Lyotropic Polymorphism of Racemic Dipalmitoylphosphatidylethanolamine. A Differential Scanning Calorimetry Study†

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ABSTRACT: Aqueous suspensions of racemic dipalmitoylphosphatidylethanolamine and L-dipalmitoylphosphatidylethanolamine studied by differential scanning calorimetry were found to have different thermotropic behavior. Two endothermic transitions (a small one at 34 °C and a great high-temperature one at 82 °C) and an exothermic transition in the range 55–65 °C were recorded in racemic dipalmitoylphosphatidylethanolamine suspensions prepared at temperatures below the high-temperature transition. The exothermic transition indicated the existence of a metastable supercooled state being a consequence of the irreversibility of the high-temperature transition. After incubation of the samples above the high-temperature transition for about 1 h, all these transitions were abolished, and instead, a single endothermic transition at 61 °C appeared. This transition is very similar to the well-known gel–liquid-crystal transition in L-dipalmitoylphosphatidylethanolamine suspensions. When suspensions of L-dipalmitoylphosphatidylethanolamine were prepared at temperatures below the gel–liquid-crystal transition, a high-temperature transition at 65 °C was recorded. This transition existed only during the first heating scan, and afterwards it was replaced by the gel–liquid-crystal transition. The high-temperature transitions and the melting of the anhydrous L- and DL-dipalmitoylphosphatidylethanolamine occurring at 105 and 120 °C, respectively, have similar enthalpies (19–21 kcal·mol^{-1}). The calorimetric results demonstrate the influence of the chirality of the lipid molecules on the properties of the lipid phases. They suggest that at a presumably low hydration achieved by preparation of the samples at low temperatures the racemic dipalmitoylphosphatidylethanolamine aqueous suspensions are in the state "racemic compound" characterized by a stronger affinity between different enantiomers, while after a high-temperature incubation of the samples (a presumably maximal hydration) a "racemic solid solution" is formed in which the affinities in the pairs of antipode and similar lipid molecules are nearly equal.

The phospholipids are another example of chiral biomolecules with at least two stereoisomers, only one of them being present in living organisms. The widely spread phospholipids phosphatidylcholine (PC)[1] and PE have only one asymmetric carbon atom. This is the central carbon atom (C-2) of the glycerol backbone of their molecules. The absence of reflective symmetry for this atom leads to the existence of two enantiomeric forms of PC and PE. In the native membranes, only the L antipodes are present due to the circumstance that their precursor in the lipid biosynthesis is always L-glycerophosphate. The chiral purity of natural compounds like animo acids and carbohydrates has been known for a long time, and its origin and biological significance have been frequently discussed and explained from different viewpoints [see, e.g., Bernal (1965) and Morozov & Fedin (1976)]. However, up to now little, if any, attention has been paid to the influence of the chiral purity of the lipid molecules on the properties of the lipid membranes. Quite recently it has been demonstrated that L-DPPC and DL-DPPC form different crystal structures at low hydration degrees (Sakurai et al., 1983). It was pointed out by these authors that the chirality of the molecules should be taken into account when considering the properties of the lipid aggregates.

It is well-known that the intermolecular interactions in a racemic modification could be divided at least into two different classes, one of them being formed by LL and DD pairs and another one by DL pairs (Eliel, 1962). In particular, if a racemic phospholipid bilayer is considered, it could be expected that its physical properties would be different from the properties of a bilayer built from the pure L antipode since in the racemic bilayer there are also interactions between L and D antipodes. In a recent report, we have checked this assumption by comparing the thermal phase transitions in aqueous suspensions of L-DPPC and DL-DPPC. By means of DSC, it was found that the so-called "subtransition" observed in L-DPPC bilayers is absent in DL-DPPC bilayers (Boyanov et al., 1983). The explanation proposed was that the mutual space orientation of the polar groups of L and D isomers of DPPC does not favor the dense packing of the acyl chains characteristic for the low-temperature L_c phase. In order to extend this observation for other phospholipids, we have now studied the thermal phase transitions in aqueous suspensions of racemic DPPE. Comparison with L-DPPE revealed a unexpectedly great difference between the themal properties of the racemic mixture and those of the pure L antipode.

Materials and Methods

Commerical-grade L-DPPE and DL-DPPE (Fluka AG Buchs, Switzerland) were used without further purification. By thin-layer and gas chromatography, both phospholipids were shown to be of over 99% purity.

The phospholipid suspensions were prepared in 50 mM borate buffer, pH 8.0. L-DPPE or DL-DPPE dissolved in $CHCl_3/CH_3OH$ (9:1 v/v) was added into a glass tube. The solvent was removed by drying under nitrogen; the appropriate amount of the buffer was added and sonicated for 15 s to remove the phospholipid from the tube walls. The phospholipid was hydrated for 24 h at a chosen temperature in the range from 4 to 90 °C, and then it was sonicated at the same tem-

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[1] Abbreviations: PE, phosphatidylethanolamine; PC, phosphatidylcholine; L-DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; DL-DPPE, *rac*-1,2-dipalmitoylglycero-3-phosphoethanolamine; L-DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DL-DPPC, *rac*-1,2-dipalmitoylglycero-3-phosphocholine; L-DLPE, 1,2-dilauroyl-*sn*-glycero-3-phosphoethanolamine; L-DMPE, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine; DSC, differential scanning calorimetry; TLC, thin-layer chromatography.

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Table I: Transition Properties of L-DPPE and DL-DPPE

	L-DPPE			DL-DPPE		
	L_β–L_α	high-temp transition	melting of anhydrous lipid[a]	L_β–L_α	high-temp transition	melting of anhydrous lipid[a]
T_{tr} (°C)	62.2 ± 0.3	65.3 ± 0.3	105	60.9 ± 0.3	81.7 ± 0.5	120
$\Delta T_{1/2}$ (°C)	1.4 ± 0.1	1.1 ± 0.1		2.5 ± 0.1	1.1 ± 0.1	
ΔH_c (kcal·mol^{-1})	9.6 ± 0.6	21.2 ± 2.3	19.8 ± 1.5	8.7 ± 0.6	20.5 ± 1.6	20.1 ± 1.5
cooperative unit (molecules)	60	34		35	39	

[a] The values of $\Delta T_{1/2}$ for the melting of the anhydrous lipids are not representative due to the broadening of the transitions inherent for the DSM-2M calorimeter.

perature for 30 s. The resulting phospholipid suspension was then transferred into the calorimeter cell or into a refrigerator at 4 °C. The phospholipid concentration in the samples was about 0.3 mg/mL. No chemical degradation of the phospholipids was detected by TLC after several successive heating–cooling cycles of the samples, but prolonged incubation at high temperatures (24 h at 90 °C) resulted in appreciable degradation of the phospholipids. However, equilibration of the samples at 70 °C for 24 h and at 85 °C for 1 h did not result in a chemical breakdown detectable by TLC or by deviations of the DSC line shapes.

Differential scanning calorimetry (DSC) over the heating range 5–100 °C was performed with a Privalov differential adiabatic scanning calorimeter (DASM-1M) with a sensitivity better than 4×10^{-6} cal·K^{-1} and a noise level less than 5×10^{-7} W (Privalov et al., 1975). The DSC traces were recorded during heating of the samples. The heating rate was 0.5 °C·min^{-1}. The cooling of the sample inside the cell is not controllable. It was found to be nearly exponential with a relaxation time of about 16 min. The transition temperatures were obtained from the maximum of the transition peaks; the transition enthalpies were calculated from the peak areas. The size of the cooperative units during the different transitions was determined by using the following approximate expression:

$$N = 6.9T_{tr}^2/(\Delta T_{1/2}\Delta H_{cal})$$

where T_{tr} is the transition temperature, $\Delta T_{1/2}$ is the transition width at half-height, and ΔH_{cal} is the transition enthalpy (Mabrey & Sturtevant, 1978).

In some cases, the cooling DSC traces were recorded at 0.5 °C·min^{-1} by using a DASM-4 apparatus—a recently developed modification of DASM-1M. It should be noted that the cooling mode of registration of DASM-4 is not supplied with calibration facilities. For this reason, the cooling curves can be used only for comparative studies, but not for quantitative determination of the transition enthalpy and temperature.

The thermograms of the anhydrous phospholipids were recorded with a DSM-2M microcalorimeter (USSR) at a heating rate of 4 °C·min^{-1}. The enthalpies were determined by a comparison of the peak areas with a reference indium sample.

The quantity of phosphorus was determined after the termination of the experiment (Kahovkova & Odavic, 1969). Double glass-distilled water was used throughout all experiments. All chemicals used were analytical grade.

Results

Most methods of preparation of aqueous phospholipid suspensions include incubation of the sample at temperatures above the gel–liquid-crystal phase transition. For DPPE, this transition is at 63 °C. After incubation of L-DPPE suspensions at 70 °C for 24 h, a single transition appears at 62.2 °C (Figure 1b, Table I). This transition is excellently reproducible, and its enthalpy coincides with the values reported earlier (Mabrey & Sturtevant, 1978). Quite different is the thermal

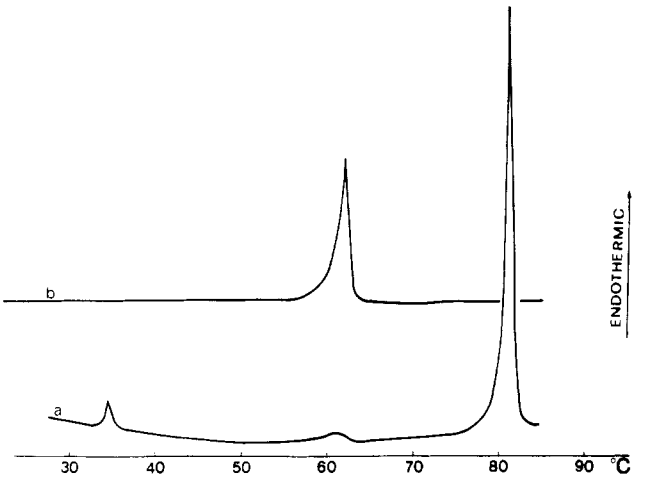

FIGURE 1: DSC traces of aqueous suspensions of DL-DPPE (a) and L-DPPE (b) incubated for 24 h at 70 °C (first scan).

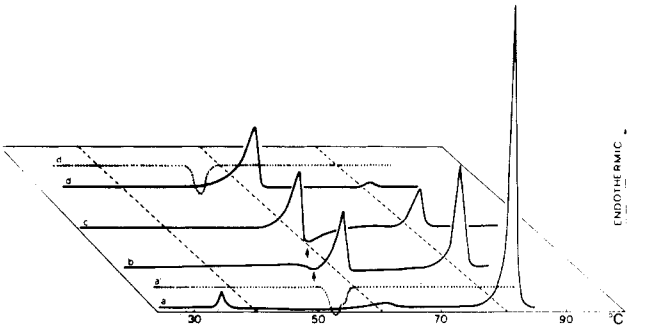

FIGURE 2: Successive DSC traces of a DL-DPPE aqueous suspension prepared at 70 °C showing the evolution of the sample with repetition of the heating–cooling cycles. Some of the cycles are omitted in the figure. The arrows indicate the positions of the exothermic transition. The dotted curves a′ and d′ are cooling curves recorded after the heating curves a and d.

behavior of a DL-DPPE suspension prepared in the same way (Figure 1a). The gel–liquid-crystal transition at 62 °C is absent or very slightly hinted. Two other endothermic transitions are seen—a small one at 34 °C and a large one at 82 °C. The DSC trace in Figure 1a is recorded during the first scan of the sample after its preparation. In order to check its reproducibility, several successive cycles of heating and cooling were performed without taking the sample out of the calorimeter, and the thermogram of each heating was recorded. It is clearly seen that the thermogram of the second scan is substantially different from the first thermogram (Figure 2b). The transition at 34 °C is absent, while the gel–liquid-crystal endotherm appears at above 61 °C and increases in size with repetition of the scans. An exothermic transition is displayed in the range 55–65 °C (indicated by arrows in Figure 2b,c). The high-temperature endotherm at 82 °C decreases monotonically until its total disappearance. After five to eight scans of the sample, the only transition left is the endotherm at 61

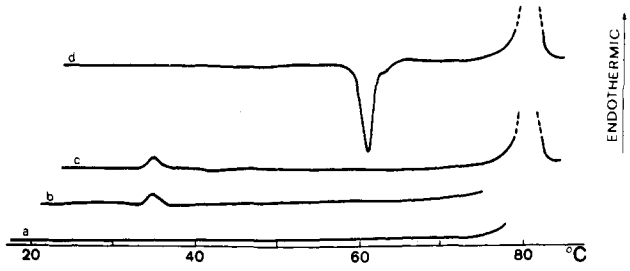

FIGURE 3: Successive DSC traces of a DL-DPPE aqueous suspension prepared at 20 °C. Scans a and b are finished immediately below the high-temperature transition; scan c is finished after completion of this transition.

°C (Figure 2d). This is the final state of the DL-DPPE suspension, and further cycles of heating and cooling of the sample do not lead to any further changes in its thermal behavior. Prolonged storage of the sample at low temperatures (4 °C, 30 days) does not lead to the restoration of the initial thermogram in Figure 2a either. The same phase transitions and a similar picture of the thermal evolution were also observed for all DL-DPPE suspensions hydrated for 24 h at lower temperatures, namely, 4, 12, 20, 40, and 55 °C. For this reason, the thermograms of these samples are only partially reproduced in the present article. A specific peculiarity of the small endotherm at 34 °C is that it is missing in the first scans of the samples hydrated at 4–55 °C but is is present in the first scan of the 70 °C samples. To provoke its appearance, a longer than 24-h hydration up to 10–15 days or a heating to temperatures between the exotherm at 55–65 °C and the endotherm at 82 °C is required (the effect of heating is shown in Figure 3a,b).

A specific feature of the picture shown in Figure 2 is that the heating of the sample is always terminated above (after the completion of) the high-temperature phase transition at 82 °C. A very different thermal behavior is observed when the heating is finished below this transition (Figure 3). In this case, the exothermic transition is absent on the next scan; the gel–liquiud-crystal transition at 61 °C does not appear at all, while the endotherm at 34 °C remains unchanged (Figure 3b,c). However, if the heating of the sample is allowed to continue above the transition at 82 °C, then on the next DSC trace the exothermic transition appears while the transition at 34 °C is absent (Figure 3c,d). The conversion of the sample between the two states characterized by the exotherm and by the endotherm at 34 °C is reversible, and it can be repeated many times simply by terminating the heating below or above the transition at 82 °C (Figure 4).

Concerning the endothermic transition at 61 °C, it has to be noted that it appears and increases in size only when the temperature during the preceding scan was raised above the high-temperature transition at 82 °C. Incubation at 85 °C for 1 h is sufficient to completely convert the sample into its final state characterized by a single endotherm at 61 °C, while incubation at 70 °C or lower temperatures for 24 h does not lead to a material conversion of the sample into this state (Figures 1a, 3a, 4a, and 5a). The rate of conversion of the samples depends on both the time and temperature of incubation above 82 °C, with higher temperatures accelerating the process. However, the kinetics of this conversion were not studied partially due to precautions necessary to avoid decomposition of the phospholipid at elevated temperatures.

The exothermic transitions on the DSC traces are currently interpreted as an indication for the existence of a metastable supercooled state. In this case, the high-temperature transition should not be reversible on cooling. This assumption was

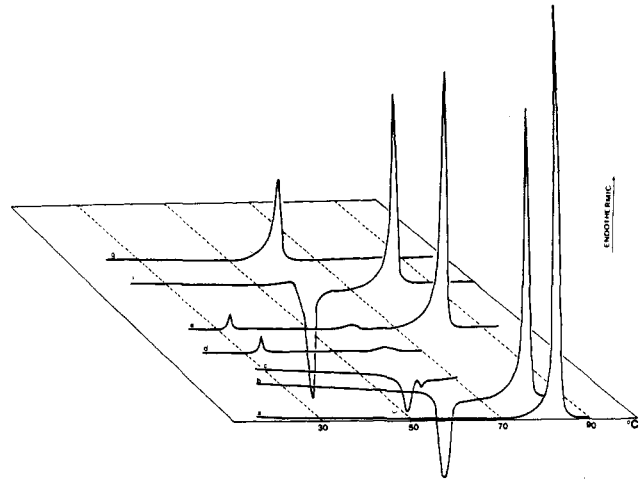

FIGURE 4: Successive DSC traces of a DL-DPPE aqueous suspension prepared at 20 °C. The figure illustrates the conversion of the sample between the two states characterized by the endotherm at 34 °C and the exothermic transition. For a detailed explanation, see the text and the diagram in Figure 8.

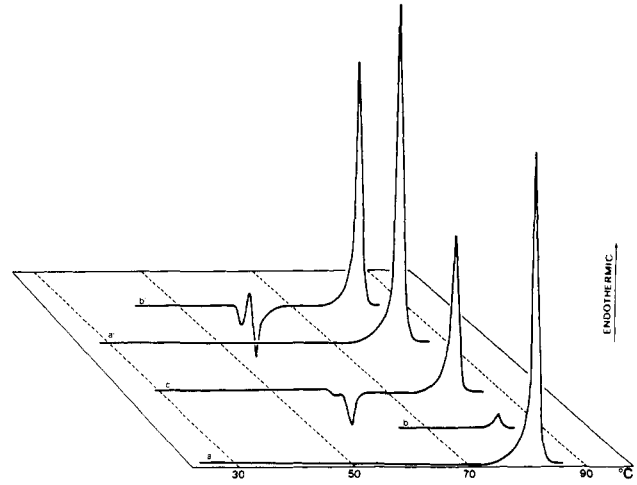

FIGURE 5: Two sequences of DSC traces of a DL-DPPE aqueous suspension prepared at 20 °C (a, b, c and a′, b′). The figure shows the irreversibility of the high-temperature transition on cooling. For a detailed explanation, see the text.

checked by registration of the cooling DSC curves of the samples (Figure 2a′,d′) and also by the following indirect experiment. The sample was heated above the transition at 82 °C and then cooled to a temperature immediately above the exotherm at 60 °C and heated again. On the second scan, the high-temperature transition is practically absent (Figure 5b). Then the same sample was cooled down to a lower temperature. During the next heating, the exothermic transition appears, and the endotherm at 82 °C is restored (Figure 5c). When the sample is cooled to any temperature below the exothermic transition, it appears on the next heating trace, and then the high-temperature transition is also present (Figures 4a,b,c and 5c,a′,b′). The position of the exothermic transition on the temperature scale is not fixed. It moves in the range 55–65 °C, and its position presumably depends on the time intervals between the preceding scans of the sample. This question was not investigated in detail in this work.

The two great endothermic transitions at 61 and 82 °C obviously reveal the existence of two states of different structure of the DL-DPPE aggregates. In order to check the stability and the principles of formation of these structures, we carried out the following experiments. Samples prepared in a manner to exhibit only one of these transitions (see, e.g.,

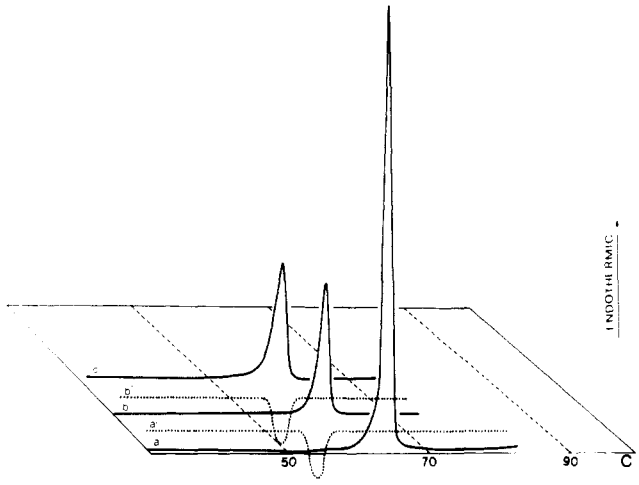

FIGURE 6: Successive DSC traces of a L-DPPE aqueous suspension prepared at temperatures below the gel–liquid-crystal phase transition. The dotted curves a′ and b′ are cooling curves recorded after the heating curves a and b.

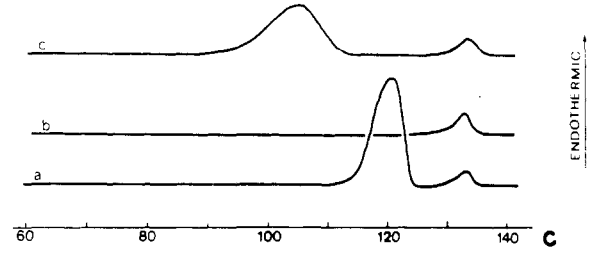

FIGURE 7: DSC traces of anhydrous PE crystallized from a chloroform solution. Scans a and b are for DL-DPPE; scan c is for L-DPPE. Scan b is taken 40 min after scan a. The heating rate was 4 °C·min^{-1}.

Figure 4a,g) were lyophilized, and the obtained anhydrous DL-DPPE was again hydrated in excess water for 24 h at 4 and 70 °C. It was found that the newly hydrated samples have exactly the same thermal behavior as their precursors before lyophilization. When the lyophilized phospholipid was recrystallized from chloroform solution before hydration, then the high-temperature transition was always seen on the first DSC trace.

We also investigated the phase transitions in L-DPPE aqueous suspensions prepared at temperatures below the gel–liquid-crystal transition (4, 12, 20, 40, and 55 °C). In all cases, the gel–liquid-crystal transition at 62 °C appears during the second scan of the samples and remains unchanged during further heating and cooling. During the first scan, this transition is replaced by another one shifted to a slightly higher temperature (65–66 °C) and about 2-fold greater enthalpy (Figure 6; Table I).

The DSC curves of the anhydrous DL-DPPE and L-DPPE show large endothermic transitions at 120 and 105 °C, respectively, followed by a smaller one at 133 °C (Figure 7a,c). The curves for DL-DPPE are similar to the ones reported earlier for some synthetic racemic PEs (Chapman et al., 1966).

Discussion

Figures 1–5 demonstrate that the thermotropic behavior of a DL-DPPE aqueous suspension strongly depends on the thermal prehistory of the sample, i.e., on the temperature and duration of hydration of the phospholipid. The borderline temperature of hydration of DL-DPPE that divides the thermograms of the samples into two distinct kinds is the temperature of the phase transition at 82 °C. When the samples are hydrated at temperatures above this transition for approximately 1 h, then cooled down to 10 °C, and scanned in the heating mode of the calorimeter, the DSC trace obtained is characterized by a single perfectly responsible endothermic transition at 61 °C (Figure 4g; Table I). By its parameters, this transition is very similar to the gel–lamellar liquid-crystalline (L_β–L_α) phase transition in L-DPPE (Mabrey & Sturtevant, 1978: Seddon et al., 1983a). Obviously the same interpretation should be proper for DL-DPPE. The slightly lower transition temperature of the racemic mixture is not unexpected. Comparison of melting points is one of the traditional methods for distinguishing between racemates and pure antipodes. Similar slight differences have been earlier registrated for the melting points of racemic mixtures and pure antipodes of triglycerides (Schlenk, 1965). However, there is not any difference of this kind between racemic DPPC and L-DPPC (Boyanov et al., 1983). In this case, the points of the gel–liquid-crystal transitions coincide within 0.1 °C. On the other side, the gel–liquid-crystal transitions in both DPPC and DPPE racemates are of 2–3 times lower cooperativity. On this basis, it could be concluded that the racemic lipid bilayers have more disordered structure in comparison to the L antipodes.

Entirely different from the transitions described above are the thermotropic properties of the DL-DPPE aqueous suspensions prepared at temperatures below 82 °C. Initially, the L_β–L_α transition at 61 °C is totally absent or very slightly hinted (only in the 70 °C sample). Instead of it, two other transitions are present—a small one at 34 °C and a very large one at 82 °C. The enthalpy of the high-temperature transition considerably exceeds the enthalpy of the L_β–L_α transition (Table I). For this reason, we assume that it also reflects the melting of the hydrocarbon core of the lipid aggregates and, in this way, it replaces the transition at 61 °C. The greater enthalpy of this transition should be due to a more ordered and less hydrated crystalline initial state (denoted as C_{rac} in the further discussion). The small endotherm at 34 °C ($\Delta H \simeq 1$ kcal·mol^{-1}) resembles very much the so-called pretransition in DPPC bilayers and probably reflects some minor modification of the solid structure formed by the DL-DPPE molecules. In order to distinguish between the two solid states connected by the endotherm at 34 °C, we denote the low-temperature one as C_{rac}'. Taking into account the small enthalpy of this transition, it is to be expected that states C_{rac} and C_{rac}' have nearly identical structures. It is beyond doubt that C_{rac} is hydrated to some extent since its melting occurs at about 40 °C lower temperature than the melting of the anhydrous compound (Table I). However, the degree of hydration should be low in comparison to other phospholipids. It was found that DL-DPPE binds about one molecule of H_2O per lipid molecule (Ladbrooke & Chapman, 1969). It was not clarified whether the degree of hydration increases with repetition of the scans, but it seems natural to assume that an additional hydration occurs during the high-temperature incubation and conversion of the samples to the state characterized by a single L_β–L_α endotherm.

The exothermic transition in the range 55–65 °C which is always present in the DSC traces after a preceding heating of the samples above the transition at 82 °C reveals the existence of a metastable low-temperature state which spontaneously converts on heating into the more stable state C_{rac}. In order to get some insight about the nature of the metastable state, it is necessary to determine whether the transition at 82 °C is reversible on cooling. By comparison of the curves a′ and d′ in Figure 2 (and also a′ and b′ in Figure 6), it becomes evident that the transitions on the cooling curves must be identified as reversed L_β–L_α transitions. No transition on

cooling corresponds to the high-temperature transitions in both DL-DPPE and L-DPPE. This conclusion is further supported by the obvious irreversibility of the transition at 82 °C demonstrated by the sequence of the curves a, b, and c in Figure 5. On these grounds, it can be concluded that the metastable state is a consequence of the supercooling of the fluid state above 82 °C. On storage at low temperatures, this metastable state should relax to a stable one, and the exothermic transition should disappear. We have found that storage between 3 and 30 days at 4 °C abolishes the exothermic transition. The existence of a metastable state is not an unusual feature of the aqueous lipid suspensions. Early investigations by means of differential thermal analysis of several synthetic racemic PEs of low water content showed the existence of exothermic transitions (Chapman et al., 1966; Ladbrooke & Chapman, 1969). The exothermic transition in DL-DPPE suspensions observed in the present work appears at the same temperatures, and in our opinion, it is exactly the same kind in spite of the very different DL-DPPE concentrations—0.03 wt % in our case compared to 80 or more wt % in the quoted articles. The new point that was not earlier realized is that it exists only in racemic DPPE suspensions but not in the pure antipodes of DPPE. However, exothermic transitions revealing metastable states have also been observed in aqueous suspensions of natural lipids. Recently, several works have been published in which metastable states have been observed in sphingolipids (Estep et al., 1980; Curatolo et al., 1982) and different kinds of cerebrosides (Freire et al., 1980; Ruocco et al., 1981; Curatolo, 1982).

The explanations proposed in these works for the existence of the metastable states especially emphasize the importance of the hydration and dehydration processes and also the formation of interlipid hydrogen bonds. It might well be that the same processes are relevant in our case. It is well-known that hydration and hydrogen bonding of the PE polar groups are two essential factors influencing the crystalline structure of the PE aggregates [for a review, see Hauser et al. (1981)]. However, in spite of these directing considerations, the physical reasons determining the extent of reversibility on cooling of the gel to fluid transitions in lipid suspensions remain at present unclear.

A high-temperature transition exists not only in DL-DPPE suspensions but also in L-DPPE suspensions when the samples are prepared at temperatures below the L_β–L_α transition (Figure 7; Table I). Quite recently, this transition has been investigated for the L antipodes of several saturated PEs including L-DLPE (Mantsch et al., 1983; Chang & Epand, 1983; Seddon et al., 1983), L-DMPE, and L-DPPE (Mantsch et al., 1983). It was shown to be due to a simultaneous acyl chain melting and hydration of the phospholipids and was interpreted as a transition from a crystalline to a lamellar liquid state (L_c–L_α transition). Our results confirm the calorimetric characteristics of the L_c–L_α transition in L-DPPE reported by Mantsch et al. (1983).

While the L_β–L_α transitions of L- and DL-DPPE are very similar, their high-temperature transitions are substantially different. First, the high-temperature transition in DL-DPPE suspensions occurs at about 16 °C higher temperature (Table I). Second, the high-temperature transition in L-DPPE suspensions is present only during the first heating of the samples since this is a transition directly to the L_α state, while the conversion of DL-DPPE suspensions to the state characterized by a single L_β–L_α endotherm requires at least five to eight heating–cooling cycles or an uninterrupted incubation for about 1 h at 85 °C. This way, the high-temperature transition in DL-DPPE is not a transition of the L_c–L_α type but rather a transition from a crystalline to an intermediate fluid state of presumably low hydration which upon incubation at high temperatures slowly converts into the fully hydrated L_α state. An intermediate state of this kind was not resolved in L-PEs where melting and hydration occur simultaneously (Mantsch et al., 1983; Seddon et al., 1983; Chang & Epand, 1983). It is relevant to remark that the L_c–L_α enthalpies of L-DLPE and L-DMPE measured in these works are very close to the enthalpies of melting of anhydrous DLPE and DMPE [13.0 and 16.2 kcal·mol^{-1}, respectively, according to Ladbrooke & Chapman (1969)]. The same is valid also for L- and DL-DPPE (Table I).

In the recent investigations of ester- and ether-saturated PEs of different chain length, it was found that in some of them a high-temperature transition occurs above the L_β–L_α transition, and during this transition, the sample converts from the L_α state to the inverted hexagonal (H_{II}) state (Harlos & Eibl, 1980; Seddon et al., 1983b). In this connection, it should be especially emphasized that in a DL-DPPE suspension the high-temperature transition does not follow the L_β–L_α transition. These two transitions are independent in the sense that they occur in two kinds of lipid aggregates of different structure. This is unambiguously demonstrated by the DSC traces in which only one of them is present (Figure 4a,g). When both transitions are present on the same curve, this means that the two kinds of the lipid aggregates are merely coexisting in the sample, and with repetition of the heating–cooling scans, one of them is converting into the other one. A conclusion of practical interest derived from here which might prove to be relevant also to the above-mentioned sequence L_β–L_α–H_{II} is that preparation of fully hydrated PEs should include equilibration not only above the L_β–L_α transition but also above the high-temperature transition.

To summarize and illustrate the calorimetric results, we propose the schematic presentation of the different transitions in a DL-DPPE aqueous suspension shown in Figure 8. The sequences of DSC scans shown in Figures 2–5 can easily be followed with this diagram. This is the simplest diagram consistent with all data obtained. It requires postulation of three different new states. They are the solid states C_{rac} and C_{rac}' and the relatively stable intermediate fluid state above the high-temperature transition. The metastable state should be similar to the fluid state since the transition at 82 °C is not reversible on cooling. It is worth noting that this metastable state cannot be identified as an L_β or L_α state because these two states do not convert to C_{rac} on storage or heating (Figure 8).

The most important result of the present work is the demonstration of the different thermal behavior of L- and DL-DPPE suspensions prepared in the same way. In principal, it is not surprising that the racemic lipid and its L antipode can form different structures. This phenomenon has been known for a long time for a number of more simple organic compounds having two stereoisomers (Eliel, 1962). The racemic modifications can exist in three principally different states determined by the relative strength of the interactions between the antipode and similar enantiomers. The so-called "racemic mixtures", "racemic compounds", and "racemic solid solutions" are characterized by different easily discernible phase diagrams (Eliel, 1962). According to this classification, the existence of a transition at a higher temperature (82 °C) than the corresponding transition temperature of the L antipode (65–66 °C) is an indication that the DL-DPPE aggregates at low hydration should be classified as a racemic compound char-

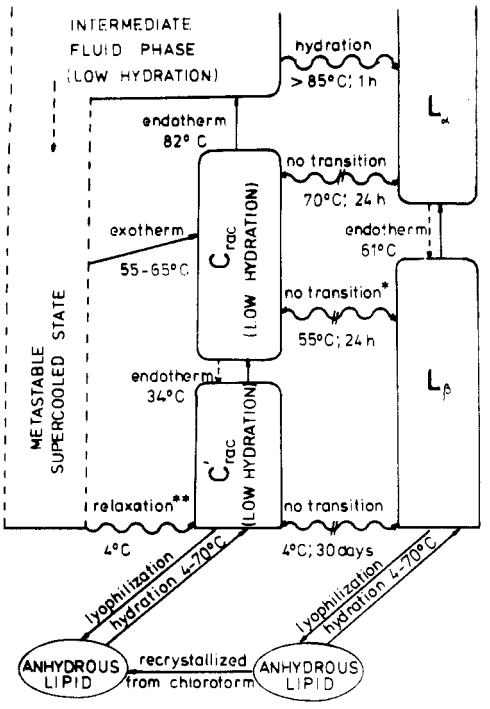

FIGURE 8: Diagram of the phase transitions and the states of different structure in a DL-DPPE aqueous suspension. (*) The absence of a transition in both directions was also established for other temperatures (20 and 40 °C) and for longer periods of storage (up to 5 days). (**) The relaxation time at 4 °C is between 3 and 30 days. No attempt to obtain a more precise value was made.

acterized by a stronger attraction in DL pairs than in LL and DD pairs. If DL-DPPE is regarded as an equimolar binary mixture, this might become, to our knowledge, the first example of nonideal mixing of two phospholipids characterized not by clustering of the like molecules, but, on contrary, by a "chessboard" type arrangement of the two molecular species. After a high-temperature equilibration where a presumably maximal hydration is reached, the close similarity of the L_β–L_α transitions in L-DPPE and DL-DPPE indicates that in this case DL-DPPE forms a racemic solid solution in which the interactions in DL, LL, and DD pairs are nearly equal. A solid solution of this kind is formed also by hydrated DL-DPPC (Boyanov et al., 1983). In these two cases, the mixing of the two components must be close to ideal.

Registry No. L-DPPE, 923-61-5; DL-DPPE, 5681-36-7.

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