Butyl paraben induced changes in the biophysical properties of dipalmitoyl phosphatidic acid vesicles

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Abstract
The perturbation in the biophysical properties of multilamellar vesicular (MLV) and unilamellar vesicular (ULV) of dipalmitoyl phosphatidic acid (DPPA) by the preservative butyl paraben (BPB) has been studied using DSC and (1H and 31P) NMR. DSC results indicate that the mechanism by which BPB interacts with the DPPA vesicles is similar in MLV and ULV. BPB affected the thermotropic phase transition and the molecular mobility of the DPPA vesicles. For all concentrations, the BPB molecules seem to get intercalated between the polar groups of the phospholipids with its alkyl chain penetrating into the co-operative region. The gel to liquid crystalline phase transition temperature (Tm) of the DPPA vesicles was decreased in the presence of BPB. This suggests increased headgroup fluidity due to reduced headgroup-headgroup interaction. In the presence of BBP additional transitions are observed whose intensity increases with increasing BPB concentration. The large enthalpy values obtained in the presence of BPB suggest that presence of BPB makes the DPPA bilayer more ordered (rigid). The effect of inclusion of cholesterol in the BPB-free and BPB-doped DPPA dispersion was also studied.

Keywords
DPPA, differential scanning calorimetry, NMR, Parabens

Introduction
Studies of dipalmitoyl phosphatidic acid (DPPA) vesicles containing salicylic acid (SA) benzoic acid (BA), aspirin (ASA), para-hydroxy benzoic acid (p-HOBA) and propyl paraben had shown that (a) these molecules are located in the neighborhood of the DPPA headgroup and (b) these molecules interact with DPPA vesicles by affecting both their thermotropic behaviour and their molecular mobility[1-3]. The aromatic compounds, SA, ASA, BA and p-HOBA have carboxylic acid (-COOH) as one of the functional groups with pKa values 2.98, 3.5, 4.2 and 4.58 respectively. Thus the –COOH group in SA is the most acidic while the –COOH group in p-HOBA is the least acidic (the acidic strength, S, of –COOH group in these drugs are as follows: SSA > SASA > SBA > Sp-HOBA). In the presence of SA, BA, ASA and p-HOBA, the gel to liquid crystal phase transition temperature (Tm) of the DPPA vesicles increases and hence reduces lipid headgroup fluidity. The DPPA headgroup becomes more rigid in the presence of SA, BA, ASA and p-HOBA molecules due to increased interaction between the lipid head groups. The maximum increase in rigidity of the DPPA headgroup was found when DPPA is doped with ASA and least when doped with SA. There seems to be good correlation between the acidity of –COOH group present in the drugs and the increase in Tm value (representing rigidity). The width (∆m) of the chain melting transition decreases with increasing concentration of SA, BA, ASA and p-HOBA, implying an enhanced co-operativity of the acyl chain. However, the
results obtained with PPB doped DPPA vesicles were different from that obtained with SA, ASA, BA and p-HOBA doped DPPA vesicles. In the presence of PPB, the gel to liquid crystalline phase transition temperature (Tm) of the DPPA vesicles decreases hence increases lipid headgroup fluidity due to reduced headgroup-headgroup interaction. The width (Δm) of the chain melting transition increases with increasing concentration of PPB implying reduced cooperativity of the acyl chain. At high PPB concentration, additional transitions are observed whose intensity increases with increasing PPB concentration. The large enthalpy values obtained at high PPB concentration suggest that presence of PPB makes the DPPA bilayer more ordered (rigid). Also presence of PPB converted the gel phase of DPPA to a metastable gel phase which on equilibration transforms to stable crystalline phase. PPB esters of p-hydroxybenzoic acid differ from p-HOBA only in the –COOR group (in PPB; R= -CH2CH2CH3 and in p-HOBA; R = H). Hence, the differences in interaction observed with PPB and p-HOBA doped DPPA dispersion seem to be related to the –COOR group.

Parabens (esters of p-hydroxybenzoic acid; methyl paraben(MPB), ethyl paraben(EPB), butyl paraben(BPB) and butyl paraben(BPB)) have both antimicrobial and antioxidant activity[4]. Parabens because of their antimicrobial activity[5-6] and their relatively low toxicity in humans are used as preservatives in foods, cosmetics toiletries and pharmaceuticals. The mechanism by which parabens acts is mainly by causing disorganization of the microbial cell membrane: (a) at low (bacteriostatic) concentrations, parabens appear to cause energy uncoupling which inhibits the uptake of metabolites and (b) at higher (bactericidal) concentrations, loss of the membrane semipermeability occurs[7-10]. Parabens thus inhibit the growth of microorganisms. However, lately the toxicity and cancerigoneous potential of parabens have been raised[11-12]. This paper describes the effect of the preservative, BPB on the thermal and dynamic properties of DPPA dispersions using DSC and (1H and 31P) NMR. BPB esters of p-hydroxybenzoic acid differ from PPB in the –COOR group (in PPB; R= -CH2CH2CH3 and in BBP; R = - CH2CH2CH2CH3).

Materials and Methods

Sample preparation

Lipid, L-a-DPPA, was purchased from Avanti Polar Lipids, Inc., Alabama, USA, and was used without further purification. The preservative, BPB(99+% purity) and cholesterol (from porcine liver, > 99 % purity) were obtained from Aldrich Chemical Company, Inc., USA and Sigma Chemical Company, USA, respectively. The buffer of (i) pH 7.4 was prepared using 10 mM di-sodium hydrogen orthophosphate (Na2HPO4) and 10 mM sodium dihydrogen orthophosphate (NaH2PO4) solutions; to this buffer 0.9 % (w/w) NaCl was added and (ii) pH 9.3 was prepared using 0.2 M boric acid and 0.05 M borax (Na2B4O7.10 H2O) solutions. The model membranes used in this investigation are in multilamellar vesicular (MLV) and unilamellar vesicular (ULV) forms. The method of preparation of the membrane sample in the MLV and ULV forms is the same as that detailed elsewhere (1, 13, 14). The weight ratio of DPPA:cholesterol(ch) is 3:1. The weight fraction of buffer to DPPA is 2.5 in MLV. In ULV the lipid concentration [lipid], used is 25 mM. The molar ratio, Rm, of BPB to DPPA is in the range, 0<Rm<0.4. From systematic study carried out with DPPA dispersion prepared at different pHs, it is found that DPPA formed stable ULV at pH 9.3 when sonicated for 10 minutes (forms translucent dispersion). However, at pH < 9.3 DPPA do not form stable ULV (the dispersion remains milky indicating presence of MLV also). Hence the ULV of DPPA is prepared using pH 9.3 buffer as it forms stable ULV at pH 9.3 (1,14).
For DSC measurements 7-12 mg (for MLV) and 15-18 mg (for ULV) of the samples were hermetically sealed in aluminum pans. To obtain NMR spectra, approximately 1 ml of ULV was taken in a conventional NMR tube. TLC studies on the samples were carried out to check the intactness of the DPPA and BPB molecules.

**Differential Scanning Calorimeter**

Mettler Toledo DSC 822 was used for thermal measurements of the membrane samples, with an empty aluminum pan as a reference. Temperature and enthalpy calibration of the instrument were done using cyclohexane and indium at a heating rate of 10 °C/min. The chain melting (CM) transition temperature, \( T_m \), was obtained by extrapolating the transition peak temperatures (obtained at scanning speed of 10, 5 and 2 °C/min) to zero scanning speed. The transition enthalpy, \( \Delta H_m \) of the endothermic curve reported is the average of 5 and 2 °C/min scans. The full width at half maximum, \( \Delta m \), used to compare the co-operativity of the CM transitions was obtained from 5 °C/min scans.

The DSC measurements were carried out for both the MLV and the ULV. Experiments were carried out immediately after the preparation (\( \tau_e \approx 0 \)) of the respective (MLV and ULV) membrane samples. Experiments were repeated after equilibrating the samples (a) for 1 day (\( \tau_e \approx 1 \) day) at 25 °C and (b) for 7 days (\( \tau_e \approx 7 \) days) at 25 °C. For each value of the molar ratio, \( R_m \), the experiment was repeated with at least three samples. Data were considered only for those samples in which weight loss was less than 0.2 mg at the end of the scanning experiments. The expected experimental error in temperature and enthalpy values was ± 0.1 °C and ± 5%, respectively.

**Nuclear Magnetic Resonance (NMR)**

\(^1H\) and \(^31P\) NMR spectra were recorded on a Bruker Avance 500 spectrometer equipped with a calibrated temperature control at 500 MHz and 202 MHz respectively. \(^1H\) NMR spectra were acquired using a 9000 Hz spectral width into 8 K data points, a 1s recycle delay, an acquisition time of 0.5 s and a \( \Pi/2 \) pulse length of 10 µs. The number of acquisitions was 512. The water signal suppression was achieved with pre-saturation of the HDO signal during the relaxation delay of 1 s. The free induction decays (FIDs) were multiplied by a 90° phase shifted sin-bell function before Fourier transformation. For \(^31P\) NMR the broadband proton-decoupled spectra were acquired using a recycle delay of 2 s, spectral width of 60,000 Hz, a \( \Pi/2 \) pulse length of 17 µs and an acquisition time of 0.67 s. The number of acquisitions was 1024. A line broadening of 10-20 Hz was applied to the FID, before Fourier transformation.

The conventional 5 mm NMR tube containing approximately 1 ml of ULV was used to record both \(^1H\) and \(^31P\) NMR spectra. D2O and H3PO4(85 %) were used as external references for \(^1H\) and \(^31P\) NMR experiments respectively. The NMR spectra were recorded in the vicinity of the chain melting transition temperatures of the ULV. At each temperature the samples were equilibrated in the NMR spectrometer for at least 10 min before recording the spectra.

**Results**

**DSC - multilamellar vesicles (MLV) \( \tau_e = 0 \)**

Fig. 1a-c represents the DSC heating scans of BBP doped DPPA vesicles obtained at a scan rate of 5 °C/min, and for equilibration time, \( \tau_e = 0, 1 \) and 7 days respectively. The corresponding molar ratio (\( R_m \))-dependence of the thermotropic parameters, the transition temperature, \( T_m \) and the transition enthalpy, \( \Delta H_m \) are given in Fig. 2a and b respectively. The \( T_m \) and \( \Delta H_m \) values for DPPA-PPB-buffer pH 7.4 system are also given in Fig. 2a and d (filled circle) [3].
Figure 1 The DSC heating profiles of DPPA-BPB-buffer pH 7.4(MLV) containing increasing amount of BPB at 5 °C/min for (a) $\tau_e = 0$, (b) $\tau_e = 1$ day and (c) $\tau_e = 7$ days. The molar ratio $R_m$ of BPB to DPPA is indicated on the curve. The dotted curve is first run after equilibration; solid curve is second run.

The DPPA ($R_m=0$) dispersion prepared in buffer pH 7.4, when heated undergoes a gel to liquid-crystalline phase transition centered at 65.5 °C and the enthalpy, $\Delta H_m$, associated with this transition is 44.5 kJ mol$^{-1}$ in agreement with the previous reports[15,16]. Presence of BPB in DPPA-buffer pH 7.4 system decreases the chain melting transition temperature and increases the width of the CM transition. DPPA dispersion with BPB concentration, $R_m \geq 0.05$, shows presence of additional transitions(approximately at 35 °C(LC2* phase) and 47 °C(LC3* phase)) and whose transition temperatures are not affected significantly with increasing BPB concentration. Similar effect was seen in DPPA-PPB-buffer pH 7.4 system[3]. However, in DPPA-PPB-buffer pH 7.4 system the new transitions were observed at approximately 39.5 °C(LC2* phase) and 50.5 °C(LC3* phase)(Fig. 2a and d (filled circle)). In DPPA-BPB-buffer pH 7.4 system the intensity of the transition seen at 35 °C increases with increasing BPB concentration. But the intensity of the transition seen at 47 °C (LC3* phase) is not changed with increasing BBP concentration. This behaviour is different from that seen with DPPA-PPB-buffer pH 7.4 system was in the intensity of both LC2* and LC3* phases increases with increasing PPB concentration [3]. The total transition enthalpy of DPPA-BPB-buffer pH 7.4 system, for $R_m=0.1$, increases with increasing BPB concentration. But for $R_m \geq 0.1$ there is no further increase in the enthalpy value. However, in DPPA-PPB-buffer pH 7.4 system the total transition enthalpy increases with increasing PPB concentration(Fig. 2d (filled circle)).

Figure 2 $R_m$-dependence of transition temperatures, $T_m/c_2/c_3$ and the total transition enthalpy ($\Delta H_{tot}$) of : (a) and (d) DPPA-BPB/PPB-buffer pH 7.4, (b) and (e) DPPA-ch-BPB-buffer pH 7.4 and (c) and (f) DPPA-BPB/PPB-buffer pH 9.3(ULV) systems respectively. For BPB : $\tau_e = 0$ (o); $\tau_e = 1$ day (?); $\tau_e = 7$ days (A) and for PPB : $\tau_e = 0$ (?). The size of the symbol has been chosen in conformity with the error bar. The lines connecting the data points have been drawn as a guide to the eye.
The effect of addition of cholesterol (ch) to the above systems was also studied. The DSC heating profiles of DPPA-ch dispersion prepared in buffer pH 7.4 both in presence and absence of BPB are presented in Fig. 3a-c for equilibration time, \( \tau_e \approx 0, 1 \) and 7 days respectively. The \( R_m \)-dependence of the thermotropic parameters, the transition temperatures and the transition enthalpies are given in Fig. 2b and e. Presence of 25% cholesterol in DPPA dispersion broadens the CM transition and shifts the transition peak to lower value (59.5 °C). The transition enthalpy, \( \Delta H_m \) associated with this broad transition is approximately 10.9 kJ.mol\(^{-1}\). These effects are similar to that seen with dipalmitoyl phosphatidylcholine (DPPC)-ch system (17-18). From Figs. 2b and 3a it is seen that presence of BPB in DPPA-ch dispersion decreases the CM transition temperature. The transition enthalpy, \( \Delta H_m \) of DPPA-ch-BPB system decreases for BPB concentration, \( R_m = 0.1 \) and with further increase in BPB concentration (\( R_m > 0.1 \)) the transition enthalpy, \( \Delta H_m \) increases (Fig. 2e). For a given concentration of BPB the reduction in the CM transition temperature is more in cholesterol-doped DPPA-BPB system than in cholesterol-free DPPA-BPB system suggesting the interaction of the BPB with DPPA is higher in the presence of the cholesterol.

\[ \tau_e \approx 1 \text{ day and } \tau_e \approx 7 \text{ days} \]

On comparing \( \tau_e = 0 \) day scans with the first (dotted curves) and the second (solid curves) scans recorded with DPPA-BPB-buffer pH 7.4 samples equilibrated at 25 °C for 1 day (\( \tau_e = 1 \) day) and 7 day (\( \tau_e = 7 \) days) (Fig. 3b and c), it is found that there is no significant perturbation in the transition temperature and enthalpy values (except slight reduction in the transition temperature of the LC2* phase (at 39.5 °C) in the first scan). Hence the thermograms obtained after 1 day and 7 days equilibration resemble to that of \( \tau_e = 0 \) day scans. However, this observations are different from that observed with PPB-doped DPPA dispersion were in for \( R_m > 0.1 \) equilibration strongly perturbs the gel phase structure [3] as indicated by the presence of additional peaks (LC2* and LC3*) in the heating scans. These additional transitions are not seen or are of less intensity in the second and the successive scans recorded. The gel phase of PPB-free DPPA dispersion is known to be a stable phase and do not form crystalline phase (LC2* and/or LC3*) on equilibration. However, the gel phase of PPB doped DPPA dispersion is metastable and on equilibration converted to stable crystalline (LC3*) phase as indicated by a large enthalpy value. The successive heating scans obtained for \( \tau_e = 1 \) and 7 days resemble that obtained for \( \tau_e = 0 \) day and the thermotropic parameters have not changed significantly as compared to their \( \tau_e = 0 \) values.

**Figure 3** The DSC heating thermograms at 5 °C/min of DPPA-ch-BPB-pH 7.4 system with increasing BPB concentration, \( R_m \) as indicated on the curve for: (a) \( \tau_e = 0 \) day (b) \( \tau_e = 1 \) day and (c) \( \tau_e = 7 \) days. First heating scans: dotted curve; Second heating scans: solid curve;

Multiple transitions in the DSC scan indicate presence of different micro-domain probably due to the: (a) presence of drug rich and drug poor region or (b) removal of water molecules bounded to the polar groups of the headgroup because of strong headgroup-headgroup interaction which results in reduced hydration of the headgroup. This could result in more ordered crystalline phase. Example saturated phosphatidylethanoamine (PE) vesicles show
such a behaviour or (c) disruption of vesicles by BPB molecules hence forming aggregates. The first (dotted curve) and the second (solid curve) heating scans recorded with cholesterol doped DPPA-BPB-buffer pH 7.4 samples equilibrated at 25 °C for τe = 1 day and τe = 7 days are shown in Fig. 3 b and c respectively. On comparing τe = 0 day and τe = 1 day and 7 days scans(Fig. 3 a - c), it is observed that (a) the thermal parameters of BPB-free DPPA-ch(Rm=0) and BPB-doped DPPA-ch (for Rm = 0.1) dispersions do not show any significant change and (b) the first scan obtained for Rm > 0.1 shows presence of additional transition and increase in transition enthalpy value. But the thermal parameters of the successive scans do not show any significant change. Similar behaviour was seen with PPB doped DPPA-ch-buffer pH7.4 system.

DSC - unilamellar vesicles

The DSC measurements were also carried out with unilamellar vesicles(ULV) of DPPA prepared in buffer pH 9.3. The DSC heating profiles of the ULV of DPPA both in presence and absence of BPB obtained at the scan rate of 5 °C/min, for τe = 0 and with increasing BPB concentrations are shown in Fig. 4. The Rm-dependence of the thermotropic parameters is given in Fig. 2c and f. The DSC heating thermogram of DPPA dispersion(Rm=0) display an endothermic chain-melting transition at temperature 54.0 °C. A hump is seen at 52 °C whose intensity reduces with time. The enthalpy, ΔHm associated with this transition is 15.1 kJ.mol⁻¹. These thermal values are smaller than the corresponding ones for the MLV. In MLV the water molecules within the bilayers are more structured than the water molecules in ULV. Hence in MLV headgroup-headgroup interaction between the neighboring DPPA molecules is stronger than that in ULV. This reduced headgroup - headgroup interaction in the ULV leads to reduction in Tm value of ULV and less tight packing of the chains than in the MLV form. Presence of BPB in DPPA dispersion decreases the CM transition temperature and increases the CM transition width (Figs. 2c and 5(solid curve)). The reduced CM transition temperature suggests that the presence of BPB reduces the effective headgroup-headgroup interaction. The transition enthalpy(Fig. 2f) associated with the CM transition increases on addition of BPB(for Rm=0.2). However for Rm>0.2 the transition enthalpy decreases. This could probably be due to (a) increase in the size of the ULV when doped with the BPB molecules and/or (b) increased rigidity of the acyl chains due to the presence of the BPB molecules. Dynamic light scattering measurements have shown that the presence of BPB increases the average size of the ULV(data not shown). Similar behaviour was observed with PPB doped ULV of DPPA(Fig. 2c and f (filled symbol)) [3]. The ULV of DPPA when prepared in buffer 7.4 displayed transition at 63.6 °C and the enthalpy associated with this transition is 31.9 kJ.mol⁻¹(Fig. 4 dotted curve). Presence of BPB(Rm=0.2) in DPPA-pH 7.4 dispersion reduces both the transition temperature (60.7 °C) and the enthalpy 28.4 kJ. mol⁻¹) values. These results suggest that the mechanism of interaction of BPB with DPPA is similar in MLV and ULV.

Figure 4 DSC heating scans at 5 °C/min. of ULV of DPPA-BPB in (a) buffer pH 9.3; solid curve and (b) buffer pH 7.4; dash curve with increasing Rm as indicated on the curves and for τe = 0.
\textbf{\textsuperscript{1}H NMR - unilamellar vesicles}

\textbf{Lipid (DPPA) resonances}

The \textsuperscript{1}H NMR spectra of DPPA molecules in DPPA-buffer pH 9.3 and DPPA-BPB-buffer pH 9.3 (Rm=0.2) systems for various temperatures in the vicinity of Tm are shown in Fig. 5a and b respectively. The inset in Fig. 5 gives the assignments for the various proton resonances in the spectra. On comparing BPB-free and BPB-doped DPPA spectra, it is seen that the DPPA resonances are sharper and better resolved for temperature, T > Tm. In presence of BPB the DPPA proton resonances were considerably broadened. Hence the resonances labeled (3) and (7) were hardly seen. The reduced mobility of lipidic protons would mean that the acyl chains become more rigid in the presence of BPB. Even though the Tm of DPPA dispersion was affected by the presence of BPB, no significant change in the chemical shifts of the various lipidic resonances was observed.

\textbf{Figure 5} \textsuperscript{1}H NMR spectra of (a) DPPA(Rm=0) and (b) DPPA-BPB(Rm=0.2) dispersions in the vicinity of Tm. Assignments for the various groups of DPPA are given in the inset. [DPPA]=25mM.

\textbf{BPB resonances}

The \textsuperscript{1}H NMR spectra of BPB in the aqueous medium, BPB-buffer at various temperatures is shown in Fig. 6a. The assignment of BPB molecule is shown in the inset of the figure. The concentration of BPB in the aqueous medium was same as that present in DPPA-BPB system with Rm=0.2. The spectra of the aromatic protons of BPB obtained from DPPA-BPB dispersions at various temperatures around Tm are given in Fig. 6b. On comparing the BPB spectra obtained with DPPA-free (Fig. 6a) and DPPA-doped (Figs. 5b and 6b) systems show perturbation in the BPB resonances (both aromatic and alkyl chain protons). In the presence of DPPA the doublet structure of the aromatic proton resonances of BPB almost disappears as the resonances was considerably broadened. The broadening effect was larger for temperature, T> Tm. These data suggests that BPB molecules interact with the DPPA molecules and the interaction is more for T> Tm. The presence of DPPA increases the chemical shift (downfield shift) of the aromatic protons of BPB. This could be due to the hydrogen bonding interaction taking place between the polar groups of BPB (-OH) and that of DPPA. The proton resonances corresponding to the -OH group of BPB was not seen due to exchange processes. From Figs. 5b and 6a, it is seen that the proton resonance labeled 15, 16, 17 and 18 (\textsuperscript{1}-COOCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3} group) of BPB observed in aqueous medium is not seen in DPPA environment. This means that the dynamics of the BPB protons are affected in the lipid environment. Hence the alkyl chain of BPB becomes more rigid in the presence of DPPA. In PBP doped DPPA system the alkyl chains (R of -COOR) resonances even though broadened are clearly seen for temperature, T< Tm. The \textsuperscript{1}H NMR results suggest that the polar group of BPB molecules is expected to be located near the lipid glycerol moiety and/or the polar headgroup, with its polar group interacting with (a) the vicinal water or (b) the P=O (DPPA) group or (c) the C=O (DPPA) group through hydrogen bonding.
Figure 6  $^1$H NMR spectra of BPB in (a) BPB-buffer pH 9.3 and (b) DPPA-BPB-buffer pH 9.3 (Rm=0.2) systems in the vicinity of Tm. Inset gives the assignment for BPB. [DPPA]=25 mM. The concentration of BPB in buffer is the same as that present in DPPA-BPB-buffer pH 9.3 system with Rm=0.2.

31P NMR - unilamellar vesicle

31P NMR experiments were carried out with BPB-free and BPB-doped unilamellar vesicles of DPPA, to see whether the polar group of BPB interacted with the phosphate group of DPPA. The 31P NMR spectra from the BPB-free and BPB-doped ULV of DPPA are presented in (Fig. 7 a) and b respectively. The BPB-free DPPA dispersion shows presence of two isotropic signals, which implies presence of two chemically different phosphorous environments. The 31P NMR resonance of BPB-free DPPA dispersion is broad for temperature T<Tm and becomes sharp for T>Tm. The broadening of the resonances is due to reduced mobility of the polar headgroup in the gel phase, due to the strong hydrogen bonding interaction between the lipid headgroup. On comparing the 31P NMR spectra obtained with BPB-free and BPB-doped DPPA dispersion, it is observed that the presence of BPB broadens the resonances and shifted the ppm values to higher value (clearly seen for T<Tm). These results suggest that the polar group of BPB interacted significantly with the phosphate group of DPPA and hence reduces the PA-PA headgroup interaction. This interaction reduces the mobility of the phosphate group resulting in a broad peak even for T>Tm. Similar behaviour was seen with PPB doped DPPA dispersion (expect presence of two isotropic signals even for temperature, T>>Tm[3]. This could be related to the length of the alkyl chain.

Figure 7 Proton decoupled 31P NMR spectra of (a) DPPA-buffer pH 9.3 and (b) DPPA-BPB-buffer pH 9.3(Rm =0.2 ) systems in the vicinity of Tm. [DPPA]=25 mM.

Discussion

The DSC results of ULV and MLV indicate that the effect of BPB on the DPPA bilayer was more or less the same in both forms. The mode of action of BPB with DPPA is also similar in cholesterol doped DPPA dispersion. The 31P NMR spectra and the decreased Tm value of the BPB-doped DPPA dispersions suggest that the presence of BPB decreases the headgroup-headgroup interaction of the neighboring DPPA molecules and the polar group of BPB probably
interacts with the phosphate group of DPPA. This is supported by the 1H NMR results that the presence of DPPA reduces the mobility of the aromatic protons of BPB. This effect is due to hydrogen bonding and or electrostatic interactions between the polar groups of BPB and DPPA molecules, which reduce the effective headgroup-headgroup interaction. The polar moiety of BPB seems to get intercalated between the polar groups of the phospholipids and the -COOCH2CH2CH3 group of BPB penetrating into the co-operative region. This is supported by the values of transition enthalpy and the chemical shift (not changed) of the various DPPA proton resonances of BPB doped DPPA dispersion. Hence BPB increases the membrane headgroup fluidity and the acyl chain rigidity. However, in PPB doped system (a) at low PPB concentration, PPB increases the membrane headgroup fluidity and decreases the acyl chain rigidity and (b) at high PPB concentration, PPB increases both the membrane headgroup fluidity and the acyl chain rigidity. Also presence of PPB converted the gel phase of DPPA to a metastable gel phase which on equilibration transforms to stable crystalline phase. This effect was not observed with BPB doped DPPA system. The differences in interaction observed with BPB and PPB doped DPPA dispersions seem to be related to the alkyl chain length (R of –COOR group). Prolonged equilibration of BPB-doped MLV of DPPA-ch resulted in the formation of a stable crystalline phase. The stable crystalline phases seem to be more ordered than Lβ phase as indicated by the large enthalpy values. The strong interaction between DPPA-ch and BPB molecules seems to facilitate the stable crystalline phase formation by sequestering out the interbilayer water. The dispersion of dipalmitoyl phosphatidylethanolamine (DPPE) is known to form crystalline subgel (LC) phase and the subgel LC phase. Lα phase transition was assigned to the simultaneous hydration and acyl chain melting of a poorly hydrated crystalline sample, which give rise to high change in total transition enthalpy [30]. While the metastable gel Lβ phase ? Lα phase transition the enthalpy obtained corresponds only to the melting of the acyl chains. However, in BPB doped DPPA-buffer pH 7.4 system equilibration did not bring about any change in the thermal values.

**Figure 8** Schematic model representing the location of BPB molecule in DPPA bilayer.

Fig. 8 gives the schematic model for the likely location of BPB molecules in DPPA bilayer. The –OH group of BPB can form hydrogen bonding with the (a) vicinal water or (b) the -P=O (DPPA) group or (c) the -C=O (DPPA) group. 31P and 1H NMR results suggest that interaction represented by (B) is most likely. As the butyl chain (of BPB) resonances in the presence of DPPA are not seen even for temperature, T<< Tm and the lipid acyl chain resonances are broadened in the presence of BPB. From the interactions represented by (A) and (C) the interaction (A) is more likely than (C). Hence, the presence of PPB molecule between DPPA molecules in the bilayer would increase the separation between headgroups thereby weakening the PA-PA interactions, hence enhances headgroup fluidity.
Conclusion

The results clearly indicate that BPB strongly interacts with DPPA bilayer and creates varied environment for DPPA molecules. BPB increases the membrane headgroup fluidity the acyl chain rigidity. These results imply that inclusion of BPB in biological membranes probably leads to alteration in membrane function. BPB seems to interact with the lipid and makes the cell wall more rigid(ordered). This could lead to loss of the membrane semipermeability hence affecting the membrane function. The perturbation in the membrane properties by the presence of preservative could be of pharmacological importance.

References


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