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Nuclear magnetic resonance and thermal studies on the interaction between salicylic acid and model membranes

Lata Panicker [a,*], K.P. Mishra [b]

[a] *Solid State Physics Division, Bhabha Atomic Research Centre, Mumbai 400 085, India*
[b] *Radiation Biology and Health Sciences Division, Bhabha Atomic Research Centre, Mumbai 400 085, India*

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Abstract

DSC and (^{1}H and ^{31}P) NMR measurements are used to investigate the perturbation caused by the keratolytic drug, salicylic acid (SA) on the physicochemical properties of the model membranes. Model membranes (in unilamellar vesicular (ULV) form) in the present studies are prepared with the phospholipids, dipalmitoyl phosphatidylcholine (DPPC), dipalmitoyl phosphatidylethanolamine (DPPE), dipalmitoyl phosphatidic acid (DPPA) and mixed lipid DPPC–DPPE (with weight ratio, 2.5:2.2). These lipids have the same acyl (dipalmitoyl) chains but differed in the headgroup. The molar ratio of the drug to lipid (lipid mixture), is in the range 0 to 0.4. The DSC and NMR results suggest that the lipid head groups have a pivotal role in controlling (i) the behavior of the membranes and (ii) their interactions with SA. In the presence of SA, the main phase transition temperature of (a) DPPE membrane decreases, (b) DPPA membrane increases and (c) DPPC and DPPC–DPPE membranes are not significantly changed. The drug increases the transition enthalpy (i.e., acyl chain order) in DPPC, DPPA and DPPC–DPPE membranes. However, the presence of the drug in DPPC membrane formed using water (instead of buffer), shows a decrease in the transition temperature and enthalpy. In all the systems studied, the drug molecules seem to be located in the interfacial region neighboring the glycerol backbone or polar headgroup. However, in DPPC–water system, the drug seems to penetrate the acyl chain region also.

Keywords: DPPC; DPPE; DPPA; Differential scanning calorimetry; NMR; Salicylic Acid

1. Introduction

The lipid acyl chain melting (CM) transition of model membrane and the associated changes in the membrane properties, are strongly dependent on factors such as the acyl chain length [1,2], the nature of the lipid headgroup [3,4], the presence of small molecules [5,6] and the pH of the solvent [7,8]. Nuclear magnetic resonance (NMR) and differential scanning calorimetry (DSC) are two out of the many important experimental techniques used in model membrane studies. Generally, ^{1}H, ^{2}H and ^{13}C NMR spectra obtained with the lipid dispersions, give information on the hydrocarbon chain region [9–11], while ^{31}P NMR spectra gives information on the polar headgroup region of the bilayer [12,13]. The NMR techniques are thus useful in determining (a) the nature of the lyotropic phases formed and (b) the dynamics and kinetics of the phase transitions in the model/bio-membranes. DSC is used to obtain the thermodynamic parameters like transition temperature, enthalpy, entropy and co-operativity of the different lyotropic phases formed in the model and bio-membranes [14,15].

In this study, the phospholipids used to form the model membranes are dipalmitoyl phosphatidylcholine (DPPC), dipalmitoyl phosphatidylethanolamine (DPPE) and dipalmitoyl phosphatidic acid (DPPA), with all of them having the same acyl (dipalmitoyl (DP)) chains but their headgroup differed. At neutral pH, the head-groups, phosphatidylethanolamine (PE) and phosphatidylcholine (PC) are zwitterionic,

Abbreviations: DSC, Differential Scanning Calorimetry; SA, Salicylic Acid; DPPC, Dipalmitoyl phosphatidylcholine; DPPE, Dipalmitoyl phosphatidylethanolamine; DPPA, Dipalmitoyl phosphatidic acid; CM, Chain melting; T_m, Chain melting transition temperature.

* Corresponding author. Tel.: +91 22 25594075; fax: +91 22 25505151.
E-mail addresses: lata@magnum.barc.ernet.in, Lata_Panicker@yahoo.com (L. Panicker).

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while the headgroup phosphatidic acid (PA) is negatively charged [16,17]. Dispersions of (a) single lipid (DPPC, DPPE, DPPA) as well as (b) mixed lipid (DPPC–DPPE), in buffer and (c) DPPC in water, are used for DSC and NMR measurements. The perturbation caused in their physicochemical properties by the presence of the kerotolytic drug, salicylic acid (SA), is investigated. SA is an antiseptic and antipruritic drug used in the treatment of wounds and parasitic skin diseases like corns, warts and acne. The objectives of these studies are to determine the (i) likely mechanism by which the drug interacts with the membrane, (ii) effect of the drug on the membrane fluidity, (iii) possible localization of the drug molecules within the bilayer and (iv) influence of drug on the thermotropic phase behavior of the membrane. The information obtained can be of help in understanding the type of interaction between the drug and the membranes in the living organisms.

2. Materials and methods

2.1. Sample preparation

Lipids, L-α-DPPC, L-α-DPPE and L-α-DPPA, were purchased from Avanti Polar Lipids, Inc., Alabama, USA, and were used without further purification. The drug, SA (+99% purity), was obtained from Aldrich Chemical Company, Inc., USA. The buffer of pH 9.3 was prepared using 0.2 M boric acid and 0.05 M borax ($Na_2B_4O_7{\cdot}10H_2O$) solution. The model membrane in unilamellar vesicular (ULV) form were made using single (DPPC/DPPE/DPPA) and mixed (DPPC–DPPE) lipid. The weight ratio of DPPC to DPPE was 2.5:2.2, in order to mimic that constituted in the outer layer of human erythrocyte membrane. To prepare ULV, first a stock solution of SA was prepared in methanol. It was then added to the weighed quantity of lipid (or mixed lipid) powder in order to get the required mole ratio, R_m, of SA in lipid (or mixed lipid). To this lipid (or mixed lipid) drug mixture, chloroform was added and mixed thoroughly with the help of a vortex mixer.

The ULV (with R_m in the range 0 to 0.4) was prepared as follows. A thin film was formed on the inner surface of a test tube, by drying the lipid (or mixed lipid) or drug–lipid (or mixed lipid)–chloroform solution with a stream of nitrogen. The traces of remaining organic solvent were removed by vacuum drying for at least 24 h. This film was hydrated with the buffer/water. Thorough dispersion of lipid (or mixed lipid) or drug–lipid (or mixed lipid) in the buffer/water, was achieved by heating in a water bath kept at a temperature 10 °C above the chain melting transition and then vorticising at room temperature. This procedure of heating and vorticising, was repeated at least ten times. The milky suspension of liposomes obtained was transferred to a sample cell maintained at temperature of 10 °C above the chain melting transition and sonicated (for approximately 20 min) until translucency set in. The translucent dispersion obtained is an indication that the vesicles formed are ULV or vesicles with a few lamella. Sonication was carried out in a vibra-cell ultrasonic processors, using a microtip. The sonication time, power and duty cycle were kept almost the same for all the preparations. This was to achieve the population distribution of the vesicles (ULV and vesicles with a few lamellae), nearly same in all the preparations. DPPE, DPPA and DPPC–DPPE, formed stable ULV when prepared in buffer pH 9.3. The buffer was replaced by doubly (glass) distilled water, for preparing DPPC–water and drug–DPPC–water membranes.

For DSC measurements 15–18 mg of the sample was hermetically sealed in aluminum pans. The concentration of lipid (or mixed lipid), [lipid (or mixed lipid)], were 50 and 25 mM for DSC and NMR experiments, respectively. Light scattering measurement was carried out with both MLV and ULV.

2.2. Differential scanning calorimeter

Perkin Elmer DSC-2C instrument was used for thermal measurements of the membrane samples, with an empty aluminum pan as a reference. Temperature calibration of the instrument was done, using cyclohexane and indium at a heating rate of 10 °C/min. The calibration constants required to calculate the enthalpy values, were obtained using cyclohexane, at heating rates of 10, 5 and 2.5 °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 2.5 °C/min) to zero scanning speed. The area under the endothermic curve, was used to obtain the transition enthalpy, ΔH_m. The scans at 5 and 2.5 °C/min were used for the calculation of CM transition enthalpies. The full width at half maximum, Δm, used to compare the co-operativity of the CM transitions, was obtained from 5 °C/min scans. For each value of the molar ratio, R_m, the experiments were repeated with at least three samples to arrive at the average values of the thermal parameters. Experimental data obtained with samples whose weight loss at the end of the DSC scans was less than 0.2 mg, were only used in the analysis of the results.

2.3. Nuclear magnetic resonance (NMR)

^1H and ^{31}P NMR spectra were recorded on a Bruker Avance 500 spectrometer equipped with a calibrated temperature control at 500 and 202 MHz respectively. ^1H NMR spectra were acquired using a 9000 Hz spectral width into 8 K data points, a 1 s recycle delay, an acquisition time of 0.5 s and a $\Pi/2$ pulse length of 10 μsec. The number of acquisitions were 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 ^{31}P 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.

Table 1
The average size of the vesicles obtained using DLS for both drug-free and drug doped (R_m=0.3) lipid dispersions

	MLV (50 mM)	ULV (50 mM)	ULV (25 mM)
DPPC–H_2O	3900 nm	310 nm	150 nm
DPPC–SA–H_2O	2800 nm	150 nm	145 nm
DPPC	2100 nm	300 nm	200 nm
DPPC–SA	4000 nm	650 nm	270 nm
DPPE	6200 nm	85 nm	65 nm
DPPE–SA	5500 nm	120 nm	120 nm
DPPA	480 nm	90 nm	95 nm
DPPA–SA	1200 nm	85 nm	85 nm
DPPC–DPPE	2000 nm	175 nm	150 nm
DPPE–DPPE–SA	2500 nm	125 nm	130 nm

The conventional 5 mm NMR tube containing approximately 1 ml of ULV solution was used to record both ^1H and ^{31}P NMR spectra. D_2O and H_3PO_4 (85%) were used as external references for ^1H and ^{31}P 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.

2.4. Dynamic light scattering (DLS)

Average sizes of the liposome dispersions were determined by dynamic light scattering at 25 °C using a Malvern 4800 Autosizer employing 7132 digital correlator. The light source was Argon-ion laser operated at 514.5 nm with a maximum power of 2 W. The measurements were made at scattering angle of 90°. The measured intensity correlation functions were analysed by CONTIN method of analysis. The average size, of the vesicles obtained with the drug-free and drug-doped (R_m=0.3) lipid dispersions (for MLV (50 mM), and ULV (50 and 25 mM)) are given in Table 1.

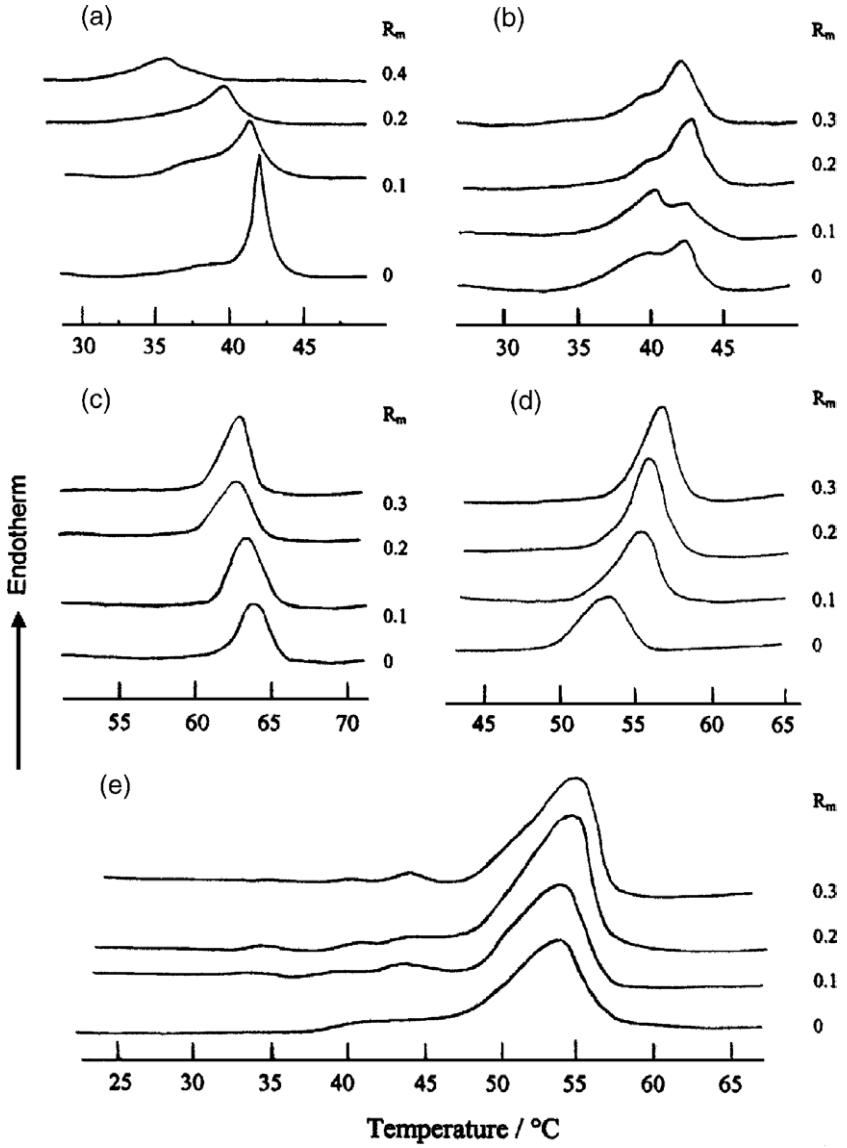

Fig. 1. DSC heating scans at 5 °C/min of (a) DPPC dispersion in water and (b) DPPC (c) DPPE (d) DPPA and (e) DPPC–DPPE dispersions in buffer, both in presence and absence of drug. The molar ratio, R_m, of SA to lipid is indicated against each curve. [lipid]= 50 mM.

3. Results

3.1. DSC

The DSC heating thermograms obtained with DPPC–water, DPPC, DPPE, DPPA and DPPC–DPPE dispersions both in presence and absence of drug, SA are shown in Fig. 1a,b,c,d and e as a function of increasing molar ratio of drug to lipid, R_m. The corresponding R_m-dependence of the thermotropic parameters—the acyl chain melting (CM) transition temperatures, T_m, and the transition enthalpies, ΔH_m,—obtained, are given in Fig. 2a and b.

As seen from Fig. 1b for the case of drug-free DPPC dispersion, the chain melting transition endotherm is broad. This consists of a primary CM transition at 41.8 °C and a broad secondary CM transition at 39.4 °C. The intensity of the primary CM transition is almost same as that of the secondary one and the total transition enthalpy, ΔH_m, associated with the CM transitions, was 7.8 kcal/mole. This characteristic of the CM transition profile suggests the coexistence of ULV with different sizes. The primary and secondary CM transitions arise from big and small size ULV, respectively [18,19]. However, when the ULV (Fig. 1a) was prepared using water instead of buffer, it was noticed that the intensity of the primary CM transition was larger than the secondary CM transition. The thermal parameters of DPPC–water system, (T_m=41.°C and ΔH_m=7.2 kcal/mole), are not significantly altered by change in the pH of the solvent. From Figs. 1b and 2a and b, it is observed that the incorporation of SA in DPPC dispersion hardly alters the CM transition temperature and its width. However, the transition enthalpy, ΔH_m increases with increasing drug concentration. The increased ΔH_m value is related to the increased intensity of the primary CM transition. This suggests the formation of large ULV, with small curvature, resembling the acyl chain order as in multilamellar vesicles (MLV) [18,19]. However, the presence of SA in DPPC–water system (Fig. 1a) broadens the CM transition and decreases the transition temperature and enthalpy. This shows that the interaction of SA with DPPC when the membrane is prepared with water, is considerably stronger than when the membrane is prepared using buffer. The DLS measurements for the drug-free vesicle prepared in water and buffer showed that the vesicle size distribution range (30 to 500 nm) is almost similar in both the case. However, the population of large sized vesicles is more when the vesicle was prepared in water. Presence of drug seems to (i) reduce the average size of the vesicle when prepared in water and (ii) increase the average size of the vesicles when prepared in buffer (Table 1). These observations are consistent with that of DSC findings. The increased number of large size vesicles formed are responsible for increased enthalpy value.

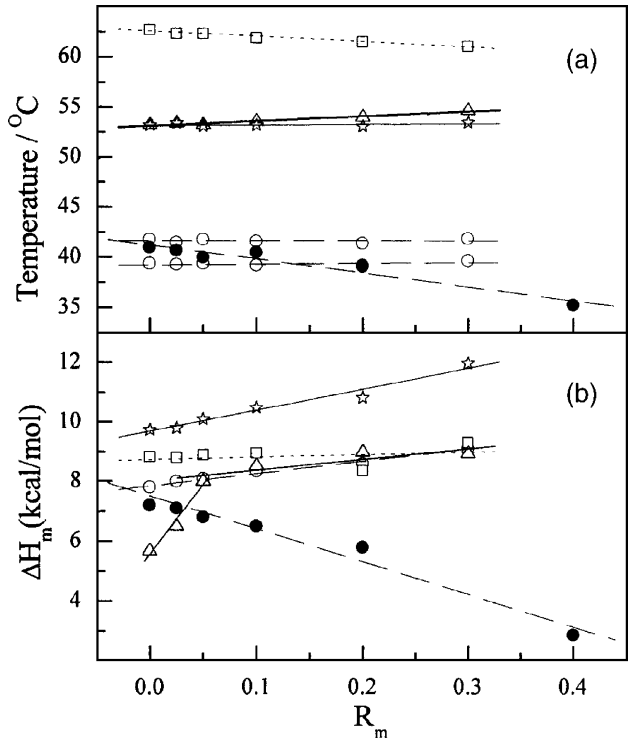

Fig. 2. R_m-dependence of transition (a) temperature, T_m and (b) enthalpy, ΔH_m. ●—DPPC–water, ○—DPPC (transition temperature corresponds to : primary (T_m) and secondary ($T<T_m$) CM transitions), □—DPPE, Δ—DPPA and ☆—DPPC–DPPE dispersions both in presence and absence of the drug. The size of the symbol has been chosen in conformity with the error bar.

The DSC heating profile of drug-free DPPE dispersion given in Fig. 1c shows an endothermic CM transition at a temperature, 62.7 °C. The transition enthalpy, ΔH_m associated with it is 8.8 kcal/mole. As observed from Figs. 1c and 2b, the presence of SA in DPPE dispersion does not change the CM transition enthalpy, ΔH_m and its width. However, the transition temperature, T_m (Fig. 2a) is reduced with increasing drug concentration.

The DPPA dispersion, when heated from ambient temperature to 65 °C, shows an endothermic CM transition in the DSC heating thermogram (Fig. 1d), with T_m and ΔH_m values 53.2 °C and 5.1 kcal/mole, respectively. The DSC thermograms obtained with drug doped DPPA dispersions, are also shown in Fig. 1d. The presence of the drug increases the CM transition temperature, T_m and the enthalpy, ΔH_m of the bilayer and this effect is concentration-dependent (Fig. 2a and b). However the transition width reduces linearly with increasing drug concentration. DLS measurements has shown that the presence of drug did not change the average size of the ULV significantly (Table 1). The increased T_m and ΔH_m values obtained indicates that the presence of drug increased the rigidity of the acyl chain.

Fig. 1e illustrates the DSC heating thermogram of drug-free DPPC–DPPE dispersion. It shows a broad endothermic CM transition at temperature 53.2 °C, with a small hump at 43 °C. The total CM transition enthalpy, ΔH_m, is 9.7 kcal/mole. It is seen that the values for T_m and ΔH_m, obtained for mixed lipid membrane are greater than their average obtained from the respective values for the individual membranes (DPPC and DPPE). This could be due to the increased acyl chain order in the mixed lipid system. From DLS measurement it is found that the size of the mixed-lipid vesicle is (a) less than that of DPPC vesicle and (b) greater than that of DPPE vesicles (Table 1). As observed from Figs. 1e and 2a and b, the incorporation of the drug, SA, in DPPC–DPPE dispersion, hardly affects the transition temperature T_m, and its width. However, the

transition enthalpy, ΔH_m, increases with increasing drug concentration. This is indicative of increased acyl chain order and is supported by the DLS result that presence of drug did not significantly change the size of the ULV (Table 1). However, some additional small humps are observed at temperatures less than CM transition temperature. The intensity of the additional humps, is hardly affected by any further increase in drug concentration.

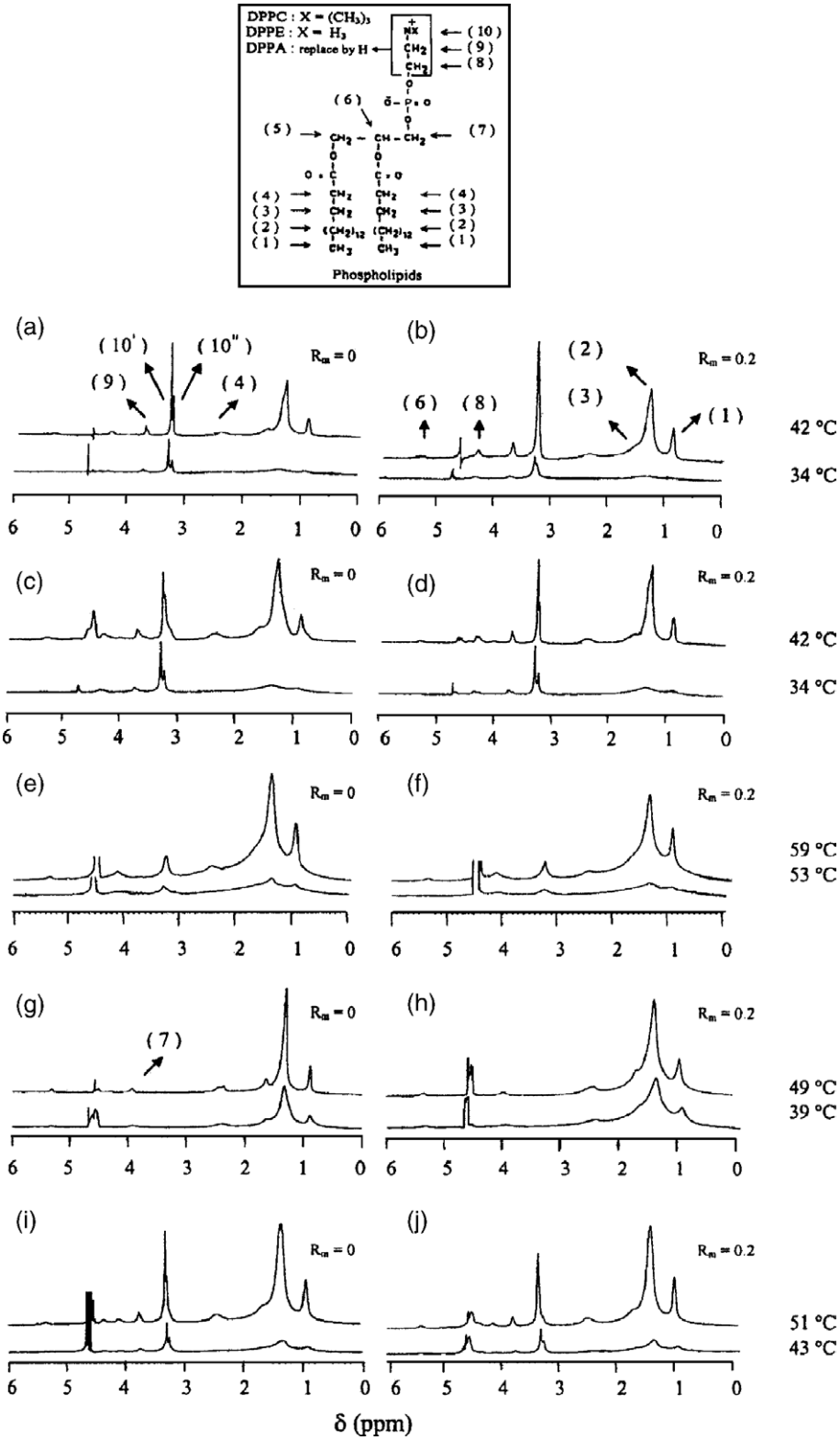

Fig. 3. ^{1}H NMR spectra of (a) DPPC–water (b) DPPC–SA–water (c) DPPC–buffer (d) DPPC–SA–buffer (e) DPPE–buffer (f) DPPE–SA–buffer (g) DPPA–buffer (h) DPPA–SA–buffer (i) DPPC–DPPE–buffer and (j) DPPC–DPPE–SA–buffer, at temperature, $T<T_m$ and $T>T_m$. [lipid]=25 mM. Assignments of the various groups of the phospholipid are given in the inset.

3.2. ^1H NMR

3.2.1. Lipid(s) resonances

^{1}H NMR spectra of DPPC–water, DPPC, DPPE, DPPA and DPPC–DPPE dispersions both in presence and absence of drug, SA, for temperatures, $T<T_m$ and $T>T_m$, are shown in Fig. 3a–j, respectively. The assignments for the proton resonances corresponding to the different groups of the lipid, are given in the insert in Fig. 3.

Important points to be noted are : (i) Except in the case of DPPA, the chain resonances (1) and (2) are seen to be very broad and unresolved at temperatures less than T_m. They get resolved and become sharp, as the temperature approaches T_m. The abrupt change in intensity observed at chain melting ($L_\beta \Leftrightarrow L_\alpha$) transition, corresponds to major changes in molecular order and dynamics, at the transition. The increased chain proton resonance intensity upon phase transition, indicates a decrease in chain order and an increase in chain fluctuation rate. The value of T_m for the membranes, can be obtained by observing the evolution of the chain resonance (1) and (2). Although the T_m of membranes, was affected by the presence of SA, no significant change was observed in the chemical shifts and resonance width of the various lipid resonances. Thus, the spectrum of the lipid in the presence and absence of the drug closely resemble each other. However, in mixed (DPPC–DPPE) lipid dispersions, the chain resonances both in presence and absence of SA, resemble more those of DPPC dispersions than those of DPPE dispersions. The chain resonances (1) and (2) of DPPC, are narrow compared to that of DPPE and are similar to that observed for DPPC–DPPE.

However, in DPPA dispersions, the chain resonances (1) and (2) are sharp even at ambient temperature, they are only slightly broadened, as the temperature is reduced. This indicates that the acyl chains are disordered even below T_m. The proton resonances of the DPPA molecules, are broadened (clearly seen with protons labeled (1), (2) and (3)) in the presence of drug, SA. However, no significant change is observed in the chemical shifts of various DPPA resonances. These data support the DSC results that the drug increases the membrane rigidity and hence probably reduces its permeability. In DPPA dispersions, the drug seems to be localized in such a fashion that it increases the headgroup–headgroup interaction. Thus the effect of SA molecules on DPPA membrane is different from that on DPPC–water, DPPC, DPPE and DPPC–DPPE membranes.

(ii) In DPPC (in water), DPPC and DPPC–DPPE dispersions both in presence and absence of drug, SA, the

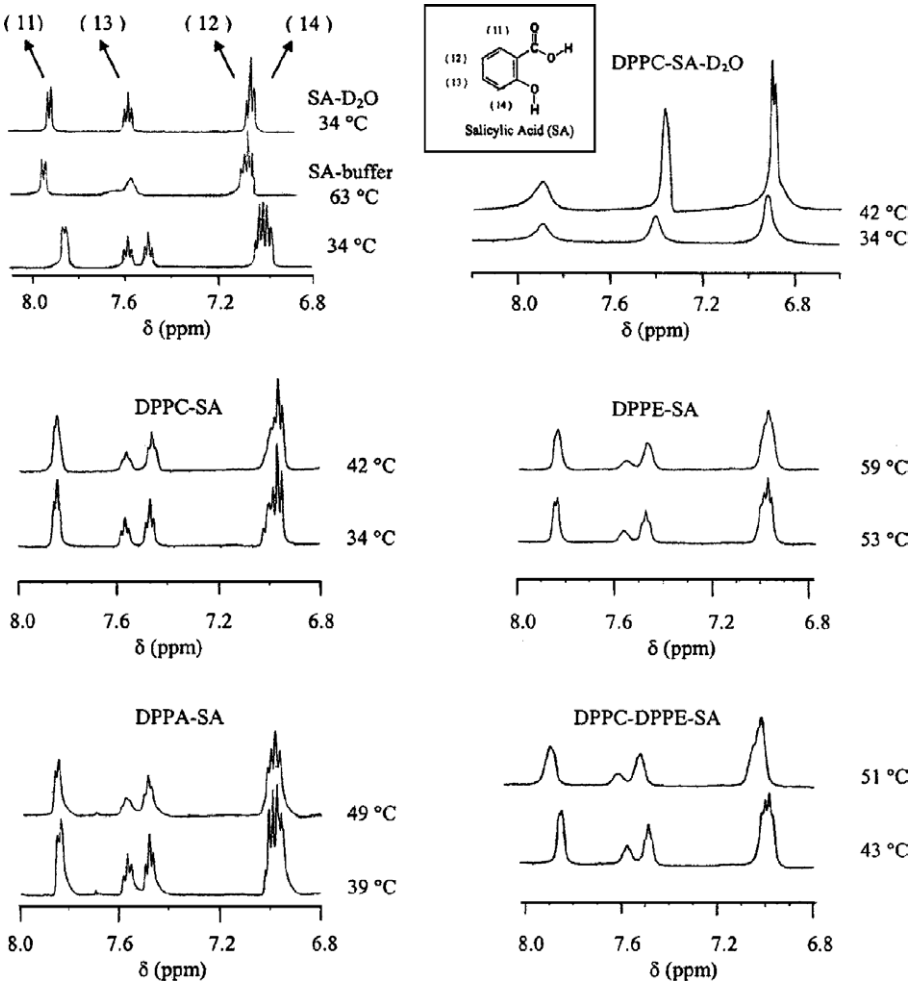

Fig. 4. ^{1}H NMR spectra of the aromatic protons of SA in SA–water and SA–buffer, DPPC–SA–water, DPPC–SA, DPPE–SA, DPPA–SA and DPPC–DPPE–SA dispersions at temperature, $T<T_m$ and $T>T_m$. Molar ratio, R_m of SA to lipid is 0.2 and [lipid]=25 mM. Inset gives the assignment for SA.

choline, ($-N^{+}(CH_3)_3$) resonance, (10), is sharp even at temperature $T<T_m$, indicating that these methyl groups are mobile not only for $T>T_m$ but also for $T<T_m$. Resonance (10), consists of two peaks, (10)′ and (10)″, corresponding to the choline groups of the outer and inner leaflets of the bilayer. The intensity and the line width of the choline resonances are hardly perturbed by the chain melting transition in drug-free and drug-doped DPPC dispersions. The drug hardly seems to be interacting with the choline group in DPPC membrane. However, in DPPC–water and mixed (DPPC–DPPE) lipid system, the choline resonances are less resolved in the presence of SA and are seen as a single peak at $T>T_m$. This suggests interaction of the drug molecules with the choline group at $T>T_m$.

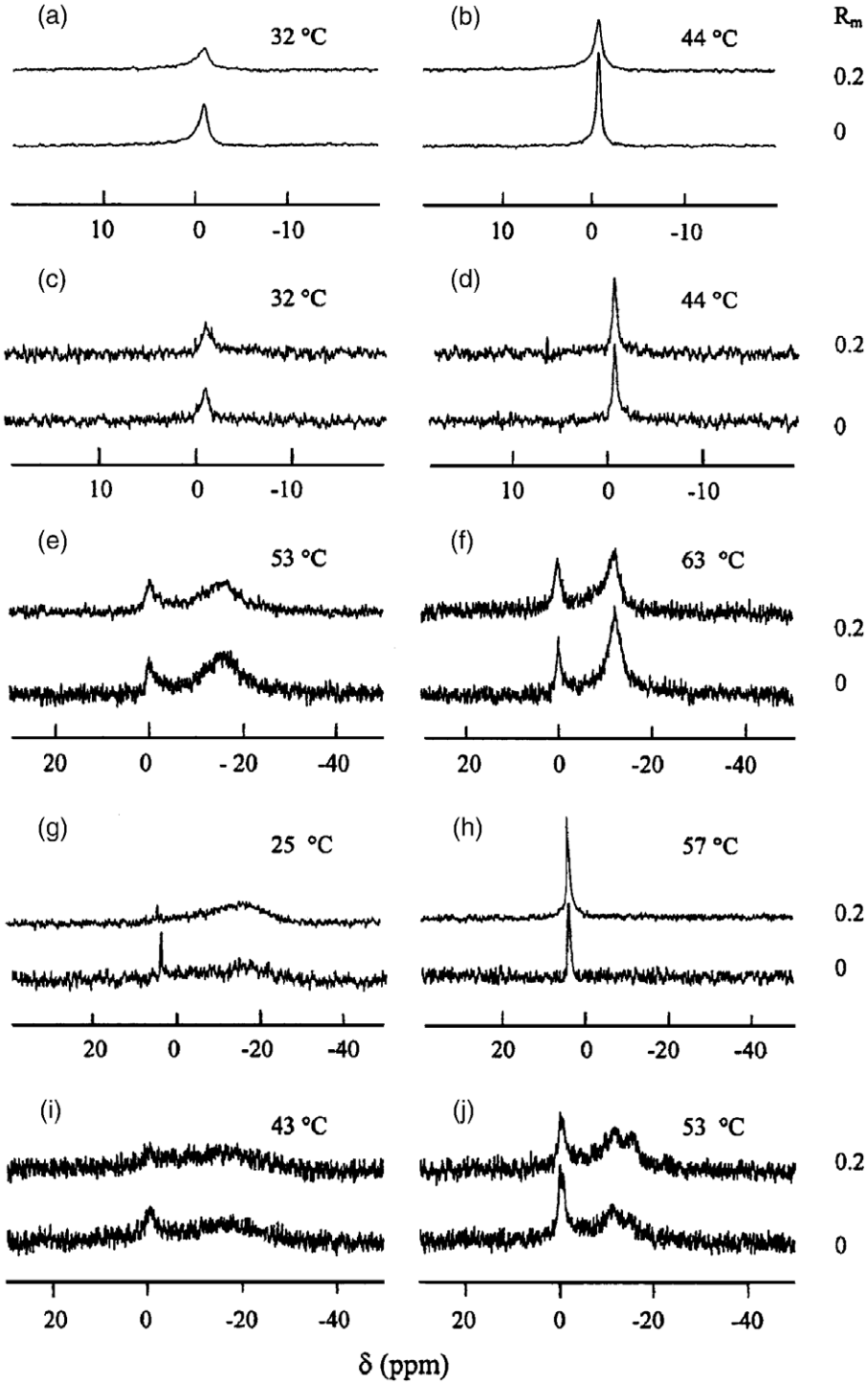

Fig. 5. Proton decoupled ^{31}P NMR spectra of (a) and (b) DPPC–water (c) and (d) DPPC–buffer (e) and (f) DPPE–buffer (g) and (h) DPPA–buffer and (i) and (j) DPPC–DPPE both in presence and absence of drug, at temperature, $T<T_m$ and $T>T_m$. The molar ratio, R_m, of SA to lipid is indicated on the curve. [lipid] = 25 mM.

3.2.2. Drug resonances

The ^{1}H NMR spectra from SA (corresponding to the protons labeled 11 to 14 of SA, as given in the inset of Fig. 4) in the aqueous medium, SA-buffer/water, is shown in Fig. 4. The corresponding aromatic proton resonances, from SA in the presence of DPPC–water, DPPC, DPPE, DPPA and DPPC–DPPE dispersions at temperatures, $T<T_m$ and $T>T_m$ are also shown in Fig. 4. Comparison of the NMR spectra of SA-buffer and SA-water brings to light the presence of two different types of SA molecules, at alkaline pH (Fig. 4). The spectra of SA (Fig. 4) in the presence of lipid(s)-buffer are also indicative of the presence of two different species of SA. The proton resonances corresponding to the –OH and –COOH group of SA, both in the presence and absence of lipid (or mixed lipid), are not observed due to the exchange processes.

From Fig. 4, it is observed that in the presence of DPPC–water, DPPE and DPPC–DPPE dispersions the fine structure of the aromatic proton resonances of SA almost disappears and the peaks are considerably broadened both above and below T_m. The presence of SA, also decreases the chemical shift values of the aromatic protons. This indicates that the SA interacts with the lipid in this range of temperatures. However, in the presence of DPPC (Fig. 4 (DPPC–SA)), the fine structure of the aromatic proton resonances of SA molecules, with lower chemical shift values is not significantly changed both above and below T_m. This indicates that these SA species hardly interact with DPPC in this range of temperatures. However, the SA molecules, with higher chemical shift values seem to interact with the DPPC molecules; the protons labeled (11) and (12) are the most affected, supporting the data obtained with DPPC–water system. It is found that in the presence of DPPA dispersions, the fine structure of the aromatic proton resonances of SA, is hardly altered in the temperature range studied. This shows that the dynamics of the aromatic protons are hardly changed in the presence of DPPA. However, the value of the chemical shift of various aromatic protons, decreased in the presence of DPPA molecules.

3.3. ^{31}P NMR

In order to find whether the polar group of the drug, interacts with the phosphorus moieties of the lipid headgroup, ^{31}P NMR experiments were carried out. The ^{31}P NMR spectra of DPPC (in water), DPPC, DPPE, DPPA and DPPC–DPPE dispersions both in the absence and presence of drug, SA, at various temperatures around T_m, is shown in Fig. 5a–j, respectively.

The ^{31}P NMR resonance (Fig. 5) is broad for temperature, $T<T_m$ and becomes sharp for $T>T_m$ in all the system studied. The broadening of the peak is due to the reduced mobility of the polar headgroup in the gel phase, due to the strong hydrogen-bonding interaction between the lipid headgroup (more clearly seen with DPPE, DPPA and DPPC–DPPE membranes). This effect is more pronounced in the presence of the drug. The sharp peak (isotropic line) obtained in the drug-doped and drug-free DPPC–water, DPPC and DPPA dispersions in the liquid crystalline phase is a characteristic ^{31}P NMR spectrum of a ULV. However, the ^{31}P NMR spectra of DPPE, DPPA (for $T<T_m$) and DPPC–DPPE dispersions both in presence and absence of the drug, show two isotropic signals (one narrow and the other broader, at higher and lower ppm values, respectively), which imply the presence of two chemically different phosphorous environments. When the ^{31}P line shapes obtained with all the systems both for lipid (or lipid mixture) and lipid (or lipid mixture)–drug, are compared, it shows that the presence of SA does significantly change neither the ^{31}P NMR resonance pattern nor its chemical shift; the presence of SA changes only the value of T_m. The experimental results obtained suggest that the polar group of SA, does not interact significantly with the phosphate (P=O) group of the lipid. However, in the case of DPPC–water membranes, the presence of the drug leads to the broadening of the peak.

4. Discussion

In this study, the interaction of drug, SA with different lipid model systems has been investigated using DSC and NMR. The different classes of phospholipids used were PC, PE and PA. The influence of SA on the thermotropic properties of the phospholipids were obtained with DSC. The effect of SA on the dynamics of the phospholipid was obtained by NMR. For the DPPC–SA system, ^{1}H NMR results suggest that the nature of the interaction of drug, SA, with DPPC membrane depends on the type of SA species. The mobility of the aromatic proton of SA species with high chemical shift values is affected but not that of SA species with low chemical shift values. This effect is clearly seen when the DPPC–SA dispersion is prepared in water, wherein the polar group of SA interacts significantly with the polar group of DPPC molecules, leading to reduced mobility of the aromatic protons of the drug molecules. These conclusions are supported by the DSC findings: (i) In DPPC–water dispersion, the presence of the drug molecules reduces the headgroup–headgroup interaction (as indicated by reduced T_m values). (ii) In the DPPC–buffer system, the incorporation of SA does not significantly change the chain melting transition temperature, T_m.

In DPPE membrane (similar to DPPC–water) system, the presence of SA reduces the headgroup–headgroup interaction and hence increases the membrane fluidity. This is supported by the ^{1}H NMR results that the presence of DPPE, leads to a reduction in the mobility of the aromatic protons of the drug molecules. This effect is due to hydrogen bonding interaction between the polar groups of SA and DPPE molecules, which reduces the effective headgroup–headgroup interaction. However, the effect of SA on DPPC–water is more than with DPPE. This differences may be related to stronger PE–PE interaction than PC–PC, allowing deeper insertion of SA molecules into DPPC than in DPPE bilayers [20,21]. This is supported by decrease in enthalpy values with increasing SA concentration in DPPC–water.

The mechanism of interaction of SA with DPPA membrane is different from that observed with DPPC–water, DPPC and DPPE membranes. The differences may be related to the

presence of negatively charged headgroup in DPPA [22,23]. The presence of the drug, SA, in DPPA membrane leads to increased T_m and ΔH_m values and reduced mobility of the methylene groups of the acyl chains. However, the fine structure of the aromatic proton resonances from the drug, SA, are not significantly changed in DPPA environment but the value of the chemical shift is reduced. The presence of SA molecules seems to help the DPPA molecules to orient in such a fashion that it increases the PA–PA headgroup interaction and the acyl chain order. This results in a better packing of the lipid chains, increasing the rigidity as well as order.

The incorporation of the drug molecules in DPPC–DPPE dispersions, does not significantly change the headgroup–headgroup interaction but increases the acyl chain order. The mobility of the aromatic protons of the drug molecules, is reduced in the presence of the DPPC–DPPE bilayer. These results indicate that the polar group of SA interacts with the polar group of the lipid molecules. The nature of interaction of the drug in this case, is similar to that observed in DPPE membrane.

In both single and mixed lipid systems, DSC (the T_m and ΔH_m values) and NMR results, suggest that the drug molecules are probably located in the lipid water interfacial region neighboring the polar headgroup or glycerol moiety of the phospholipid. However, in the case of DPPC membrane prepared with water (instead of buffer), the drug molecules seem to penetrate the co-operative region also (as indicated by reduced ΔH_m value). The drug SA is able to induce a fluidizing effects on lipidic bilayers made of DPPC–water and DPPE. However, presence of SA in DPPA, DPPC and DPPC–DPPE bilayers induced rigidity to the lipidic bilayer.

The DSC and NMR results have shown that lipid headgroup have a pivotal role in controlling the behavior of the membranes and their interactions with drug, SA, which is located at the lipid–water interface region neighboring the polar headgroup or glycerol moiety.

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