermotropic Properties of (R_P)-DPPsC. Figure 6, and b, compares the normal thermotropic behavior of DPPC, (S_P)-, and (R_P + S_P)-DPPsC with that of (R_P)-DPPsC. (R_P)-DPPsC differs from the other isomers and from DPPC in that the stepwise transitions $L_\epsilon \rightarrow L_\beta \rightarrow P_\beta \rightarrow L_\alpha$ do not occur in the heating cycle. Instead, heating leads to a conversion from L_ϵ (subphase) directly to L_α (liquid crystalline phase), with $T_s \simeq T_{pt} \simeq T_m \simeq 46$ °C.

Similar behavior has also been observed for two particular diastereomers of the cyclopentanoid analogues of DPPC, the glycerol backbones of which are replaced by cyclopentanetriol rings. The "all-trans" isomer showed a single transition at a high T_m (46 °C) with a large ΔH (18 kcal/mol).[25] Although no metastable state was observed, the authors demonstrated that when the size of the head group was increased by introducing additional methylene groups in the choline side chain, the analogues regained a subtransition at 20 ± 3 °C and the ΔH in the main transition was reduced to <10 kcal/mol (the pretransition was not observed in these cases).[26] The importance of the polar group in the phase-transition properties has also been suggested in the various metal complexes of cardiolipin.[9b]

Configurational Effect on Subtransition. Whereas the substitution of oxygen by sulfur affects all three transitions of DPPC, to different degrees, the configuration at phosphorus seems to have an especially dramatic impact on T_s and on the stability of the subphase.

The subphase has been shown to possess "more ordered molecular and hydrocarbon chain-packing modes in the crystal bilayer form" by X-ray diffraction studies.[27] This was fully supported by FT-IR studies,[28] which showed that prolonged incubation of DPPC at 2 °C results in a reduction in bandwidth and an increase in peak height of various CH_2 and C=O bands and the O—P—O stretching bands near 830 and 770 cm^{-1}, indicative of a large reduction in the mobility of the various functional groups. Since a decrease in the hydration of the head group (presumably at the C=O or the phosphate) has also been observed by X-ray[29] and FT-IR[28] studies, it has been proposed that hydration is the main driving force in the subphase → gel transition.[30,31] Evidence against this argument is the report by Lipka et al.[11] that hydration with D_2O instead of H_2O does not affect the subtransition temperature and enthalpy of DPPC, while it induces significant changes in the properties of the pre-

(22) (a) Chang, H.; Epand, R. M. *Biochim. Biophys. Acta* **1983**, *728*, 319–324. (b) Seddon, J. M.; Harlos, K.; Marsh, D. *J. Biol. Chem.* **1983**, *258* 3850–3854.
(23) Wilkinson, D. A.; Nagle, J. F. *Biochemistry* **1984**, *23*, 1538–1541.
(24) Nagle, J. F.; Wilkinson, D. A. *Biochemistry* **1982**, *21*, 3817–3821.
(25) Singer, M. A.; Jain, M. K.; Sable, H. Z.; Pownall, H. J.; Mantulin, W. W.; Lister, M. D.; Hancock, A. J. *Biochim. Biophys. Acta* **1983**, *731*, 373–377.
(26) Jain, M. K.; Singer, M. A.; Crecely, R. W.; Pajouhesh, H.; Hancock, A. J. *Biochim. Biophys. Acta* **1984**, *774*, 199–205.
(27) Ruocco, M. J.; Shipley, G. G. *Biochim. Biophys. Acta* **1982**, *684*, 59–66.
(28) Cameron, D. G.; Mantsch, H. H. *Biophys. J.* **1982**, *38*, 175–184.
(29) Ruocco, M. J.; Shipley, G. G. *Biochim. Biophys. Acta* **1982**, *691*, 309–320.
(30) Casal, H. L.; Mantsch, H. H. *Biochim. Biophys. Acta* **1984**, *779*, 381–401.
(31) Casal, H. L.; Mantsch, H. H.; Cameron, D. G.; Gaber, B. P. *Chem. Phys. Lipids* **1983**, *33*, 109–112.

transition and main transition. It is also interesting to note that while T_{pt} and T_m are affected to a substantial extent by the deuteriation of the acyl chains, the T_s is independent of such deuteriation.[31] This implies that while the mobility and packing of the acyl chains change greatly in the subtransition, it could be controlled by a factor other than the acyl chains. On the basis of ^{31}P NMR and X-ray studies, Fuldner[32] suggested that during the subtransitions a conformational change in the head-group region could possibly accompany the parallel changes in the lateral acyl chain packing and the rate of reorientation of the head group. The importance of such a conformational change (i.e., a change in the orientation of the O-P-O group and therefore the whole head group) is strongly supported by the large configurational effect on the subtransition properties of DPPsC.

Chiral Discrimination in DPPsC Membranes. From the stereochemical point of view, chiral discrimination is expected to be most important in the more condensed phases with crystalline character. The large configurational effect on the subtransition not only supports the tight packing in the subphase but also suggests that the packing may involve a stereospecific interaction between the phosphate group and the neighboring choline group. Such an intermolecular interaction has been well documented by nuclear Overhauser effects in ^{31}P NMR for small unilamellar vesicles of DPPC,[33] DPPsC,[18] and other phospholipids.[34,35] Our results could lead to a hypothesis that in the subphase the quaternary ammonium ion interacts more favorably with one of the two diastereotopic oxygen atoms at the phosphate group of DPPC. Such a possibility warrants detailed investigation by ^{31}P NMR, ^{2}H NMR, FT-IR, X-ray diffraction, etc., on the isomers of DPPsC. Several of these studies have already demonstrated discernible differences between isomers of DPPsC in the liquid crystalline phase.[18-20] The results on the subphase should provide a structural basis for the DSC results presented above.

Experimental Section

Materials. DPPC was purchased from Avanti and was used without further purification. (R_P + S_P)-DPPsC was synthesized chemically and separated into R_P and S_P isomers based on the stereospecific hydrolysis of (R_P)-DPPsC by bee venom phospholipase A_2 as described previously.[14,15] In order to obtain high diastereomeric purity of (R_P)-DPPsC, the phospholipase A_2 reaction was quenched with EDTA before 80% of (R_P)-DPPsC was hydrolyzed, and the resulting lyso-DPPsC was reacylated. The unreacted DPPsC from the phospholipase A_2 reaction was further digested exhaustively with phospholipase A_2 to give pure (unreacted) (S_P)-DPPsC. The diastereomeric purity was then determined by ^{31}P NMR in CD_3OD on a Bruker WM-300 NMR spectrometer. Both isomers obtained by the above procedure were considered >99% in diastereomeric purity since no contaminating isomer was detectable when the signal/noise ratio was >100. The chemical purity of lipid samples was monitored by ^{1}H NMR at 200 MHz on a Bruker WP-200 NMR spectrometer and by TLC on silica gel (EM Science, silica gel 60 F-254) with the solvent system $CHCl_3/CH_3OH/H_2O$, 66:33:4, with visualization by phosphomolybdic acid or I_2 vapor. The R_f values of DPPsC and DPPC were 0.5 and 0.25, respectively. Final purification of DPPsC was accomplished by six to seven precipitations from acetone/ethanol (ca. 10:1, v/v).

DSC Studies. DSC traces were obtained on a MicroCal scanning microcalorimeter Model MC-1 (Amherst, MA). Phospholipid samples dried in vacuo (12 h, 50 °C) were weighed and transferred to the calorimeter cell with chloroform. The chloroform was then removed in vacuo overnight at 50 °C, and the phospholipid was suspended in 20 mM Pipes buffer, pH 7.4, by incubation at 50-60 °C for 10 min with occasional shaking. This means of sample preparation gave reproducible DSC traces. All samples consisted of 2-6 mg of lipid in triply distilled water (5% wt/wt). Scanning rates for the pretransitions and main transitions were approximately 17 °C/h. Phase-transition enthalpies were determined by cutting and weighing the papers and were estimated to be accurate to ±10%.

In subtransition studies, samples prepared as described above were incubated at 0 °C by storage on ice in a refrigerator for the specified time periods. Scanning rates were 27-29 °C/h for subtransition studies.

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(32) Füldner, H. H. *Biochemistry* **1981**, *20*, 5707-5710.
(33) Viti, V.; Minetti, M. *Chem. Phys. Lipids* **1981**, *28*, 215-225.
(34) Burns, R. A., Jr.; Stark, R. E.; Vidusek, D. A.; Roberts, M. F. *Biochemistry* **1983**, *22*, 5084-5090.
(35) Yeagle, P. L. *Acc. Chem. Res.* **1978**, *11*, 321-327.

Synthesis of "Iso-EPSP" and Evaluation of Its Interaction with Chorismate Synthase

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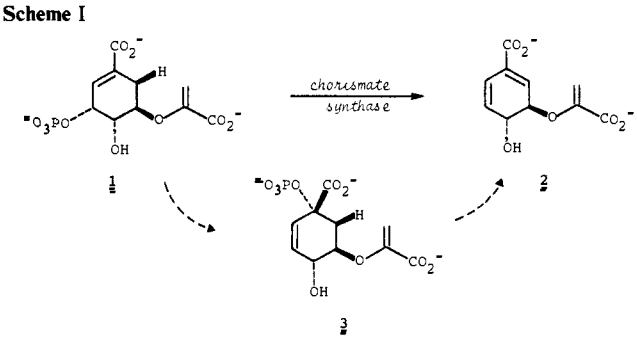
Abstract: A synthesis of "iso-EPSP" (**3**), the allylic phosphate isomer of EPSP (**1**), has been developed, starting with (-)-quinic acid. A key intermediate is the differentially protected triol **7**. Iso-EPSP is not an alternative substrate for chorismate synthase isolated from *Neurospora crassa*, although it is a good inhibitor (K_i = 8.7 μM). It thus appears that the enzymatic conversion of EPSP to chorismate does not involve allylic rearrangement followed by 1,2-elimination.

The shikimate-chorismate biosynthetic pathway is mediated by a number of enzymes which catalyze unique or unusual transformations.[1] One of these enzymes, chorismate synthase, catalyzes the conversion of 5-enolpyruvylshikimate 3-phosphate (EPSP, **1**) to chorismate (**2**) in a process which is formally a trans-1,4-elimination (Scheme I).[2,3,4] In view of the preference which such transformations frequently show for the cis-1,4-stereochemistry,[5] a number of mechanisms other than direct

Scheme I



elimination have been suggested for the enzymatic process.[1,3] An intriguing possibility that has been proposed by Ganem involves

(1) Ganem, B. *Tetrahedron* **1978**, *34*, 3353.
(2) Hill, R. K.; Newkome, G. R. *J. Am. Chem. Soc.* **1969**, *91*, 5893.
(3) Onderka, D. K.; Floss, H. G. *J. Am. Chem. Soc.* **1969**, *91*, 5894. Floss, H. G.; Onderka, D. K.; Carroll, M. *J. Biol. Chem.* **1972**, *247*, 736.
(4) Overton, K. H. *Chem. Soc. Rev.* **1979**, *8*, 447-474.
(5) Hill, R. K.; Bock, M. G. *J. Am. Chem. Soc.* **1978**, *100*, 637-639. Toromanoff, E. *C. R. Seances Acad. Sci., Ser. C.* **1980**, *290*, 81-84.

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