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Significant stabilization of the phosphatidylcholine bilayer structure by incorporation of small amounts of cardiolipin

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Abstract

The effects of the negatively charged phospholipid cardiolipin on the structural properties of egg-yolk phosphatidylcholine (EyPC) liposomal membranes were studied by monitoring the water permeability of the liposomes caused by osmotic shrinkage in hypertonic glucose solution. Incorporation of small amounts of bovine heart cardiolipin (BhCL) into the EyPC membranes caused a significant decrease in their water permeability associated with stabilization of the membrane structure. Much evidence obtained by attenuated total reflection IR spectroscopy suggested that incorporation of BhCL into the EyPC membranes causes a cooperative conformational change in the EyPC polar head groups, but does not alter the fluidity of the bilayer structure in the fluid liquid crystalline state. Incorporation of small amounts of BhCL stabilized the intermolecular hydrogen-bonded network including water molecules of the hydration layers at the bilayer surface that are important for the stable bilayer configuration of the EyPC molecules. The antisymmetric PO_2^- frequencies of the EyPC membrane with incorporated BhCL suggested that the BhCL content of 50 mol% induced a change in the phase behaviors of mixed BhCL/EyPC membranes.

Key words: Cardiolipin; Liposome; Permeability; Zeta potential; FTIR

1. Introduction

Cardiolipin is a structurally unique, naturally occurring phospholipid, because it contains 4 fatty acyl chains and two negatively charged phosphates at neutral pH. The charged backbone of caldiolipins, as well as the ester carbonyl groups, contribute to the stability of an intra- and intermolecular hydrogen-bonded network that includes water molecules of the hydration layer [1,2]. This network of cardiolipin polar groups is thought to conduct protons laterally in membranes [3]. The precise role of cardiolipin in the coupling process of phosphorylation and electron transport is not known [4], but the membrane lipid composition is reported to be well correlated with oxidative phosphorylation in mitochondria [5], and regulation of membrane enzymes [6]. Probably enzymatic digestion of cardiolipins, but not phosphatidylcholine or phosphatidylethanolamine is related to modification of the membrane structure [7].

In the present paper, we report results on the effects of BhCL on the water permeability of EyPC liposomes measured by monitoring their osmotic shrinkage in hypertonic glucose solution and on the structural properties of the liposomal membranes determined by FTIR spectroscopy and zeta potential measurements. The extents and manners of interaction of the components were determined by analysis of regions of the IR spectrum displaying on their hydrocarbon components and polar head groups and interfacial regions of the lipid structure.

2. Materials and methods

BhCL was isolated as its sodium salt from bovine heart by the methods of Faure and Morelec-Coulon [8] and Pangborn [9]. EyPC was from Nichiyu Liposome Co. (Japan). Concentrations of phospholipids were determined in terms of phosphorus (P_i) by the method of

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Abbreviations: EyPC, egg-yolk phosphatidylcholine; BhCL, bovine heart cardiolipin; LUV, large unilamellar vesicle; P_i, orthophosphate; FTIR, Fourier transform infrared.

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Ames [10]. Other reagents were commercial products. LUV suspensions of EyPC with incorporated BhCL were prepared by reverse phase evaporation [11] in 10 mM Tris-HCl buffer (pH 7.3) and filtered through 0.4 μm and 0.2 μm filters (Nuclepore Co., Pleasanton, CA). The mean diameter of the vesicles was 220 nm, determined with a Nicomp (Santa Barbara, CA) model 370 particle sizer. A BhCL bilayer dispersion was prepared by adding an appropriate amount of the buffer (pH 7.3) to the dry lipid. The sample was then sonicated briefly and extensively vortexed.

Osmotic shrinkage was determined essentially by the method of Blok et al. [12]. LUVs with a P_i concentration of 0.5 mM were suspended in 10 mM Tris-HCl buffer (pH 7.3) in a total volume of 2.94 ml at 30°C. A typical example of the time course of absorbance change of liposomes is depicted in Fig. 1. A volume of 60 μl of 1.0 M glucose solution was rapidly added (within less than 0.5 s) to 2.94 ml of liposome suspension with stirring by a microsyringe fitted into the sample chamber of the spectrophotometer, and the rapid change in absorbance of the liposome suspension with time was monitored at 450 nm with a Shimadzu double beam/dual wavelenngth spectrophotometer, model UV 3000. Output signals were stored in a microcomputer at a sampling rate of 80 ms, and were recorded on an X-Y plotter. The initial velocity of shrinkage was determined by the least-squares method.

The zeta potential of liposomes was measured as their electrophoretic mobility in a Laser-Zee, model 500 apparatus (Pen Kem, New York) at 20°C.

Spectra of lipid bilayer dispersions (60 mM) were measured in a Perkin-Elmer model 1720 FTIR spectrometer equipped with a triglycine sulfate detector with KBr windows at 22°C. Data were collected over the 900–4000 cm^{-1} infrared region with 2 cm^{-1} resolution at 1 cm^{-1} intervals. Cast-dry films of BhCL and EyPC were prepared by spreading the stock solutions uniformly on CaF_2 plates (40 × 10 × 1 mm) under dry nitrogen. For lipid dispersion, a cylindrical attenuated total reflectance cell (Circle cell; Spectratech, Stanford, CT) with a ZnSe crystal at a 45° angle of incidence was employed. The final infrared spectra of liposome samples were acquired by subtracting the spectrum of the buffer solution from that of the sample solution. Peak positions were determined by the peak-picking routine supplied with the Perkin-Elmer FTIR software.

The peak positions, peak areas, and bandwidths of the overlapping bands were evaluated as follows. First, the peak positions were determined by Fourier self-deconvolution. Then the bandwidths, positions, intensities and percent Lorenntzian/Gaussian shape were varied to obtain the best fit for the band shapes. The parameters were varied until the residual spectrum obtained by subtraction of the synthetic composite curve from the original data was minimal [13,14].

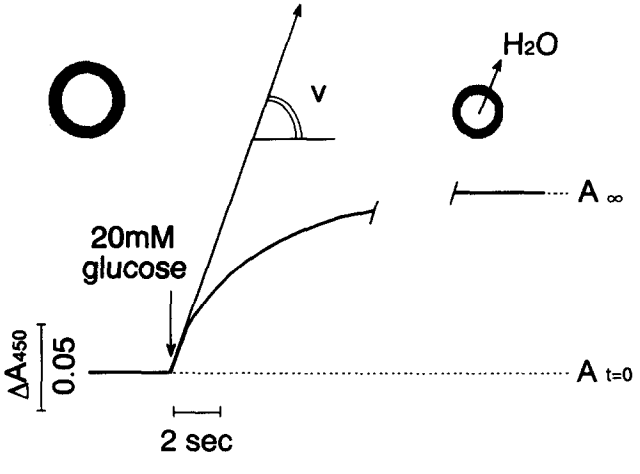

Fig. 1. Typical time-courses of shrinkage of EyPC liposomes with and without BhCL after addition of hypertonic glucose solution, measured as absorbance increase at 450 nm. Liposomes (1.5 mM P_i) were suspended in an isotonic solution of 10 mM Tris-HCl buffer (pH 7.3) in a total volume of 2.94 ml, at 30°C. Then 60 μl of glucose was added rapidly to a final concentration of 20 mM. Change in the absorbance was monitored, and $A_{t=0}$ (absorbance extraporated to the time of glucose addition) were determined.

3. Results

3.1. Permeability of liposomal membranes to water

Osmotic volume changes of liposomes can easily be followed optically. To relate volume changes in liposomes to water flow across the liposomal membrane we assumed that liposomes behave as ideal osmometers: that is, liposomes are permeable only to water and not to solute. As the reciprocal of absorbance change at 450 nm increased linearly with the reciprocal of the change in glucose concentration at the equilibrium of osmotic shrinking, the liposomes were concluded to behave as ideal osmometers (data not shown).

The initial water permeability velocity (v) through liposomal membranes induced by the hypertonic stress of glucose was determined from Eq. (1) [12,15,16]:

$$v = (\mathrm{d}(1/A)/\mathrm{d}t)_{t=0}/(1/A_{t=0}) \quad (1)$$

where $A_{t=0}$ is the absorbance of the liposome suspension extrapolated to the time of glucose injection($t = 0$). Since v is proportional to the initial rate of volume change of the liposomes, $(\mathrm{d}V/\mathrm{d}t)_{t=0}$, the following relationship holds [16]:

$$v = k(dV/dt)_{t=0} = kP_wSRT\Delta C_{glc} \quad (2)$$

where P_w is the water permeability coefficient, S is the surface area of the membrane, R is the gas constant, T

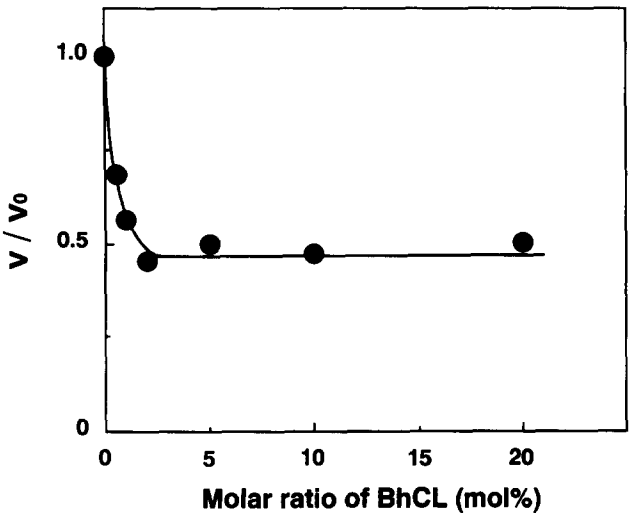

Fig. 2. Change in water permeability of liposomal membranes induced by osmotic shock on addition of 20 mM glucose with change in the molar ratio of BhCL in BhCL/EyPC liposomes. The ordinate indicates the relative initial velocity of shrinkage of EyPC liposomes with (v) and without (v_0) BhCL. Experimental conditions were as for Fig. 1.

is the absolute temperature, ΔC_{glc} is the difference between the concentrations of glucose outside and inside the liposome membrane and k is a constant.

Fig. 2 shows the relative initial velocity, v/v_0, of shrinkage of EyPC liposomes with (v) and without (v_0) BhCL induced by osmotic shock upon addition of 20 mM glucose at 30°C, as a function of the molar ratio of BhCL in the liposomes. Incorporation of small amounts of BhCL caused a significant decrease in v, but incorporation of more BhCL had little additional effect. The decrease in v was dependent on P_w and S (Eq. (2)), because other parameters were constant under the present experimental conditions [16]. As described in the Materials and methods section, the mean diameter of all the BhCL/EyPC liposomes used in this study was constant at 220 nm. Therefore, the change in v upon incorporation of BhCL into the EyPC membrane can be regarded as due to decrease in the water permeability of the liposomal membranes.

Fig. 3 shows the relationship between the zeta potential of liposomes and the molar ratio of BhCL to EyPC in the liposomes. The zeta potential became more negative with increase in the proportion of BhCL, suggesting a homogeneous distribution of BhCL in EyPC bilayer membranes. The slight negative value of the zeta potential of EyPC liposomes (BhCL/EyPC = 0/100, mol%) was due to the exposed PO_2^- of the polar head in medium of low ionic strength [17] and the value becomes zero when the ionic strength was increased.

3.2. FTIR spectrum

The vibrations of individual groups of lipid membranes in the IR spectrum provide structural information on highly localized regions of the lipid bilayer. A frequency shift of the specific vibrational mode and a change in the line shape indicate changes in a particular interaction between components in the membranes and in hydrogen bonding of the water to lipid polar groups [18,19]. The phosphate stretchings of the lipid polar head group and the carbonyl stretchings of the interfacial region are readily distinguished from the nonpolar CH_2 stretching vibration [20]. Fig. 4 shows the absorbance spectra of EyPC and BhCL liposomes together with data on dry films. Vibrational bands of the PO_2^- and C-O-C groups of phospholipids are seen in the 1000–1300 cm^{-1} region of the spectrum [21]. The ester C = O stretching band is observed in the 1600–1800 cm^{-1} range. The vibrational spectrum of the hydrocarbon chains is characterized by the two intense bands in the 2800–3000 cm^{-1} range, which arise from the CH_2 stretching bands of the fatty acyl chains. The antisymmetric stretching vibration of the CH = CH at 3010 cm^{-1} is expected since EyPC and BhCL contain large amounts of unsaturated fatty acid residues [22]. BhCL contains two two-chain phospholipids linked through a polar group to form a four chain phospholipid. The spectrum of BhCL looks very similar in line shape to that of EyPC, except for the symmetric and antisymmetric stretching vibrations (ca. 1070 and ca. 1175 cm^{-1}) of the C-O-C signal [21]. Assignments of the major absorption bands of EyPC and BhCL are summarized in Table 1. Compared with

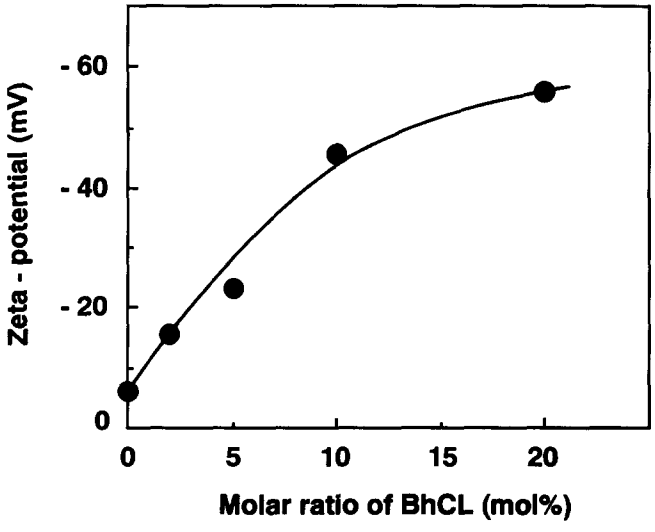

Fig. 3. Zeta potential of liposomes as a function of the molar ratio of BhCL in BhCL/EyPC liposomes suspended in 10 mM Tris-HCl buffer (pH 7.3).

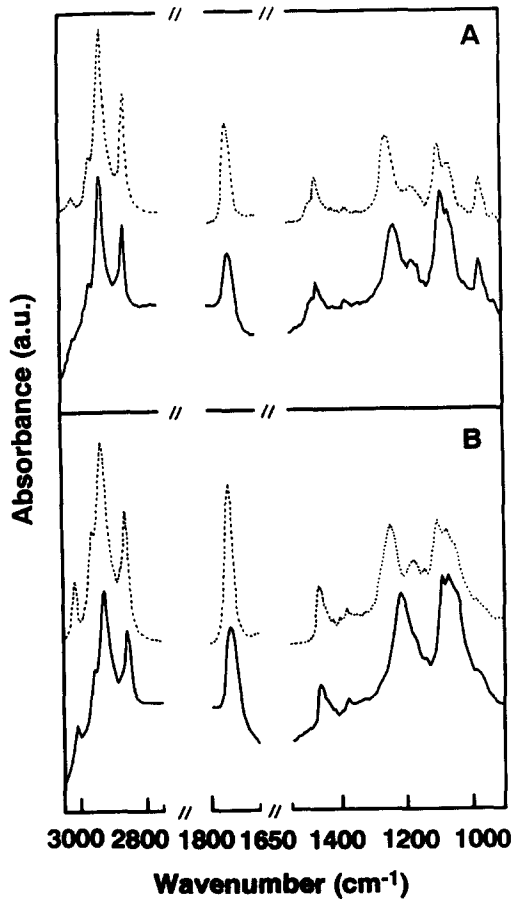

Fig. 4. Absorbance infrared spectra of EyPC (A) and BhCL (B): full line, bilayer suspensions; dotted line, dry films.

the spectra of dry films for the bilayer suspensions, the antisymmetric PO_2^- stretching bands detected as the most prominent change in the polar bands of EyPC and BhCL were shifted by 19 cm^{-1} for electrically neutral EyPC and 30 cm^{-1} for negatively charged BhCL to lower frequencies, indicating that in both lipids the PO_2^- groups are involved in very strong hydrogen bonds [2]. The polar bands of the symmetric PO_2^- stretching and the ester C = O stretching vibrations are also shifted to lower frequencies and broadened during hydration [23]. The antisymmetric and symmetric C-O-C stretching vibrations (1172 and 1073 cm^{-1}) of BhCL are shifted to lower frequencies, whereas those of EyPC are shifted to higher frequencies (1176 and 1067 cm^{-1}). A hydrogen-bond interaction between water and these lipid molecules causes the change in orientation of the polar groups in the lipid bilayer membranes [21].

The phase behavior of fatty acyl chains in phospholipid molecules is important for stabilization of the membrane structure. The spectra of mixed BhCL/EyPC membranes in the CH_2 stretching vibration region were very similar to those of membranes with only BhCL or EyPC (data not shown). The frequencies of the symmetric and antisymmetric CH_2 stretching bands (ca. 2855 and ca. 2925 cm^{-1}) of these membranes are characteristic of disordered acyl chains of lipids in the liquid crystalline state [2,24]. The results are consistent with fluidity data on mixed BhCL/EyPC membranes determined by the fluorescene polarization method [25].

A. Phosphate stretching region

Fig. 5 shows the infrared spectra in the polar group region between 900–1300 cm^{-1} of EyPC liposomal membranes containing 10 and 50 mol% BhCL, to-

Table 1
Assignment major absorption bands of dry films and bilayer suspensions of EyPC and BhCL

Assignment	Wavenumber (cm^{-1})			
	EyPC		BhCL	
	film	suspension	film	suspension
Hydrocarbon region				
CH = CH stretching band	3010	–	3010	3008
Asymmetric stretching band of terminal CH_3	2955	2953	2958	2956
Antisymmetric CH_2 stretching band	2921	2923	2927	2926
Symmetric CH_2 stretching band	2853	2853	2856	2855
CH_2 scissoring band	1467	1466	1465	1465
Symmetric bending band of terminal CH_3	1378	1379	1378	1378
Interfacial region				
C = O stretching band	1738	1734	1740	1736
Antisymmetric C-O-C stretching band	1168	1176	1175	1172.5 *
Symmetric C-O-C stretching band	1059	1067	1076	1073
Phosphate region				
Antisymmetric PO_2^- stretching band	1248	1229	1245	1215
Symmetric PO_2^- stretching band	1091	1085	1104	1092
Asymmetric $N^+-(CH_3)_3$ stretching band	968	973	none	none

* Determined by curve-fit analysis

gether with those of liposomes containing only EyPC and only BhCL. The strong absorption bands for EyPC at ca. 1230 cm^{-1} assigned to antisymmetric PO_2^- stretching vibrations of the polar head group decreased in frequency and increased in bandwidth with increase in BhCL content and another strong band in the lower 1050–1100 cm^{-1} frequency region assigned to symmetric PO_2^- stretching vibrations (ca. 1085 cm^{-1}) also tends to broaden in line shape, indicating that the two (antisymmetric and symmetric) PO_2^- stretching vibrations are perturbed by the incorporation of BhCL.

The spectra of binary membranes composed of EyPC and BhCL involve the two PO_2^- vibrations of EyPC and BhCL that are apparently superpositions of two separate individual bands. Therefore, quantitative information on the extent of hydration of the two phosphate PO_2^- groups was extracted by simulating the observed band shapes with Gaussian-Lorentzian functions. As typical examples, the observed and the calculated infrared spectra in the antisymmetric PO_2^- 1150–1300 cm^{-1} stretching region for bilayers composed of the BhCL/EyPC binary components (10 and 50 mol% BhCL) are shown in Fig. 6. The dashed line is the calculated line shape. The results show excellent agreement between the calculated and observed spectra. The observed antisymmetric PO_2^- stretching vibration was clearly split into two individual bands. The calculated frequency band at ca. 1175 cm^{-1} arises from the C-O-C antisymmetric stretching vibration (Table 1). Table 2 summarizes the observed and calculated frequencies for the antisymmetric PO_2^- vibrations of the mixed BhCL/EyPC liposomes. For example, although the peak of the antisymmetric PO_2^- vibrations for EyPC membranes containing 10 mol% BhCL was observed at 1225 cm^{-1}, the frequencies used in the simulated spectrum were 1230 and 1211 cm^{-1}. Since the frequency bands of the antisymmetric PO_2^- vibrations are at 1229 cm^{-1} for EyPC and at 1215 cm^{-1} for BhCL, at first glance the incorporation of BhCL appears to have little affect on the structural integrity of EyPC bilayers. However, we found that on increase in incorporation of BhCL into EyPC liposomal membranes, their permeability to water decreased (Fig. 2). Furthermore, as the calculated values for antisymmetric PO_2^- vibrations of BhCL in mixed membranes were shifted to lower frequency than that of membranes with BhCL only (Table 2), it is reasonable to expect

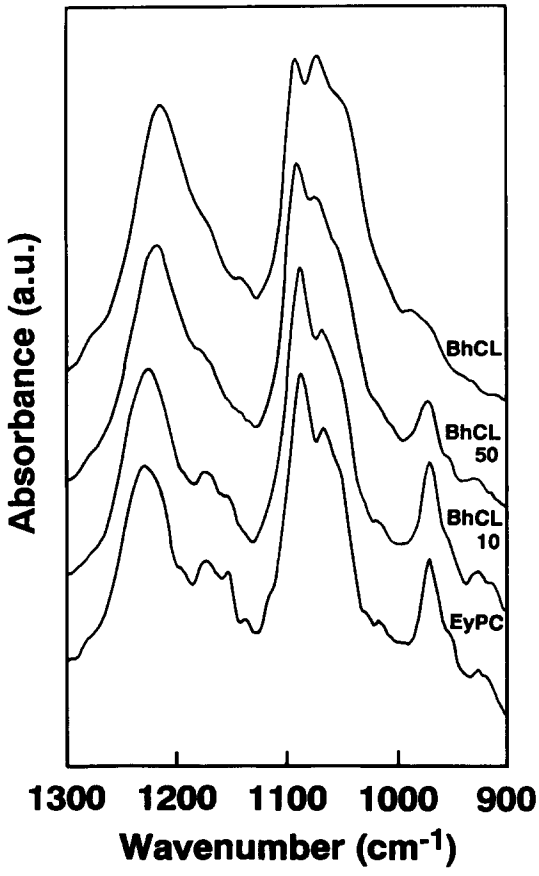

Fig. 5. Infrared spectra of mixed BhCL/EyPC (10 and 50 mol% BhCL), pure EyPC and pure BhCL bilayer suspensions in the polar group region between 900–1300 cm^{-1}.

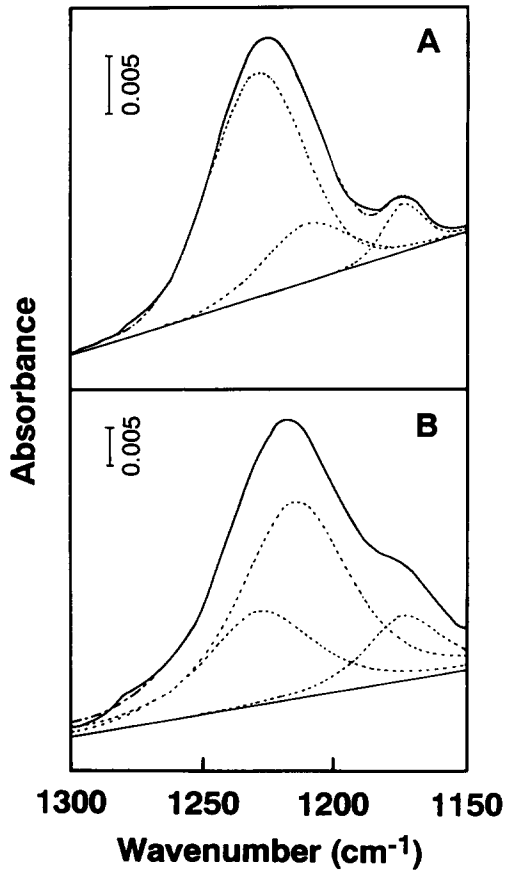

Fig. 6. Observed and calculated infrared spectra in the PO_2^- 1150–1300 cm^{-1} stretching region for mixed BhCL/EyPC liposomes (10 (A) and 50 (B) mol%): full line, observed; dotted lines, calculated. Dashed line, sum of the individual calculated lines.

Table 2
Observed and calculated antisymmetric PO_2^- stretching vibrations of mixed EyPC/BhCL liposomes

	Wavenumber (cm^{-1})					
	EyPC	BhCL (5 mol%)	BhCl (10 mol%)	BhCl (20 mol%)	BhCl (50 mol%)	BhCL
Observed frequency	1229	1226	1225	1220	1217	1215
Calculated frequency						
EyPC component		1230.1	1229.6	1228.5	1228.7	
BhCL component		1211.9	1210.8	1212.5	1215.3	

that incorporation of BhCL into EyPC membranes promoted and stabilized the intermolecular, hydrogen-bonded network that includes water molecules of the hydration layer at the membrane surface. In EyPC membranes with 50 mol% BhCL, the frequencies in the calculated spectrum were at 1229 and 1215 cm^{-1}, which are in fair agreement with those of membranes with EyPC or BhCL only. This suggests that a BhCL content of 50 mol% induces a change in the phase behaviors of the mixed BhCL/EyPC membranes.

B. C = O stretching region

To obtain further information on the hydration of EyPC molecules in mixed membranes, we next focused attention on the ester C = O stretching bands of the interfacial region of the lipid molecules. Fig. 7 shows the observed spectra for C = O stretching in the 1650–1800 cm^{-1} region of EyPC liposomes containing 10 mol% BhCL (Fig. 7A), together with the observed and calculated spectra of those containing only EyPC or BhCL. The C = O stretching bands for fully hydrated EyPC and BhCL are broad and no longer symmetric (Fig. 7C and D) due to the overlapping of the two ester C = O stretching vibrations [13,20]. Thus, we determined the C = O frequencies of the *sn*-1 and *sn*-2 C = O groups of EyPC and BhCL by curve-fit analysis. The dashed lines in Fig. 7C and D are the calculated spectra based on the sum of the individual bands. The C = O vibrational bands are split into 1737 and 1726 cm^{-1} for EyPC and 1741 and 1725 cm^{-1} for BhCL. For dry films, the C = O vibrational bands were at 1742 (*sn*-1) and 1729 (*sn*-2) cm^{-1} for EyPC and 1743 (*sn*-1) and 1733 (*sn*-2) cm^{-1} for BhCL (data not shown). The shifts to lower frequencies of their spectra in liposomes imply that the ester C = O groups in the interfacial region of the lipid molecules, and the PO_2^- groups in the polar head region, are important as sites of hydrogen bonding of water to bilayer membranes. The difference between the frequencies of the *sn*-1 C = O and the *sn*-2 C = O groups of the fully hydrated EyPC is not very large. It seems likely that in the liquid crystalline phase, both C = O groups become more or less equally accessible to water due to the expansion of the lipid lattice, which facilitates the intrusion of water molecules between the head groups and the acyl chains [26]. For BhCL, on the other hand, the *sn*-2 C = O group is more hydrated than the *sn*-1 C = O group, thus exposing the *sn*-2 C = O group to the interface. The difference in the C = O frequency of the *sn*-1 (high frequency) and *sn*-2 (low frequency) C = O groups arises from the conformational nonequivalence of these ester groups in the bilayer phase. The C = O vibrations of the mixed BhCL/EyPC (10 mol% BhCL) membranes observed at 1734 cm^{-1} are apparently due to superpositions of four separate individual bands. It is difficult to simulate the two C = O bands for the two lipids. Since curve-fit analysis creates artifacts when the frequencies of the components become too close, we decided to use the difference spectrum (Fig. 7B) rather than the simulated one. Subtraction of the spectrum of BhCL from that of the mixed BhCL/EyPC membrane provides that of EyPC. The C = O vibration of the subtracted EyPC spectrum was shifted to lower frequency from that of the mixed BhCL/EyPC mem-

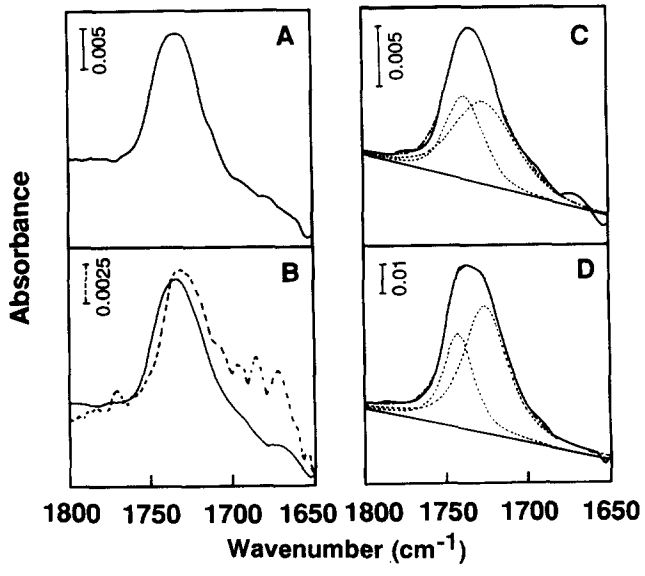

Fig. 7. Observed (A) and subtracted (B) spectra of EyPC liposomes with 10 mol% BhCL of the C = O stretching in the 1650–1800 cm^{-1} region together with the spectra of pure EyPC (C) and pure BhCL (D). (B) Full line, observed EyPC spectrum; broken line, difference spectrum of 10 mol% BhCL from that of mixed BhCL/EyPC (BhCL, 6 mM; EyPC, 54 mM); (C) and (D), full line, observed spectrum; dotted lines, calculated lines. Dashed line, sum of the individual calculated lines.

brane, indicating that the hydrations around the C = O groups in the interfacial region of EyPC molecules were strengthened by the intrusion of BhCL into the bilayer membrane.

4. Discussion

The effects of BhCL on the structural properties of EyPC bilayers in the liquid crystalline state were examined by monitoring the water permeability induced by osmotic stress. The incorporation of small amounts of BhCL (up to 20 mol%) into the EyPC bilayers significantly increased the barrier ability of the liposomal membranes against water permeation (Fig. 2). The negative zeta potential values, which reflected a structural property of the membrane/water interface, increased with increase in incorporation of BhCL (Fig. 3), suggesting that the negative charge of the polar head groups is important for its barrier ability.

From the vibrations of individual groups of the lipid membrane in the IR absorption spectrum, the PO_2^- stretchings of the polar head group and the ester C = O stretchings of the interfacial region of the lipid molecules are readily distinguished from the nonpolar CH_2 stretching vibrations. The fatty acyl chains of phospholipids undoubtedly play an important role in the structural stability of the membrane bilayer. However, the IR data obtained showed that the frequencies of the symmetric and antisymmetric CH_2 stretching vibrations of the mixed BhCL/EyPC membrane under the present experimental conditions were characteristic of disordered acyl chains of lipids in the liquid crystalline state, irrespective of the content of incorporated negatively charged BhCL [25]. It can, therefore, be presumed that strengthening of the membrane barrier ability arises from conformational change in lipid polar groups, including rearrangement of the hydrogen bonding of water to the membrane, due to the incorporation of BhCL into the EyPC membrane, rather than a change in the phase behavior of the fatty acyl chains.

The spectra clearly indicated a change in the state of the membrane surface caused by BhCL (Fig. 5). Incorporation of up to 20 mol% of BhCL into the EyPC bilayers perturbed the antisymmetric PO_2^- stretching vibrations of the lipid polar head groups, which depended on the BhCL content (Table 2). The calculated antisymmetric PO_2^- vibrations of BhCL in the mixed BhCL/EyPC membranes shifted to lower frequency from that of pure BhCL (1215 cm^{-1}) and on the other hand, the calculated vibrations of EyPC, were equal in frequncy to that of the pure EyPC (1230 cm^{-1}). It is difficult to explain the slight change in the antisymmetric PO_2^- frequency of EyPC in the mixed BhCL/EyPC membranes. However, the subtracted stretching vibrations on the ester C = O group in the interfacial region of EyPC molecules, as another potential site for hydrogen bonding with water, shifted to lower frequencies compared with that of pure EyPC (Fig. 7B), indicating that the hydrations around the C = O groups of the EyPC molecules were strengthened by the intrusion of BhCL into the bilayer membrane. In EyPC liposomes, the polar head PO_4^--N^+ moiety of the EyPC molecules is thought to be oriented coplanarly to the bilayer [21,27]. Therefore, BhCL may cause a cooperative conformational change in the EyPC head groups in such a way that the negative charge of BhCL moves the N^+ end of the P-N dipole of EyPC toward a more parallel orientation to the membrane surface [28]. This change of the P-N dipole caused by BhCL incorporation should promote the rearrangement of the water bridges at the bilayer surface that are important for the stable bilayer configuration of the EyPC molecules [29].

We found that for an EyPC bilayer with 50 mol% BhCL, the antisymmetric PO_2^- frequencies (1229 cm^{-1} for EyPC and 1215 cm^{-1} for BhCL) used in the calculated spectrum agreed with those of pure EyPC and pure BhCL (Fig. 6). The BhCL concentration of 50 mol% may induce a change in the phase behaviors of the mixed BhCL/EyPC membranes.

Cardiolipin can adopt both the bilayer structure on hydration and hexagonal H_{II} arrangement under certain strict conditions [30–32]. The H_{II} structure should be unfavorable for the permeability barrier of the bilayer membrane, hence increasing water permeability. We found that the incorporation of up to 20 mol% BhCL resulted in progressive stabilization of the bilayer of mixed BhCL/EyPC liposomes. Thus, it is expected that cardiolipin plays a role in stabilizing the structure of various biomembranes in which its content is low, as well as that of the cardiolipin-rich inner mitochondrial membrane. The molecular mechanism of this effect is important for understanding the role of cardiolipin in various biological membranes.

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