Biochimica et Biophysica Acta, 985 (1989) 199–210
Elsevier

BBAMEM 74582

Interdigitation of phosphatidylcholine and phosphatidylethanolamine mixed with complexes of acidic lipids and polymyxin B or polymyxin B nonapeptide

Joan M. Boggs [1], Hai-Ying Wang *, Godha Rangaraj [1] and Burkhard Tümmler [2]

[1] *Department of Biochemistry, Hospital for Sick Children, and Department of Clinical Biochemistry, University of Toronto, Toronto (Canada) and* [2] *Abteilung Biophysikalische Chemie Medizinische Hochschule, D-3000 Hannover 61 (F.R.G.)*

(Received 26 April 1989)

Key words: Spin label; Interdigitation; Lipid bilayer; Phase transition; Acidic lipid; Polymixin B; Polymyxin B nonapeptide

A fatty acid spin label, 16-doxyl-stearic acid, was used to determine the percent interdigitated lipid in mixtures of a neutral phospholipid and an acidic phospholipid. Interdigitation of the acidic lipid was induced with polymyxin B (PMB) at a mole ratio of PMB to acidic lipid of 1:5. This compound does not bind significantly to neutral lipids or induce interdigitation of the neutral lipids by themselves. The neutral lipids used were dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), or dipalmitoylphosphatidylethanolamine (DPPE), and the acidic lipids were dipalmitoylphosphatidylglycerol (DPPG) or dipalmitoylphosphatidic acid (DPPA). The percent interdigitated lipid was determined from the percent of the spin label which is motionally restricted, assuming that the spin label is homogeneously distributed in the lipid. Assuming further that 100% of the acidic lipid is interdigitated at this saturating concentration of PMB, the percentage of the neutral lipid which can become interdigitated along with it was calculated. The results indicate that about 20 mole % DPPC can be incorporated into and become interdigitated in the interdigitated bilayer of PMB/DPPG at 4°C. As the temperature approaches the phase transition temperature, the lipid becomes progressively less interdigitated; this occurs to a greater degree for the mixtures than for the single acidic lipid. Thus the presence of DPPC promotes transformation of the acidic lipid to a non-interdigitated bilayer at higher temperatures. At the temperature of the lipid phase transition little or none of the lipid in the mixture is interdigitated. Thus the lipid phase transition detected by calorimetry is not that of the interdigitated bilayer. The shorter chain length DMPC can be incorporated to a greater extent than DPPC, 30–50 mol%, in the interdigitated bilayer of PMB-DPPG. This may be a result of reduced exposure of the terminal methyl groups of the shorter myristoyl chains at the polar/apolar interface of the interdigitated bilayer. Less than 29% of the total lipid was interdigitated in a DPPC/DPPA/PMB 1:1:0.2 mixture indicating that none of the DPPC in this mixture becomes interdigitated. This is attributed to the lateral interlipid hydrogen bonding interactions of DPPA which inhibits formation of an interdigitated bilayer. DPPE was found to be incorporated into the interdigitated bilayer of PMB-DPPG to a similar extent as DPPC if the amount of PMB added is sufficient to bind to only the DPPG in the mixture. Differential scanning calorimetry showed that the remaining non-interdigitated DPPE-enriched mixture phase separates into its own domain. However, if enough PMB is added to bind to both the DPPE and the DPPG, the amount of DPPE which is interdigitated increases. Mixing of DPPE with other lipids apparently allows PMB to bind to DPPE and directly induce interdigitation of a portion of it. Polymyxin B nonapeptide did not cause interdigitation of DMPC or DPPE in mixtures with DPPG as well as PMB did.

* Present address: Physics Teaching and Research Section, Department of Basic Courses, Huazhong Agricultural University, Wuhan, Hubei Province, People's Republic of China.

Abbreviations: PMB, polymyxin B; PMBN, polymyxin B nonapeptide; DPPG, dipalmitoylphosphatidylglycerol; DPPA, dipalmitoylphosphatidic acid; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DPPE, dipalmitoylphosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PA, phosphatidic acid; ESR, electron spin resonance; NMR, nuclear magnetic resonance; DSC, differential scanning calorimetry.

Correspondence: J.M. Boggs, Biochemistry Department, Hospital for Sick Children, 555 University Ave., Toronto, Ontario, Canada M5G 1X8.

Introduction

The antibiotic polymyxin B (PMB) has been shown by X-ray diffraction to induce interdigitation of acidic phospholipids, to which it binds in a 1 : 5 mole ratio of PMB to lipid [1,2]. In the preceding paper [3], we used spin labels to estimate the percentage of interdigitated lipid at nonsaturating concentrations of PMB added to dipalmitoylphosphatidylglycerol (DPPG) and dipalmitoylphosphatidic acid (DPPA). Long chain spin labels with the nitroxide group near the terminal methyl of the chain become motionally restricted in the interdigitated bilayer [4–7]. The percentage of interdigitated lipid can be estimated from the percentage of the spin label which is motionally restricted, assuming that it is evenly distributed in the interdigitated and non-interdigitated domains. We showed that significant amounts of PMB-unbound lipid can be incorporated into the interdigitated bilayer of PMB-bound lipid provided that the acidic lipid, such as DPPG, does not participate in lateral interlipid hydrogen bonding interactions [3]. If the lipid can participate in these interactions, as is the case for DPPA at neutral pH, unbound lipid becomes phase separated from the interdigitated PMB-bound lipid into its own domain of non-interdigitated bilayer, where interlipid hydrogen bonding can take place.

In the present paper we similarly use the spin label 16-doxyl-stearic acid to determine whether a lipid which does not bind to PMB, phosphatidylcholine (PC), can be incorporated into the interdigitated bilayer of the complexes of PMB with the acidic phospholipids, dipalmitoylphosphatidylglycerol (DPPG) and dipalmitoylphosphatidic acid (DPPA). We also investigate the effect of the chain length of the PC on its ability to become interdigitated with the DPPG, using dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC). A similar study was carried out using mixtures of DPPG with dipalmitoylphosphatidylethanolamine (DPPE) at pH 6, with the initial assumption that PMB does not bind to DPPE. PMB has no effect on pure DPPE at this pH, supporting this assumption. However, we found that PMB causes a greater percentage of the lipid to become interdigitated in DPPG/DPPE mixtures than in DPPG/DPPC mixtures, if enough PMB is added to saturate both the DPPE and the DPPG. This suggests that it binds to DPPE when mixed with other lipids, although not to pure DPPE at this pH.

Other groups have also investigated the ability of dissimilar lipids to be incorporated into an interdigitated bilayer. An X-ray diffraction study showed that at an equimolar ratio of DMPC to DPPG, 50 mol% DMPC can be incorporated into the interdigitated bilayer of PMB/DPPG [2]. However, higher concentrations of DMPC were not studied, nor was the effect of the chain length of the PC. X-ray diffraction studies also showed that 30–50 mol% of the normally non-interdigitating DPPC can be incorporated into an interdigitated bilayer of the dialkyl form of PC, dihexadecylphosphatidylcholine [8,9]. However, only 11 mol% of the hydrogen bonding lipid dilauroylphosphatidylethanolamine can be incorporated into the interdigitated bilayer of DPPC when interdigitation is induced with ethanol, a substance which does not cause interdigitation of pure PE [10,11].

Materials and Methods

Dipalmitoylphosphatidylglycerol (DPPG) was from Calbiochem, La Jolla, CA, and dipalmitoylphosphatidic acid (DPPA) was from Avanti Polar Lipids, Birmingham, AL. Dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) were from Sigma Chemical. Dipalmitoylphosphatidylethanolamine (DPPE) was from Fluka. Polymyxin B sulfate was from Burroughs Wellcome, Inc., Kirkland, Quebec, Canada. Polymyxin B Nonapeptide (PMBN) was prepared as described previously [7] and was 47% pure peptide and 53% NaCl. The fatty acid spin label, 16-doxyl-stearic acid was from Syva, Palo Alto, CA.

Spin-labeled (16-doxyl-stearic acid) complexes of PMB with DMPC/DPPG and DPPC/DPPG mixtures at pH 7.4, and with DPPE/DPPG and DPPC/DPPA mixtures at pH 6 were prepared and studied by electron spin resonance (ESR) spectroscopy and differential scanning calorimetry (DSC) as described in the preceding paper [3]. Some samples were also prepared by dissolving the lipids and PMB together in benzene, freezing the benzene solution and lyophilizing the benzene [20]. Similar results were obtained as when the samples were prepared by evaporating chloroform/methanol solutions of the mixtures. Before measuring ESR spectra the samples were first heated to a temperature above their phase transition temperatures. The temperature chosen for measurement of most of the spectra was 4°C, lower than in the preceding paper, because of the lower transition temperature of DMPC. At this temperature, the probe in the lipids in the absence of PMB was a little more motionally restricted than at 9°C, so that the contribution of non-interdigitated lipid to the spectra of the samples containing PMB was not as obvious on visual inspection of the spectra as at 9°C. However, it was still possible to resolve the spectra into a motionally restricted component with T_{max} value of 29–31 G, characteristic of an interdigitated bilayer, and a more mobile component, characteristic of the pure lipid mixture. Addition of the mobile and motionally restricted components by varying the mole fraction of each, F^X and F^Y, in increments of 0.05 as described in the preceding paper [3] showed that all the result spectra obtained were clearly different from each other.

The percent PC or PE which becomes interdigitated was determined as the ratio of $F^{Y}_{PC,PE}$ to the mole fraction of PC or PE in the mixture, multiplied by 100, where $F^{Y}_{PC,PE}$ is the contribution of PC or PE to the mole fraction of the total lipid which is interdigitated. The latter is given by $F^{Y}_{PC,PE} = F^{Y} - F^{Y}_{PG}$ where F^{Y}_{PG} is the contribution of the DPPG-PMB to the mole fraction of the total lipid which is interdigitated and is assumed to be equal to the mole fraction of DPPG-PMB in the mixture. The mol% PE or PC which becomes interdigitated and incorporated into the interdigitated bilayer of the DPPG/PMB was determined as the ratio of $F^{Y}_{PC,PE}$ to F^{Y}, multiplied by 100, where F^{Y} is the mole fraction of the total lipid which is interdigitated and determined as described in the preceding paper [3].

Results

Mixtures of DPPG-PMB with DPPC or DMPC

DPPG is ideally miscible with DPPC [22] and gives a single sharp transition at an equimolar ratio as shown in Fig. 1b. It is nearly ideally miscible with DMPC and at an equimolar ratio gives a somewhat broader gel to liquid crystalline phase transition at a temperature about halfway in between those of the individual components (Table I). When PMB is added to these mixtures at DPPG/DPPC/PMB and DPPG/DMPC/PMB 1:1:0.2 ratios, it results in the double-peaked transition characteristic of the 1:5 complex of PMB with DPPG [2,3,5] (Fig. 1c and h). However, at higher concentrations of DPPC or DMPC, only a single peak can be detected as indicated in Fig. 1 and Table I. Peak 2 of the thermogram of the DPPG/DPPC/PMB 1:1:0.2 sample could be equivalent to peak 2 of the DPPG/PMB 5:1 sample, i.e. a result of hydrophobic and electrostatic interactions of PMB with the lipid mixture, or it could be due to a domain of DPPC phase separated from the mixture. However, its temperature is less than that of DPPC (Table I) supporting the former conclusion. The transition temperature of the complex of PMB with all DPPG/DPPC mixtures is less than those of DPPG, DPPC, or combinations of these lipids in the absence of PMB, suggesting that PMB does not cause phase separation of a domain of pure DPPC from a domain of pure DPPG-PMB, at least not at the temperature of the phase transition. PMB has no effect on the transition of DPPC at a 1:5 mole ratio.

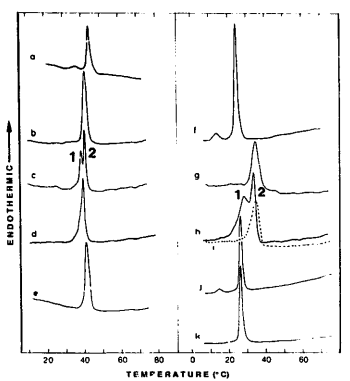

Fig. 1. DSC heating thermograms of (a) DPPC; (b) DPPG/DPPC/PMB 1:1:0; (c) DPPG/DPPC/PMB 1:1:0.2; (d) DPPG/DPPC/PMB 1:2:0.2; (e) DPPG/DPPC/PMB 1:4:0.2; (f) DMPC; (g) DPPG/DMPC/PMB 1:1:0; (h) DPPG/DMPC/PMB 1:1:0.2; (i) DPPG/DMPC/PMBN 1:1:0.2 (dashed line); (j) DPPG/DMPC/PMB 1:4:0; (k) DPPG/DMPC/PMB 1:4:0.2. For a scan of DPPG see the preceding paper [3] (Fig. 1a). All samples at pH 7.4, heated at 10 C°/min. Sensitivity settings in mcal/s were (a,c,e,g–i) 0.3; (b) 0.5; (d,j) 0.8; (f,k) 1.5. Different amounts of sample were used so the peak areas of different scans cannot be compared.

TABLE I

Effect of PMB and PMBN on the phase transition temperatures of mixtures of DPPG or DPPA with phosphatidylcholine containing palmitoyl or myristoyl chains [a]

Sample (mole ratio)	T_m (°C)
DPPG/DPPC/PMB	
1:1:0 [b]	41.6
1:1:0.2	39.9, 41.4
1:2:0.2	39.8
1:3:0.2	40.4
1:4:0.2	40.9
1:4:1	40.9
1:0:0	41.1
0:1:0	43.3
0:1:0.2	43.0
DPPG/DMPC/PMB	
1:1:0	34.8
1:1:0.2	28.3, 33.5
1:4:0	15.3, 26.2 [c]
1:4:0.2	26.3
0:1:0	24.0
DPPG/DMPC/PMBN	
1:1:0.2	34.4
DPPA/DPPC/PMB	
1:1:0	57.5
1:1:0.2	45.4, 54
1:0:0	65.7
1:0:0.2	49.3

[a] At a heating rate of 10 C°/min, pH 7.4 for DPPG and pH 6 for DPPA.

[b] 1:2:0, 1:3:0, and 1:4:0 samples have similar T_m values.

[c] Major peak is underlined - the lower temperature peak is probably the premelt.

TABLE II

Distribution of lipid on a sucrose density gradient

Sample (mole ratio)	% lipid at different sucrose levels			
	0–20% sucrose	25% sucrose	30% sucrose	35% sucrose
DPPG	88.2	6.4	3.0	2.4
DPPC	91.6	3.7	1.9	2.8
DPPG/DPPC/PMB 1:1:0.2	4.3	91.8	2.7	1.1
DPPG/DMPC/PMB 1:1:0.2	2.3	96.5	0.7	0.6
DPPG/PMB 1:0.2	0	0	1.1	98.3
DPPG/DPPE/PMB 1:1:0	13.5	85.0	1.5	0
DPPG/DPPE/PMB 1:1:0.2 [a]	0.1	0.1	99.6	0.2
DPPG/DPPE/PMB 1:3:0	98.3	1.7	0	0
DPPG/DPPE/PMB 1:3:0.2	1.7	97.4	0.9	0

[a] The 1:1:0.4 sample behaves similarly.

The transition temperature of the complex of PMB with DPPG/DMPC is also less than or similar to that of the lipid mixture, in contrast to results reported by Theretz et al. [2]. The peaks are broader and the difference in transition temperatures of the two peaks for the DPPG/DMPC/PMB 1:1:0.2 sample (Fig. 1h) is greater than for the sample containing DPPC (Fig. 1c). Although the thermogram in Fig. 1h has a low temperature shoulder that could be due to phase separated DMPC, there is no corresponding upward shift due to a phase separated domain enriched in DPPG. Indeed, peak 2 of this scan occurs at a lower temperature than for the lipid mixture in the absence of PMB. This suggests that PMB does not cause phase separation of a DPPG-enriched domain from DMPC. Centrifugation of the DPPG/DPPC/PMB and DPPG/DMPC/PMB 1:1:0.2 samples on a discontinuous sucrose gradient gave only 1 band at a density intermediate between that for DPPC or DPPG alone and the DPPG-PMB 1:0.2 complex (Table II). However, this does not rule out the occurrence of macroscopic phase separation since both DPPC or DMPC bilayers could occur trapped within the same multilayered vesicles as DPPG/PMB bilayers. For the DPPG/DMPC/PMB 1:4:0.2 sample no transition at a lower or higher temperature appears (Fig. 1k) also suggesting that PMB does not cause phase separation of the DPPG from the DMPC. Addition of the nonapeptide of PMB (PMBN) results in only a single transition at a similar temperature as the lipid mixture (Fig. 1i). This is identical to the effect of PMBN on pure DPPG [3,5] and indicates that PMBN also does not cause phase separation of these two lipids.

ESR spectra of 16-doxyl-stearic acid in complexes of DPPG/DPPC/PMB at 1:1:0, 1:1:0.2, and 1:4:0.2 ratios at 4°C are compared to that in DPPG/PMB at a 1:0.2 ratio in Fig. 2. Both spectra of the DPPG/DPPC/PMB samples (Fig. 2B,C) contain significant amounts of a motionally restricted component, indicating that a large percentage of the lipid is interdigitated. In order to quantitate the percentage of interdigitated lipid, the amount of motionally restricted component was determined by pairwise spectral subtraction as described in the preceding paper [3]. The results of subtracting the 1:1:0.2 and 1:4:0.2 spectra (Fig. 2B and C) from each other are shown in Fig. 2D and E, and

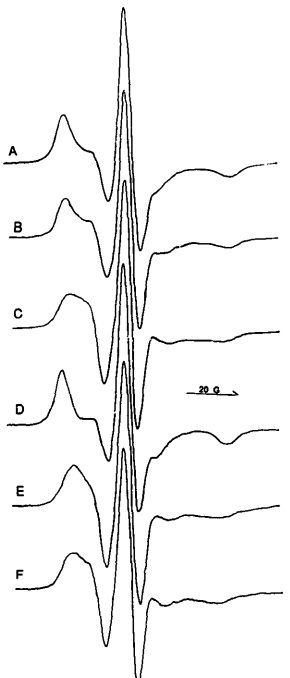

Fig. 2. ESR spectra of 16-doxyl-stearic acid at 4°C in (A) DPPG/PMB 1:0.2; (B) DPPG/DPPC/PMB 1:1:0.2; (C) DPPG/DPPC/PMB 1:4:0.2; (D) the result of subtracting the spectrum of the 1:4:0.2 sample from that of the 1:1:0.2 sample; (E) the result of subtracting the spectrum of the 1:1:0.2 sample from that of the 1:4:0.2 sample; (F) DPPG/DPPC/PMB 1:1:0. All samples are at pH 7.4. All spectra shown are normalized to the same center peak height for visual comparison. They were not normalized before spectral subtraction.

resemble, although are not identical to, the spectra of the pure lipid mixture (Fig. 2F) and the DPPG-PMB 1 : 0.2 complex (Fig. 2A), respectively. The T_{max} value of the motionally restricted component in these spectra is 30.1 G. T_{max} of the fluid component is 25.8 G in contrast to 26.0 G for the pure lipid mixture (Fig. 2A). The percentage of the lipid which is interdigitated is given in Table II and is somewhat greater than the maximal amount of lipid which is PMB-bound, as found for DPPG with varying concentrations of PMB [3]. Assuming that the DPPG in the mixture is 100% interdigitated in all cases, and using the midpoint of the

TABLE III

Percentage of lipid which is interdigitated in mixtures of PC or PE with PMB- or PMBN-DPPG complexes [a]

Sample (mole ratio)	Mol% bound lipid [b]	% Total inter-digitated lipid [c]	% PC or PE inter-digitated [d]	Mol% inter-digitated PC or PE [e]
DPPG/DPPC/PMB				
1:1:0.2	50	56–69	24	19
1:2:0.2	33	35–41	8	13
1:3:0.2	25	26–38	9	22
1:4:0.2	20	19–31	6	20
DPPG/DMPC/PMB				
1:1:0.2	50	72–78	50	34
1:4:0.2	20	41–52	32	56
DPPG/DPPE/PMB				
1:1:0.2	50	65–71	36	26
1:3:0.2	25	31–32	8	19
1:1:0.4	[f]	77–79	56	36
1:3:0.8	[f]	42–47	25	43
DPPC/DPPE/PMB				
1:1:0.2	[g]	0	0	0
DPPG/DMPC/PMBN				
1:1:02	50	55	7	7
DPPG/DPPE/PMBN				
1:1:0.2	50	60.3–61.4	22	18
1:3:0.2	25	20.2–20.4	0	0
1:1:0.4	[f]	65.8–69.5	36	26
1:3:0.8	[f]	21.8–22.0	0	0

[a] At 4°C as determined by percentage of 16-doxyl-stearic acid which is motionally restricted.

[b] Which is maximally bound to PMB or PMBN, assuming they bind to the acidic lipid in a 1:5 ratio.

[c] Percentage of the total lipid which is interdigitated.

[d] Percentage of the PC or PE which is interdigitated, assuming that 100% of the DPPG is. Calculated using the midpoint of the range of values given in the middle column.

[e] Mol% of PC or PE incorporated into the interdigitated bilayer of DPPG. Calculated using the midpoint of the range of values given in the previous column.

[f] There is enough PMB or PMBN to bind to 100% of the DPPG plus DPPE in the sample. However, it is not known how much is actually bound.

[g] There is enough PMB to bind to all the DPPE but it is not known how much is actually bound.

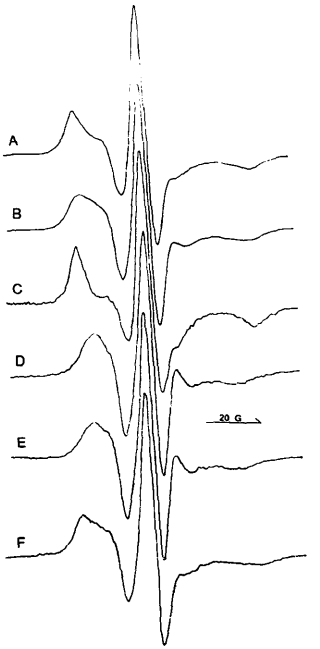

Fig. 3. ESR spectra of 16-doxyl-stearic acid at 4°C in (A) DPPG/DMPC/PMB 1:1:0.2; (B) DPPG/DMPC/PMB 1:4:0.2; (C) the result of subtracting the spectrum of the 1:4:0.2 sample from that of the 1:1:0.2 sample; (D) the result of subtracting the spectrum of the 1:1:0.2 sample from that of the 1:4:0.2 sample; (E) DPPG/DMPC/PMB 1:1:0; (F) DPPG/DMPC/PMBN 1:1:0.2. All samples are at pH 7.4. All spectra shown are normalized to the same center peak height for visual comparison. They were not normalized before spectral subtraction.

range of values given in Table III for the percent interdigitated lipid, the mol% DPPC interdigitated and incorporated into the interdigitated DPPG/PMB bilayer was calculated (Table III). The results show that about 20 mol% DPPC can be incorporated into the interdigitated PMB/DPPG bilayer at 4°C. The rest must phase separate into a non-interdigitated domain at this temperature.

At 29°C, the percentage of the spin label which is motionally restricted in the DPPG/DPPC/PMB

1 : 1 : 0.2 sample is reduced from about 61% at 4°C to 36% in contrast to a reduction from 66% at 9°C to 56% at 29°C for the DPPG/PMB 1 : 0.1 sample (data not shown). At 34°C, the percentage of motionally restricted spin label is reduced further, suggesting that little or no lipid in the mixture is interdigitated at the temperature of the lipid phase transition.

ESR spectra of DPPG/DMPC/PMB mixtures at 4°C are shown in Fig. 3. Qualitative comparison of the spectra of the 1 : 1 : 0.2 and 1 : 4 : 0.2 samples (Fig. 3A,B) with the corresponding spectra of DPPG/DPPC/PMB mixtures in Fig. 2B,C suggests that a greater percentage of the lipid is interdigitated in the DPPG/DMPC mixtures than in DPPG/DPPC mixtures. This is confirmed by resolution of the spectra of the DPPG/DMPC/PMB samples into a motionally restricted component (T_{max} value 30.2 G) (Fig. 3C) and a more mobile component (T_{max} 24.5 G) (Fig. 3D) similar to the spectrum of the pure lipid mixture (Fig. 3E) (T_{max} 24.3 G). The percentage of the lipid which is interdigitated in these samples at 4°C is given in Table III and indicates that considerably more lipid than that which is maximally bound to PMB is interdigitated. 34–56 mol% DMPC can be incorporated into the interdigitated bilayer of PMB-bound DPPG (Table III), in contrast to DPPC, even though the temperature of measurement, 4°C, is closer to the phase transition temperature of the DPPG/DMPC mixture than the DPPG/DPPC mixtures.

The ability of PMBN to induce interdigitation of DPPG/DMPC was compared with that of PMB. The spectrum of 16-doxyl-stearic acid in DPPG/DMPC/PMBN 1 : 1 : 0.2 at 4°C is shown in Fig. 3F and clearly indicates that less lipid is interdigitated than in the presence of PMB (compare to Fig. 3A). This is confirmed by obtaining the motionally restricted component in the spectrum by subtracting the spectrum of the lipid mixture (Fig. 3E) from that in Fig. 3F. The result shown in Table III indicates that in the presence of PMBN, very little lipid other than that which is PMBN-bound is interdigitated. Only 7 mol% DMPC can be incorporated into the interdigitated bilayer of PMBN-bound DPPG (Table III).

Mixture of DPPA-PMB with DPPC

DSC scans of DPPA/DPPC 1 : 1 with and without PMB (20 mol% with respect to DPPA) at pH 6, prepared by lyophilization from benzene, are shown in Fig. 4. DPPA is relatively miscible with DPPC, although mixing is not ideal [23]. Thus an equimolar mixture has a single broad asymmetric gel to liquid crystalline phase transition at 57.5°C. This is a little more than halfway between the transition temperatures of the individual components (Fig. 4a and Table I). DPPC-enriched and DPPA-enriched domains are present contributing to the width of the transition. On addition of PMB the transition narrows some and is lowered to 45.4°C with a small shoulder at higher temperatures. There are no transitions at the temperatures of pure DPPC or the DPPA-PMB 5 : 1 complex (43°C and 49.3°C, respectively). However, the peak in Fig. 4b is broad enough to encompass transitions due to a DPPC-enriched domain containing some DPPA thus raising its T_m a little, and a DPPA-PMB-enriched domain containing some DPPC, thus lowering its T_m. Scanning at a slower rate (2.5 C°/min) did not resolve this broad peak into two. The shoulder at higher temperatures may be due to some DPPA/DPPC which is not bound to PMB.

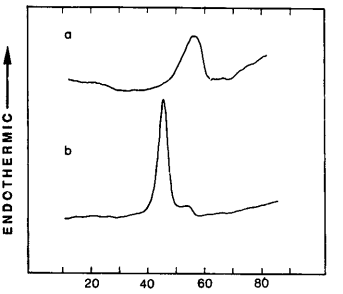

Fig. 4. DSC heating thermograms at 10 C°/min of DPPA/DPPC/PMB at pH 6 and at mole ratios (a) 1 : 1 : 0; (b) 1 : 1 : 0.2. For a scan of DPPA at pH 6 see the preceding paper [3] (Fig. 1g). Sensitivity settings in mcal/sec were 0.3 and 0.8, respectively. Different amounts of sample were used so the peaks areas of different scans cannot be compared.

ESR spectra of 16-doxyl-stearic acid in these samples at 8°C are shown in Fig. 5A,B and reveal that PMB has only a small effect on the spectrum. Subtraction of the spectrum of the lipid mixture from that of its complex with PMB gave the spectral component shown in Fig. 5C. This component is 29% of the spectrum of the PMB complex. The probe giving this component is more motionally restricted than in the pure lipid mixture; the T_{max} value is 27.2 G in contrast to 22.8 G for the lipid mixture. This is not as large a T_{max} value as expected if the lipid is interdigitated. A value of 28.4 G was obtained for the DPPA-PMB 1 : 0.1 sample [3]. However, it is difficult to envision any other mechanism which could cause this large degree of motional restriction. If this component is due to a population of interdigitated lipid, the amount of this population must be less than 29% since the spectrum in Fig 5C also contains another more fluid component which could not be removed. Thus less DPPA is induced to interdigitate in the pres-

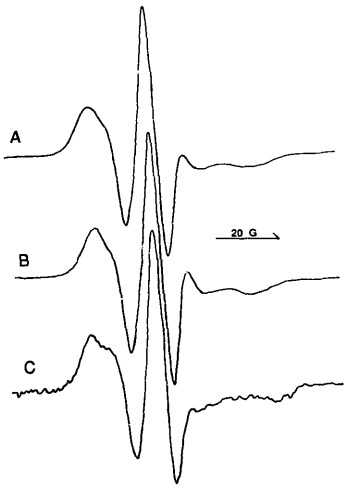

Fig. 5. ESR spectra of 16-doxyl-stearic acid at 8°C, pH 6, in (A) DPPA/DPPC/PMB 1:1:0.2; (B) DPPA/DPPC/PMB 1:1:0; (C) the result of subtracting the spectrum of the 1:1:0 sample from that of the 1:1:0.2 sample. All spectra shown are normalized to the same center peak height. They were not normalized before subtraction.

ence of DPPC than in its absence in contrast to results found for DPPG in the presence of DPPC.

Mixtures of DPPG-PMB with DPPE

DSC scans of DPPG/DPPE 1:1, 1:2, and 1:3 mixtures at pH 6, with and without PMB are shown in Fig. 6. Phase diagrams of DPPG/DPPE mixtures have not been previously reported. The fact that only one peak is seen for all three mixtures in the absence of PMB indicates that the two lipids are relatively miscible (Fig. 6a,c,e). Surprisingly, the transition temperature is relatively close to that of pure DPPE (Fig. 6k), even for the 1:1 mixture (Table IV). In contrast, the 1:1 mixture of DPPC/DPPE which are less miscible than PG/PC or PA/PC [24], has a broad transition with a midpoint at a temperature about 7 C° lower (Fig. 6i). The high phase transition temperature of PE has been attributed to intermolecular hydrogen bonding between the phosphate and amine of neighboring molecules, while the low phase transition temperature of DPPG by itself indicates that intermolecular hydrogen bonding does not occur, even though PG has hydrogen donating (glycerol hydroxyls) and accepting (phosphate oxygens) groups (reviewed in Ref. 12). The high transition temperature of the DPPG/DPPE mixture suggests not only that they mix, but also that intermolecular hydrogen bonding between PG and PE may taken place (between the glycerol of PG and the phosphate of PE, and between the phosphate of PG and the amine of PE). Mixing of the neutral PE with the negatively charged PG apparently decreases charge repulsion sufficiently that the PG can participate in hydrogen bonding with the PE.

In the presence of PMB (20 mol% with respect to DPPG), however, phase separation of DPPG and DPPE occurs, at least at the temperature of the phase transition. A broad double-peaked transition at 39–41°C is seen for all three mixtures, corresponding to the DPPG-PMB complex (Fig. 6b,d,f). The percentage of the heat absorbed in this double transition is 50, 28, and 24% for the 1:1:0.2, 1:2:0.2, and 1:3:0.2 mixtures, respectively, (Table IV) suggesting that only the DPPG-PMB complex contributes to this transition. Transitions at higher temperatures are also seen indicating the presence of DPPE-enriched domains. However, except for the 1:3 complex, the temperatures of all of these are

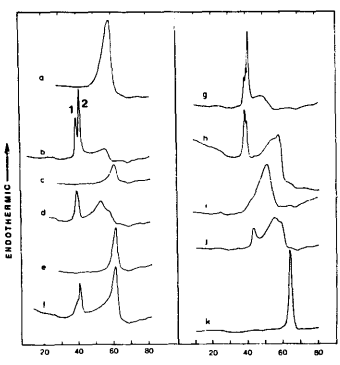

Fig. 6. DSC heating thermograms of DPPG/DPPE/PMB at mole ratios of (a) 1:1:0; (b) 1:1:0.2; (c) 1:2:0; (d) 1:2:0.2; (e) 1:3:0; (f) 1:3:0.2; (g) 1:1:0.4; (h) 1:3:0.8; (i) DPPC/DPPE/PMB 1:1:0; (j) DPPC/DPPE/PMB 1:1:0.2; and (k) DPPE only. For scans of DPPG and DPPG/PMB 1:0.2 see the preceding paper [3] (Fig. 1a,b). All samples at pH 6 and heated at 10 C°/min. Sensitivity settings in mcal/s were (c–f,h,i) 0.3; (a,b,g,j) 0.5; (k) 0.8. Different amounts of sample were used so the peak areas of different scans cannot be compared.

TABLE IV

Effect of PMB and PMBN on the phase transition temperatures of mixtures of DPPE with DPPG or DPPC [a]

Sample (mole ratio)	T_m (°C)	% of heat observed in transitions at lower temperature [c]
DPPG/DPPE/PMB		
1:1:0	59	–
1:1:0.2	39.4, 41.4, 55.6 [b]	50
1:2:0	60.6	–
1:2:0.2	39.3, 53.1, 58	28
1:3:0	61.3	–
1:3:0.2	39.3, 40.4, 54, 60.8 [b]	24
1:1:0.4	39.3, 40.2, 41.2, 48.6 [b]	64
1:3:0.8	39.4, 40, 56, 58.7	23
0:1:0	65.5	–
1:0:0.2	39.8, 41.5	100
DPPC/DPPE/PMB		
1:1:0	51.9	–
1:1:0.2	44, 56.2, 58.5 [b]	–
DPPG/DPPE/PMBN		
1:1:0.2	43.3, 55.5	38
1:3:0.2	41.4, 55.8, 60.9 [b]	7
1:1:0.4	43.3, 55.5	38
1:3:0.8	41.4, 55.8, 60.9 [b]	7
1:0:0.2	42.1	100

[a] At a heating rate of 10 °C/min, pH 6.
[b] Major peaks are underlined.
[c] Temperature characteristic of DPPG-PMB or DPPG-PMBN.

less than those of pure DPPE or of the original lipid mixtures without PMB, suggesting that some PMB is also complexed with the DPPE-enriched domains. In the case of the 1:3:0.2 complex, half of the lipid has a similar transition temperature as the original lipid mixture, indicating that it is not complexed to PMB. PMB has no effect on the phase transition of pure DPPE at pH 6. However, when the DPPG/DPPE/PMB samples were centrifuged on a sucrose gradient, all of the lipid sedimented in a single sharp band with a density intermediate between that of the lipid mixture in the absence of PMB and that of the DPPG-PMB 1:0.2 complex (Table II). The 1:1:0.2 complex had a greater density than the 1:3:0.2 complex.

Resolution of the ESR spectra of these DPPG/DPPE/PMB samples, shown in Fig. 7, into motionally restricted and fluid components indicates that at 20 mol% PMB relative to the DPPG, the total amount of interdigitated lipid is similar to that found for DPPG/DPPC mixtures (Table III). The T_{max} value of the motionally restricted component (Fig. 7F) is 30.9 G. The spin label in the fluid component found in Fig. 7A-C after spectral resolution (shown in Fig. 7G) has a larger T_{max} value (26.7 G) than DPPE (25.8 G) (Fig. 7E). Assuming that all the DPPG is interdigitated, the results indicate that 19–26 mol% DPPE can be incorporated into the interdigitated PMB-DPPG bilayer, similar to DPPC.

At higher concentrations of PMB (20 mol% with respect to each of DPPG and DPPE), however, more of the spin label is motionally restricted as indicated by the appearance of the spectrum in Fig. 7C for a 1:3:0.8 sample (compare to Fig. 7B). Spectral resolution shows that more lipid is interdigitated than at concentrations of PMB sufficient to bind to only the DPPG, indicating that PMB also binds to some of the DPPE causing more of it to become interdigitated. Assuming that all of the DPPG is interdigitated, the results in Table III indicate that at a 1:1:0.4 ratio, 56% of the DPPE also becomes

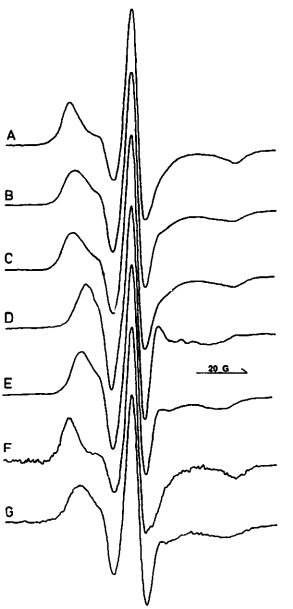

Fig. 7. ESR spectra of 16-doxyl-stearic acid at 4°C in DPPG/DPPE/PMB (A) 1:1:0.2; (B) 1:3:0.2; (C) 1:3:0.8; (D) 1:3:0; (E) DPPE only; (F) the result of subtracting the spectrum of the DPPG/DPPE/PMB 1:3:0.2 sample from that of the 1:3:0.8 sample; and (G) the result of subtracting the spectrum of the 1:3:0.8 sample from that of the 1:3:0.2 sample. All samples are at pH 6. All spectra shown are normalized to the same center peak height. They were not normalized before subtraction.

interdigitated in contrast to 56% at a 1:1:0.2 ratio. At a 1:3:0.8 ratio 25% of the DPPE is interdigitated in contrast to a value of 8% at a 1:3:0.2 ratio. The DSC scans also indicate that more of the lipid is affected by PMB at the higher concentration (Fig. 6g and h, respectively). For the 1:1:0.4 sample, 64% of the lipid has a transition temperature at 39–41°C. Indeed, three peaks can be seen in this range, in contrast to two for all other mixtures studied, suggesting that some DPPE-PMB may contribute to these transitions. The remaining lipid has a broad transition centered at 49°C, significantly lower than the original lipid mixture, indicating that it is DPPE-enriched lipid bound to PMB. Indeed, the transition temperature is similar to that of the complex of PMB with DPPA [3]. The 1:1:0.4 complex behaved similarly to the 1:1:0.2 complex on the sucrose gradient. For the 1:3:0.8 sample, the peak at 39–40°C still contains only 23% of the heat absorbed suggesting that only the PMB-DPPG contributes to this transition. However, the rest of the lipid has transitions at 56–59°C, a lower temperature than in the 1:3:0.2 sample, indicating that it results from a DPPE-enriched mixture bound to some PMB.

PMB has no effect on the spectrum of 16-doxylstearic acid in DPPE at pH 6. The significant effect of PMB on DPPE in DPPG/DPPE mixtures in contrast to its lack of effect on pure DPPE at pH 6, suggests that binding of PMB to DPPE may be increased in the presence of other lipids. Therefore, the effect of PMB on a DPPC/DPPE mixture was studied. The DSC scan of DPPC/DPPE/PMB 1:1:0.2 is shown in Fig. 6j.

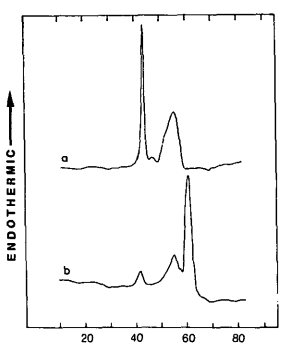

Fig. 8. DSC heating thermograms at 10 C°/min of DPPG/DPPE/PMBN, pH 6, at mole ratios of (a) 1:1:0.4 and (b) 1:3:0.8. Sensitivity setting for both samples was 0.5 mcal/s.

The mixture is clearly affected much more by PMB than either lipid by itself. Instead of the single transition at 52°C found for this lipid mixture in the absence of PMB (Fig. 6g), two transitions at 44°C and 56–59°C are observed. The first is a little greater than that of pure DPPC while the second is at a similar temperature as the transitions observed for the DPPG/DPPE/PMB samples (Table IV). Indeed this scan resembles that of the DPPG/DPPE/PMB 1:3:0.8 sample in Fig. 6h. There is no transition at the temperature of pure DPPE. Thus PMB binds to and affects more of the DPPE in the mixture with DPPC than in pure DPPE, and causes its phase separation from a DPPC-enriched domain in the mixture. Spectral subtraction showed that the spectrum of the DPPC/DPPE/PMB sample does not contain any motionally restricted component. Thus the presence of the DPPC may inhibit interdigitation of the PMB-bound DPPE even though it apparently increases the binding of PMB to DPPE. These results support the conclusion that in the DPPG/DPPE/PMB sample, PMB also binds to more of the DPPE than in the absence of DPPG.

Effect of PMBN on DPPG/DPPE mixtures

PMBN does not induce interdigitation of as much lipid in DPPG/DPPE mixtures as PMB, particularly at high DPPE concentrations (Table III). At a concentration of 20 mol% with respect to the DPPG only, it causes interdigitation of more than 50% of the total lipid for the DPPG/DPPE 1:1 mixture indicating that some of the DPPE is also interdigitated. However, for the 1:3 mixture, less than 25% of the lipid is interdigitated suggesting that only the DPPG in the mixture becomes interdigitated. In contrast to PMB, addition of enough PMBN to bind to both lipids (20 mol% with respect to each lipid) did not increase the amount of interdigitated lipid. These results suggest that PMBN does not bind to as much of the DPPE in the mixture as PMB does.

This is supported by DSC scans of 1:1:0.4 and 1:3:0.8 samples shown in Fig. 8. Scans of 1:1:0.2 and 1:3:0.2 samples were virtually identical to these. At a 1:1:0.4 ratio there are two peaks at 43.3°C and 55.5°C. The former is similar to the transition temperature of the DPPG-PMBN complex [3]. However, the heat absorbed is only 38% of the total indicating that some DPPG must be mixed with the DPPE giving the second peak. Therefore the higher temperature transition must be that of a DPPG/DPPE/PMBN mixture. At a 1:3:0.8 ratio, however, there is a large peak at 60.9°C, the same temperature as the original lipid mixture. The area is 55–60% of the total. Thus half of the lipid is not bound to PMBN. Furthermore, only 7% of the total heat is absorbed at the temperature of the DPPG-PMBN complex. The remainder of the lipid undergoes a transition at 55.8°C, the temperature of the DPPG/DPPE/

PMBN complex. This indicates that PMBN does not bind to this mixture as well as PMB does and also has less ability to cause phase separation of the DPPG from the mixture.

Discussion

In the preceding paper we showed that significant amounts of PMB-unbound DPPG can be incorporated into an interdigitated bilayer of PMB-bound DPPG, although most of the unbound DPPG is not interdigitated. In the present study, using mixtures of dissimilar lipids, PMB-bound DPPG with PMB-unbound PC, we show that the amount of DPPC which becomes interdigitated is somewhat less, particularly as the ratio of DPPC to acidic lipid increases. However using a shorter chain length species of PC, DMPC, we found that a considerably greater amount of PC becomes interdigitated. Indeed, the amount of DMPC which can be incorporated into the interdigitated bilayer of DPPG-PMB is even greater than that found for PMB-unbound DPPG [3]. Theretz et al. [2], found that at an equimolar ratio of DPPG/DMPC most of the DMPC was interdigitated, in contrast to the value of 50% obtained using the spin label. It is possible that determination of the % interdigitated lipid from the % spin label which is motionally restricted underestimates the amount of interdigitated lipid because of somewhat greater partitioning in a non-interdigitated bilayer of PC or PG [3]. However, even if this occurs it should not affect the relative differences found for the different lipid compositions studied here.

As argued earlier for myelin basic protein [4] and other amphipathic substances [21], PMB probably induces interdigitation by causing lateral separation of the lipids following penetration of its hydrophobic amino acid side chains and fatty acyl tail partway into the lipid bilayer. The fact that PMBN without the fatty acyl tail, can also cause interdigitation [7] indicates that only hydrophobic amino acid side chains are necessary, although the acyl tail of PMB may help maintain the interdigitated bilayer, especially at higher temperatures or in the presence of PMB-unbound lipids [3]. Interdigitation of the lipid fatty acid chains restores the van der Waals interactions between the lipid molecules and stabilizes the bilayer, which would otherwise be disordered by the amphipathic substance. The presence of the amphipathic substance at the hydrophobic-polar interface may help shield the hydrophobic terminal methyl groups of the lipid fatty acid chains from exposure to water in the interdigitated bilayer. This protection may be less important for the shorter chain length DMPC than DPPC in interdigitated bilayers of DPPG or DPPA, since the terminal methyl of the shorter myristoyl chain on the PC will not protrude as closely to the apolar-polar interface of the interdigitated DPPG bilayer as the longer palmitoyl chain. This may account for the fact that more DMPC can be incorporated into the interdigitated bilayer than DPPC.

The fact that most of the DPPC and at least half of the DMPC in mixtures with PMB-DPPG is not interdigitated, means that it must be phase separated into its own non-interdigitated domain, at least at the low temperatures where interdigitation occurs. This phase separation may be into large domains within the interdigitated bilayer or into a separate bilayer structure. The latter seems more likely but could not be confirmed using sucrose density gradient centrifugation to separate the two structures. However, non-interdigitated DPPC bilayers could be trapped within interdigitated bilayers in the multilayered vesicles. Freeze-fracture [7] and freeze-etch [13] micrographs of PA/PC/PMB mixtures have been interpreted to indicate the presence of domains of PA-PMB complex within the PC bilayer. However, it is not known if the PA-PMB complex was interdigitated under the conditions used in these studies.

Although lateral phase separation must occur at low temperatures, it is not apparent from the DSC scans and thus may not occur at the temperature of the phase transition. The two lipids may mix more randomly when the lipid gradually transforms to a non-interdigitated bilayer as the temperature approaches the phase transition temperature. The DPPG/DPPC/PMB sample transforms to a noninterdigitated bilayer more completely than DPPG/PMB, indicating that the presence of DPPC promotes transformation of the DPPG to a non-interdigitated bilayer at higher temperatures. Alternatively, phase separation may still be present at the temperature of the phase transition but undetectable by DSC because of the similarity of the phase transition temperatures of the PMB-bound lipid and the PC-enriched populations. An NMR study indicated that PMB causes phase separation of a DMPG-enriched domain from a DMPC-enriched domain in the liquid crystalline phase [14]. Phase separation in the gel phase, however, should result in a small increase in the T_m of the DPPG/DPPC/PMB samples due to a DPPC-enriched domain, and should result in a transition at a higher temperature for the DPPG/DMPC/PMB samples due to a DPPG-PMB-enriched domain. This is not observed.

The effect of PMB on DPPA/DPPC and DPPG/DPPE mixtures was studied in order to determine the behavior of lipids which can interact intermolecularly by hydrogen bonding. Since lateral interlipid hydrogen bonding can not take place in an interdigitated bilayer, it should stabilize the non-interdigitated bilayer, as found in the preceding paper for PMB-unbound DPPA at pH 6, when nonsaturating concentrations of PMB are added to DPPA [3]. In mixtures of DPPA with DPPC, although some of the DPPA may have become interdig-

itated, much of it did not as found for complexes of DPPA-PMB alone [3]. The low maximum percentage of the total lipid which could have been interdigitated indicates that the DPPC in the mixture did not become interdigitated. Thus intermolecular hydrogen bonding of the DPPA inhibited interdigitation in the mixture as in pure DPPA.

Surprisingly, however, in the case of DPPG/DPPE mixtures, the results indicated that DPPE is incorporated into the interdigitated bilayer of PMB-DPPG to a similar extent as PMB-unbound DPPG at least at equimolar ratios of DPPG/DPPE. Furthermore, if enough PMB is added to bind to both the DPPG and the DPPE, even more of the DPPE becomes interdigitated. This indicates that PMB can bind directly to DPPE when mixed with other lipids, resulting in inhibition of its participation in interlipid hydrogen bonding and direct induction of its interdigitation. Results from numerous studies suggest that mixing of DPPE with other lipids, such as DPPC, disrupts the intermolecular hydrogen bonding of PE with itself (reviewed in Ref. 12). This apparently allows increased binding of PMB to DPPE in the DPPE/DPPC mixture. When PE mixes with PG it may hydrogen bond with the PG, as inferred from the relatively high transition temperature of the mixture. However, the amino groups of PMB must have a higher affinity for the phosphate of PE than the hydroxyl groups of PG. When PMB binds to the hydrogen accepting phosphate group of either of these lipids, interlipid hydrogen bonding between PE and PG is also inhibited; thus the lipids can become interdigitated.

The ability of DPPE to bind PMB and become interdigitated along with the DPPG decreases as the DPPG/DPPE ratio decreases. The unbound DPPE phase separates into its own non-interdigitated domain where intermolecular hydrogen bonding can occur as found for DPPA at pH values below 9 and at less than saturating concentrations of PMB [3]. This phase separation can be detected by DSC in contrast to PG/PC mixtures. However, the spin label results indicate that DSC overestimates the degree of phase separation for DPPG/DPPE. Phase separation of the PMB-DPPE domain from the PMB-DPPG domain may occur after transformation to the non-interdigitated bilayer has taken place. Since transformation of these mixtures to non-interdigitated bilayers occurs below the phase transition temperature, these phase transitions are those of non-interdigitated bilayers. As argued earlier for DPPA-PMB, the higher phase transition temperature of the PMB-DPPE domain, relative to the PMB-DPPG domain, must be due to retention of some intermolecular hydrogen bonding for the DPPE when it is no longer interdigitated. This interlipid hydrogen bonding causes it to phase separate from the PMB-DPPG. Therefore, the relative areas of the two transitions observed by DSC do not reflect the relative amounts of DPPG and DPPE which are interdigitated at a low temperature. Some of the DPPE-enriched lipid giving higher temperature transitions must have been interdigitated at low temperatures.

As for the PG/PC mixtures, macroscopic phase separation into separate bilayers could not be detected by sucrose density centrifugation. Since lateral phase separation can apparently occur readily with increase in temperature as the interdigitated domains become non-interdigitated, the domains of interdigitated and non-interdigitated lipid may indeed be in the same bilayer as suggested by the sucrose density sedimentation results. This may also be true of the PG/PC mixtures for which increased mixing apparently occurs as the temperature increases. If the interdigitated and non-interdigitated domains were in separate bilayer structures, changes in the degree of lateral phase separation with increase in temperature could not occur so readily.

PMBN does not bind as well to the DPPE in the mixtures as PMB and has less ability to cause its interdigitation. This suggests that it does not disrupt the hydrogen bonding interactions between these lipids as well as PMB. These results are consistent with those in the previous paper using DPPA [3], and support the conclusion that interlipid hydrogen bonding inhibits interdigitation.

Binding of PMB to acidic phospholipids and/or the lipid A region of lipopolysaccharide in the outer membrane of Gram-negative bacteria must be the initial event in the mechanism of its bactericidal action [15,16]. It may then translocate to the cytoplasmic membrane where it exerts its effect. Since PE is the major phospholipid in these bacteria, in both the inner and outer membranes [17,18], the present results showing that mixing of PG with PE allows PMB to also bind to PE (as deduced from the fact that it affects its transition temperature and causes it to become interdigitated), supports a role for the phospholipids as sites of both initial binding and the bactericidal effect.

The ability of PMB to cause interdigitation may or may not be involved in the mechanism of its bactericidal effect. It can cause interdigitation of DPPG at a concentration of 2 μM, close to the minimum concentration which effectively inhibits bacterial growth [3]. However, interdigitation has only been detected in the gel phase although saturated fatty acids are not required (Boggs, J.M., Tümmler, B., unpublished results). Gram-negative bacteria contain a relatively high content of saturated fatty acids and a population of di-saturated lipids [17], although it is not known if the latter is large enough to permit domains of gel phase lipids. If interdigitation does not occur in the bacterial membrane in the presence of PMB, the bilayer may be very fluid and disordered. This, rather than interdigitation, may be the mechanism of its bactericidal effect. However, the differences in ability of PMB and PMBN

to cause interdigitation of both PG and PE when these lipids are mixed together, and the inhibition of interdigitation caused by PC, correlates with the bactericidal effect of PMB, the lack of bactericidal effect for PMBN, and the lack of effect of PMB on mammalian cells, which have high contents of PC and lack PG. It also correlates with the changes in lipid composition which occur when PMB-resistance is induced in some strains of bacteria [18,19]. This is accompanied by a large decrease in the amount of PG and PE in the membrane, as well as an increase in the content of unsaturated fatty acids, and an increase in cardiolipin and free fatty acids.

Acknowledgements

This study was supported by a grant from the Medical Research Council of Canada. J.M.B. acknowledges a Scientist Award from the Medical Research Council. H-Y.W. was supported by the Ministry of Education of the People's Republic of China.

References

1 Ranck, J.L. and Tocanne, J.F. (1982) FEBS Lett. 143, 175–178.
2 Theretz, A., Ranck, J.L. and Tocanne, J.G. (1983) Biochim. Biophys. Acta 732, 499–508.
3 Wang, H-Y., Tümmler, B. and Boggs, J.M. (1989) Biochim. Biophys. Acta 985, 182–198.
4 Boggs, J.M., Stamp, D. and Moscarello, M.A. (1981) Biochemistry 20, 6066–6072.
5 Boggs, J.M. and Rangaraj, G. (1985) Biochim. Biophys. Acta 816, 221–233.
6 Boggs, J.M. and Mason, J.T. (1986) Biochim. Biophys. Acta 863, 231–242.
7 Kubesch, P., Boggs, J.M., Luciano, L., Maass, G. and Tümmler, B. (1987) Biochemistry 26, 2139–2149.
8 Lohner, K., Schuster, A., Degovics, G., Muller, K. and Laggner, P. (1987) Chem. Phys. Lipids 44, 61–70.
9 Kim, J.T., Mattai, J. and Shipley, G.G. (1987) Biochemistry 26, 6599–6603.
10 Rowe, E.S. (1987) Biochemistry 26, 46–51.
11 Rowe, E.S. (1985) Biochim. Biophys. Acta 813, 321–330.
12 Boggs, J.M. (1987) Biochim. Biophys. Acta 906, 353–404.
13 Hartmann, W., Galla, H-J. and Sackmann, E. (1978) Biochim. Biophys. Acta 510, 124–139.
14 Sixl, F. and Watts, A. (1985) Biochemistry 24, 7906–7910.
15 Teuber, M. and Bader, J. (1976) Antimicrob. Agents Chemother, 9, 26–35.
16 Storm, D.R., Rosenthal, K.S. and Swanson, P.E. (1977) Annu. Rev. Biochem. 46, 723–763.
17 Ishinaga, M., Kanamoto, R. and Kito, M. (1979) J. Biochem. 86, 161–165.
18 Gilleland, H.E., Champlin, F.R. and Conrad, R.S. (1984) Can. J. Microbiol. 30, 869–873.
19 Conrad, R.S. and Gilleland, H.E. (1981) J. Bacteriol. 148, 487–497.
20 Silvius, J.R. and Gagne, J. (1984) Biochemistry 23, 3232–3240.
21 McIntosh, T.J., McDaniel, R.V. and Simon, S.A. (1983) Biochim. Biophys. Acta 731, 109–114.
22 Graham, I., Gagne, J. and Silvius, J.R. (1985) Biochemistry 24, 7123–7131.
23 Findlay, E.J. and Barton, P.G. (1978) Biochemistry 17, 2400–2405.
24 Blume, A., Wittebort, R.J., Das Gupta, S.K. and Griffin, R.G. (1982) Biochemistry 24, 6243–6253.