Although the antibody is of low titer, serum containing the activity is readily available. Therefore, we plan to purify the antibody by affinity chromatography on a receptor B column as has been done for a low titer, low affinity calmodulin antibody (Dedman et al., 1978). This will permit us to perform experiments with unlabeled receptor and to isolate the portion of the receptor containing the antigenic site.

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Acyl Chain Order and Lateral Domain Formation in Mixed Phosphatidylcholine–Sphingomyelin Multilamellar and Unilamellar Vesicles†

Barry R. Lentz,* Mathias Hoechli, and Yecheskael Barenholz

ABSTRACT: The phase behavior of mixtures of dimyristoylphosphatidylcholine (DMPC) with *N*-palmitoylsphingosinephosphorylcholine (C_{16}SPH) has been investigated in both small unilamellar and large multilamellar vesicles. The steady-state fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) has been used to detect temperature-induced structural changes in these membranes. In addition, electron microscopy has revealed vastly different fracture-face morphologies for large multilamellar vesicles "jet-frozen" from different temperatures. These data have been interpreted in terms of proposed phase diagrams for this lipid mixture. The shapes of the proposed phase diagrams have led us to conclude that phosphatidylcholine and sphingomyelin species of similar acyl chain length mix freely in both highly curved and uncurved bilayers, except at temperatures at which both lipids are in low-temperature, ordered phases. In addition, the similarity of these phase diagrams to phase diagrams for analogous mixtures of pure phosphatidylcholines suggested that sphingomyelin and phosphatidylcholine species might substitute for each other in supporting the lamellar phase necessary to cell membrane structure. Finally, the anisotropy of DPH fluorescence was found to be essentially invariant with sphingomyelin content at temperatures just above and below the solid–liquid phase separation in small unilamellar vesicles. This demonstrates that the sphingomyelin backbone, per se, does not order the membrane bilayer. These results are discussed in terms of the possible role of sphingomyelin in controlling acyl chain order within mammalian cell membranes.

The phase behavior of many of the component phospholipids of mammalian membranes has been studied extensively in recent years with a view toward defining the role of different phospholipid species in establishing membrane structure. While the ability of some species to form nonlamellar phases may be important to specialized membrane functions (Cullis & DeKruijff, 1979), those species that spontaneously form lamellar mesomorphic phases in water should stabilize the essential bilayer structure of cell membranes. The choline phosphatides phosphatidylcholine and sphingomyelin are major components of mammalian membranes that form lamellar phases in excess water. Changes in the ratio of these two lipids have been correlated with the occurrence of various tissue malfunctions (Barenholz & Thompson, 1980) such as aging (Rouser & Solomon, 1969), atherosclerosis (Small & Shipley,

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1974), and infantile respiratory distress syndrome (Gluck et al., 1971) as well as with changes in the nonspecific permeability of red blood cells (Deuticke, 1977). A reciprocal relationship between the levels of sphingomyelin and phosphatidylcholine has also been noted by de Gier & van Deenen (1961) in the membranes of red blood cells from several animal species. Furthermore, sphingomyelin mixtures isolated from mammalian tissue are unique among the cell's phospholipid components in undergoing a lamellar phase separation over a temperature range that encompasses the cell growth temperature of 37 °C (Shipley et al., 1974; Shinitzky & Barenholz, 1974). This implies that the ratio of sphingomyelin to phosphatidylcholine may control the extent of gel-phase lipid domain formation or the degree of acyl chain order in mammalian cell membranes. However, less is known about the tendency of phosphatidylcholine and sphingomyelin to mix within the plane of a bilayer membrane than is known about the mixing tendencies of pairs of phosphatidylcholine species. This is largely due to the unavailability of pure synthetic sphingomyelins for use in model membrane studies. With a notable exception (Calhoun & Shipley, 1979), investigations of sphingomyelin–phosphatidylcholine interactions have been confined to mixtures of naturally obtained lipids (Schmidt et al., 1977; Untracht & Shipley, 1977; Yeagle et al., 1978). These and other investigations have led to the suggestion that strong interactions may exist between sphingomyelin molecules as a result of special features of the sphingosine backbone. The notion that sphingomyelin might order a membane bilayer (Shinitzky & Barenholz, 1974; Schmidt et al., 1977) is one derivative of this point of view. However, before these ideas can be seriously tested, it will be necessary to investigate the mixing of sphingomyelin and phosphatidylcholine species with similar acyl chains. It has been reported that the mixing of dimyristoylphosphatidylcholine (DMPC)[1] and dipalmitoylphosphatidylcholine (DPPC) is nearly ideal in both small unilamellar and large multilamellar vesicles (Lentz et al., 1976). In order to directly compare with this system, we have studied the mixing of DMPC with C_{16}SPH in both types of bilayer vesicles, using a combination of fluorescent probe and freeze–fracture electron microscopic techniques. The results of our investigation have led us to three principle conclusions. First, that sphingomyelin and phosphatidylcholine species with similar acyl chains show no anomalous mixing behavior in either highly curved or (essentially) flat bilayers except in the gel or "solidlike" phases. Second, the similarity of phase diagrams for DMPC/C_{16}SPH and DMPC/DPPC mixtures in both types of model membranes suggests that sphingomyelin and phosphatidylcholine might be interchangeable in supporting the lipid-bilayer structure of biological membranes. Finally, we conclude that any ordering effect of sphingomyelin in mammalian membranes is likely to be due to the saturated acyl chain composition of the naturally occurring species rather than to the sphingomyelin backbone, per se.

Materials and Methods

Lipids. 1,2-Dimyristoyl-3-*sn*-phosphatidylcholine (DMPC) was purchased from either Avanti Biochemicals (Birmingham, AL) or Berchtold Labor (Bern, Switzerland). Samples were recrystallized from chloroform–acetone mixtures (Lentz et al., 1976) and determined by thin-layer chromatography to contain greater than 99% of a single phosphorus-containing component (Lentz et al., 1976). Gas–liquid chromatography of the trans-methylated (Stoffel et al., 1959) acyl chains showed greater than 99.5 mol % methyl myristate. Quantitation of DMPC in lipid mixtures was accomplished by inclusion of a small amount (<0.1 mol %) of 1,2-[^{14}C]dimyristoyl-3-*sn*-phosphatidylcholine (Applied Science, lot B0141, State College, PA) in the chloroform stock of pure DMPC. This stock was stored under an argon atmosphere at −70 °C in the dark at a concentration of 12 mM.

DL-*erythro*-*N*-Palmitoylsphingosinephosphorylcholine (C_{16}SPH) was the kind gift of Professor D. Shapiro (Weizmann Institute of Science, Rehovot, Israel) and was further purified by silicic acid chromatography. It was stored in argon-saturated chloroform/methanol (1:1 v/v) at 9.5 mM at −70 °C in the dark. Previous analysis of this material (Barenholz et al., 1976) revealed 2 mol % dihydrosphingosine and less than 0.3 mol % fatty acids other than palmitic acid. The limited available quantity (11 μmol) of this highly purified synthetic sphingomyelin dictated the use of sensitive fluorescent-probe procedures for characterization of phospholipid phase transitions and acyl chain motion. Whenever possible, unused vesicle samples were extracted (Bligh & Dyer, 1959); phosphatidylcholine was alkaline hydrolyzed (0.4 N KOH in methanol for 2 h) and C_{16}SPH recovered for reuse by preparative thin-layer chromatography on silica gel preformed glass plates (Analtech GHL Uniplates).

Vesicle Preparation. Large multilamellar vesicles were prepared by mixing appropriate quantities of stock solutions as previously described (Lentz et al., 1980). Dried lipid was dispersed into 50 mM KCl solution to a total concentration of 0.1 mM at 48 °C, and vesicles were formed by swirling at this temperature (Lentz et al., 1980).

Small unilamellar vesicles were prepared by sonicating 4 mL of 0.12 mM large multilamellar vesicle preparations at 48 °C (Barrow & Lentz, 1980). Fractionation of the sonicate by high-speed centrifugation (Barenholz et al., 1977) resulted in recovery of 50–70% of the total lipid as small vesicles except for samples containing 70 and 86 mol % C_{16}SPH, which yielded only 10% of total phospholipid as small vesicles. Samples were maintained at 40–50 °C at all times before use and were used within 24 h of preparation.

The exact composition of all vesicle samples was determined by liquid scintillation counting of [^{14}C]DMPC and by high-sensitivity total phosphate analysis by using a modification of the procedure of Goodwin et al. (1958).

Fluorescence Measurements. Zone-purified 1,6-diphenyl-1,3,5-hexatriene (DPH) was introduced into vesicle suspensions by injection of a small quantity (0.2–0.4 μL) of 2 mM DPH dissolved in tetrahydrofuran. The final dye to lipid ratio was 1:500 for multilamellar vesicles, 1:380 for most small vesicles, and 1:70 for the 70 and 86 mol % C_{16}SPH small vesicle samples. The low ratio for the latter samples was necessitated by low yields of small vesicles. Controls have shown that energy-transfer fluorescence depolarization was not detected at DPH to lipid ratios as low as 1:50 (Lentz et al., 1976).

Measurements of DPH fluorescence and fluorescence anisotropy were made in heating and cooling scans at rates of ±18 °C/h with either a modified Perkin-Elmer MPF3 or a T-system SLM 4000 spectrofluorometer. Details of the measurements as well as the calculation of the microviscosity and microviscosity activation energy parameters are given in the literature (Lentz et al., 1978, 1980). "Microviscosity" is used here not as a measure of lipid dynamics but only as a membrane structural parameter that has the convenient property of linear Arrhenius behavior in the absence of membrane phase transitions. "Microviscosity activation

[1] Abbreviations used: DPH, 1,6-diphenyl-1,3,5-hexatriene; DMPC, 1,2-dimyristoyl-3-*sn*-phosphatidylcholine; DPPC, 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine; C_{16}SPH, DL-*erythro*-*N*-dipalmitoylsphingosine-phosphorylcholine.

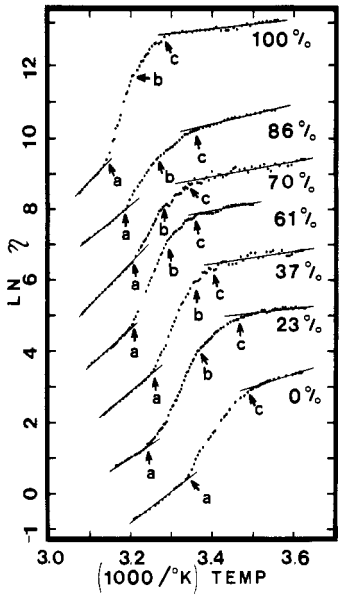

FIGURE 1: Arrhenius plots of DPH-derived bilayer microviscosity (η) for small unilamellar vesicles composed of various mixtures of DMPC and C_{16}SPH. The compositions (in mol % C_{16}SPH) are indicated beside each plot. The curve for 0% is plotted according to the units on the ordinate, while other curves are displaced increasingly upward by 1.25 units per data set. These data were obtained during heating scans (scan rate = 18 °C/h). Lines have been drawn through linear segments to illustrate our method of analysis. Points a and b delimit the region of most rapid change in microviscosity while points b and c delimit the region of slower change.

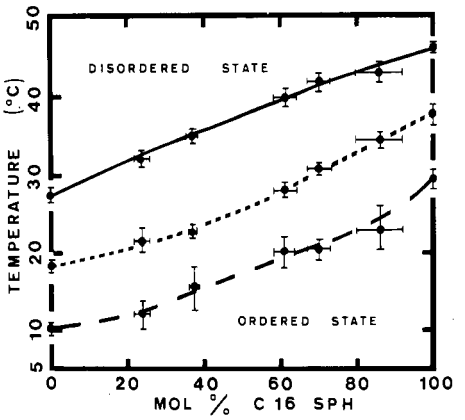

FIGURE 2: Temperature–composition diagram summarizing the phase behavior of small unilamellar vesicles composed of mixtures of DMPC and C_{16}SPH. The delimiting temperatures were determined from Arrhenius plots such as those in Figure 1. Vertical and horizontal bars represent uncertainties in break temperatures and compositions, respectively. The upper line (—) is determined by breaks of the type labeled by a in Figure 1, the middle line (- - -) point b, and the lower line (– –) point c.

energy" is obtained by numerical differentiation of the logarithm of microviscosity with respect to reciprocal temperature and increases dramatically at phospholipid phase transitions (Lentz et al., 1978). The validity of these techniques as procedures for detecting phospholipid phase transitions has been established for a variety of lipid mixtures by comparison with calorimetric data (Lentz et al., 1976, 1978, 1980). Phase-transition profiles obtained by fluorescence measurements are somewhat broader than those obtained calorimetrically (Lentz et al., 1978). This broadening probably reflects an increased sensitivity of the fluorescence techniques to changes in acyl chain order in small local environments as opposed to the somewhat larger cooperative units detected by calorimetry (Lentz et al., 1978).

Freeze–Fracture Electron Microscopy. Aliquots (0.5 mL) of multilamellar vesicle suspensions (0.5 mM) were pelleted in plastic centrifuge tubes with a Beckman microfuge B and then slowly cooled (7 °C/h) from 55 °C to temperatures from which freeze-quenching was desired. Samples were further equilibrated at these temperatures for at least 48 h before freeze-quenching. Careful equilibration was warranted by the previously noted slow equilibration of phospholipid gel phases (Lentz et al., 1980). Rapid jet-freezing of vesicle samples was accomplished as previously reported (Lentz et al., 1980) except that a copper electron microscope grid was not used as a spacer between the opposing copper sheets that held the vesicle suspension. A very thin film of Dow Corning silicone grease was used along two sides of the copper sheets to limit evaporation from the thin sandwich of sample between the sheets. These modifications allowed more reliable incubations in the copper sandwich at high temperatures and resulted in more uniform appearance of fracture faces. Other details of procedures are as previously described (Lentz et al., 1980).

Results

Phase Behavior of Small Vesicles. In Figure 1 are presented Arrhenius plots of the DPH microviscosity parameter derived from heating scans performed on small vesicle samples composed of several mixtures of DMPC and C_{16}SPH. Cooling scans performed on the same samples yielded nearly identical curves, indicating that negligible disruption of the small vesicle structure occurred during the initial cooling and subsequent heating scans. The curves in Figure 1 demonstrate that all mixtures studied underwent a broad phase change characteristic of small unilamellar vesicles (Lentz et al., 1976; Lentz & Litman, 1978). As previously observed (Lentz et al., 1976), this broad curve could be subdivided into two segments. The high-temperature segment (between points a and b in the scans of Figure 1) reflects substantial changes in microviscosity over a fairly narrow temperature range. This contrasts with the less rapid changes seen in the low-temperature segment (between points b and c in Figure 1). This two-segment behavior is substantially identical with that previously observed in small vesicles composed of mixtures of DMPC with DPPC (Lentz et al., 1976).

In Figure 2, we have summarized in a temperature–composition diagram the delimiting temperatures of the phase separations for each of the C_{16}SPH/DMPC small vesicle mixtures studied. The delimiting temperatures in Figure 2 were obtained from Arrhenius plots for both heating and cooling scans (e.g., see Figure 1). Above the high-temperature line in Figure 2 (—, defined by break points labeled a in Figure 1), the membrane is in a liquidlike state characterized by low DPH fluorescence anisotropy (i.e., low microviscosity parameter). Below the low-temperature line (– – in Figure 2, corresponding to break points labeled c in Figure 1), the membrane is in a more ordered state in which the motion of DPH is severely restricted, resulting in a large fluorescence anisotropy (i.e., high microviscosity parameter). The significance of the intermediate line (determined by point b in Figure 1 and indicated by - - - in Figure 2) is unclear. However, an analogous intermediate line has been reported for the temperature–composition diagram for small vesicles composed of DMPC/DPPC mixtures (Lentz et al., 1976).

From Figure 1, it is clear that the motional freedom of DPH varies considerably through the phase-separation region defined by Figure 2. Figure 3 illustrates the variation of DPH motional freedom with membrane sphingomyelin content at temperatures that are 2–3 °C above and below the high-temperature and low-temperature lines, respectively, of Figure 2. The essential invariance of DPH fluorescence anisotropy across

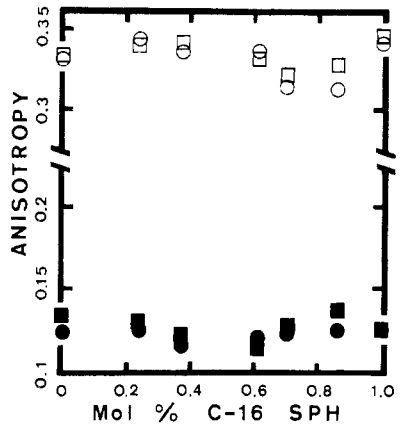

FIGURE 3: Variation with sphingomyelin content of the anisotropy of DPH fluorescence at temperatures 2–3 °C above (●, ■) and below (○, □) the phase separation in small unilamellar vesicles composed of DMPC/C_{16}SPH mixtures. Data from both heating (■, □) and cooling (●, ○) scans are presented.

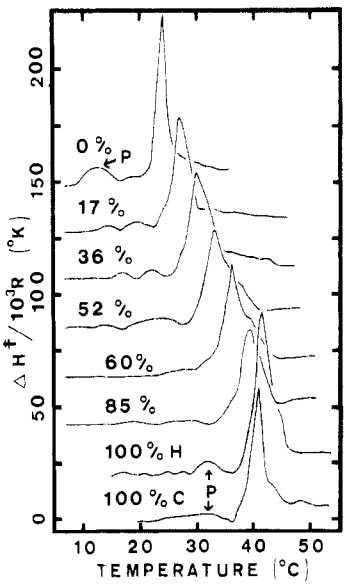

FIGURE 4: Temperature dependence of the DPH-derived microviscosity activation energy in large multilamellar vesicles composed of several representative mixtures of DMPC and C_{16}SPH. The sample compositions are indicated next to each plot (mol % C_{16}SPH). Data for heating scans only are shown for all compositions except pure C_{16}SPH, for which both heating (100% H) and cooling (100% C) results are shown. The 100% C curve is plotted according to the units on the ordinate, while other curves are displaced increasingly upward by 2 units per data set. The location of the low-temperature or pretransition phase change is indicated by P for both the pure DMPC and pure C_{16}SPH samples.

the entire range of sphingomyelin content, both above and below the phase separation, demonstrates that sphingomyelin effects no change in acyl chain order within the bilayer, at least for mixtures of species with similar acyl chain length. A similar invariance of DPH anisotropy with composition has been noted for mixtures of different phosphatidylcholine species (Lentz et al., 1976).

Phase Behavior of Large Multilamellar Vesicles. Figure 4 presents DPH fluorescence data displayed as microviscosity activation energy plots obtained from a series of heating scans performed on large multilamellar vesicles composed of several different mixtures of C_{16}SPH and DMPC. These results show only one major peak for all compositions. This suggests that gel and liquid-crystalline phases separate uniformly at all compositions as the temperature is varied through the phase-transition region. Therefore, these results are inconsistent with the existence of phase inhomogeneities at temperatures just above or below the gel/liquid-crystalline phase separation.

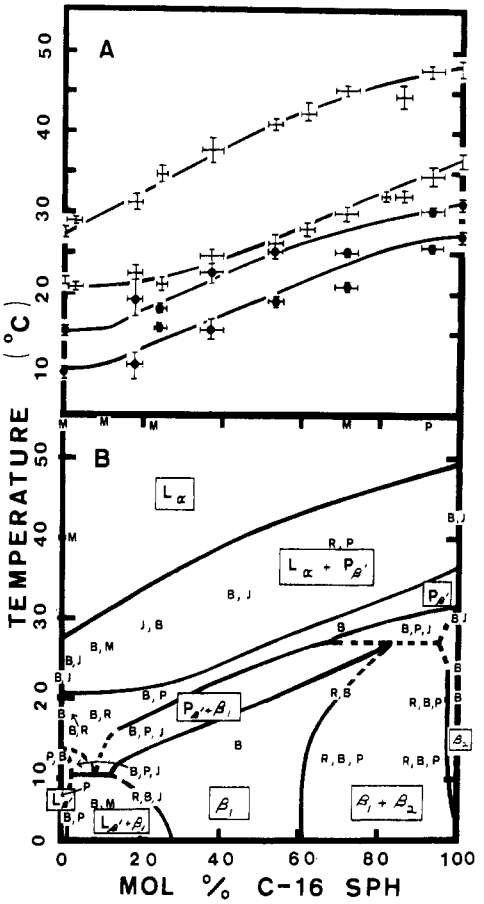

FIGURE 5: Temperature–composition diagram for DMPC/C_{16}SPH multilamellar vesicles. (A) Temperature extremes of the main phase-separation region are indicated by crossed bars, while those of the pretransition are indicated by dotted crossed bars. Uncertainties in the temperatures and compositions are indicated by the size of the vertical and horizontal bars, respectively. (B) Summary of freeze–fracture electron microscopy results together with our interpretation in terms of a phase diagram for this lipid mixture. Observed morphologies are indicated as follows: P, planar; M, mottled; R, rivuletted; B, banded; J, jumbled. Proposed phase assignments[2] are shown in large letters. Dashed curves at very low and high C_{16}SPH content indicate the theoretical phase-diagram topology in these regions, consistent with the rules of phase equilibrium (Gordon, 1968).

In addition to the main peak, most of the plots in Figure 4 display a smaller peak (P in Figure 4) at temperatures slightly below the major one. In pure DMPC, this corresponds to the so-called "pretransition" (Lentz et al., 1978) between $L\beta'$ and $P\beta'$ phases[2] (Janiak et al., 1976). This minor transition occurred in nearly every DMPC/C_{16}SPH mixture we studied and even in pure C_{16}SPH (Figure 4). As for phosphatidylcholine (Lentz et al., 1978), cooling scans revealed this transition in C_{16}SPH vesicles at slightly lower temperatures and broadened relative to heating scans. In pure phosphatidylcholines, this hysteresis has been attributed to the slow rate of transition between phospholipid gel phases (Lentz et al., 1978). No such low-temperature transition has been reported in published differential-scanning calorimetric studies of the

[2] Lipid phase nomenclature is after Tardieu et al. (1973) except for β_1 and β_2. $L\alpha$, lamellar, disordered acyl chains; $L\beta'$, lamellar, acyl chains extended at an acute angle to the bilayer normal; $P\beta'$, pleated, acyl chains extended at an acute angle to the bilayer normal. β_1 and β_2 are phases of banded morphology but unknown structure.

phase behavior of C_{16}SPH (Barenholz et al., 1976; Calhoun & Shipley, 1979).

The delimiting temperatures of both the major and minor peaks observed in heating and cooling scans of DMPC/C_{16}SPH multilamellar vesicles are summarized in the temperature–composition diagram given in Figure 5A.

Morphological changes associated with the phase transitions of pure C_{16}SPH multilamellar vesicles are evident in the freeze–fracture electron micrographs shown in Figure 6a–e. The fracture-face morphologies of these vesicles as well as of vesicles composed of mixtures of DMPC and C_{16}SPH are summarized in the temperature–composition diagram in Figure 5B. At 55 °C, C_{16}SPH vesicles produced slightly textured but planar (P, Figure 6a) fracture faces characteristic of phosphatidylcholine vesicles jet-frozen from the Lα phase (Lentz et al., 1980). In the range of the phase transition, we observed a mixed banded (B) and planar or mottled[3] (M) morphology (Figure 6b) indicative of coexisting phases. At temperatures just below the principle phase transition of C_{16}SPH vesicles, the bilayer fracture faces displayed a distinctly banded morphology (Figure 6c) reminiscent of the Pβ' fracture-face morphology seen in pure DMPC vesicles (Figure 6f). At 30 °C (the temperature of the minor peak for pure C_{16}SPH multilamellar vesicles), freeze–fracture electron micrographs displayed a mixed banded and jumbled[3] (J) appearance characteristic of a membrane undergoing a phase change (Figure 6d). At a slightly lower temperature (20 °C), the C_{16}SPH sample also produced clearly banded fracture faces (Figure 6e) but with a distinctly different appearance from those observed in samples quenched from 35 °C (Figure 6c). These observations support our contention that the minor peak in the C_{16}SPH microviscosity activation energy plots results from a transition between gel phases, analogous to but not identical with the pretransition observed in pure phosphatidylcholine multilayers.

In mixtures of C_{16}SPH and DMPC, we observed, at temperatures above and within the main phase-separation region, morphologies appropriate to Lα or mixed Lα plus Pβ' phases (Figure 6g), respectively. Just below the phase-separation temperature range, mixed vesicles displayed either the banded or rivuletted (R) morphologies characteristic of the Pβ' phase in pure phosphatidylcholine (Lentz et al., 1980) (Figure 6h). At even lower temperatures, we observed another region of mixed morphology corresponding to the temperature range of the minor transition seen in the microviscosity activation energy plots of Figure 4 (Figure 6i). At yet lower temperatures, the morphology of mixed DMPC/C_{16}SPH fracture faces was extremely complex. At low sphingomyelin content, we observed mixed morphologies at temperatures below 10 °C. The mixed banded and planar morphology at 2% C_{16}SPH and the widely spaced bands at 10% C_{16}SPH are especially noteworthy (Figure 6j,k, respectively). At intermediate sphingomyelin content, a simple banded pattern was seen (not shown). Finally at high C_{16}SPH content, a chaotic pattern was typically observed, with small rivuletted areas arranged at sharp angles to each other and interrupted by small regions of jumbled, planar, or banded morphology (e.g., Figure 6l).

To provide a reference point for understanding our results for mixtures of C_{16}SPH and DMPC, we have studied the fracture-face morphology of DMPC/DPPC mixed vesicles. The temperature–composition phase diagram for these mixed vesicles has previously been shown to have two phase-separation regions (Lentz et al., 1976; Mabrey & Sturtevant, 1976). Large multilamellar vesicles composed of DMPC/DPPC mixtures displayed fracture-face morphologies (summarized in Figure 7) suggestive of the phase assignments shown in Figure 7. These observations are consistent with the results of Luna & McConnell (1978).

Discussion

We have interpreted our results for mixed sphingomyelin–phosphatidylcholine multilamellar vesicles in terms of the phase diagram shown in Figure 5B. The phase assignments made in this figure are projections based on the known phase behavior (Janiak et al., 1976) and fracture-face morphology (Luna & McConnell, 1977) of pure phosphatidylcholines. Except at low C_{16}SPH content, the shape of our phase diagram in the Lα and Lα + Pβ' regions is qualitatively similar to that obtained in a previous calorimetric study (Calhoun & Shipley, 1979). In addition, the peak temperature for the C_{16}SPH order–disorder phase transition obtained by DPH fluorescence (40.4 °C) is in excellent agreement with calorimetrically obtained values of 40.5 °C (Calhoun & Shipley, 1979) and 41.3 °C (Barenholz et al., 1976). Below the main phase-separation region, our results extend the observations of Calhoun and Shipley. Both our DPH fluorescence data (Figure 4) and our freeze–fracture electron micrographs (Figure 5) provide evidence for a transition between two gel phases in pure C_{16}SPH and its mixtures with DMPC. Unlike the P$\beta' \rightleftarrows$ Lβ' pretransition in phosphatidylcholines, this sphingomyelin pretransition converts one banded phase (presumed by our phase assignments to be Pβ') to another (termed β_2 in Figure 5B). The similarity of this gel-to-gel transition to the pretransition observed in mixtures of DMPC and DPPC may be seen by comparing the phase diagrams of Figures 5B and 7. Below the pretransition region in C_{16}SPH/DMPC mixtures, however, the similarity with DPPC/DMPC bilayers ends due to the distinctly different low-temperature gel phases formed by C_{16}SPH and DMPC. This difference should result in gel-phase inhomogeneities. Our freeze–fracture electron micrographs indicate that the expected inhomogeneous or two-phase regions occur at both low and high C_{16}SPH content, as we have noted in Figure 5B. At intermediate C_{16}SPH content, we found no evidence for coexisting phases but rather a single, banded phase (Figure 6g) that we have called β_1. This uniform intermediate phase may be related to the formation of gel-phase compounds previously reported in mixtures of egg phosphatidylcholine with bovine-brain sphingomyelin (Untracht & Shipley, 1977). Our proposed phase diagram is in disagreement with the report of Calhoun & Shipley (1979) of no gel-phase inhomogeneities in C_{16}SPH/DMPC mixtures as investigated by X-ray scattering. It may be that the X-ray scattering patterns of the two banded phases we have observed are sufficiently similar to obscure phase inhomogeneities. In addition, it may be that the low-temperature transition in pure C_{16}SPH or C_{16}SPH/DMPC mixtures is associated with so small an enthalpy change as to be undetectable by the calorimetric methods used by Calhoun & Shipley.

Certain cell membranes are thought to contain some separated gel phases, the formation of which is dependent upon membrane sphingomyelin content (Rintoul et al., 1979). For this reason, the special properties of sphingomyelin-rich gel-phase bilayers, as evidenced by the phase diagram in Figure 5B, could have significance in determining the properties of sphingomyelin-rich lateral domains within cell membranes.

[3] "Mottled" describes a morphology that is planar in the sense that it lacks bands or rivulettes but is grainy rather than perfectly smooth. An extremely mottled fracture face is described as "jumbled" in that disoriented swirls or "wormy" regions are present. Mottled and jumbled morphologies can occur in the same sample and may be interpreted as reflective of the same phase structure trapped at slightly different freezing rates.

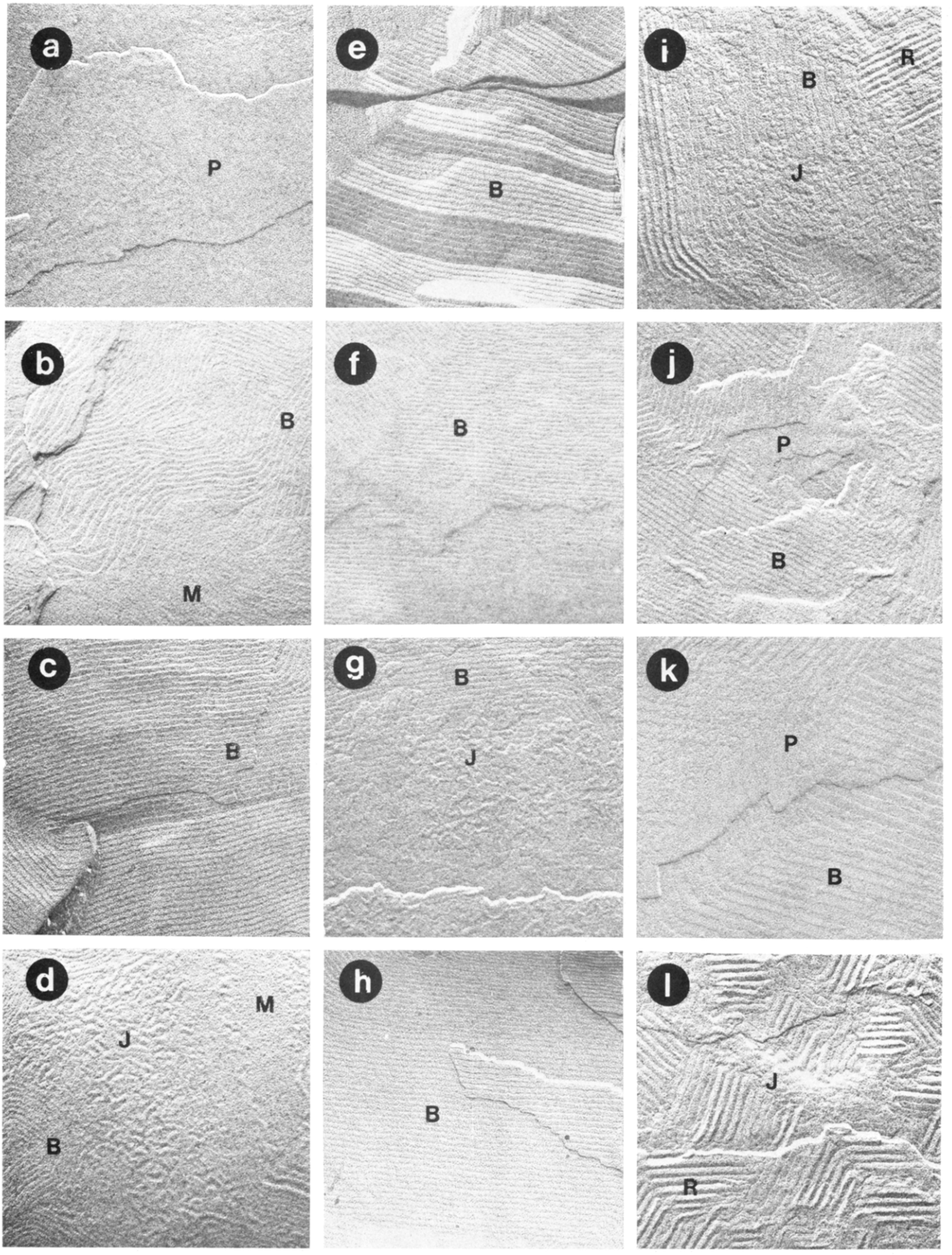

FIGURE 6: Electron micrographs of freeze–fracture replicas prepared from DMPC/C_{16}SPH large multilamellar vesicles quenched from several temperatures. Magnification 75 000×. Samples shown are the following: pure C_{16}SPH quenched from (a) 55 °C, (b) 43 °C, (c) 34.5 °C, (d) 30 °C, and (e) 20 °C; (f) pure DMPC from 18 °C; (g) 23 mol % C_{16}SPH from 28 °C; (h) 46 mol % C_{16}SPH from 22 °C; (i) 92 mol % C_{16}SPH from 29 °C; (j) 2 mol % C_{16}SPH from 5 °C; (k) 10 mol % C_{16}SPH from 7 °C; (l) 92 mol % C_{16}SPH from 13 °C. Fractured samples were platinum shadowed at an angle of 45°. The micrographs are shown with the shadowing from below.

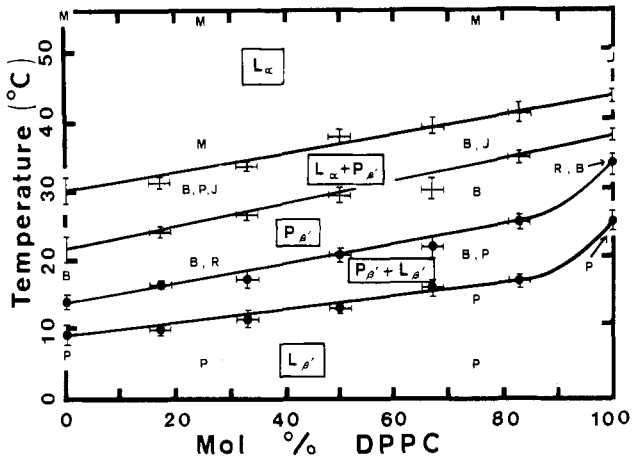

FIGURE 7: Temperature–composition diagram for DMPC/DPPC multilamellar vesicles. The delimiting-temperature data are reproduced from Lentz et al. (1976) with symbols consistent with Figure 5. The fracture-face morphologies obtained in this study are indicated as described for Figure 5. Proposed phase assignments[2] are shown in large letters.

Our results indicate that phosphatidylcholine and sphingomyelin species of nearly equal acyl chain length are completely miscible in the liquidus phase within both highly curved and essentially uncurved bilayers. It is especially interesting that the temperature–composition diagrams summarizing our results (Figures 2 and 5B) are quite similar to the corresponding small vesicle (Lentz et al., 1976) and large vesicle (Lentz et al., 1976; Mabrey & Sturtevant, 1976; Luna & McConnell, 1978) phase diagrams proposed for mixtures of DMPC with DPPC. Along with the observed invariance of acyl chain order with C_{16}SPH content (Figure 3), these observations suggest that phosphatidylcholine and sphingomyelin species of similar chain length make very similar contributions to membrane structure. The major distinction between the two lipids occurs in the difference between low-temperature gel phases. However, within fluid membrane domains, our results indicate that sphingomyelin and phosphatidylcholine are interchangeable with respect to their effect on membrane acyl chain order.

It has long been recognized that the total membrane content of sphingomyelin plus phosphatidylcholine often remains constant while the ratio of these two lipids may vary considerably with the physiological state of a cell (Barenholz & Thompson, 1980). The results reported here suggest a possible reason for this observation. We have shown that sphingomyelin is not likely to alter the acyl chain order of a phosphatidylcholine bilayer except as it alters the bilayer acyl chain content. The highly saturated acyl chain content of natural sphingomyelins is well documented (Barenholz & Thompson, 1980) and probably reflects the different enzymatic pathways involved in forming the ester linkages of glycerophosphatides as opposed to the amide bond of sphingomyelins. In addition, we have shown that sphingomyelin–phosphatidylcholine mixtures behave similarly to phosphatidylcholine–phosphatidylcholine mixtures. Taken together, these observations suggest that varying the sphingomyelin to phosphatidylcholine ratio would afford the cell the opportunity to vary membrane acyl chain order without affecting other bilayer structural features.

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