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DRUG-LIPID INTERACTIONS IN THE MODEL MEMBRANE, DPPC-WATER: A DSC AND PROTON NMR STUDY.

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Abstract The interactions of the keratolytic drug Salicylic Acid (SA) and the antifungal drugs Methyl Paraben(MPB) and Propyl Paraben(PPB) with the model membrane, DPPC-Water, have been studied using DSC and ^{1}H NMR. The results show that these drug molecules are situated near the membrane interface with their polar group(s) interacting with the vicinal water, while the aromatic regions and the methyl and propyl groups of MPB and PPB interact with the neighbouring hydrophobic regions of the DPPC molecule. The strength of interaction of the aromatic group is in the order PPB > MPB > SA.

INTRODUCTION

It is well known that several drugs, commonly used as medicines, interact with biomembranes, changing their physical characteristics and hence their functions. However, at a molecular level, these interactions are not well understood, since biomembranes are extremely complex in nature, and in many cases, consist of several co-existing phases. In a naive way, the membrane can be pictured as a lipid bilayer matrix (in the lyotropic phase, L_α) in which are embedded proteins, sugars etc. In order to gain some understanding of the drug-biomembrane system, one often studies the drug interactions with model membranes. These are made up of one or more phospholipids which are important membrane components. DiPalmitoyl PhosphatidylCholine (DPPC) -Water is one of the model systems which has been used often for such studies, since PhosphatidylCholines (phospholipids) are important components of mammalian membranes.

Many small drug molecules such as Diamino Diphenyl Sulfone (DDS) and Acetyl Salicylic Acid (Aspirin or ASA) (Figure 1), contain aromatic as well as polar groups. Studies of DPPC-water membranes, containing some of these drugs (DDS[1] and ASA[2] and Δ^8-tetrahydrocannabion[3]), have shown that (a) the drug molecules are located in the neighbourhood of the lipid headgroup, (b) their aromatic group(s) interact with the glycerol moiety and the hydrophobic parts of the DPPC headgroup and (c) their polar group(s) interact with those of DPPC. In order to observe whether other similar drug molecules interact in a similar way with DPPC, three such drugs, Salicylic Acid (SA), Methyl Parahydroxy Benzoate (Methyl ParaBen or MPB) and Propyl Parahydroxy Benzoate (Propyl ParaBen or PPB),

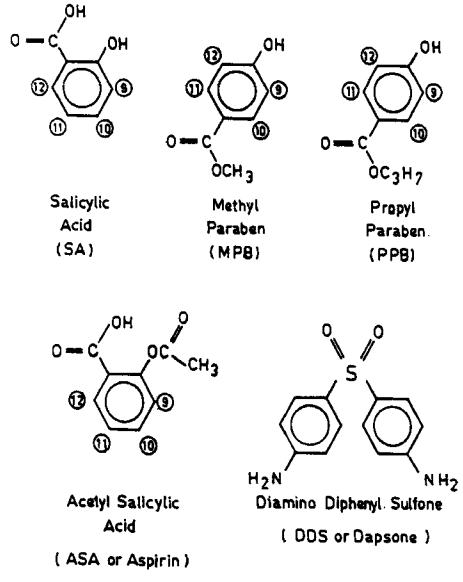

FIGURE 1 Structure of the drug molecules

were used in studies of DPPC-drug-water membrane systems. SA, which is keratolytic, is used in the treatment of corns and MPB and PPB are used as antifungal preservatives. This paper describes DSC and ^{1}H NMR studies of interaction of these drugs in the DPPC-Water system. The results are compared to those obtained with DPPC-ASA-Water system[2].

EXPERIMENTAL DETAILS

L_α-DPPC was purchased from Sigma Chemical Company, USA, and was used without further purification. The drug, SA, was purchased from S.D.Fine Chemical Pvt. Ltd., India and was used after recrystallising with absolute alcohol. The drugs, MPB and PPB were a gift from Nicholas Piramal, India Ltd. The method of preparation of the membrane samples was the same as that detailed elsewhere[4,5], with the following difference: the stock solutions of the drugs, MPB and PPB were prepared in chloroform instead of methanol. The molar ratio, R_m, of drug to lipid was 0.1. In the case of stacked bilayer membranes, the weight ratio, X, of water to lipid was 2.5 and for vesicular membranes, the DPPC concentration was 50 mM. The DSC measurements were made using a Perkin Elmer DSC-2C instrument, with stacked bilayer samples weighing between 8-10 mg. The ^{1}H-NMR experiments were carried out on a Bruker WH-270 FT-NMR spectrometer with the membrane in vesicular form.

RESULTS AND DISCUSSION

DSC Results

DPPC-H_2O exhibits two transitions when heated from ambient temperature, -a pretransition (PT), ($L_{\beta'}$ -> $P_{\beta'}$) at T_{PT} and a Chain Melting (CM) transition, ($P_{\beta'}$ -> L_α) at T_{CM}[6]. The chain melting transition is brought about by the melting (disordering) of the lipid acyl chains. It is of

physiological importance, since it determines the permeability of the biomembranes. The DSC heating scans for the CM transition in DPPC-H_2O as well as for DPPC-drug-H_2O systems are shown in Figure 2. Given for comparison is the scan for DPPC-ASA-H_2O. The presence of the drug molecules in the membrane (a) broadens the CM transition and (b) inhibits the pretransition. These factors indicate that the drugs interact with the membrane and probably with the vicinal water, changing its gel phase structure. The presence of SA leads to the CM transition peak developing a high temperature hump, indicating a phase separation in the bilayer into drug-rich and drug-poor regions, as in the case of the drug, DDS[1]. Table I gives the transition

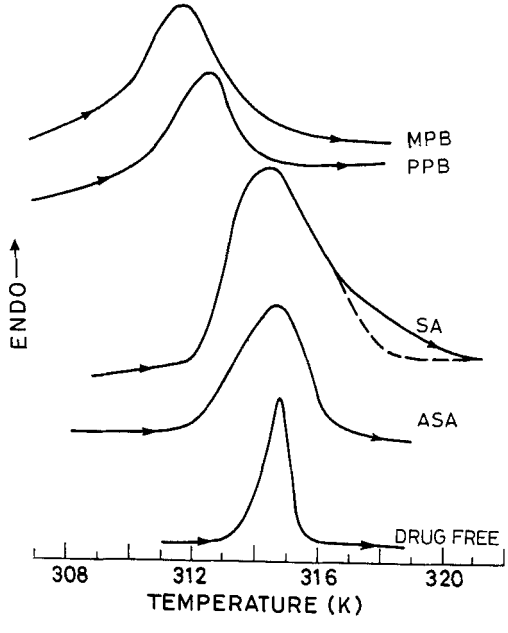

FIGURE 2 DSC heating scans at 2.5 K/min for DPPC-drug-H_2O for X = 2.5 and R_m = 0.1

TABLE I T_{CM}, Δ_{CM} AND ΔH_{CM} for DPPC-drug-H_2O.

Drug	T_{CM} (K)	Δ_{CM} (K)	ΔH_{CM} (K Cal/mole)
None	314.2	0.9	7.7
SA	313.5 315.4	3.4	8.2
MPB	311.6	2.7	5.6
PPB	312.8	2.2	5.7
ASA	314.9	2.7	7.5

temperatures, the transition widths (Δ_{CM}, the full width at half maximum of the transition peak) and the transition enthalpies, ΔH_{CM}. The presence of MPB and PPB leads to a small reduction in T_{CM} (by $\simeq$ 2 K) whereas in the case of SA, the drug-poor and drug-rich regions undergo CM transitions at $T_{CM1} < T_{CM}$ (DPPC - H_2O) and $T_{CM2} > T_{CM}$ (DPPC-H_2O) respectively. The transition width is largest in the case of SA, possibly due to the phase separation. ΔH_{CM} of the membrane is hardly changed by SA, whereas a significant decrease in ΔH_{CM} is brought about by MPB and PPB. ΔH_{CM} is related to the drop in the acyl chain order parameter[7], η , at T_{CM}. Assuming that for T just above T_{CM}, η is almost the same in the case of all the membranes, the reduction in ΔH_{CM} indicates that the presence of the two homologous drugs, MPB and PPB, in the membrane, leads to a reduction in η just below T_{CM}.

^{1}H NMR Results

DPPC Resonances

The gross features of the ^{1}H NMR spectra of DPPC in DPPC-D_2O solution with and without the drugs are similar but a close examination reveals the following: (a) The chain resonances broaden slightly, the broadening being the largest in the case of PPB (b) all resonances show an upfield shift, although the extent of the shift is not the same for all of them. The two choline CH_3 resonances seen for DPPC-D_2O, due to the inner and outer leaflets of the bilayer, are also observed in the DPPC-drug-D_2O systems, showing that there is no significant interaction between the drug molecule and the choline group of DPPC. A preliminary ^{31}P NMR study in the case of SA has shown that this drug interacts with the P=O group of DPPC. The resonance corresponding to the methyl group in ASA occurs at $\sim$ 2.54 ppm and is seen as a sharp peak superimposed on the broad resonance of the lipid acyl chain protons. On the other hand, the methyl group resonance of MPB is broader and is seen at $\sim$ 3.97 ppm. The chain resonances of PPB are broadened and overlap with those of DPPC and hence are not observed.

Drug Resonances

The common features observed in the resonances of all the drugs, incorporated into DPPC - D_2O, are the following: (1) The proton resonances corresponding to the OH group of MPB and PPB, and OH and COOH groups of SA, were not observed, due to the exchange processes. (2) The aromatic proton resonances of all three drugs broaden (Figure 3), showing that this group interacts with the hydrophobic region of the bilayer. These resonances are shown as a function of $\Delta T(=T-T_{CM})$ in the vicinity of T_{CM}, for all the three drugs, in Figure 3. The corresponding spectra

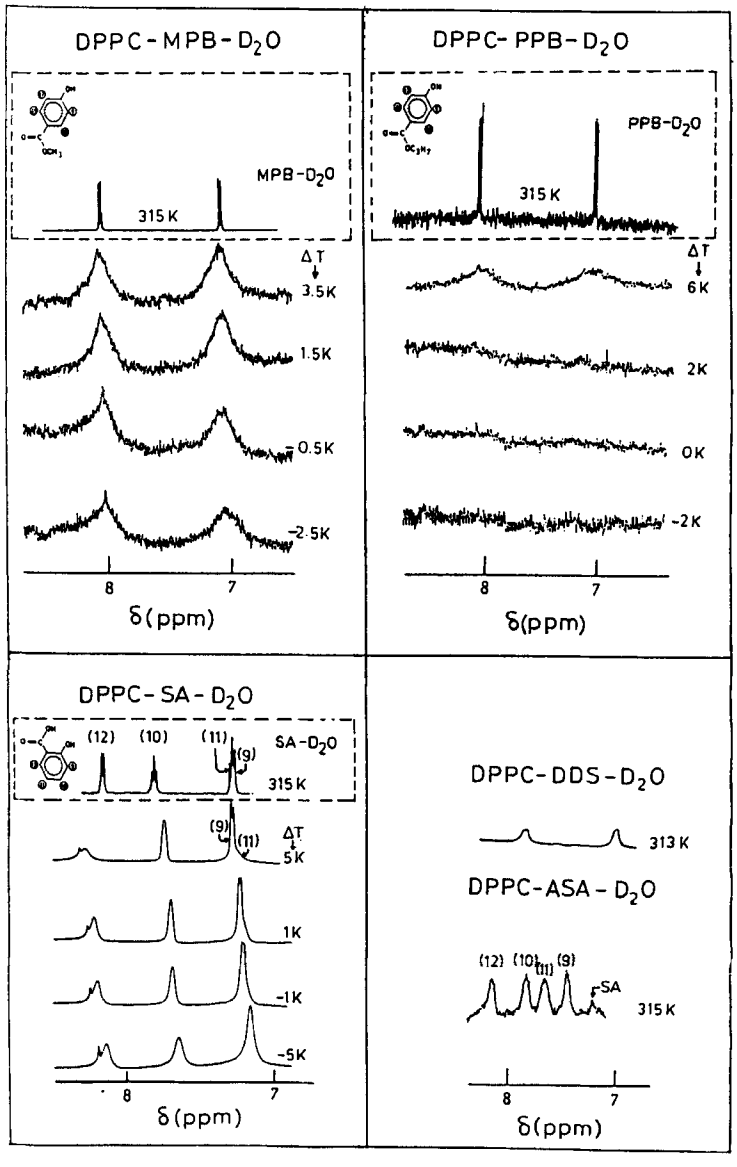

FIGURE 3 ^{1}H NMR spectra of aromatic proton resonances of drugs in DPPC-D_2O as a function of temperature.

for drug-D_2O, are also included in the figure. These resonances do not undergo any significant change at T_{CM}, showing that the aromatic group does not reside in the acyl chain region of the lipid bilayer. The aromatic proton resonances for DDS in DPPC-DDS-D_2O and ASA in DPPC-ASA-D_2O are also given in Figure 3 for comparison. In both cases, the resonances are broadened in the presence of DPPC.

Salicylic Acid

As mentioned earlier, the only drug resonances observed in DPPC-SA-D_2O are those from the aromatic protons. As the temperature increases above T_{CM} ($=T_{CM1}$) (ΔT=1 to 5K), the resonances of the protons 11 and 12 broaden whereas those of the protons 9 and 10 sharpen (Figure 3). These results indicate stronger interaction in the neighbourhood of protons 11 and 12 compared to that of protons 9 and 10. This shows that the drug molecule is located in the interfacial region and above T_{CM} its orientation changes in such a way that the protons 11 and 12 approach closer to the hydrophobic groups of DPPC.

Methyl Paraben

The methyl resonance of MPB is broadened in the DPPC-MPB-D_2O system, as stated earlier. The aromatic resonances too are broadened and this broadening is larger than that observed for SA (Figure 3). The chemical shifts and the widths of these resonances do not change appreciably with increasing temperature, showing that the conformation and mobility of this molecule, within the membrane, do not change significantly in the vicinity of T_{CM}.

Propyl Paraben

The aromatic proton resonances of Propyl Paraben in DPPC-PPB-D_2O system broaden so much below T_{CM}(Figure 3) that they are not observed. Highly broadened aromatic proton resonances are observed only for $T > T_{CM} + 4K$ and the line

widths are nearly two orders of magnitude larger than those observed in PPB-D_2O. The resonances of the propyl group are not observed due to broadening and overlap with DPPC resonances, indicating that this group interacts with the hydrophobic regions of the DPPC bilayer.

The above results indicate that all three drugs interact with DPPC. The transition temperature, T_{CM}, and enthalpy, $\triangle H_{CM}$, are significantly smaller in the case of MPB and PPB than for the drug-free membrane, showing that the drugs introduce some degree of structural disorder in the gel phase of the membrane. The strength of the interaction of the aromatic group of the drugs with DPPC, is greatest for PPB and smallest for SA, as can be seen from the widths of the aromatic proton resonances. In the case of SA, the higher temperature transition CM2, seen in DSC, may be related to the broadening of the resonances of the aromatic protons 11 and 12, which in turn is due to an orientational change of the SA molecule vis-a-vis the membrane interface. In contrast to this behaviour, the presence of ASA (structurally very similar to SA) in the DPPC membrane, leads to just a single CM transition and in addition, none of the aromatic proton resonances of ASA, broaden with increasing temperature. This difference may be attributed to the difference in temperature-dependent orientational changes of SA and ASA with respect to the membrane interface. The broadening of the aromatic proton resonances in the case of MPB and PPB, which is much larger than that of SA and ASA may be explained in the following way. In MPB and PPB molecules, (i) the OH group interacts with either the DPPC polar group or vicinal water, (ii) the aromatic protons interact with the glycerol moiety of DPPC molecule and (iii) their alkyl groups (in the para position) are embedded in the hydrophobic (acyl chain) region of the lipid bilayer. This leads to a considerable reduction in the mobility of these molecules compared to

that of SA and ASA, which remain near the membrane interface. A greater penetration of the alkyl chain of PPB into the bilayer, compared to that of MPB, is indicated by the very large-line widths observed for PPB.

CONCLUSIONS

(1) The drugs, SA, MPB and PPB interact with the model membrane, DPPC-Water, changing its structure, thus inhibiting the pretransition, $L_{\beta'} \rightarrow P_{\beta'}$.
(2) MPB and PPB reduce the transition temperature and transition enthalpy significantly, showing that they probably reduce the order parameter at T_{CM}.
(3) The SA molecule leads to a phase separation in the membrane to drug-rich and drug-poor regions. Above T_{CM} this molecule changes its orientation vis-a-vis the membrane interface. Such a change is not observed with MPB and PPB.
(4) The location and the aromatic group interaction of these three drugs in DPPC-Water, are similar to those of other drugs containing aromatic as well as polar groups, such as DDS, ASA and Δ^8-tetrahydrocannabion.

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