Lipid Chain Motion in an Interdigitated Gel Phase: Conventional and Saturation Transfer ESR of Spin-Labeled Lipids in Dipalmitoylphosphatidylcholine–Glycerol Dispersions

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ABSTRACT: The lipid chain dynamics in the interdigitated gel phase of dipalmitoylphosphatidylcholine (DPPC) dispersed in glycerol and in the fully hydrated noninterdigitated gel phase in aqueous buffer were compared by using conventional and saturation transfer electron spin resonance (ESR) spectroscopy. Twelve different positional isomers of phosphatidylcholine spin-labeled in the *sn*-2 chain were used to characterize the chain motion. The outer hyperfine splittings of the conventional ESR spectra and the line height ratios at the diagnostic spectral positions in the saturation transfer ESR spectra were taken as indices of the rotational mobility of the labeled chain segments in the gel phase (0–40 °C). The conventional spin label ESR spectra revealed a gradient of increasing mobility on proceeding down the chain toward the terminal methyl end in the fully hydrated DPPC gel phase bilayer structure. This gradient was absent in the interdigitated gel phase, i.e., the rotational mobility throughout the length of the lipid chain was comparable to that near the polar interface, on the conventional ESR time scale. Values of the outer hyperfine splitting for spin labels at the 5- and 14-C atom positions in the chain were 65.5 and 61.0 G in buffer, respectively, and 67.0 G for both positions in glycerol, at 0 °C. At 35 °C, still in the gel phase, these differences between the two systems were much greater. Saturation transfer ESR measurements revealed that the motion throughout the chain was restricted on the microsecond time scale in the interdigitated phase. The motional anisotropy was less than in the normal gel phase, and the onset of rapid long axis rotation at the pretransition of bilayers in water was absent in the presence of glycerol. Effective correlation times for long axis rotation recorded by the 5-position spin label were approximately 20–25 μs in both systems at 5 °C and 10 and 2 μs in glycerol and water, respectively, at 35 °C. The effective rotational correlation times for the off-axial chain motion were considerably longer (ca. 40–70 μs) and were much less affected by temperature. There was no evidence for chain interdigitation in the fluid phase (43–50 °C) of DPPC dispersed in glycerol. The conventional spin label ESR spectra indicated a chain flexibility gradient similar to that obtained in normal liquid-crystalline lipid bilayers, although the segmental mobility was uniformly reduced throughout the chain in the presence of glycerol, relative to the DPPC bilayers in water.

Although the basic structural arrangement of biological membranes is the lipid bilayer, phospholipid dispersions in water are able to adopt a variety of different phases other than the conventional lipid bilayer lamellae. Among these other phases, it has been found that under various conditions the opposing lipid monolayers may interpenetrate each other to form an interdigitated gel phase in which the terminal methyl groups of the lipid chains are located near the interfacial region on the opposite side of the lipid lamella [for a review, see Slater and Huang (1988)]. Indeed, above a certain threshold concentration, various compounds such as glycerol (McDaniel et al., 1983; O'Leary & Levin, 1984; Boggs & Rangaraj, 1985; Veiro et al., 1987), small surface-active molecules (McIntosh et al., 1983), alcohols (Boggs & Rangaraj, 1985; Simon & McIntosh, 1984; Rowe, 1985; Nambi et al., 1988; Tenchov et al., 1989), and ions (Cunningham et al., 1989) are able to induce complete interdigitation in the gel phase of saturated, symmetrical-chain phosphatidylcholines. Such molecules also will have a corresponding action at the lipid–water interface in biological membranes, even though not normally giving rise to a membrane structure with interdigitated chains in this instance. Thus, studies on the formation of interdigitated phases are generally of interest with respect to interfacial interactions in membranes, and a comparison of both fluid and gel phases is of especial relevance. Additionally, studies on the lipid chain mobility in interdigitated phases extends the range of chain dynamics that might conceivably be encountered, albeit for other reasons, in both native and reconstituted membranes.

The interdigitated system DPPC[1]/glycerol has been well characterized both structurally by X-ray diffraction and calorimetrically (McDaniel et al., 1983; Boggs & Rangaraj, 1985). Additionally, Raman (O'Leary & Levin, 1984) and ESR (Boggs & Rangaraj, 1985) spectroscopic techniques have provided information complementary to the structural data, but on different time scales. In particular, Boggs and Rangaraj

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[1] Abbreviations: DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; *n*-PCSL, 1-acyl-2-(*n*-(4,4-dimethyloxazolidine-*N*-oxy)stearoyl)-*sn*-glycero-3-phosphocholine; *n*-SASL, *n*-(4,4-dimethyloxazolidine-*N*-oxy)stearic acid; ESR, electron spin resonance; STESR, saturation transfer ESR; V_1, first harmonic ESR absorption signal detected in-phase with respect to the field modulation; V_2', second harmonic ESR absorption signal detected 90° out-of-phase with respect to the field modulation; $L_{\beta'}^i$, lamellar gel phase with interdigitated chains; $L_{\beta'}$, lamellar gel phase with tilted and noninterdigitated chains; $P_{\beta'}$, rippled gel phase of oblique lattice with noninterdigitated chains; L_α, fluid lamellar phase.

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(1985) have investigated the behavior of two spin-labeled stearic acids, *n*-SASL, in the interdigitated gel phase of the DPPC/glycerol system by means of conventional ESR spectroscopy. They found that the mobility of a fatty acid segment labeled near to the terminal methyl end of the chain (16-SASL) is restricted to a degree similar to that of a segment labeled much closer to the polar headgroup region (5-SASL) in the interdigitated gel phase.

In this paper, we have investigated the interdigitated DPPC system in considerably greater detail by using both conventional and saturation transfer ESR spectroscopy to compare the mobility throughout the entire length of the lipid chains for dispersions both in glycerol and in aqueous buffer. For this purpose we have used positional isomers of phosphatidylcholine that are spin-labeled at 12 different positions down the *sn*-2 acyl chain (*n*-PCSL, where $n = 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,$ and 16). Saturation transfer ESR spectroscopy is used because it is particularly appropriate for studying the slow molecular motions of lipids in the interdigitated gel phase, since it extends the motional sensitivity of conventional spin label ESR spectroscopy (corresponding to rotational correlation times $\tau_R \approx 10^{-11}$–10^{-8} s) (Freed, 1976; Marsh, 1989) down to the T_1 time scale (corresponding to $\tau_R \approx 10^{-7}$–10^{-3} s) (Thomas et al., 1976; Hemminga & De Jager, 1989). Previous measurements have indicated the suitability of STESR spectroscopy for investigation of the dynamics of spin-labeled lipid chains in normal lamellar gel phases (Marsh, 1980; Marsh & Watts, 1980; Watts & Marsh, 1981).

In particular, the STESR measurements described here are able to demonstrate that the motion of the *n*-PCSL labels is uniformly slow in the interdigitated gel phase of DPPC dispersed in glycerol and lies in the time regime of the spin–lattice relaxation of the spin label (i.e., of $T_1 \approx 10$ μs). The anisotropy of the chain motion (i.e., a preferentially faster rotation about the long axis on the STESR time scale) is found to be reduced and less affected by temperature in the interdigitated system, as compared with the normal lipid gel phase. Conventional ESR measurements show that, although the chains of DPPC dispersed in glycerol are interdigitated in the gel phase, there is no evidence for chain interdigitation in the fluid phase. Indeed, a chain flexibility gradient is present in the fluid phase of DPPC dispersed both in glycerol and in aqueous buffer, although the mobility of the labels is uniformly reduced throughout the lipid chains for the dispersions in glycerol, relative to those in water.

MATERIALS AND METHODS

Materials. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was obtained from Fluka (Buchs, Switzerland), and the purity was checked by thin-layer chromatography. Phosphatidylcholine spin labels with the nitroxide group on the C-*n* atom of the *sn*-2 chain, 1-acyl-2-[*n*-(4,4-dimethyloxazolidin-*N*-oxy)stearoyl]-*sn*-glycero-3-phosphocholine (*n*-PCSL, with $n = 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,$ or 16) were synthesized as described in Marsh and Watts (1982). Glycerol was from Merck, Darmstadt, FRG. Other chemicals used were of analytical grade purity. Water was double-distilled from quartz.

Sample Preparation. Lipid dispersions were prepared by first mixing the lipids with 0.5% by weight of the spin label in dichloromethane solution, evaporating off the solvent with a stream of dry nitrogen, and then placing them under vacuum overnight. The dried lipids were dispersed in 20 mM Hepes, pH 7.4, buffer to a final concentration of 10 mg/mL by vortex mixing at a temperature of 45 °C, i.e., above the gel to fluid phase transition. Samples in glycerol were prepared similarly by adding 25 μL of glycerol to 1 mg of the dry DPPC/spin label mixture. The suspensions were transferred to 1-mm (i.d.), 100-μL glass capillaries and centrifuged on a bench centrifuge at 2000 rpm for 15 min. Excess supernatant was removed to obtain pellets of 5-mm length. This standard sample configuration was used in all STESR experiments (Fajer & Marsh, 1982; Hemminga et al., 1984). The sealed sample capillary tube was accommodated in a standard 4-mm quartz tube containing light silicone oil for thermal stability and was centered in the Varian TE102 cavity. Samples were incubated at 10 °C for 24 h and then were measured immediately, starting at low temperature.

Electron Spin Resonance Measurements. ESR spectra were recorded on a Varian Century Line 9-GHz spectrometer equipped with nitrogen gas flow temperature regulation and interfaced to a PDP 11/10 laboratory computer. Conventional, first harmonic in-phase, absorption ESR spectra (V_1 display) were recorded at a modulation frequency of 100 kHz and a modulation amplitude of 1.25 G peak-to-peak, at the same microwave power as used for recording the STESR spectra. Saturation transfer ESR spectra were recorded in the second harmonic, 90° out-of-phase, absorption mode (V_2' display) with a modulation frequency of 50 kHz and a modulation amplitude of 5 G peak-to-peak. The microwave power was set for each sample and at each temperature, so as to give an average microwave field over the sample of $\langle H_1^2 \rangle^{1/2} = 0.25$ G, according to the standardized protocol given in Fajer and Marsh (1982) and in Hemminga et al. (1984). Calibration of the diagnostic STESR line height ratios, L″/L, C′/C, and H″/H, in terms of the rotational correlation times of spin-labeled hemoglobin were taken from Horváth and Marsh (1988).

RESULTS AND DISCUSSION

Conventional ESR Spectra in the Gel Phase. The in-phase, first harmonic absorption ESR spectra (V_1 display) of different phosphatidylcholine spin label positional isomers (*n*-PCSL) incorporated in DPPC dispersions, either in aqueous buffer or in glycerol, are given in Figure 1. Spectra recorded in the gel phase at 0 and 35 °C are compared in Figure 1, panels A and B, respectively. At 0 °C, the spectra in both environments display a high degree of immobilization but nevertheless show differences in chain mobility between the two systems that become greater on proceeding toward the terminal methyl end of the chain. This can be seen by comparing both the splittings and the widths of the outer hyperfine lines of the spectra in Figure 1, since these are parameters that are characteristic of the spin label dynamics in the slow motional regime of conventional spin label ESR spectroscopy [cf. Freed (1976) and Marsh (1981)]. In fact, the most striking feature in Figure 1A is that the spin labels in the DPPC/glycerol system remain immobilized on the conventional ESR time scale, throughout the entire length of the chain. In contrast, some motion, in the slow regime of conventional ESR spectroscopy, takes place for spin labels in the DPPC/buffer system (evident from $n = 7$), leading to a decrease in the outer hyperfine splitting with increasing *n*.

A larger difference between the conventional ESR line shapes for any given position of labeling is seen for the two systems at 35 °C (Figure 1B). In the DPPC/glycerol system, the spectra of the positional isomers near the methyl terminus of the chain show a spectral splitting still similar to that found for those close to the polar headgroup region. In the DPPC/water system, however, the spectral anisotropy decreases

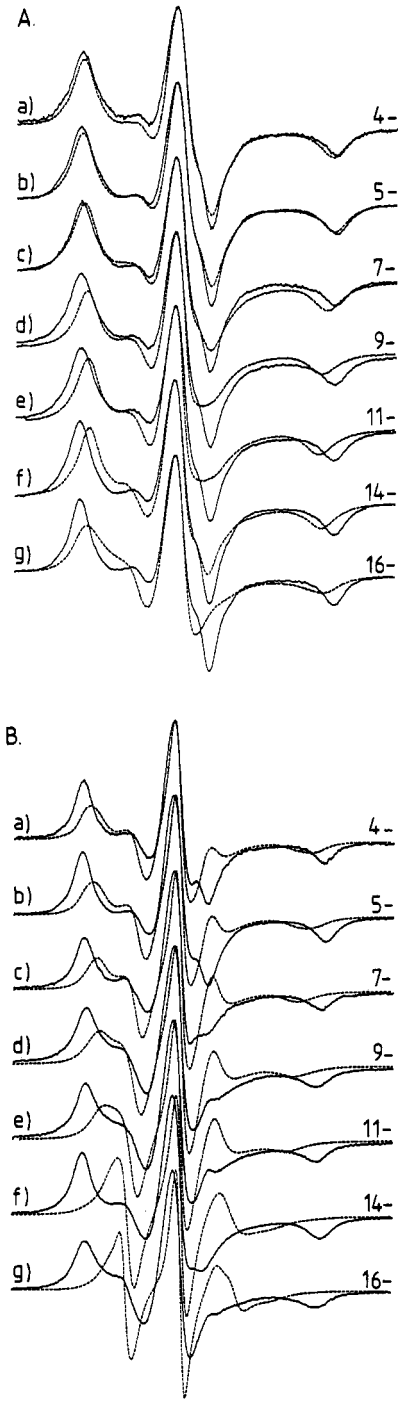

FIGURE 1: Conventional, in-phase, absorption ESR spectra (V_1 display) of phosphatidylcholine spin label positional isomers (0.5 mol %) (a) 4-PCSL, (b) 5-PCSL, (c) 7-PCSL, (d) 9-PCSL, (e) 11-PCSL, (f) 14-PCSL, and (g) 16-PCSL, in dispersions of DPPC in glycerol (full line) and in aqueous buffer (dashed line). (A) Spectra recorded at 0 °C. (B) Spectra recorded at 35 °C. Total scan range = 100 G.

continuously on proceeding toward the terminal methyl end of the chain. For the spin label positional isomers, *n*-PCSL, closer to the polar headgroup end of the chain ($n \approx 4$–7), the difference in outer hyperfine splitting between dispersions in glycerol and in aqueous buffer is much larger for the spectra recorded at 35 °C than for those recorded at 0 °C. For the spin label isomers positioned toward the terminal methyl end of the chain ($n \approx 11$–14), the spectra from dispersions in aqueous buffer exhibit extensive motional narrowing of the hyperfine anisotropy on the conventional spin label ESR time scale, whereas those from dispersions in glycerol are motionally restricted and still lie exclusively in the slow motional regime.

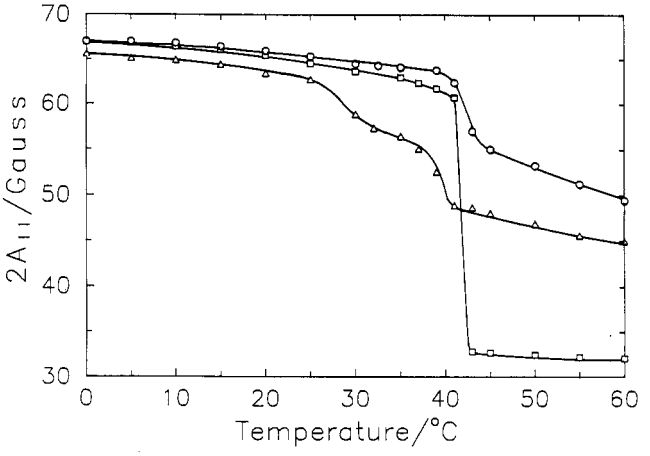

FIGURE 2: Temperature dependence of the outer hyperfine splitting, $2A_{\parallel}$, of the 5-PCSL phosphatidylcholine spin label in dispersions of DPPC in aqueous buffer (△) and in glycerol (○) and of 14-PCSL in DPPC/glycerol (□).

The chain motions observed in the normal gel phase for DPPC dispersed in aqueous buffer therefore display a mobility gradient that is quite strongly affected by temperature: it is already evident in the $L_{\beta'}$ phase at 0 °C and is much more pronounced in the $P_{\beta'}$ phase at 35 °C. In addition to the more rapid rotation about the long axis of the lipid chains that takes place in the $P_{\beta'}$ phase (Marsh, 1980), this mobility gradient corresponds to a limited degree of chain segmental motion which has been found previously both by ^{2}H NMR (Davis, 1979; Moser et al., 1989) and by spin label ESR (Moser et al., 1989) for different phosphatidylcholines in the normal noninterdigitated gel phase.

In contrast, for the interdigitated phase, L_{β}^{i}, induced by glycerol, the motion of phosphatidylcholines labeled near the terminal methyl end of the chains is restricted to an extent similar to that for positional isomers located much closer to the polar headgroup region. This behavior reflects the similar locations of the methyl terminal ends and the carboxyl heads of the lipid chains in the interdigitated phase. Boggs and Rangaraj (1985) have exploited this fact previously in comparing the behavior of the 5-SASL and 16-SASL spin-labeled stearic acids in the $L_{\beta'}$ phase of DPPC/buffer and in the interdigitated phase of DPPC/glycerol at 9 °C. A similar restriction of the chain motion was found in other interdigitated systems also investigated with the spin label ESR technique, both for saturated mixed-chain phosphatidylcholines (Boggs & Mason, 1986; Boggs et al., 1989) and for acidic phospholipid complexes with polymyxin (Boggs & Rangaraj, 1985; Kubesch et al., 1987).

Quite generally, it is found that the chain motion for any label position is much less affected by temperature in the interdigitated phase than in the noninterdigitated gel phase. The temperature dependence of the outer hyperfine splitting ($2A_{\parallel}$) of the 5-PCSL and of the 14-PCSL labels in DPPC/glycerol dispersions is compared with that of the 5-PCSL label in aqueous DPPC dispersions in Figure 2. In the normal gel phase (i.e., DPPC/water), as is well known, a discontinuity is observed in the temperature dependence of $2A_{\parallel}$, corresponding to the pretransition between the $L_{\beta'}$ and $P_{\beta'}$ phases at ca. 25–30 °C in the gel phase. This occurs before the main chain-melting phase transition which takes place at ca. 40 °C. In the interdigitated gel phase (i.e., DPPC/glycerol),

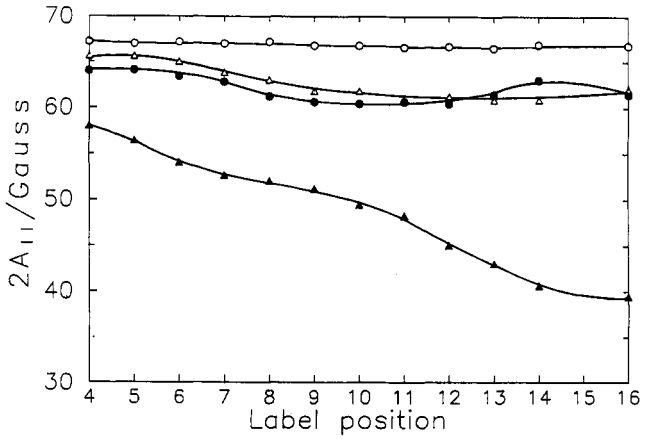

FIGURE 3: Outer hyperfine splitting, $2A_{\parallel}$, as a function of the label position, n, in the *sn*-2 chain for phosphatidylcholine spin labels (*n*-PCSL) in dispersions of DPPC in aqueous buffer (△) and in glycerol (○). Open symbols correspond to $T = 0$ °C, and filled symbols to $T = 35$ °C.

however, not only are the values of $2A_{\parallel}$ greater for both the 5-PCSL and 14-PCSL labels than for the 5-PCSL label in the noninterdigitated gel phase but also the values remain uniformly high throughout the gel phase, with no indication of any pretransitional behavior. Appreciable changes in the values of $2A_{\parallel}$ are seen only at the chain-melting transition which is found to occur at 43 °C, in agreement with other measurements (McDaniel et al., 1983; Boggs & Rangaraj, 1985). The lack of a pretransition involving the L_{β}^{i} phase is in agreement with previous findings, not only with DPPC/glycerol (McDaniel et al., 1983; Boggs & Rangaraj, 1985) but also with interdigitated phosphatidylcholine systems induced either by short-chain alcohols (Veiro et al., 1987) or by ions (Cunningham et al., 1989). The present findings with the DPPC/glycerol system suggest that a uniformly tight packing of the interdigitated chains is maintained at all temperatures in the L_{β}^{i} phase, up to the chain-melting transition at ca. 43 °C (see Figure 2).

The profile of chain segmental mobility in the interdigitated and noninterdigitated gel phases is compared in terms of the outer hyperfine splittings, $2A_{\parallel}$, in the conventional ESR spectra of the *n*-PCSL phosphatidylcholine spin labels, in Figure 3. For DPPC dispersions in glycerol at 0 °C, the values of the outer hyperfine splitting remain constant at a level of 66.8 G throughout the entire length of the lipid chain, while the values for DPPC dispersed in buffer are somewhat smaller and decrease steadily with the position of the spin label down the chain. At 35 °C, the values of $2A_{\parallel}$ vary somewhat with the position, n, of chain labeling for DPPC dispersions in glycerol but certainly do not decrease systematically on proceeding down the chain. It is likely that these limited changes with chain position reflect, on the one hand, differences in the ease with which the various spin label positional isomers can be accommodated in the interdigitated phase (for labels in the middle of the chain) and, on the other hand, the slight difference in chain length between the host DPPC and spin-labeled PCSL lipid molecules (for labels near to the terminal methyl end of the chain). In contrast, the values of the outer hyperfine splitting for DPPC dispersions in aqueous buffer at 35 °C are considerably smaller and decrease rapidly with position of labeling down the lipid chain, corresponding to the extensive motional narrowing of the hyperfine anisotropy in the conventional ESR spectra of the labels situated close to the terminal methyl end of the chain. The results of Figure 3, therefore indicate, with considerable detail, the differences

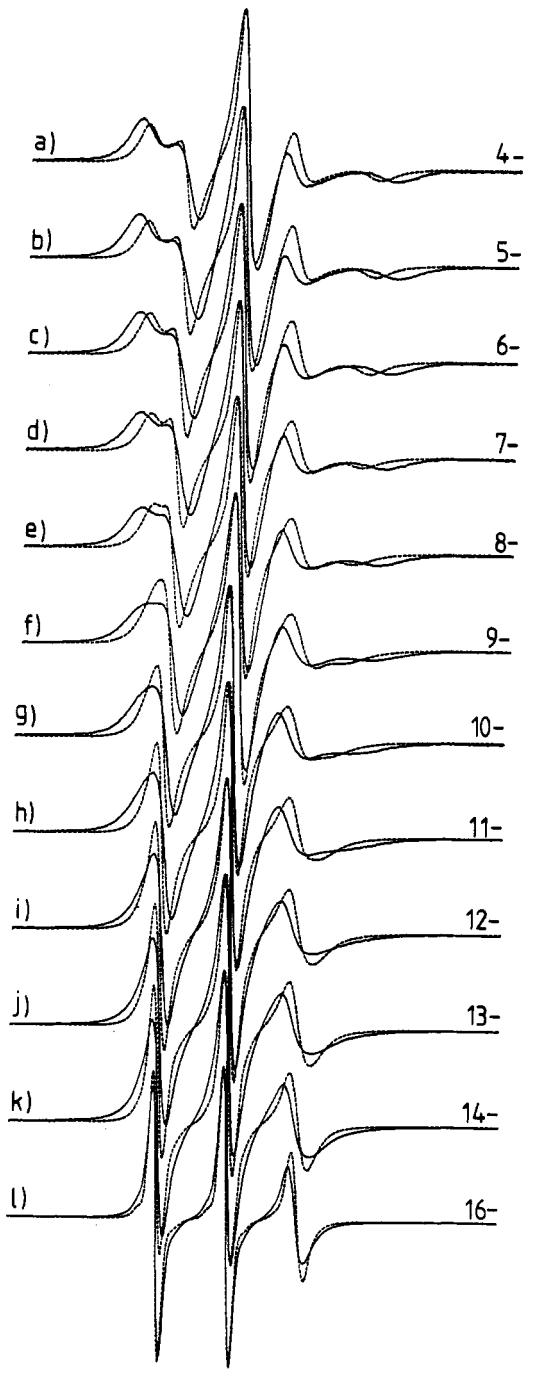

FIGURE 4: Conventional, in-phase, absorption ESR spectra (V_1 display) of different positional isomers of the phosphatidylcholine spin label (a) 4-PCSL, (b) 5-PCSL, (c) 6-PCSL, (d) 7-PCSL, (e) 8-PCSL, (f) 9-PCSL, (g) 10-PCSL, (h) 11-PCSL, (i) 12-PCSL, (j) 13-PCSL, (k) 14-PCSL, and (l) 16-PCSL, in dispersions of DPPC in glycerol (full line) and in aqueous buffer (dashed line), recorded in the fluid phase at 50 °C. Total scan range = 100 G.

in chain segmental mobility that result from the different modes of chain packing in the interdigitated and normal gel phases [cf. O'Leary and Levin (1984)].

Conventional ESR Spectra in the Fluid Phase. For the fluid phase (at 50 °C), the conventional ESR spectra of the different spin label positional isomers in dispersions of DPPC in glycerol are compared with those in aqueous buffer, in Figure 4. The spectra from both environments are principally in the fast motional regime and possess axial anisotropy, characteristic of a liquid crystalline L_{α} phase in each case. For the dispersions in glycerol, the spectral anisotropy

decreases monotonically with label position down the chain from the polar headgroup region toward the terminal methyl end. This indicates that chain interdigitation is absent for the fluid phase in glycerol, just as it is for the fluid phase in aqueous buffer. However, for a given label position, the spectral anisotropy is considerably greater for the DPPC dispersions in glycerol than for those in aqueous buffer, when spectra from the two systems are compared. For instance, the spectrum of 11-PCSL in the aqueous lipid dispersion is already nearly isotropic, whereas that from 11-PCSL in the glycerol dispersion is still markedly anisotropic. The origin of this effect lies most probably in a somewhat higher chain packing density of the lipid bilayers in glycerol than of those in water, corresponding to the different hydration potentials of these two solvents. Additionally, the effects of the higher solvent viscosity for the dispersion in glycerol may be propagated from the surface, hence affecting the chain mobility deeper in the bilayer. An unusually large restriction of the mobility at the chain ends is not observed for the fluid phase of DPPC in glycerol, in contrast to the situation in the interdigitated gel phase.

Saturation Transfer ESR Spectra in the Gel Phase. Since it is found that the motions of the spin-labeled lipid chains in the interdigitated phase lie close to the limits of motional sensitivity of conventional spin label ESR spectroscopy, the chain mobility can be defined more precisely by the use of saturation transfer ESR spectroscopy. It has been found previously, for instance, that the rotational mobility of the lipid chains in normal gel phase lipid bilayers lies in a region to which STESR spectroscopy is optimally sensitive (Marsh & Watts, 1980; Watts & Marsh, 1981; Fajer et al., 1992). Therefore, the saturation transfer ESR spectra of the *n*-PCSL labels are likely to reflect, in even greater detail, the differences in chain dynamics between the interdigitated and noninterdigitated phases that were found above by conventional ESR spectroscopy.

The second harmonic, 90° out-of-phase, absorption STESR spectra (V_2' display) from the 5-PCSL spin label in DPPC dispersions both in glycerol and in aqueous buffer are given in Figure 5, where spectral recording was made at various temperatures in the gel phases. The two sets of spectra indicate that the rotational motion of the spin-labeled lipid chains lies in the saturation transfer ESR regime for both systems at the lower temperatures in the gel phase. The STESR spectra of the 5-PCSL spin label change relatively little and only gradually with increasing temperature for the interdigitated DPPC bilayers in glycerol. On the other hand, the STESR spectra from 5-PCSL in aqueous DPPC bilayers change considerably, relative to those in glycerol, at temperatures above 25 °C, in agreement with previous findings on the effect of the pretransition in the normal, noninterdigitated gel phase of DPPC (Marsh, 1980).

The temperature dependence of the diagnostic line height ratios, L″/L, C′/C, and H″/H in the low-field, central, and high-field hyperfine manifolds, respectively, of the STESR spectrum [see Thomas et al. (1976) for a definition] are given for the 5-PCSL label in dispersions of DPPC in aqueous buffer and in glycerol, in Figure 6, panels A and B. The temperature dependence in the noninterdigitated gel phase (see Figure 6A) is in agreement with previous measurements on aqueous DPPC bilayers, in that the line height ratios L″/L and H″/H change relatively little with increasing temperature, but the C′/C ratio changes rather abruptly at ca. 25–30 °C, corresponding to the onset of a more rapid rotation around the long axis of the lipid chains at the bilayer pretransition (Marsh,

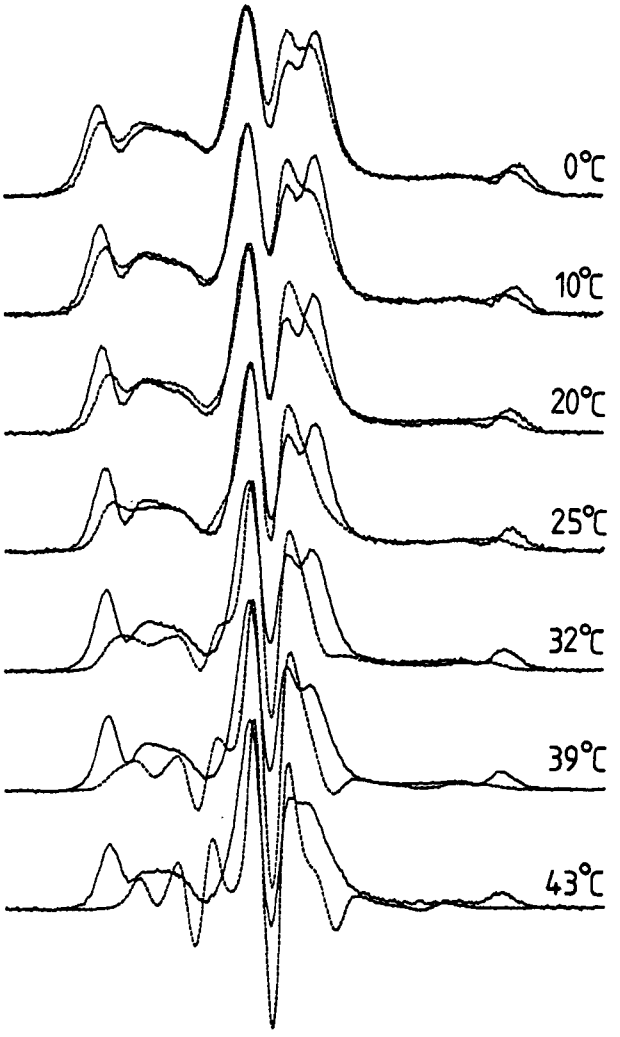

FIGURE 5: Second harmonic, 90° out-of-phase, absorption STESR spectra (V_2' display), recorded at different temperatures, from the 5-PCSL phosphatidylcholine spin label in dispersions of DPPC in glycerol (full line) and in aqueous buffer (dashed line). Total scan range = 100 G.

1980). In contrast, all three diagnostic STESR line height ratios vary relatively little throughout the gel phase for the interdigitated bilayers of DPPC in glycerol and give no evidence for any cooperative increase in the rate of rotation about the chain long axis (Figure 6B). Interestingly, the line height ratios at low temperatures are greater for DPPC in aqueous buffer than they are for DPPC in glycerol.

The effective rotational correlation times for 5-PCSL in the two systems, which are obtained from the STESR calibrations for isotropic rotational diffusion reported by Horváth and Marsh (1988), are given separately for the three diagnostic regions of the spectrum in Table I. The different values obtained from the C′/C and H″/H, L″/L ratios evidence anisotropy in the rotational diffusion of the spin-labeled lipid chains in both gel phases [cf. Marsh (1980) and Fajer and Marsh (1983)]. It is seen that the effective rotational correlation time for long axis rotation, τ(C′/C), decreases with increasing temperature in the noninterdigitated gel phase, from a value of 38 μs at 0 °C to a value of 2.4 μs at 37 °C, whereas it varies only from 19 to 9 μs over the same temperature range in the interdigitated phase. The effective rotational correlation times for the off-axial motion, τ(L″/L) and τ-(H″/H), are considerably longer and less affected by temperature in both systems. In general, the values obtained

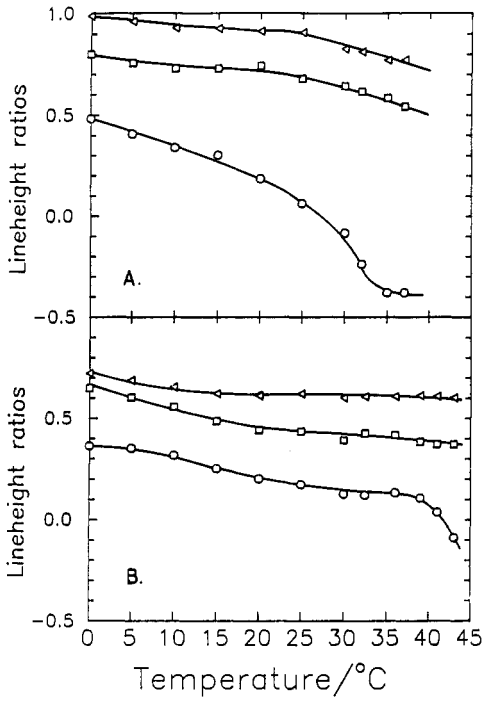

FIGURE 6: Temperature dependence of the low-field line height ratio L″/L (△), the central line height ratio C′/C (○), and the high-field line height ratio H″/H (□), in the V_2' STESR spectra of the 5-PCSL phosphatidylcholine spin label in dispersions of DPPC in (A) aqueous buffer and (B) glycerol.

Table I: Effective Rotational Correlation Times Derived from the Low-Field, Central, and High-Field Line Height Ratios [τ(L″/L), τ(C′/C), and τ(H″/H), Respectively] at Different Temperatures in the V_2' STESR Spectra of 5-PCSL in DPPC Liposomes Dispersed in (A) Aqueous Buffer and (B) in Glycerol

temperature (°C)	τ(L″/L) (μs)	τ(C′/C) (μs)	τ(H″/H) (μs)
(A) water			
0	58	38	73
5	54	24	65
10	51	17	60
15	50	15	60
20	49	10	63
25	48	7.7	52
30	41	5.8	46
32	40	4.0	42
35	36	2.4	37
37	36	2.4	32
(B) glycerol			
0	33	19	47
5	30	18	40
10	27	16	34
15	25	12	26
20	24	11	22
25	25	10	22
30	23	8.8	19
32.5	24	8.7	21
36	24	9.0	20
39	24	8.4	18
41	24	7.3	17
43	23	5.7	17

from the L″/L and H″/H ratios are of the same order of magnitude, as would be expected, since both reflect the off-axial motion. The values of τ(L″/L) and τ(H″/H) obtained for the noninterdigitated phase are consistently higher than those obtained for the interdigitated phase. This presumably has certain implications regarding the chain packing in the two phases. The larger area per lipid headgroup in the interdigitated phase may conceivably allow somewhat greater motional freedom of the lipid chains than in the normal gel

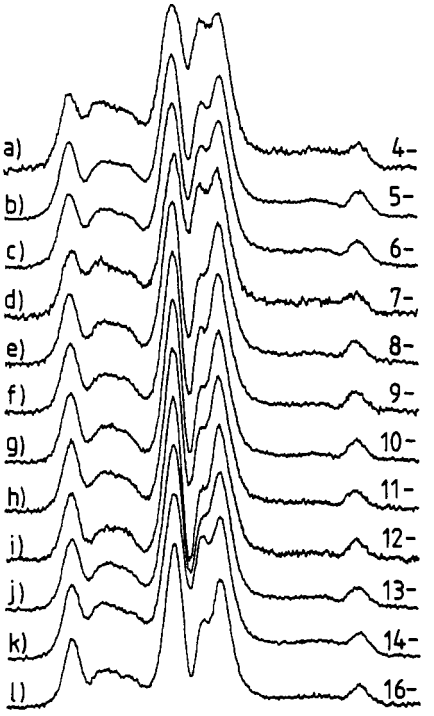

FIGURE 7: Second harmonic, 90° out-of-phase, absorption STESR spectra (V_2'-display) of different positional isomers of the phosphatidylcholine spin label (a) 4-PCSL, (b) 5-PCSL, (c) 6-PCSL, (d) 7-PCSL, (e) 8-PCSL, (f) 9-PCSL, (g) 10-PCSL, (h) 11-PCSL, (i) 12-PCSL, (j) 13-PCSL, (k) 14-PCSL, and (l) 16-PCSL, in DPPC dispersed in glycerol, at 0 °C. Total scan range = 100 G.

phase, including not only the off-axial motions but also the axial rotations at temperatures below the pretransition of the noninterdigitated gel phase. In this connection, it is interesting to note that the lipid chains are untilted and pack on a hexagonal lattice in the interdigitated phase of DPPC dispersed in glycerol, whereas the chains are tilted and the hexagonal packing is distorted in the noninterdigitated $L_{\beta'}$ phase of DPPC in water (McDaniel et al., 1983). However, the net area per chain in the plane of the chain packing is slightly smaller in the interdigitated phase of DPPC in glycerol (19.4 Å^2, at 20 °C) than in the noninterdigitated $L_{\beta'}$ phase (20.1 Å^2, at 20 °C).

The dependence of the STESR spectra on the position of the nitroxide group down the chain of the *n*-PCSL spin labels is given for DPPC dispersions in glycerol in Figure 7. The spectra indicate that the rotational mobility for all segments of the spin-labeled lipid chains lies in the saturation transfer ESR regime of motional sensitivity. In general, the overall STESR line shape does not change very greatly with the position of labeling, although there are quantitative differences which are considered below. At 0 °C, the STESR spectra of the 5-, 6-, and 7-PCSL spin label isomers, which are positioned closer to the lipid headgroups, are rather similar to that of the 14-PCSL label, which is positioned closer to the terminal methyl end of the chains. This could suggest that the 14-C segment of one lipid chain is situated close the 5-C segment of the chain directed from the opposite surface of the interdigitated bilayer. Recent experiments on the paramagnetic relaxation of the spin-labeled lipid chains induced by Ni^{2+} ions in the solvent phase have found that the C-16 chain segment from one side of the layer is positioned approximately in register with the C-3 segment from the opposite surface in the interdigitated phase of DPPC dispersions in glycerol (Páli et al., 1992).

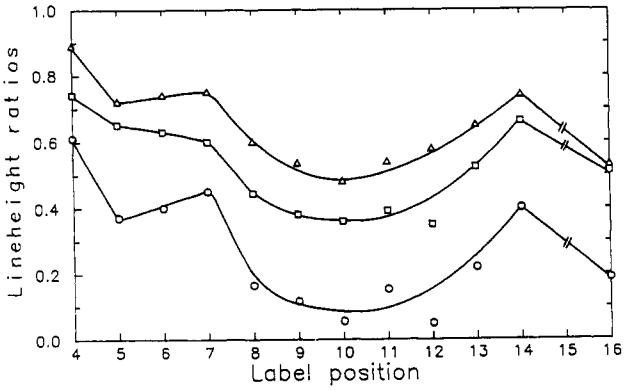

FIGURE 8: Low-field line height ratio L″/L (△), central line height ratio C′/C (○), and high-field line height ratio H″/H (□), for the V_2' display STESR spectra of the *n*-PCSL phosphatidylcholine spin label isomers, in dispersions of DPPC in glycerol at 0 °C, as a function of the label position, *n*, in the *sn*-2 chain.

The chain profiles of the diagnostic STESR line height ratios, L″/L, C′/C, and H″/H, for the different *n*-PCSL spin label positional isomers in the interdigitated phase of DPPC dispersed in glycerol at 0 °C are given in Figure 8. The behavior is complex, in that the dependence on position of labeling is nonmonotonic; any chain flexibility gradient that might exist in the interdigitated phase, on the saturation transfer ESR time scale, is at least biphasic. In detail, it is seen that there is a decrease in the line height ratios from the $n = 4$ position, which is rather close to the position of acylation of the *sn*-2 chain to the glycerol backbone, to the $n = 5$–7 positions, where the line height ratios are approximately constant. Beyond the $n = 7$ position, there is a dip in the profile of line height ratios with a minimum at $n = 10$ and then a subsequent increase to the $n = 14$ position. This latter behavior is somewhat similar to that found previously in the normal gel phase of DPPC (Fajer et al., 1992), where a decrease in the central line height ratios was attributed to the effects of spin–spin broadening, arising from a partial segregation only of those spin label positional isomers that are labeled close to the center of the chain. It is possible that the same explanation holds also for spin labels positioned in the central region of the chain in the interdigitated phase of DPPC in glycerol. Beyond the $n = 14$ position, the line height ratios decrease again to the $n = 16$ position. This decrease most probably arises from a true motional effect (since spin–spin interactions are absent) and corresponds to the labeled segment of 16-PCSL being situated close both to the terminal methyl end of the chain and to the surface of the opposing layer.

The effective rotational correlation times for the different labeled chain segments in the interdigitated gel phase of DPPC dispersed in glycerol have been deduced from the diagnostic STESR line height ratios that are given in Figure 8. This was done by using the spectral calibrations obtained for isotropically diffusing spin-labeled hemoglobin by Horváth and Marsh (1988), and the results are given in Table II. The values of the effective rotational correlation times reflect the trends in the diagnostic line height ratios discussed above. A significant result is that the effective correlation times all remain on the time scale of tens of microseconds, throughout the entire length of the chain, in the interdigitated phase. It will be noted that the effective correlation times tend to a minimum toward the center of the chain. This could be due to the possibility that, as discussed above, the values deduced for the segments in the center of the chain are artifactually low as a result of spin–spin broadening. Alternatively, the minimum could reflect a true motional effect, implying a flexibility gradient on approaching the center of the chain from either end, in the interdigitated gel phase. Corresponding results for the normal noninterdigitated gel phase of DPPC in water have been presented by Fajer et al. (1992) and were interpreted in terms of limited torsional oscillations increasing from the polar end of the lipid chains to the terminal methyl groups. This systematic torsional gradient throughout the length of the chain appears to be reduced for the interdigitated phase in glycerol, as judged from the net changes in the C′/C line height ratios between the chain ends (cf. Table II).

Table II: Effective Rotational Correlation Times Deduced from the Low-Field, Central, and High-Field Line Height Ratios [τ(L″/L), τ(C′/C), and τ(H″/H), Respectively] of the Phosphatidylcholine Spin Label Positional Isomers *n*-PCSL in DPPC Dispersed in Glycerol at 0 °C

label position, *n*	τ(L″/L) (μs)	τ(C′/C) (μs)	τ(H″/H) (μs)
4	47	103	62
5	32	20	47
6	34	23	44
7	35	32	40
8	23	9.7	23
9	19	8.6	18
10	15	7.6	17
11	19	9.4	19
12	22	7.5	16
13	27	11	30
14	34	24	49
16	18	10	29

Conclusions. Interdigitated bilayers have been characterized both thermodynamically and structurally for several different lipid systems (Serrallach et al., 1983; Hui et al., 1984; Ruocco et al., 1985; Kim et al., 1987; Mattai et al., 1987; Haas et al., 1990). Here we have chosen to study the chain dynamics in detail for the interdigitated phase of DPPC dispersions in glycerol, in order to make a direct comparison with the corresponding noninterdigitated phase of DPPC in water [cf. Marsh (1980) and Fajer et al. (1992)]. Both conventional and saturation transfer ESR have been used with phosphatidylcholines spin-labeled at 12 different segments throughout the length of the *sn*-2 chain.

The most salient conclusions of this study can be summarized as follows. At 0 °C, in the interdigitated phase, the conventional ESR spectra of all spin-labeled lipid chain segments reflect immobilization on the conventional ESR time scale, i.e., they resemble powder spectra. Saturation transfer ESR spectroscopy demonstrates that the residual chain motion corresponds to effective rotational correlation times of tens of microseconds or longer in the interdigitated L_{β}^{i} phase and that the systematic torsional flexibility gradient which is characteristic of the normal noninterdigitated $L_{\beta'}$ phase is reduced in the L_{β}^{i} phase. However, it is possible that a limited flexibility gradient toward the center of the chain might exist in the interdigitated gel phase, although this point is not certain. The changes in the conventional ESR spectra that are associated with the pretransition of the normal gel phase of DPPC, and the cooperative onset of more rapid rotation about the long molecular axis that is observed with STESR on entering the normal, noninterdigitated $P_{\beta'}$ phase, are not observed in the interdigitated L_{β}^{i} phase. Finally, it is found by conventional ESR spectroscopy that, on entering the fluid (L_{α}) phase, the lipid chains of DPPC dispersions in glycerol become no longer interdigitated, although they remain so throughout the gel phase, up until the chain-melting transition temperature. The influence of glycerol, and presumably other surface-adsorbing molecules, is primarily on the chain packing

in the gel phase, where the area per headgroup would otherwise be lower, and is not so appreciable in the fluid phase.

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