ELSEVIER

Biochimica et Biophysica Acta 1238 (1995) 42–48

BBA Biochimica et Biophysica Acta

Molecular miscibility of phosphatidylcholine and phosphatidylethanolamine in binary mixed bilayers with acidic phospholipids studied by ^{2}H- and ^{31}P-NMR

Kyong-hwa Shin, Hiroyuki Maeda, Toshimichi Fujiwara, Hideo Akutsu *

Department of Bioengineering, Faculty of Engineering, Yokohama National University, Hodogaya-ku, Yokohama 240, Japan

Received 24 January 1995; revised 12 April 1995; accepted 26 April 1995

Abstract

The intermolecular interactions and microscopic miscibility of the lipid bilayers of single component and binary mixtures with high content of saturated fatty acids were investigated by ^{2}H- and ^{31}P-NMR for phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and cardiolipin (CL). Their glycerol backbones were selectively deuterated by biosynthesis and chemical synthesis. Deuterium quadrupole splittings and phosphorus chemical shift anisotropies provided the consistent information for the molecular miscibility of each phospholipids. PE was found to be completely miscible with PG and CL. Since deuterium quadrupole splittings and phosphorus chemical shift anisotropy are identical for two components in the mixed bilayer, the dynamic structure from the glycerol backbone to phosphate group should be uniform in the binary mixture of these phospholipids. In contrast to PE, PC was not fully miscible with PG and CL at molecular resolution. The dynamic structure from the glycerol backbone to phosphate group is different for two components in the binary mixed bilayers. In the case of the mixed bilayers of PC and PE, both phospholipids are microscopically immiscible with each other. Thus, while PE, PG and CL can adapt to a new situation to form a uniform dynamic structure in mixed bilayers, PC has no ability for adaptation. The molecular miscibility in lipid bilayers was shown to depend on the molecular species and the nature of the molecular interactions. The biological significance of this result was discussed.

Keywords: Miscibility; Phospholipid bilayer; Phosphatidylcholine; Phosphatidylethanolamine; Acidic phospholipid; Specific deuteration; NMR, ^{2}H-; NMR, ^{31}P-

1. Introduction

Biological membranes can exert their functions in the presence of a wide variety of phospholipids. To elucidate the necessity of a variety of phospholipids, extensive works have been carried out from the biochemical and physicochemical aspects. Although some of the specific roles of phospholipids have been reported, they are not yet well understood. Especially, physicochemical elucidation of the specificity of each phospholipids is very poor except for the propensity of the phase transition to the Hexagonal II. The nature of intermolecular interactions among different phospholipids is one of important physicochemical properties of phospholipids. The intermolecular interactions have been investigated in either macroscopic or microscopic ways. In the former, the calorimetry has been the most popular method [1–3], which provides us with phase diagrams and macroscopic miscibility of the phospholipids. In the latter, one of the most powerful methods is nuclear magnetic resonance (NMR) [4–8], which provides us with local information.

Glycerophospholipid bilayers can be divided to three parts, namely, hydrophobic, hydrophilic and interface regions. A wide variety of hydrocarbon chains are present in the hydrophobic region. The hydrophilic region consists of various polar head groups. Thus, both of them are chemically heterogeneous in natural membranes. NMR parameters obtained from the hydrocarbon chains and polar head groups are sensitive to fluidity and specific interpolarhead-group interactions, respectively. In contrast to them, the

Abbreviations: ^{2}H-NMR, deuterium magnetic resonance; ^{31}P-NMR, phosphorus-31 nuclear magnetic resonance; PE, phosphatidylethanolamine (1,2-diacyl-*sn*-glycero-3-phosphoethanolamine); PC, phosphatidylcholine (1,2-diacyl-*sn*-glycero-3-phosphocholine); CL, cardiolipin; PG, phosphatidylglycerol; Pipes, 1,4-piperazinediethanesulfonic acid; EDTA, ethylenediamine tetraacetic acid; DSC, differential scanning calorimetry.

* Corresponding author. Fax: +81 45 3316143.

SSDI 0005-2736(95)00111-5

interface region is chemically identical, since the only constituent of the interface region is the glycerol moiety. The glycerol backbone is known to be the most rigid part in the bilayer and this region has been thought to be similar for all bilayers. If the bilayer structure is homogeneous as expected, the backbone conformation should be the same even for different phospholipids. This can be defined as ideal mixing of different phospholipids. If the mixing is not ideal, different molecules will retain their individuality even in the mixture. Therefore, the backbone conformation could be different for different phospholipids in non-ideal mixing. Thus, the structure of this part is potentially sensitive to the miscibility of phospholipids. The quadrupole splittings of the backbone deuterons may be used directly to examine the microscopic miscibility of different phospholipid molecules in mixed phospholipid bilayers. The information from the glycerol backbone is quite unique in comparison with that from hydrocarbon chains and polar head groups. Extensive works on the hydrocarbon chains and head groups have been carried out by NMR. In contrast to them, NMR studies on the glycerol backbones are few. We have investigated the effect of intermolecular interactions on the quadruple splittings of the glycerol backbones of a variety of phospholipids in this work.

Phospholipids of most biomembranes are composed of zwitter-ionic and acidic phospholipids. Phosphatidylethanolamine (PE) and phosphatidylcholine (PC) are the two major zwitter-ionic phospholipids, which are used in this work. In addition to them, cardiolipin (CL) and phosphatidylglycerol (PG) were used as acidic phospholipids. They are the major acidic phospholipids in bacterial membranes. ^{2}H- and ^{31}P-NMR spectra of the lipid bilayers of single phospholipid and binary mixture of phospholipids were examined. The results showed that the deuterium quadrupole splitting can be used to investigate molecular miscibility of phospholipids and that the molecular miscibility of phosphatidylcholine is very different from that of other phospholipid species examined.

2. Materials and methods

2.1. Preparation of phospholipids selectively deuterated in the glycerol backbone

Specific deuteration of *Escherichia coli* phospholipids was performed as described elsewhere [6]. Perdeuterated glycerol ([$^{2}H_5$]glycerol) was synthesized chemically according to the reported methods [6]. The deuterated glycerol was put in the medium and incorporated into a *E. coli* mutant requiring glycerol and unsaturated fatty acids (*E. coli* K-12 UFA^{ts}GRA). The requirement for unsaturated fatty acids is temperature-sensitive (typically, at 42°C). Under high osmotic pressure (for example, 2% KCl in this work), it can grow even without unsaturated fatty acids at 42°C. The cells grown under such conditions accumulate phospholipids with extremely high content of saturated fatty acids (about 90%), which were used in this work. The phospholipid composition and fatty acid compositions of each phospholipids were well characterized [9]. Phospholipids were extracted from the cells according to the reported method. Purity was confirmed by silicic acid thin-layer chromatography (TLC). The extent of deuteration was estimated from a ^{1}H-NMR spectrum of phosphatidylethanolamine (PE) [6]. It was about 65% in average.

Enough amount of deuterated PE and cardiolipin (CL) was obtained from the *E. coli* cells. Phosphatidylglycerol (PG) was obtained by transphosphatidylation of purified PE using cabbage phospholipase D [6]. Phosphatidylcholine (PC) was synthesized by methylation of PE purified from the cells described above according to a modified method of reported one [10,11]. The reaction mixture, PE (400 mg), CH_3I (3.2 g) and $KHCO_3$ (2 g) in $CHCl_3/CH_3OH$ (1:1, v/v, 25 ml), was refluxed at 40°C. To ensure 100% yield, more amount of CH_3I than usual was added. The reaction was monitored by TLC. After 16 h, the reaction was accomplished. After unsolved $KHCO_3$ was removed by filtration, the reaction mixture was applied to a cation exchange column (AMBERLITE 200C), and to an anion exchange column (AMBERLITE IRC-50/AMBERLYST A-21) to remove K^+, CO_3^{2-}, and I^- ions. The obtained lipid fraction was loaded on a silicic acid column (30 × 300 mm), which was eluted with chloroform/methanol/water (65:25:4, v/v). A Yamazen fast flow liquid chromatography system was used with the flow rate of 1 ml/min. The purified PC was confirmed by a ^{1}H-NMR spectrum. The spectra of PC and PE were identical except for the polar group signals, suggesting that hydrocarbon chains of PC are intact. The obtained PC was about 240 mg. Specific deuteration of the glycerol backbone of PC has been carried out by using specifically deuterated PE.

2.2. Preparation of NMR samples

50 mg of phospholipid was dissolved in chloroform/methanol (1:1, v/v) and washed with 0.5 vol. of a 0.5 M Na_2SO_4, 2.0 mM EDTA solution (pH 7.2) to remove polyvalent metal ions. The chloroform fraction was transferred to a 5 mmϕ tube then dried to a film. It was further dried under high vacuum. The phospholipid was dispersed into a buffer containing 0.1 M Pipes, 2 mM EDTA, pH 7.2 at 55°C (in the liquid-crystalline state) and was peletted by centrifugation at 4°C. In the case of a binary mixture, 50 mg of deuterated lipid was mixed with the defined amount of nonlabeled the other lipid in chloroform/methanol (1:1) and treated in the same way.

2.3. NMR measurement

^{1}H-NMR spectra were obtained with a Bruker AM-400 NMR spectrometer. ^{2}H-NMR spectra were measured with

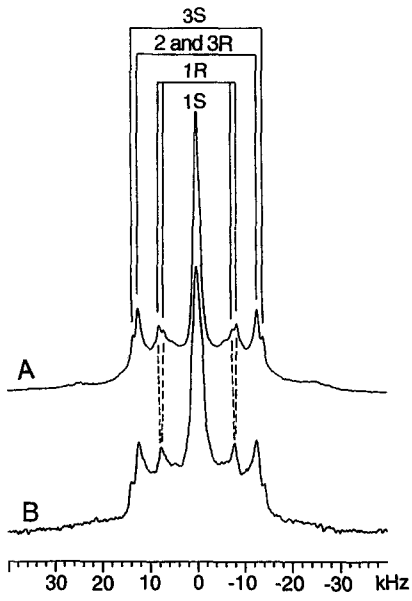

Fig. 1. ^{2}H-NMR spectra (61.1 MHz) of the perdeuterated glycerol backbone of phosphatidylethanolamine (A) and phosphatidylcholine (B) bilayers at 50°C. The assignments are given on the top. For example, 1S stands for the pro-S deuteron at C1 site of the glycerol backbone.

Chemagnetics CMX-400 NMR spectrometer operating at 61.1 MHz equipped with a CP/MAS probe for a 5 mmϕ sample tube. The 90° pulse width was 4 μs. The quadrupole echo pulse sequence (90_x-τ_1-90_y-τ_2) was employed with $\tau_1 = 30$ μs, $\tau_2 = 20$ μs and 0.5 s of recycle time. ^{31}P-NMR spectra were recorded on a Chemagenetics CMX-400 spectrometer operating at 161.15 MHz under proton decoupling. The 90° pulse width was 3.5 μs.

2.4. Differential scanning calorimetry (DSC)

The thermograms of the lipid samples were recorded on a Rigaku DSC-8230 to confirm the phase transition of the samples used for NMR measurements. The scan rate was 3 K/min.

3. Results

^{2}H-NMR spectra of the phosphatidylethanolamine (PE) and phosphatidylcholine (PC) bilayers with the perdeuterated glycerol backbones in the liquid-crystalline state (at 50°C) are presented in Fig. 1A and B, respectively. They are overlapped powder patterns characteristic for nuclei with $I = 1$ under strong magnetic fields. The assignments for the highest peaks were given on the basis of the reported ones [12,13]. In the spectrum of PE, each C1 deuterons of the glycerol backbone gave rise to two quadrupole splittings, suggesting the presence of two stable conformations. This was discussed in detail elsewhere [6]. In contrast to PE, PC bilayers gave rise to only one quadrupole splitting for C1R deuteron. Since PC was synthesized from PE, they should have identical fatty acid composition. Nevertheless, the backbone conformation of PC is homogeneous in the bilayers in contrast to PE.

At first, PE was mixed with cardiolipin (CL) or phosphatidylglycerol (PG). Fig. 2A, B and C shows ^{2}H-NMR spectra of CL*, PE/CL* and PE*/CL bilayers, respectively, at 50°C, where the asterisk (*) stands for the phospholipid perdeuterated in the glycerol moieties. The mixing ratio was one to one by weight. As will be shown later, whole systems are in the liquid-crystalline lamellar phase in the temperature range examined on the basis of ^{31}P-NMR spectra. A strong signal pair with the quadrupole splitting of about 14 kHz in Fig. 2A and B was attributed to the five deuterons of the head group of CL [14,15]. Some of the quadrupole splittings have changed on mixing. The quadrupole splittings of PE/CL bilayers are plotted as a function of temperature in Fig. 3 along with those of PE and CL bilayers. In Fig. 3A and C, the quadrupole splittings of the mixed bilayers were compared with those of the corresponding single lipid bilayers. In Fig. 3B, the quadrupole splittings of PE* and CL* in the mixed bilayers were compared with each other. In the single lipid bilayers, the quadrupole splittings of C2 and C3 deuterons of PE are larger than those of CL. On mixing, they have changed (Fig. 3A and C) and became identical for PE and CL (Fig. 3B). In the case of C1R deuteron, the quadrupole splittings were similar for PE* and CL* bilayers. Therefore, the change on mixing was small. The result shows that PE and CL are taking the same dynamic structures in the mixed bilayers even though they take different ones originally.

The ^{2}H-NMR and ^{31}P-NMR spectra of PG*, PE/PG* and PE*/PG bilayers were also observed at five different

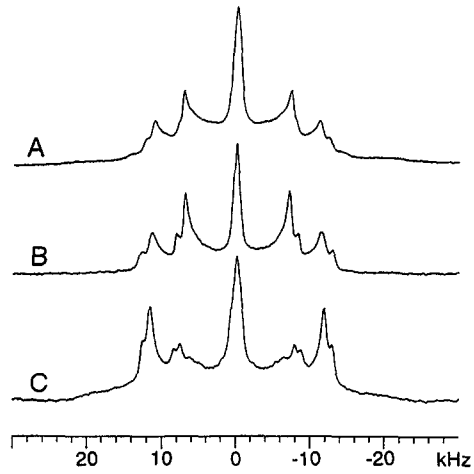

Fig. 2. ^{2}H-NMR spectra (61.1 MHz) of the perdeuterated glycerol moieties of cardiolipin (CL) and phosphatidylethanolamine(PE)/CL bilayers at 50°C. A, deuterated CL; B, a binary mixture of nondeuterated PE and deuterated CL; C, a binary mixture of deuterated PE and nondeuterated CL. The mixing ratio is one to one by weight. A pair of strong peaks with the quadrupole splitting of about 14 kHz in A and B, which are not present in C, were assigned to the five deuterons of the head group of CL [15,16].

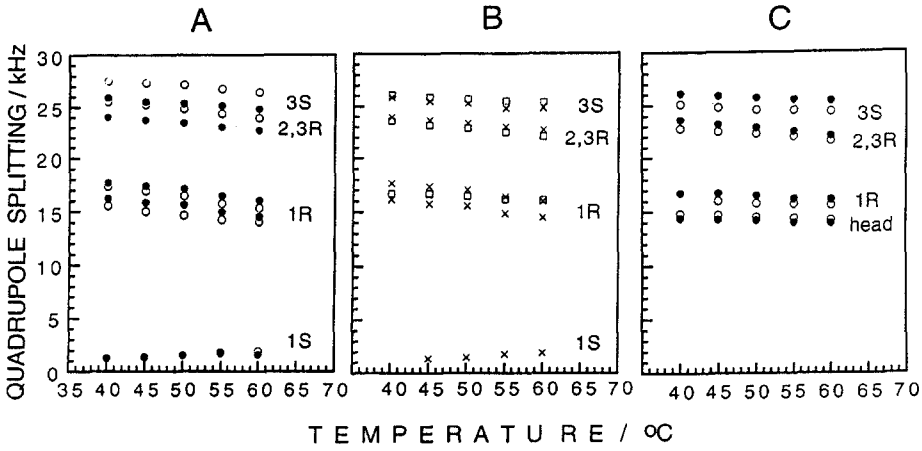

Fig. 3. Temperature dependence of the quadrupole splittings of deuterated phosphatidylethanolamine (PE*) and deuterated cardiolipin (CL*), PE*/CL and PE/CL* bilayers. The mixing ratio is one to one by weight. A, PE* (open circle) and PE*/CL (closed circle) bilayers; B, PE*/CL (cross) and PE/CL* (square) bilayers; C, CL* (open circle) and PE/CL* (closed circle) bilayers.

temperatures as in the case of PE/CL system. The mixing ratio is one to one. The observed quadrupole splittings of C2 and C3 deuterons at 50°C are given in Table 1 along with those of PE* bilayers. The standard deviations were obtained from the least-squares fitting to the observed values at five different temperatures. The quadrupole splittings of the glycerol backbones of PE and PG changed on mixing in the way similar to those of the PE/CL mixture. Namely, the quadrupole splittings of PE* and PG* in PE/PG bilayers were identical although they were different in the single lipid bilayers. Therefore, it can be concluded that PE and PG also take the same dynamic structure in the mixed bilayers. The results of PE/CL and PE/PG bilayers indicate that the deuterium quadrupole splittings are sensitive to the bilayer structure and this parameter can be used to examine how homogeneous the bilayer structures are. The latter is closely connected with the miscibility of the phospholipid molecules. Consequently, it can be said that PE molecules are completely miscible with CL and PG molecules in the binary mixture. The changes in the quadrupole splittings of C2 and C3 deuterons on mixing were about 1.5 and 1 kHz for PE and acidic phospholipids (CL and PG), respectively. This suggests that PE has to change its dynamic conformation more extensively than the acidic phospholipids. PE was also mixed with CL or PG at different ratio (2 to 1 and 4 to 1, respectively, by weight). The characteristics in the change of quadrupole splittings are essentially the same as those for the mixture at one to one ratio.

Then, PC was mixed with CL or PG. The ^{2}H-NMR spectra were also measured at five different temperatures as well as ^{31}P-NMR spectra. The mixing ratio was one to one. The quadrupole splittings of C2 and C3 deuterons of the glycerol backbone at 50°C were summarized in Table 1. The behavior of the quadrupole splittings of the glycerol backbone was different from those of PE/acidic phospholipids systems. While the quadruple splittings of C2 and C3 deuterons of CL changed on mixing, those of PC do

Table 1
Deuterium quadrupole splittings (kHz) of the glycerol backbones in the single and binary mixed lipid bilayers at 50°C

Combination A/B	Deuterated site	A [a]	(A + B) [b]		B [a]
			A	B	
PE/CL	3*S*	27.0 ± 0.1	25.4 ± 0.1	25.6 ± 0.1	24.6 ± 0.1
	2,3*R*	24.7 ± 0.1	23.3 ± 0.1	23.0 ± 0.1	22.2 ± 0.1
PE/PG	3*S*	27.0 ± 0.1	25.3 ± 0.1	25.6 ± 0.1	24.5 ± 0.1
	2,3*R*	24.7 ± 0.1	22.9 ± 0.1	23.0 ± 0.1	22.4 ± 0.1
PC/CL	3*S*	28.3 ± 0.1	28.5 ± 0.1	26.7 ± 0.1	24.4 ± 0.1
	2,3*R*	25.2 ± 0.2	25.3 ± 0.1	23.6 ± 0.1	21.8 ± 0.1
PC/PG	3*S*	28.3 ± 0.1	28.2 ± 0.1	26.3 ± 0.1	24.5 ± 0.1
	2,3*R*	25.2 ± 0.2	24.8 ± 0.1	23.6 ± 0.1	22.5 ± 0.1
PC/PE	3*S*	28.3 ± 0.1	28.3 ± 0.1	26.8 ± 0.1	26.7 ± 0.1
	2,3*R*	25.6 ± 0.1	25.7 ± 0.1	24.0 ± 0.1	24.3 ± 0.1

Standard deviations were obtained from the least-squares fitting to the values at five different temperatures (see Fig. 3). A and B stand for the components given in the 'combination' column.
[a] Single lipid bilayers.
[b] Binary mixed lipid bilayers.

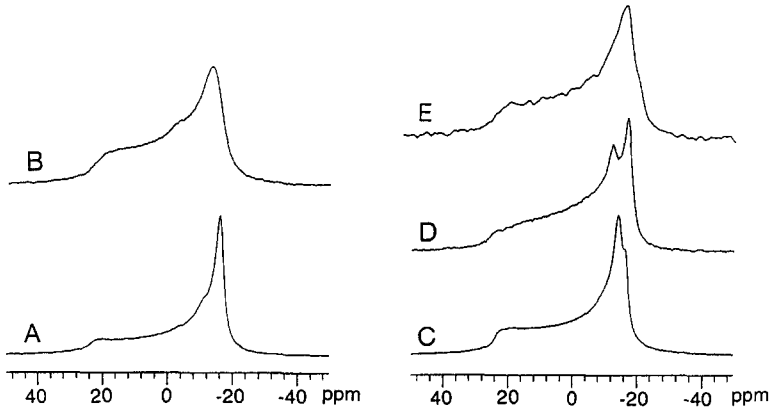

Fig. 4. ^{31}P-NMR spectra (161.15 MHz) of the binary mixed bilayers. The mixing ratio is one to one by weight. A, phosphatidylethanolamine (PE)/phosphatidylglycerol (PG); B, PE/cardiolipin (CL); C, phosphatidylcholine (PC)/PG; D, PC/CL; E, PC/PE. 85% phosphoric acid was used as an external reference.

not change at all. Furthermore, the quadrupole splittings were not identical for PC* and CL* in the mixed bilayers. These facts indicate that the mixing of the phospholipids induces a change in the dynamic structure of the glycerol backbone only for CL and the backbone conformation is not homogeneous in these mixed bilayers. Similar results were obtained for the quadrupole splittings of PC/PG* and PC*/PG bilayers as shown in Table 1. The changes of the quadrupole splittings of C2 and C3 deuterons were smaller for PG than for CL on mixing with PC. It can be concluded from these results that PC is not fully miscible with CL and PG in the binary mixture in contrast to PE.

Finally, the binary mixture of PE and PC (1:1, w/w) was examined at five different temperatures, since both are present in mammalian membranes. The results are summarized in Table 1 as well. Surprisingly, the quadrupole splittings of C1, C2 and C3 deuterons of PE and PC did not change at all on mixing. Consequently, the quadrupole splittings were not identical for PE* and PC* in the mixed bilayers. Apparently, there are no interactions between PE and PC molecules.

To monitor the polymorphic structure of the lipid samples and the effect of intermolecular interactions on the polar head groups, ^{31}P-NMR spectra were obtained for the same samples that were used for ^{2}H-NMR measurements. The ^{31}P-NMR spectra of PE/PG*, PE*/CL, PC/PG*, PC/CL* and PC/PE* systems at 50°C are presented in Fig. 4. Their powder patterns show that there is no other phase than the liquid-crystalline bilayers in all samples. While the spectra of PE/PG and PE/CL bilayers are single powder patterns typical for the molecules undergoing axially symmetric motions, those of PC/PG, PC/PL and PC/PE bilayers are not typical ones. This suggests that the phosphate groups of PE and PG molecules in PE/PG bilayers and those of PE and CL molecules in PE/CL bilayers take uniform dynamic conformations, respectively. It is consistent with the results obtained by ^{2}H-NMR. The chemical shift anisotropies of PE/CL and PE/PG bilayers were −40 and −41 ppm, respectively. Since those of PE, PG and CL bilayers at 50°C are −43, −38 and −33 ppm, respectively, the values for the mixed bilayers are the in-between of those of two components.

On the other hand, the ^{31}P-NMR spectra of PC/PG, PC/CL and PC/PE bilayers can be explained by the superposition of two axially symmetric powder patterns. A similar superimposed spectrum was reported for DOPC/CL bilayers [16], POPC/*E. coli* PE bilayers [17] and DMPC/DMPG/DDAB bilayers [18]. DOPC, POPC and DMPC stand for dioleoyl-, palmitoyloleoyl- and dimyristoylphosphatidylcholines, respectively. DMPG and DDAB denote dimyristoylphosphatidylglycerol and didodecyldimethylammonium bromide, respectively. In every case, two axially symmetric powder patterns were ascribed to each phospholipid species. Therefore, the powder pattern with a larger chemical shift anisotropy can be ascribed to PC in Fig. 4C, D and E. The apparent chemical shift anisotropy of the larger component of PC/PG and PC/CL bilayers was about −43 ppm. That of PC bilayers was −47 ppm. This suggests that the conformation of the phosphate group of PC has changed on mixing with acidic phospholipid. It is well known that the polar head group of PC changes its conformation in the presence of membrane surface charge [8,19,20]. In contrast, the apparent chemical shift anisotropy of the larger component of PC/PE bilayers was about −46 ppm. It is close to the original value of PC bilayers, which is consistent with ^{2}H-NMR data of PC/PE bilayers. In conclusion, ^{31}P-NMR spectra showed that in the binary mixtures including PC, the dynamic conformations of phosphate groups are not uniform as those of the glycerol backbones were not.

4. Discussion

It has turned out in this study that the microscopic miscibility is different depending on the combination of

phospholipid species. Since the microscopic miscibility is dominated by intermolecular interactions, it can be called molecular miscibility. PE is totally miscible with PG and CL. Since deuterium quadrupole splittings and phosphorus chemical shift anisotropy are identical for two components in the mixed bilayer, the dynamic structure from the glycerol backbone to phosphate group should be uniform in the binary mixture. These molecules exert the ability of adaptation to form an uniform bilayer structure on mixing. Such ability is a kind of molecular adaptation. This would come from strong interpolarheadgroup interactions including hydrogen bonding among these molecules. Actually, in the binary mixed bilayers of PE and PG, significant changes in the deuterium quadrupole splittings of the polar head groups on mixing were reported for both molecular species [21].

In contrast to PE, PC is not fully miscible with PG and CL. Judging from the conformation of the glycerol backbone, PC has no ability for molecular adaptation. However, PG and CL are trying to adapt to PC in the mixed bilayers. Nevertheless, the dynamic structure from the glycerol backbone to phosphate group is different for two components. Significant changes in deuterium quadrupole splittings of the polar head groups of PG and CL on mixing with PC are also reported [14,21]. However, the conformation of the polar head groups of PC is also known to change just by the electrostatic interactions as already mentioned. Therefore, the information from the polar head groups is not straightforward from the view point of miscibility. This shows the advantage of the information from the glycerol backbone to examine the miscibility.

In the case of PC/PE bilayers, both of PC and PE are microscopically immiscible. Non-ideal mixing in PC/PE bilayers from the macroscopic point of view has been reported on the basis of ^{13}C- and ^{2}H-NMR experiments [4] and calorimetry [1]. The changes in the deuterium and nitrogen-14 quadrupole splittings and phosphorus chemical shift anisotropy of the polar head groups of PC and PE on mixing were reported to be small [17,21], suggesting that the interpolarheadgroup interactions between PC and PE are weak. The dynamic structure from the glycerol backbone to phosphate group in PC/PE bilayers is not uniform.

What is the molecular view of the miscibility? Although the deuterium quadruple splittings of PE changed on mixing with either PG or CL, they did not change at all in the mixture with PC. This means that PE molecules still retain the PE–PE interactions in the mixture, suggesting the existence of PE domains in the PC/PE bilayers. Since the mixed bilayers are macroscopically homogeneous, the domain size should be very small, namely, a kind of microdomains. Actually, a working hypothesis on the basis of microdomain formation for PE, PG and CL can explain all the results mentioned above. Phospholipid molecules in each domains are assumed to take on uniform backbone conformations. The basic assumptions for PC/CL and PC/PG bilayers are that the acidic phospholipids retain their own domains, but some of them are dissolved in the PC regions in the binary mixture, and that there is a rapid exchange of the acidic phospholipid molecules between their own domains and PC regions. Since the backbone conformations of the former and latter should be similar to the original one in the single-acidic phospholipid bilayers and to that in PC bilayers, respectively, the deuterium quadrupole splittings would give rise to intermediate values as were observed in this work. The quadrupole splittings of PC would not change if PC molecules are not dissolved in PE, CL and PG domains at all. In contrast, PE forms homogeneously mixed domains with PG and CL in the binary mixed bilayers. The motive force of such domain formation should be hydrogen bonding interactions among the polar head groups. Although PE, PG and CL can form hydrogen bonding, PC cannot. The origin of the conformational adaptation may be the hydrogen bonding interactions as well. This also explains the fact that the quadrupole splittings of PC did not change at all in the binary mixtures, even though some of acidic phospholipid molecules would be dissolved in PC regions. Since there is no hydrogen bonding between PC and acidic phospholipid molecules, the backbone conformation of PC would not be affected by the dissolved acidic phospholipid molecules. Therefore, the hydrogen bonding interactions may be one of the most important factors, which regulate the molecular miscibility. The presence of microdomains of PE with saturated fatty acids in PE bilayers was confirmed by us [22].

Distinct difference in the molecular miscibility between PC and PE could have biological significance. As it is well known, phospholipid distribution in mammalian plasma membranes is asymmetric [23]. Aminophospholipids such as PE and phosphatidylserine are mainly located in the inner leaflet, while PC is mainly located in the outer leaflet of the plasma membrane. Many of bacterial membranes contain only PE as zwitter-ionic phospholipids and contain PG and CL as major acidic phospholipids. Therefore, PE is the major zwitter-ionic phospholipids in the inner leaflet of the plasma membranes regardless of the biological species. The acidic phospholipids coexisting with PE also have the hydrogen bonding ability. These facts suggest that the inner leaflets of the plasma membranes are always composed of mainly fully miscible phospholipids. Since the inner side of the plasma membrane is biochemically most active, the miscibility of the phospholipids could play a role in exerting the membrane functions. By contrast, PC is mainly located in the side where biological activities are relatively low.

Acknowledgements

We are grateful to Rigaku Co. Ltd. for letting us use their facilities.

References

[1] Silvius, J.R. (1986) Biochim. Biophys. Acta 857, 217–228.
[2] Epand, R.M. and Bottega, R. (1988) Biochim. Biophys. Acta 944, 144–154.
[3] Wiedmann, T., Salmon, A. and Wong, V. (1993) Biochim. Biophys. Acta 1167, 114–120.
[4] Blume, A., Wittebort, R.J., Das Gupta, S.K. and Griffin, R.G. (1982) Biochemistry 21, 6243–6253.
[5] Boden, N., Jones, S.A. and Sixl, F. (1991) Biochemistry 30, 2146–2155.
[6] Yoshikawa, W., Akutsu, H., Kyogoku, Y. and Akamatsu, Y. (1988) Biochim. Biophys. Acta 944, 321–328.
[7] Marassi, F.M., Shivers, R.R. and Macdonald, P.M. (1993) Biochemistry 32, 9936–9943.
[8] Pinheiro, T.J.T., Duralski, A.A. and Watts, A. (1994) Biochemistry 33, 4896–4902.
[9] Uehara, K., Akutsu, H., Kyogoku, Y. and Akamatsu, Y. (1977) Biochim. Biophys. Acta 466, 393–401.
[10] Stockton, G.W., Polnaszek, C.F., Leitch, L.C., Tulloch, A.P. and Smith, I.C.P. (1974) Biochem. Biophys. Res. Commun. 60, 844–850.
[11] Akutsu, H., Suezaki, Y., Yoshikawa, W. and Kyogoku, Y. (1986) Biochim. Biophys. Acta 854, 213–218.
[12] Gally, H.U., Pluschke, G., Overath, P. and Seelig, J. (1981) Biochemistry 20, 1826–1831.
[13] Strenk, L.M., Westerman, P.W. and Doane, J.W. (1985) Biophys. J. 48, 765–773.
[14] Allegrini, P.R., Pluschke, G. and Seelig, J. (1984) Biochemistry 23, 6452–6458.
[15] Yoshikawa, W., Akutsu, H., Kyogoku, Y. and Akamatsu, Y. (1984) Chem. Lett., 105–108.
[16] Spooner, P.J. and Watts, A. (1992) Biochemistry 31, 10129–10138.
[17] Ghosh, R. (1988) Biochemistry 27, 7750–7758.
[18] Marassi, F.M. and Macdonald, P.M. (1991) Biochemistry 30, 10558–10566.
[19] Akutsu, H. and Seelig, J. (1981) Biochemistry 20, 7366–7373.
[20] Macdonald, P.M., Leisen, J. and Marassi, F.M. (1991) Biochemistry 30, 3558–3566.
[21] Sixl, F. and Watts, A. (1983) Proc. Natl. Acad. Sci. USA 80, 1613–1615.
[22] Shin, K., Nagamori, T., Kimura, Y., Tomoi, M., Fujiwara, T. and Akutsu, H. (1995) Chem. Phys. Lipids, in press.
[23] Devaux, P.F. (1991) Biochemistry 30, 1163–1172.