Spin-Label Electron Spin Resonance Studies on the Dynamics of the Different Phases of *N*-Biotinylphosphatidylethanolamines

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ABSTRACT: The chain dynamics and phase behavior of a homologous series of diacyl-*N*-biotinylphosphatidylethanolamines of chain lengths from C(12:0) to C(20:0) were investigated by spin-label electron spin resonance (ESR) spectroscopy using both phosphatidylcholine and *N*-biotinylphosphatidylethanolamine spin-label probes. Chain-melting phase transition temperatures determined from the ESR spectral measurements, for all the five lipids in the presence as well as in the absence of 1 M NaCl, correlate with the endothermic phase transition temperatures determined by differential scanning calorimetry [Swamy, M. J., Angerstein, B., & Marsh, D. (1994) *Biophys. J. 66*, 31–39], confirming that the latter correspond to chain-melting transitions. ESR spectra obtained in the gel phase from phosphatidylcholine probes with the spin-label near the terminal methyl of the hydrocarbon chain showed a similar degree of immobilization (as reflected by the outer hyperfine splittings) to that for spin-labels positioned close to the glycerol backbone, indicating that the biotin-lipids of chain lengths from 14 to 20 carbon atoms form interdigitated gel phases in the presence of salt, as also do those of chain lengths between 16 and 20 carbon atoms in the absence of salt. For dispersions of the C(16:0) chain length biotin-lipid in 1 M NaCl, continuous monitoring of the central ESR peak intensity as a function of temperature detects a cooperative decrease in mobility in the fluid phase at ca. 65 °C with a spin-label in the lipid head group and an accompanying increase in mobility at the same temperature with spin-labels positioned toward the terminal methyl of the hydrocarbon chain. These changes in mobility correspond to the transition from the novel fluid isotropic (I_M) phase, which has been identified also for the biotin-PEs of shorter chain lengths in 1 M NaCl [Swamy, M. J., Würz, U., & Marsh, D. (1993) *Biochemistry 32*, 9960–9967], to the fluid lamellar (L_α) phase and demonstrate that the surfaces of the lipid aggregates in the I_M phase are positively curved. Comparison of the lipid chain flexibility profiles shows that the ESR spectra of spin-labels in the region of the 5 C-atom of the *sn*-2 chain in dispersions of the different biotin-PEs in the fluid phase are significantly less anisotropic in the absence of salt than they are in the presence of 1 M NaCl, whereas those from labels close to the chain ends were somewhat more motionally hindered. This indicates that the fluid phase in the absence of salt has a chain packing characteristic of (normal) micelles, whereas that in the I_M phase is more similar to that of a fluid lamellar phase.

Biotinylated phospholipids in which the vitamin, biotin, is attached via a covalent link to the head group of a phospholipid such as phosphatidylethanolamine or phosphatidylserine, have been found to be widely applicable in various areas of biological and biomedical interest (Bayer & Wilchek, 1984; Rivnay et al., 1987). These include targeting of drug-loaded liposomes to tumor cells (Urdal & Hakomori, 1980), noncovalent attachment of antibodies to liposomes (Loughrey et al., 1987), large-scale purification of avidin from egg white extracts (Guzman et al., 1990), and localization of liposomes in studies on cell–liposome interactions (Bayer et al., 1979). In addition, it has been found that monolayers containing biotinylated lipids are a suitable substrate for the formation of two-dimensional protein crystals and consequently for the structural characterization of bound streptavidin and avidin (Ahlers et al., 1989; Blankenburg et al., 1989; Darst et al., 1991). Formation of two-dimensional crystals generally can be of use for proteins which are difficult to crystallize by conventional methods, in order to determine their three-dimensional structure by electron crystallography (Kornberg & Darst, 1991).

For all of the above applications, and for their use in studying molecular recognition at membrane surfaces, the aggregation state of the biotin lipids is likely to play a significant role. In this context, it is therefore important to study the behavior of the different phases formed by the various biotinylated phospholipids in aqueous dispersion. In order to characterize the polymorphic phase behavior of these lipids, studies have been made previously on a homologous series of diacyl-*N*-biotinylphosphatidylethanolamines (biotin-PEs)[1] with chain lengths from C(12:0) to C(20:0) by using differential scanning calorimetry, ^{31}P-NMR spectroscopy and low-angle X-ray diffraction (Swamy et al., 1993, 1994). It was found that, in

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[1] Abbreviations: ESR, electron spin resonance; NMR, nuclear magnetic resonance; DSC, differential scanning calorimetry; PC, phosphatidylcholine; PE, phosphatidylethanolamine; biotin-PE, *N*-biotinylphosphatidylethanolamine; DLBPE, DMBPE, DPBPE, DSBPE, and DABPE, 1,2-dilauroyl-, 1,2-dimyristoyl-, 1,2-dipalmitoyl-, 1,2-distearoyl-, and 1,2-diarachinoyl-*sn*-glycero-3-(*N*-biotinyl)phosphoethanolamine; DMPC and DMPE, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine and -phosphoethanolamine; *n*-PCSL and *n*-PESL, 1-acyl-2-[*n*-(4,4-dimethyloxazolidine-*N*-oxyl)stearoyl]-*sn*-glycero-3-phosphocholine and -phosphoethanolamine; *n*-BPESL, 1-acyl-2-[*n*-(4,4-dimethyloxazolidine-*N*-oxyl)stearoyl]-*sn*-glycero-3-(*N*-biotinyl)phosphoethanolamine; acetamido-PESL, *N*-[4-acetamido-2,2,6,6-tetramethylpiperidine-*N*-oxyl]phosphatidylethanolamine (*N*-TEMPO-acetamidophosphatidylethanolamine); EDTA, ethylenediaminetetraacetic acid; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid.

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the main, the various biotin-PE dispersions exhibited unusual phases, other than the lamellar gel and fluid phases that are found normally with long-chain diacyl phospholipids. In most cases, the gel phases of the biotinyl lipids appear to be of an interdigitated type (L_{β}^{i}). Further, whereas the fluid phases in the absence of salt are of a normal micellar type, it appears that, depending on chain length, those in 1 M NaCl may be of a novel isotropic form consisting of aggregated micelles (I_M), which hitherto had not been reported for diacyl phospholipids. For the special case of the C(16:0) derivative DPBPE in 1 M NaCl, a thermotropic transition occurs from this I_M phase to a normal fluid lamellar phase at higher temperatures.

It is therefore of considerable interest to characterize these various phases and their transitions in more detail, by using spectroscopic methods that are sensitive to the dynamics of the lipid chains. In addition to identifying transitions in the state of mobility of the lipid molecules, such studies also should provide valuable additional information on the dynamic properties of the individual phases that will aid further in their identification and characterization. In the present study, electron spin resonance investigations on the dynamic phase behavior of biotin-PEs are reported for chain lengths of C(12:0) to C(20:0), both in the presence and in the absence of 1 M NaCl, by using phosphatidylcholine or biotin-PE probes spin-labeled at different positions in the *sn*-2 chain. Details of the structures of the different phases are obtained from the profile of the chain dynamics with position of labeling and from the differential changes in molecular mobility that occur at the transition between the I_M and L_{α} phases above the chain-melting transition of the biotin-PE derivative with C(16:0) chain length.

MATERIALS AND METHODS

Materials. Dilauroyl-, dimyristoyl-, dipalmitoyl-, distearoyl-, and diarachinoylphosphatidylethanolamines were obtained from Fluka, Buchs, Switzerland. Biotin *N*-hydroxysuccinimide ester was purchased from Molecular Probes, Eugene, OR. Biotin-PEs were prepared from the corresponding phosphatidylethanolamines according to Bayer et al. (1979). Phosphatidylcholines and phosphatidylethanolamines bearing the spin-label group on the *sn*-2 chain (*n*-PCSL and *n*-PESL) were synthesized as described in Marsh and Watts (1982). Spin-labeled biotin-PEs (*n*-BPESL) were synthesized from the corresponding spin-labeled phosphatidylethanolamines in a manner analogous to that for the biotin-PEs. The composition of the *sn*-1 chain of all spin labels was that of egg yolk phosphatidylcholine, i.e., predominantly palmitoyl.

Sample Preparation. Samples for ESR spectroscopy were prepared as follows. One milligram of the lipid plus 1 mol % of the spin-label were codissolved in CH_2Cl_2 and a thin film was formed by evaporating the solvent under a gentle stream of dry nitrogen gas at room temperature. The solubility of DABPE was rather low in CH_2Cl_2 alone; therefore a 2:1 mixture (v/v) of CH_2Cl_2/MeOH was used to dissolve this lipid and the sample tube was partially immersed in a warm water bath (ca. 40 °C) while the solvent was evaporated with nitrogen gas. The samples were then subjected to vacuum drying for a minimum of 3 h. The dry thin film of lipid was then hydrated at ca. 10 deg above the chain-melting phase transition of the lipid and transferred to a 100-μL (1-mm diameter) capillary, and the lipid dispersions were pelleted at room temperature in a benchtop microcentrifuge (samples in the presence of salt) or at 4 °C in a Beckman L7-55 ultracentrifuge (Ti-50 rotor at 15 000 rpm; samples in the absence of salt). The lipids of shorter chain lengths [C(12:0) and C(14:0)] could not be pelleted in the absence of salt even in the ultracentrifuge and therefore for these lipids the measurements were made directly on the aqueous dispersions. For these lipids, only 30–40 μL of buffer/mg of lipid was used for hydration, whereas for the lipids of longer chain lengths, 100–250 μL of buffer/mg of lipid was used. After pelleting, the excess supernatant was removed with a drawn-out Pasteur pipette and the capillary was flame-sealed.

Electron Spin Resonance Spectroscopy. ESR spectra were recorded on a Varian E-12 Century Line or E-line 9-GHz spectrometer equipped with nitrogen gas flow temperature regulation system. Samples in 1-mm diameter 100-μL glass capillaries were inserted into regular 4-mm quartz tubes containing silicone oil for thermal stability. Spectral data were collected digitally on a IBM personal computer using the software written by Dr. M. D. King of this institute. Continuous temperature scans were performed by locking the spectrometer to the central peak maximum of the ESR spectrum and monitoring its intensity as a function of temperature as described previously (Watts et al., 1978).

RESULTS AND DISCUSSION

ESR spectroscopy of spin-labeled lipid probes is capable of giving information not only on the local rotational mobility of the labeled probes but also, in favorable circumstances, on the dynamic structure of different lipid phases. In the latter case, comparison between spin-labels located at different positions in the lipid molecule can be particularly valuable. Here, lipid probes spin-labeled at different C-atoms in the *sn*-2 chain, and also in the lipid head group, are used. Useful additional information is also obtained by studying the changes taking place at cooperative thermotropic transitions between the different phases: not only the chain-melting transition but here also a transition taking place within the fluid phase of DPBPE. The chain-melting transitions are treated first, and then the results obtained on the dynamic structures of the gel phases and fluid phases are presented separately. The biotin-PEs are negatively charged lipids at neutral pH and therefore are expected to exhibit strong effects of electrostatic interactions, which, however, should be screened out at high ionic strength. Since the biotin-PEs have, in fact, been found to exhibit different phase properties in the presence and absence of salt (Swamy et al., 1993, 1994), experiments were performed in buffers containing 1 M NaCl as well as in those without salt.

Chain-Melting Phase Transitions. The chain-melting behavior of the aqueous dispersions of *N*-biotinylphosphatidylethanolamines was investigated by means of the characteristic changes occurring in the ESR spectra of a phosphatidylcholine probe (5-PCSL) spin-labeled on the 5 C-atom of the *sn*-2 chain. Representative ESR spectra of the 5-PCSL spin-label incorporated in DPBPE dispersions in 1 M NaCl (with 1 mM EDTA and 10 mM Hepes, pH 7.4), recorded at various temperatures are shown in Figure 1. The spectra at low temperatures approximate a powder pattern, characteristic of a rather rigid phase, and change relatively little with increasing temperature until 36 °C. Above this temperature, there is an abrupt change and the spectrum at 39 °C displays a considerably lower (axial) anisotropy that is characteristic of a fluid liquid-crystalline phase. The temperature at which the abrupt change in the ESR spectral anisotropy occurs (38 °C) correlates reasonably well with the endothermic phase transition at 40.7 °C detected for the same system by differential scanning calorimetry (Swamy et al., 1994). The

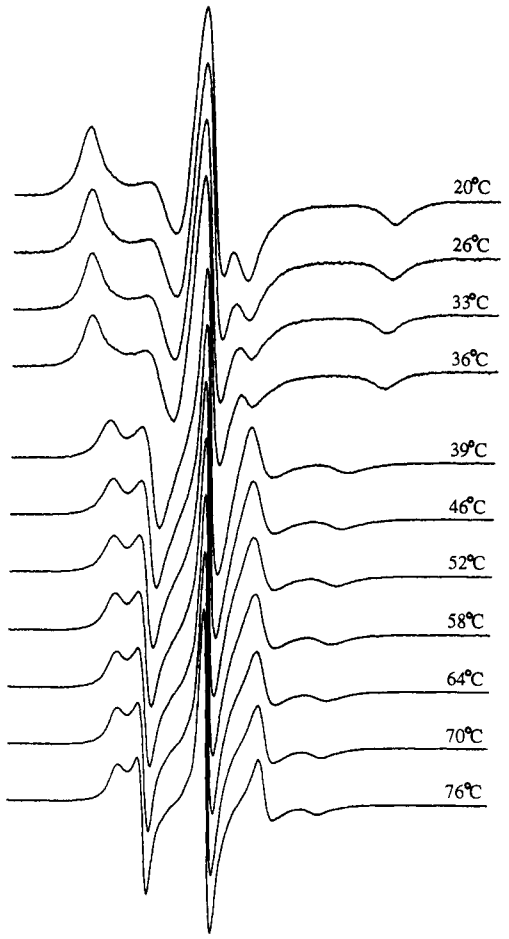

FIGURE 1: ESR spectra of the 5-PCSL spin label in aqueous dispersions of dipalmitoyl-*N*-biotinyl-PE. Spectra were recorded at the temperatures indicated. Buffer: 10 mM Hepes, 1 M NaCl, and 1 mM EDTA, pH 7.4. Spectral width: 100 G.

temperature dependence of the chain dynamics is most conveniently monitored by the hyperfine splitting, $2A_{max}$, between the two outer extreme peaks in the ESR spectrum of the spin-labeled phospholipid. In general, this quantity is sensitive both to the mobility and to the amplitude of motion of the spin-labeled chain for spectra that are, at least partially, in the slow motional regime of conventional nitroxide ESR spectroscopy [cf. Marsh and Horváth (1989)]. The temperature dependences of the outer hyperfine splitting constant, A_{max}, for the 5-PCSL spin-label in dispersions of DLBPE, DMBPE, DPBPE, DSBPE, and DABPE in 1 M NaCl are shown in Figure 2. The values given were obtained with increasing temperature, but those obtained on cooling indicate a complete reversibility without any appreciable hysteresis (data not shown). The data in Figure 2 very clearly reflect the cooperative thermotropic chain-melting transitions of the diacylbiotin-PEs of different chain lengths in 1 M NaCl. The transition temperatures for the biotin-PEs, determined from the sharp discontinuities in the temperature dependence of A_{max}, are −7.0, 18.5, 37.6, 52.5, and 61.0 °C for DLBPE, DMBPE, DPBPE, DSBPE, and DABPE, respectively, in 1 M NaCl. These values are in reasonably good agreement with the corresponding transition temperatures determined by differential calorimetry, which were found to be −9.5, 18.3, 40.7, 55.0, and 65.5 °C for DLBPE, DMBPE, DPBPE, DSBPE, and DABPE, respectively (Swamy et al., 1994). In general, the transition temperatures recorded by ESR are somewhat lower than those obtained from calorimetry, partly because of a freezing point depression by the spin-label and

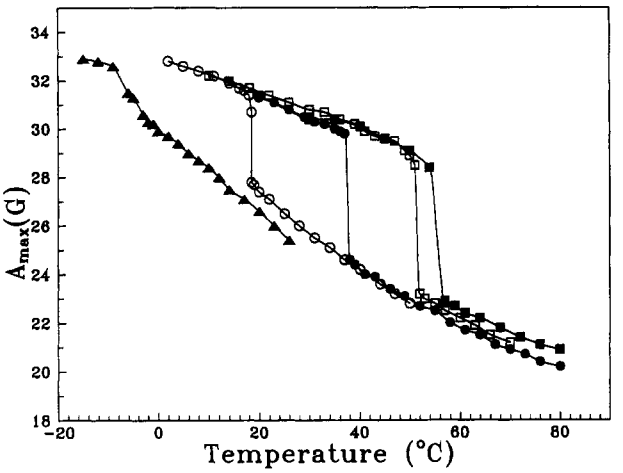

FIGURE 2: Temperature dependence of the outer hyperfine splitting constant, A_{max}, for the 5-PCSL spin label in dispersions of biotin-PEs of different chain lengths in the presence of 1 M NaCl. (▲) DLBPE; (○) DMBPE; (●) DPBPE; (□) DSBPE; (■) DABPE. Buffer: 10 mM Hepes, 1 M NaCl, and 1 mM EDTA, pH 7.4.

also because of nonvanishing temperature gradients in the ESR dewar for the lipids of longer chain length, which have the higher transition temperatures. Thus, within these limits, the ESR results reported here give direct confirmation that the transitions that are detected calorimetrically correspond to chain-melting transitions.

It will be noted that, with the exception of DLBPE, the chain length of which is considerably shorter than that of the spin-label, the temperature dependences of the values of A_{max} for the lipids of different chain length in 1 M NaCl lie approximately on a single line, in the gel phase. This would tend to suggest that the gel phase of these particular lipids has a rather uniform structure in 1 M NaCl. By contrast, there are significant differences in the temperature dependences in the fluid phase, possibly pointing to different mesomorphic structures for the various lipids above the chain-melting temperature. Only DMBPE and DPBPE appear to have a common temperature dependence, suggesting that in 1 M NaCl these two lipids have structurally, or at least dynamically, similar properties. This latter point will be returned to later.

ESR spectra also were recorded at various temperatures for dispersions of all five biotin-PEs in the absence of salt (10 mM Hepes and 1 mM EDTA, pH 7.4) with the 5-PCSL spin-label. The temperature dependence of the values of A_{max} for 5-PCSL in dispersions of the different lipids in the absence of salt are shown in Figure 3, for heating as well as for cooling experiments. As for the samples in the presence of salt, there is a sharp discontinuity in the anisotropy of the spectra (given by A_{max}) at the chain-melting transition temperature. There is a large hysteresis between the heating and the cooling scans, with the transition occurring approximately 6–13 deg lower in temperature in the cooling scans. This hysteresis has been observed also by calorimetry (Swamy et al., 1994) and is a particular feature of the samples in the absence of salt, because the temperature dependences given in Figure 2 for samples in 1 M NaCl essentially are fully reversible. The transition (on heating) for each lipid in the absence of salt is found to occur at a lower temperature than that in the presence of salt (cf. Figure 2). The chain-melting transition temperatures derived from Figure 3 are −1.0, 5, 28.5, 42, and 56–57 °C for DLBPE, DMBPE, DPBPE, DSBPE, and DABPE, respectively, as compared with corresponding values of 7–8, 29, 45.5, and 60 °C obtained for the calorimetric endotherms of DMBPE, DPBPE, DSBPE, and DABPE, respectively (Swamy

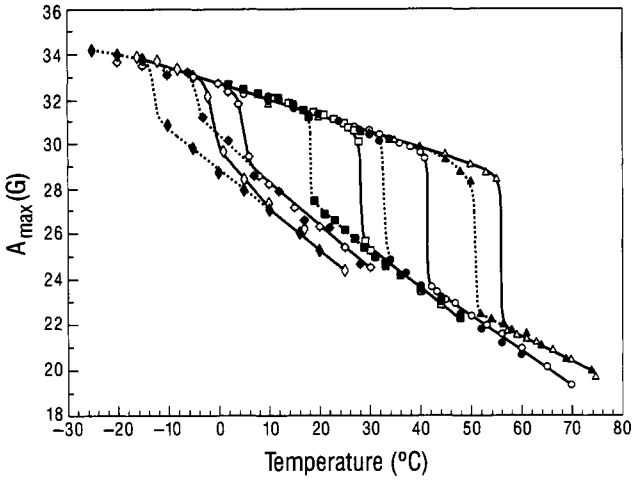

FIGURE 3: Temperature dependence of the outer hyperfine splitting constant, A_{max}, for the 5-PCSL spin label in dispersions of biotin-PEs of different chain lengths in the absence of salt. (◇, ◆) DLBPE; (◇, ◆) DMBPE; (□, ■) DPBPE; (○, ●) DSBPE; (△, ▲) DABPE. Open and filled symbols represent heating and cooling data, respectively. Buffer: 10 mM Hepes and 1 mM EDTA, pH 7.4.

et al., 1994). Again the transition temperatures recorded from the spin-label ESR spectra are somewhat lower than those from calorimetry, but the rather close correlation between the two confirms that the calorimetric endotherms observed for the lipids in the absence of salt correspond to chain-melting transitions. For DLBPE in the absence of salt, it was not possible to determine the transition temperature by calorimetry because, as demonstrated here, it lies very close to the ice-melting transition.

It is striking that the temperature dependence of the values for the outer hyperfine splitting constant, A_{max}, in the gel phase is continuous between the lipids of different chain length in Figure 3. This suggests that the structures formed in the gel phases in the absence of salt are rather similar for all the biotin-PEs investigated. There is also a rather uniform trend in the temperature dependence above the transition, possibly indicating that the structures of the fluid phases in the absence of salt are also rather similar. It will be noted that these conclusions, and those made above for samples in the presence of salt, would not hold if comparisons were made on a reduced temperature scale. From the regularities in Figures 2 and 3, it seems likely, however, that the dynamic properties (and kinetic energies, etc.) are determined by the absolute temperature rather than by the reduced temperature.

Structures and Dynamics of the Gel and Fluid Phases: (*A*) *Gel Phases*. The structure of the gel phases of the biotin-PEs in the presence and in the absence of salt have been investigated by X-ray diffraction and ^{31}P-NMR spectroscopy (Swamy et al., 1993). The X-ray diffraction data have suggested that for the biotin-PEs of chain length between 14 and 20 carbons in the presence of salt the lipid bilayer is interdigitated in the gel phase. In the absence of salt, measurements on DSBPE at controlled hydration indicated that the gel phase of this lipid also has an interdigitated lamellar structure, at least at low hydration. Because the evidence for interdigitation of the gel phase in 1 M NaCl was somewhat indirect, and because interdigitation in the absence of salt was established only for one lipid and at low hydration, it is desirable to have additional information from studies on the lipid chain dynamics to support these conclusions further and to extend them both to the lipids of other chain lengths and to excess water content for the lipids in the absence of salt. ESR spectroscopy of lipids spin-labeled in the acyl chain offers

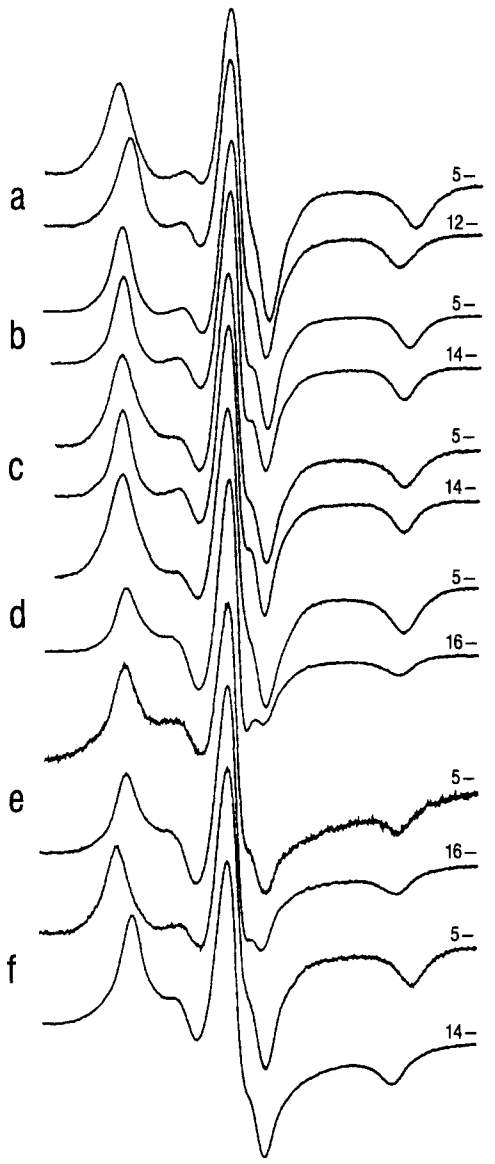

FIGURE 4: ESR spectra of *n*-PCSLs with the spin label positioned near the glycerol backbone ($n = 5$) or near the chain methyl terminus (n = 12, 14, or 16) in dispersions of different biotin-PEs and of dimyristoyl-PE. The position, n, of the nitroxide moiety in the *sn*-2 chain is indicated on the right of the figure. (a) DLBPE at −15 °C; (b) DMBPE at 5 °C, (c) DPBPE at 5 °C, (d) DSBPE at 10 °C, (e) DABPE at 20 °C, and (f) DMPE at 2 °C. Buffer: 10 mM Hepes, 1 M NaCl, and 1 mM EDTA, pH 7.4. Spectral width is 100 G.

a suitable means to do this, because it has been demonstrated previously by using this method that the chain dynamics, and in particular their positional dependence, differ very significantly between noninterdigitated and interdigitated lamellar phospholipid gel phases (Boggs & Rangaraj, 1985; Bartucci et al., 1993).

The ESR spectra from a spin-label probe positioned close to the carboxyl head of the phospholipid chain (i.e., 5-PCSL) are compared in Figure 4 with those from a spin-label positioned close to the terminal methyl end of the chain (i.e., 12-PCSL, 14-PCSL, or 16-PCSL) in the gel phase of the different lipids. Spectra are given for dispersions of biotin-PEs of different chain lengths from C(12:0) to C(20:0) in the presence of salt, and also for the normal gel phase of dimyristoyl-PE as a comparison. For each lipid, the spectra of both spin-labels were recorded at the same low temperature, well below the chain-melting transition. In DMPE, which has a noninterdigitated lamellar gel phase [see, e.g. Seddon

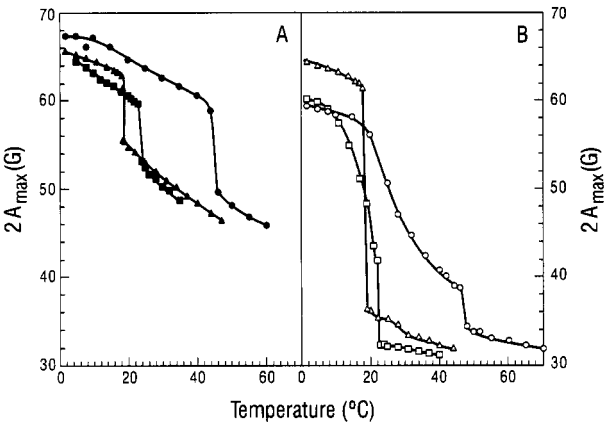

FIGURE 5: Temperature dependence of the outer hyperfine splitting, $2A_{max}$, for (A) 5-PCSL spin-label (filled symbols) and (B) 14-PCSL spin-label (open symbols), in dispersions of DMBPE (▲, △), DMPE (●, ○), and DMPC (■, □) in the presence of salt. Buffer: 10 mM Hepes, 1 M NaCl, and 1 mM EDTA, pH 7.4.

et al. (1984)], the separation of the outer hyperfine peaks is considerably smaller for the 14-PCSL spin label than for the 5-PCSL label (Figure 4f). This is also the case for DLBPE (Figure 4a), but in the spectra for the biotin-PEs of all other chain lengths, in the presence of 1 M NaCl, the hyperfine splittings of the spin labels close to the end of the chain are very similar to those of the 5-PCSL label (Figure 4b–e). These spectral effects indicate clear differences in the chain dynamics between dispersions of DMBPE, DPBPE, DSBPE, or DABPE and either DLBPE or DMPE. The biotin-PEs of chain lengths C(14:0) to C(20:0) in the presence of salt have the dynamic characteristics of interdigitated lamellar gel phases, whereas the C(12:0) chain length biotin-PE has the characteristics of a normal, lamellar gel phase, as exemplified by DMPE. Further evidence to support these structural assignments comes from the temperature dependence and spin label positional dependence of the ESR spectra in the gel phase that are described immediately following.

The temperature dependences of the outer hyperfine splitting, $2A_{max}$, for the 5-PCSL and 14-PCSL labels are shown in Figure 5, panels A and B, respectively, for dispersions of DMBPE, DMPE, and DMPC. The values of A_{max} for 5-PCSL and 14-PCSL in DMBPE dispersions are comparable at all temperatures in the gel phase, whereas in dispersions both of DMPE and of DMPC, the values of A_{max} for 14-PCSL are significantly smaller than those for 5-PCSL throughout the gel phase. Additionally, the values of A_{max} for 14-PCSL in DMPC and DMPE dispersions in the gel phase region decrease considerably as the temperature is increased, whereas those for 14-PCSL in DMBPE dispersions decrease only marginally with increases in temperature. The latter again are characteristics that would be expected for an interdigitated lamellar phase because the chain ends are located in a region close to the polar–apolar interface and therefore do not possess the degrees of freedom that are possible at the bilayer midplane.

The values of A_{max} for different positional isomers of the *n*-PCSL spin labels and of the *n*-BPESL biotin-PE spin-labels in gel-phase dispersions of the different biotin-PEs, both in the absence and in the presence of 1 M NaCl, are given in Table 1. The variation in the values of A_{max} with position of the spin-label group down the chain are also given by the differences, ΔA_{max}, obtained by subtracting the values of A_{max} for each spin-label from that for 5-PCSL. Data obtained for DMPE and DMPC are also shown in the table for comparison, as examples of normal gel-phase bilayers. Comparable values

Table 1: Outer Hyperfine Splitting Constants, A_{max}, for Different Positional Isomers of Phosphatidylcholine and *N*-Biotinylphosphatidylethanolamine Spin-Labels (*n*-PCSL and *n*-BPESL) in Dispersions of *N*-Biotinylphosphatidylethanolamines in the Presence and Absence of Salt[a]

lipid	spin-label	T (°C)	A_{max} (G)	ΔA_{max}[b] (G)
		1 M NaCl		
DLBPE	5-PCSL	−15	32.9	0.0
	10-PCSL	−15	31.6	1.3
	12-PCSL	−14	30.8	2.1
DMBPE	4-PCSL	5	32.3	0.3
	5-PCSL	5	32.6	0.0
	5-BPESL	5	32.5	0.1
	6-PCSL	5	32.3	0.3
	7-PCSL	5	32.5	0.1
	8-PCSL	5	31.9	0.7
	9-PCSL	5	31.7	0.9
	10-PCSL	5	31.5	1.1
	11-PCSL	5	31.9	0.7
	12-PCSL	5	31.8	0.7
	13-PCSL	5	31.6	1.0
	14-PCSL	5	32.2	0.4
	14-BPESL	5	32.0	0.6
DPBPE	5-PCSL	5	32.4	0.0
	13-PCSL	5	32.1	0.3
	14-PCSL	5	32.3	0.1
	16-PCSL	5	31.5	0.9
DSBPE	5-PCSL	10	32.2	0.0
	14-PCSL	10	31.3	0.9
	16-PCSL	10	31.2	1.0
DABPE	5-PCSL	20	31.4	0.0
	16-PCSL	20	31.0	0.4
DMPE	5-PCSL	2	33.7	0.0
	12-PCSL	2	30.3	3.4
	14-PCSL	2	29.7	4.0
DMPC	5-PCSL	5	32.2	0.0
	14-PCSL	5	29.9	2.3
		No Salt		
DMBPE	5-PCSL	2	32.4	0.0
	5-BPESL	2	32.7	−0.3
	14-PCSL	2	30.5	1.9
DPBPE	5-PCSL	2	32.5	0.0
	10-PCSL	2	32.2	0.3
	12-PCSL	2	31.8	0.7
	14-PCSL	2	32.2	0.3
DSBPE	5-PCSL	5	32.3	0.0
	10-PCSL	5	31.8	0.5
	12-PCSL	5	31.7	0.6
	14-PCSL	5	31.8	0.5
DABPE	5-PCSL	20	31.4	0.0
	16-PCSL	20	30.5	0.9

[a] Buffer: 10 mM Hepes and 1 mM EDTA, pH 7.4. Data for DMPC and DMPE in 1 M NaCl are given for comparison. [b] Decrease in A_{max} relative to 5-PCSL.

are obtained for the *n*-PCSL and *n*-BPESL labels in the same system, which validates using the much wider range of label positions available for the former. The values of ΔA_{max} determined at 5 °C for the different positional isomers of the spin labels in DMBPE dispersions in 1 M NaCl are all relatively small, in the range of 0.1–0.7 G, except for the spin-labels positioned in the middle of the chain (9-PCSL and 10-PCSL), which gave values of 0.9 and 1.1 G, respectively. Presumably these latter spin labels cannot be accommodated into the interdigitated bilayer as readily as those probes that bear the spin-label near the head group or at the end of the chain [cf. Bartucci et al. (1993)]. The values of ΔA_{max} for the spin-label close to the methyl end of the chain that are obtained in the gel phases of DPBPE, DSBPE, and DABPE are also in the low range of 0.4–1.0 G, whereas DLBPE had a value of ΔA_{max} of 2.1 G, even at the very low temperature of −15 °C. For comparison, the values of ΔA_{max} obtained for 14-

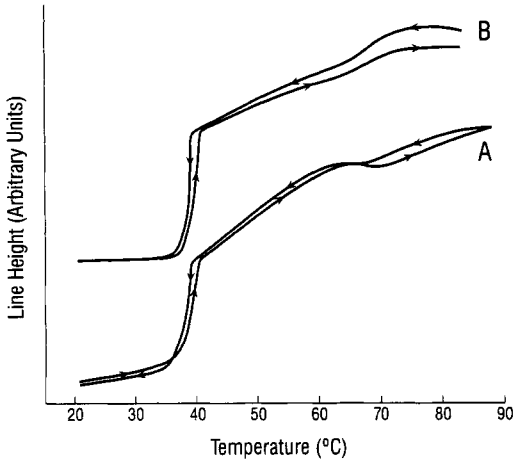

FIGURE 6: Continuous recording of the central line height of spin-labeled phospholipid probes in dispersions of DPBPE during temperature scans. (A) Head-group spin-label, acetamido-PESL; (B) chain spin-label, 14-PCSL and buffer: 10 mM Hepes, 1 M NaCl, and 1 mM EDTA, pH 7.4. Scan rate: 0.5 °C/min. The direction of the temperature scan is indicated by the arrows.

PCSL in dispersions of DMPE and DMPC in the gel phase are 4.0 and 2.3 G, respectively. These data suggest strongly that, in the presence of 1 M NaCl, the different biotin-PEs (with the possible exception of DLBPE, which had a somewhat larger value of ΔA_{max}) form interdigitated bilayers in the gel phase. In the absence of salt, the values of ΔA_{max} are 0.3, 0.5, and 0.9 G for DPBPE, DSBPE, and DABPE, respectively, whereas DMBPE has a value of 1.9 G. From this it can be concluded similarly that DPBPE, DSBPE, and DABPE form interdigitated gel phases also in the absence of salt, although whether this is the case for DMBPE is less likely.

(B) Fluid Phases. Dispersions of the biotin-PE of intermediate chain length, DPBPE, in 1 M NaCl were found, in the fluid phase, to display a behavior intermediate between those of the derivatives of shorter and longer chain lengths (Swamy et al., 1993). The phase formed immediately above the chain-melting transition exhibits characteristics in X-ray diffraction and ^{31}P-NMR that are similar to those of the fluid phases of DLBPE and DMBPE dispersed in 1 M NaCl, which were proposed to be of the isotropic aggregated micellar type, i.e., I_M. At high tempratures, in the fluid phase, a broad transition of the DPBPE dispersions in 1 M NaCl takes place to a normal lamellar, L_α, phase. This transition is not detected in the temperature dependence of the outer hyperfine splitting of the 5-PCSL label in DPBPE dispersions (Figure 2), indicating that there is no large difference in the chain rotational isomerism between the two phases, since both are condensed fluid phases. A similar result has been observed for the transition between a cubic phase and an inverted-hexagonal fluid phase in a phosphatidylcholine–fatty acid mixture (Rama Krishna & Marsh, 1990). More sensitive registration of the changes in the spin-labeled lipid chain dynamics in the fluid phase can be made by recording the ESR line height continuously as the temperature is scanned [see Marsh and Watts (1981)].

Continuous temperature scans of the central peak height in the ESR spectra of spin labels with the nitroxide positioned either in the phospholipid head group (acetamido-PESL) or close to the methyl terminal of the lipid chains (14-PCSL) are given, for DPBPE dispersions in 1 M NaCl, in Figure 6. An abrupt increase is observed in the peak height of each spin label, corresponding to the increase in rotational mobility of the lipid molecules at the chain-melting transition, at 39.5 °C. A broad thermotropic transition is also observed in the ESR peak height of each spin label at ca. 65 °C, in the fluid phase. However, whereas this transition corresponds to an increase in mobility at the lipid chain ends (Figure 6B, and also for 16-PCSL, not shown), the mobility of the spin-labeled lipid head groups is found to decrease at the transition (Figure 6A). This result indicates that a transition in the structure of the fluid phase that is accompanied by a change in curvature occurs in the region of 65 °C. Apparently, the lipid head groups are somewhat more loosely packed, and the chain ends are somewhat more tightly packed, below this transition than above it. Since the fluid phase at high temperature is known—both from X-ray diffraction and ^{31}P-NMR—to be of the lamellar L_α type, the lower temperature fluid phase from which it is formed must be one of normal rather than inverted curvature, in order to account for the relative signs of the responses observed by the acetamido-PESL and 14-PCSL spin labels in Figure 6. Combination with the previous results from ^{31}P-NMR and X-ray diffraction (Swamy et al., 1993) therefore suggests that the fluid isotropic phase, I_M, that is found with biotin-PEs in 1 M NaCl is composed of aggregates of normal micelles.

In the absence of salt at neutral pH, the biotin-PEs give clear solutions. They also give sharp isotropic ^{31}P-NMR spectra in the fluid phase (Swamy et al., 1993). These observations taken together suggest that the biotin-PEs form micellar solutions in this phase. The increase in head group size as a result of the attachment of the biotin moiety, as well as the electrostatic repulsion in the absence of salt, would both tend to favor the formation of normal micellar structures. The ESR data presented here below give further support to this notion.

The ESR spectra of the 5-PCSL spin-label in dispersions of all five biotin-PEs, both in the absence and in the presence of 1 M NaCl, and recorded at a temperature in the fluid phase, are shown in Figure 7. It can be seen from this figure that for each lipid the spectra recorded in the absence of salt exhibit smaller hyperfine anisotropies than those recorded in the presence of salt at the same temperature. The outer hyperfine splitting constants, A_{max}, and the increase in the A_{max} values, ΔA_{max}, obtained by subtracting the value obtained in the absence of salt from that obtained in the presence of 1 M NaCl, are presented in Table 2. The values of ΔA_{max} obtained for the different biotin-PEs are in the range of 0.8–1.6 G and reflect a considerably higher motional disorder in the regions of the chain close to the lipid head groups in the dispersions without salt, when compared to those which are hydrated with 1 M NaCl. This difference results from the looser packing of the region of the lipid chains close to the head groups in the micellar structure with normal curvature.

The profile of the outer hyperfine splitting constant, A_{max}, with spin-label position in the lipid chain is given in Figure 8, for samples of DSBPE dispersed with and without 1 M NaCl, in the fluid phase. For further comparison, data from DPBPE in 1 M NaCl at the same temperature, i.e., in the I_M phase, is also included in the figure. The chain length of the different spin-labels is comparable to that of these two lipids. Comparison of the two profiles for DSBPE shows clearly that (as in Figure 7D) the labels in the upper part of the chain (from 4-PCSL to 8-PCSL) experience a smaller restriction of motion in the dispersions in the absence of salt than in those in the presence of salt. However, the labels closer to the terminal methyl end of the chain experience a significantly larger motional restriction, for the dispersions in the absence of salt, relative to that for the dispersions in the presence of

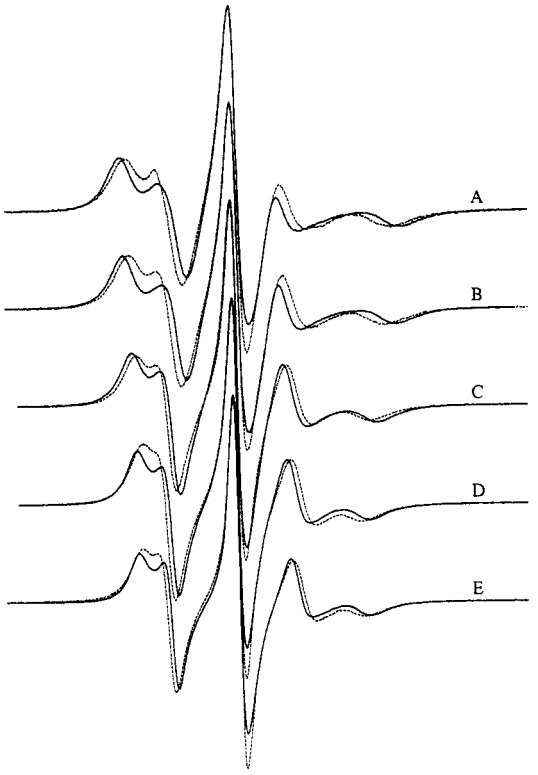

FIGURE 7: ESR spectra of the 5-PCSL spin-label in the fluid phase of biotin-PEs in the presence (solid lines) and in the absence (dashed lines) of 1 M NaCl. (A) DLBPE at 20 °C; (B) DMBPE at 28 °C; (C) DPBPE at 48 °C; (D) DSBPE at 60 °C; and (E) DABPE at 65 °C. Buffer: 10 mM Hepes and 1 mM EDTA, pH 7.4. Spectral width is 100 G.

Table 2: Outer Hyperfine Splittings Constants, A_{max}, for the 5-PCSL Spin-Label in Dispersions of Different *N*-Biotinylphosphatidylethanolamines[a]

lipid	[NaCl] (M)	T (°C)	A_{max} (G)	ΔA_{max}[b] (G)
DLBPE	1.0	20	26.6	1.3
	0.0	20	25.3	0.0
DMBPE	1.0	28	26.0	1.6
	0.0	28	24.4	0.0
DPBPE	1.0	48	23.4	1.0
	0.0	48	22.4	0.0
DSBPE	1.0	60	22.2	1.2
	0.0	60	21.0	0.0
DABPE	1.0	65	22.0	0.8
	0.0	65	21.3	0.0

[a] In 10 mM Hepes buffer and 1 mM EDTA, pH 7.4, with and without 1 M NaCl. [b] Increase in A_{max} in 1 M NaCl relative to the absence of salt.

salt. This indicates that the overall tumbling motion of the micelles does not affect the ESR spectra appreciably but again would be consistent with the formation of normal micelles in the absence of salt and most probably reflects the difficulty in packing of the chain ends in normal micelles that are roughly isometric in shape. These results, taken together with the results from ^{31}P-NMR and X-ray diffraction (Swamy et al., 1993), therefore indicate that the biotin-PE lipids form normal micelles when dispersed in water at neutral pH without salt and, in addition, yield further information on the nature of the chain dynamics in micellar structures of normal (rather than inverted) curvature. Additionally, it is seen from Figure 8 that the chain profile of the mobility in DPBPE in 1 M NaCl at the same temperature (60 °C), but in the I_M phase immediately below the transition to the L_α phase, is rather similar to that for DSBPE in 1 M salt, i.e., in the L_α phase. This is consistent with the I_M phase being composed of larger

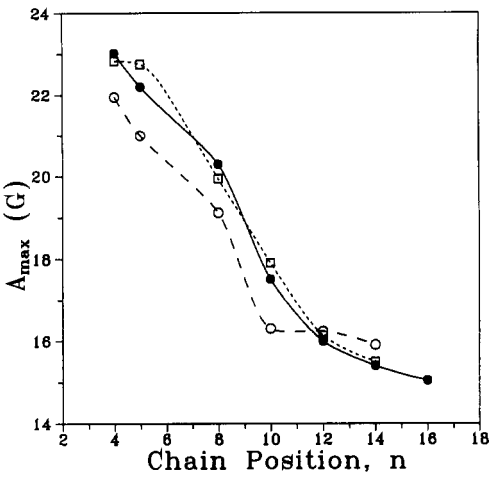

FIGURE 8: Dependence of the outer hyperfine splitting constant, A_{max}, on the position, n, of chain labeling for the n-PCSL spin labels in dispersions of DSBPE in 1 M NaCl (●), of DPBPE in 1 M NaCl (□), and of DSBPE in the absence of salt (○), all at 60 °C.

aggregated micellar structures than those present in the absence of salt, so that the chain mobility profile resembles more closely that of an extended lamellar fluid phase.

It will be noted that the bulk of the biotin-PE head group itself will tend to favor the formation of structures with normal curvature. From the shift in the chain-melting transition temperature induced by salt it was calculated that the surface area per lipid head group in the fluid phase in the absence of salt was 1.29 times larger than that in the interdigitated gel phase below the transition (Swamy et al., 1994). From this value of the surface area per head group ($A_0 \approx 1.04$ nm^2) in the fluid phase, it can be estimated that the biotinyl lipid packing parameter is $V/A_0l_c \approx 0.4$, where V and l_c are the hydrophobic volume and the maximum extended length of the lipid chains, respectively (Israelachvili et al., 1977). This value of the packing parameter suggests the formation of normal micellar structures intermediate between those of regular spherical shape ($V/A_0l_c \leq 1/3$) and asymmetric rodlike shapes ($1/3 \leq V/A_0l_c \leq 1/2$). Such considerations of lipid packing also lend support to the suggestion of a structure composed of aggregated normal micelles for the I_M phase formed by the biotin-PEs of shorter chain lengths in the presence salt.

Conclusions. With the possible exception of DLBPE, and of DMBPE in the absence of salt, the diacylbiotin-PEs of all chain lengths studied form interdigitated lamellar gel phases both in the presence and in the absence of salt. The overall chain dynamics of the shorter chain length biotin-PEs in the presence of salt, which form isotropic fluid phases (I_M), are rather similar to those of the longer chain length biotin-PEs which form normal lamellar fluid phases (L_α) in the presence of salt. However, sensitive recording of the chain dynamics by continuous temperature scans reveals differential changes in the dynamics of the lipid head group region relative to the chain ends at the cooperative transition from the I_M-type structures to the L_α-type structures in the fluid phase of DPBPE. The head groups are relatively more loosely packed and the chain ends are more tightly packed in the I_M phase than in the L_α phase, indicating that the former corresponds to structures with normal (rather than inverted) curvature. In the absence of salt above the chain-melting transition, the biotin-PEs disperse as normal micelles in which the chain motion is greater in the region close to the lipid head groups and somewhat less at the chain ends than in the more condensed L_α and I_M phases found in the presence of salt.

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