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INTERACTION OF ASPIRIN WITH DPPC IN THE LYOTROPIC, DPPC-ASPIRIN-H_2O/D_2O MEMBRANE

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Abstract In recent years, the analgesic, aspirin, has been found to be a platelet aggregation inhibitor. Its interaction with membranes at a molecular level is not yet understood. In this paper, we describe DSC and proton NMR work, carried out to study aspirin-DPPC interaction in the lyotropic, DPPC-water model membrane. Our results show that (1) the aromatic group of aspirin interacts significantly with the glycerol moiety of DPPC and (2) aspirin decreases the chain melting transition point, thus enhancing the permeability of the membrane.

INTRODUCTION

Aspirin(Acetyl Salicylic Acid or ASA(fig.1)) has been well known as an analgesic for several decades. However, it is only recently that it was found[1] to be useful in the prevention and treatment of heart ailments, because it can prevent blood clots in arteries and veins. Aspirin is known to enhance the rectal permeability of drugs[2], suggesting that this drug affects membranes at the molecular level. As a first step towards gaining some understanding of this effect, it is useful to examine its interaction with model membranes made up of lipids which are important components of biomembranes. This paper describes DSC and ^1H NMR studies of aspirin-DPPC interactions in one such model membrane, - DiPalmitoyl Phosphatidyl Choline(DPPC) - water.

EXPERIMENTAL DETAILS

Lα-DPPC was purchased from Sigma Chemical Company, USA and used without further purification. Aspirin was synthesised and purified by the standard procedure of Vogel[3]. DSC

measurements were made on a TA instrument(DSC 2000), using scanning speeds of 5K and 2.5K/min., with the model membrane in the stacked bilayer form. ^1H NMR studies were carried out on a Brucker WH 270 instrument with the membrane in vesicular form in D_2O. The method of preparation of the membrane samples is detailed elsewhere[4,5] with just one change: the stock solution of the drug was prepared using chloroform instead of methanol. The molar ratio, R_m, of ASA to DPPC was 0 and 0.1 in the DSC work and R_m was in the range $0 \leq R_m \leq 0.2$ in the NMR studies. The weight ratio of DPPC to H_2O, was 1 in the stacked bilayer form of the membrane and for DPPC vesicles, the lipid concentration was 50mM in DPPC-D_2O.

RESULTS AND DISCUSSIONS

DSC results

DPPC-H_2O shows two transitions when heated from room temperature: a pretransition ($L_{\beta'} \rightarrow P_{\beta'}$) at 307K and a chain melting(CM) transition($P_{\beta'} \rightarrow L_{\alpha}$) at 313.2K, brought

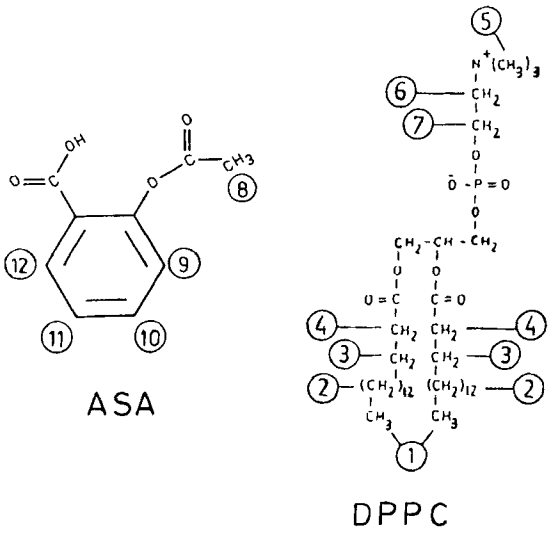

FIGURE 1 Schematic diagrams of ASA(aspirin) and DPPC.

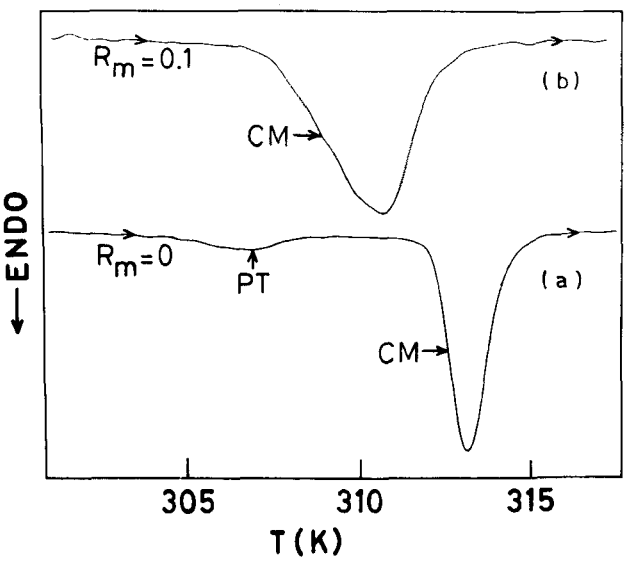

FIGURE 2 DSC heating scans at 2.5 K/min. for DPPC-ASA-H_2O with (a) R_m = 0 and (b) R_m = 0.1.

about by the melting(disordering) of the lipid(DPPC) acyl chains. These are seen in the DSC heating scan(fig.2a) for DPPC-H_2O. The DSC scan for this model membrane containing aspirin(fig.2b) shows that (1) the pretransition disappears, (2) the temperature, T_{CM}, of the chain melting transition decreases and (3) the transition width increases by about a factor of two. The disappearance of the pretransition is related to the change in the structure of the gel phase, brought about by aspirin. This is similar to what is observed when the antileprosy drug, dapsone, is incorporated in this membrane[4]. Table I gives the transition temperature, widths(Δ_{CM}, the full width at

TABLE I T_{CM}, Δ_{CM} and ΔH_{CM} for the model membranes.

Membrane System	T_{CM} (K)	Δ_{CM} (K)	ΔH_{CM} (K Cal/Mole)
DPPC-H_2O	313.1	1.1	12.1
DPPC-ASA-H_2O	310.9	2.8	10.5

half-maximum of the transition peak) and the transition enthalpies, ΔH_{CM}, for the pure and drug-doped membrane. From the DSC results alone, it is hard to ascertain whether the drug is located at the interface or within the bilayer or both. However, the relatively small decrease in T_{CM} points to the drug being at the interface.

^{1}H NMR results

DPPC resonances: Fig.3 shows the ^{1}H NMR spectra for DPPC(fig.1) in the model membranes with R_m = 0, 0.025 and 0.2, for various temperatures in the vicinity of T_{CM}. The assignments[6] for the resonances corresponding to the different groups of DPPC are given in fig.1. It should be noted that in the aspirin doped membranes one also observes the resonance (8) of the methyl group of aspirin in the observed spectra, in the region of 2.4 to 2.6 ppm. This peak which is quite sharp, is superimposed on the rather broad CH_2CO resonance of the acyl chains of DPPC. The values of T_{CM} for the membranes have been obtained by observing the evolution of the chain resonances, (1) and (2). Fig.4 gives T_{CM} as a function of the molar ratio, R_m. There is a linear reduction of T_{CM} with increasing concentration of aspirin(R_m). A good agreement is seen between the values of T_{CM} obtained by ^{1}H NMR and DSC, for R_m = 0 and 0.1. Although the transition temperature of DPPC is affected by the presence of aspirin, no change is observed in the chemical shifts of the various DPPC resonances even for R_m = 0.2.

The temperature dependence(fig.5) of the choline CH_3 resonance, is very interesting. For $T<T_{CM}$, this resonance is asymmetric, indicating that it consists of two resonances due to choline methyl groups of the inner and outer leaflets of the bilayer[7]. Since these groups are not sufficiently mobile at these temperatures, the resonances are not resolved. As T approaches T_{CM}, the asymmetry increases, culminating in the appearance of two resonances, (5') and (5''), with $\Delta\delta(= \delta(5') - \delta(5''))$ being about

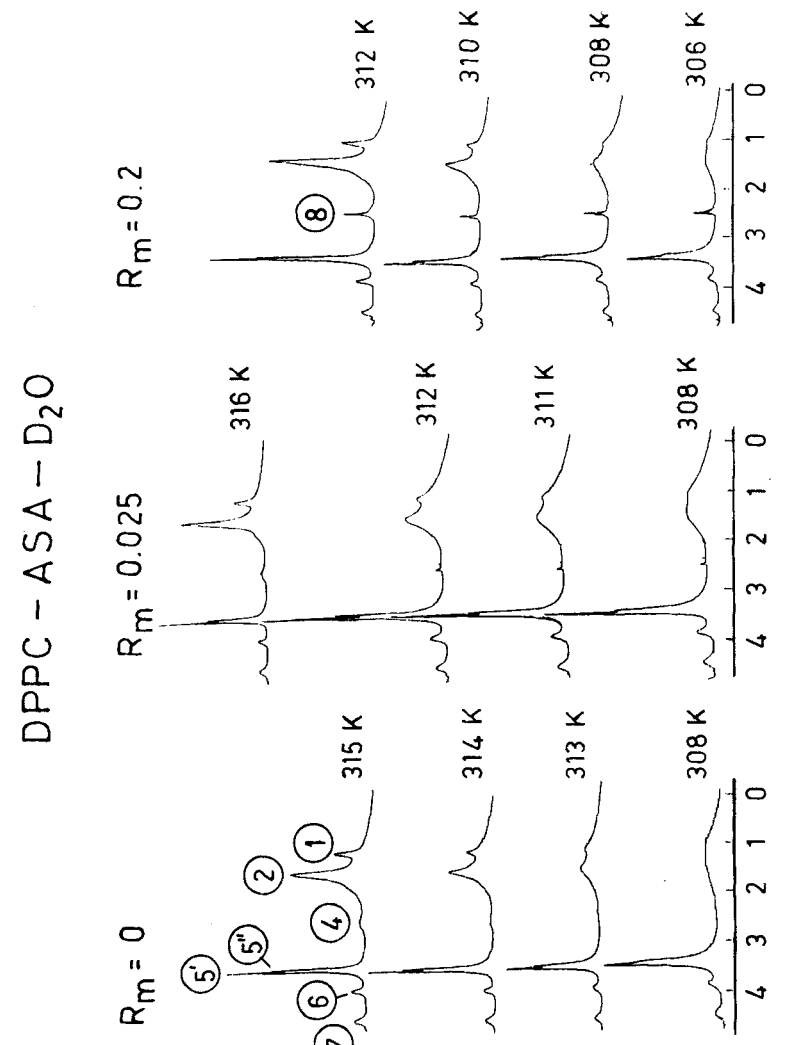

FIGURE 3 ^1H NMR spectra of DPPC for R_m = 0, 0.025 and 0.2.

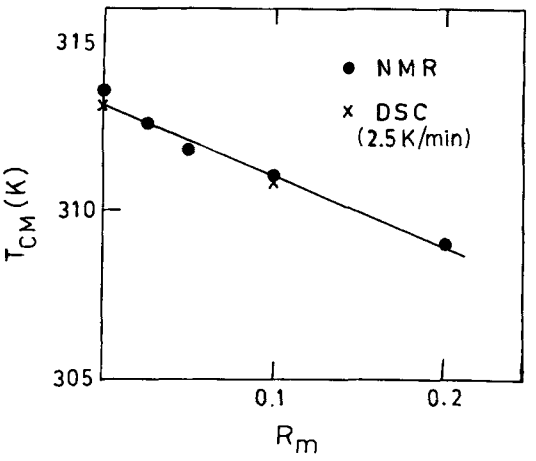

FIGURE 4 T_{CM} as a function of R_m.

0.05 ppm in the vicinity of T_{CM}. The resonance (5') is much stronger than (5''), showing that these are due to the choline groups of the outer and inner leaflets respectively. The difference in the chemical shifts show that the choline groups in the two leaflets sense slightly different environments, leading to a difference in their averaged conformations. As T increases above T_{CM}, one observes that these two resonances become sharper due to increased motions of the methyl groups. This leads to rapid conformational changes, resulting in the two resonances approaching each other and appearing as a single peak with hardly any asymmetry, for temperatures sufficiently above T_{CM}. These features are clearly seen in fig.5a for the drug-free membrane. The presence of aspirin even at fairly high concentration does not change this behaviour, as can be seen in fig.5b, indicating that the drug does not interact with the choline group of DPPC.

Aspirin resonances: The acetyl CH_3 group resonance is hardly affected when aspirin is incorporated in the membrane showing that this group is located in the interlamellar water of the model membrane. The ^1H NMR resonances of the aromatic protons of aspirin in D_2O are

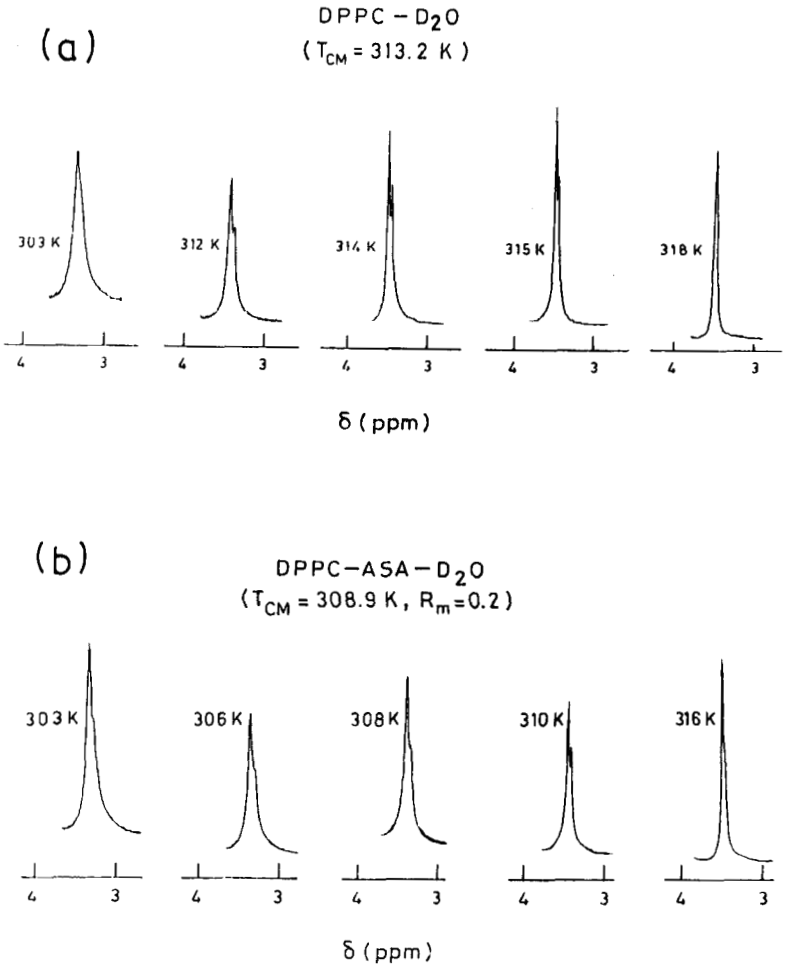

FIGURE 5 Choline methyl proton resonance for (a) R_m = 0 and (b) R_m = 0.2.

shown in fig.6. The assignments of the resonances corresponding to different protons are given in fig.1. The aspirin resonances in the model membrane for various temperatures around T_{CM} at R_m=0.2, are seen in fig.7. It is observed that in the presence of DPPC, the fine structure of the aromatic proton resonances of aspirin almost disappears and the peaks are considerably broadened. This occurs both above and below T_{CM}, showing that the aromatic group interacts significantly with the lipid in this range

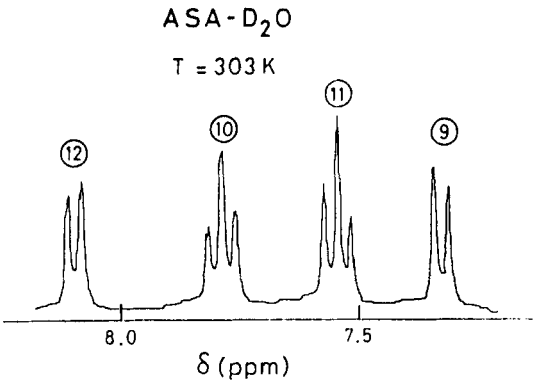

FIGURE 6 Aromatic proton resonances of ASA in D_2O.

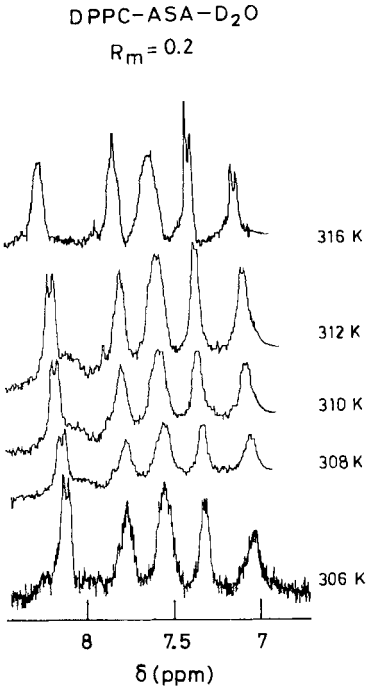

FIGURE 7 Aromatic proton resonances of ASA in DPPC-D_2O as a function of temperature.

of temperature. It should be noted that in the presence of DPPC the aromatic region shows five resonances whereas only

four are observed in ASA-D_2O. The reason for this is not clear at present. These five resonances are present for drug concentrations in the range, $0.025 < R_m < 0.2$. This is observed in fig.8 which gives the spectra for different R_m values for a $\Delta T(=T-T_{CM}) \gtrsim 3.5K$. On the other hand, the relative intensities of the resonances and their structures are R_m-dependent. This could be because a change in R_m leads to a change in the conformation of the aspirin molecule relative to the DPPC headgroup, resulting in a change in interaction between the different aromatic

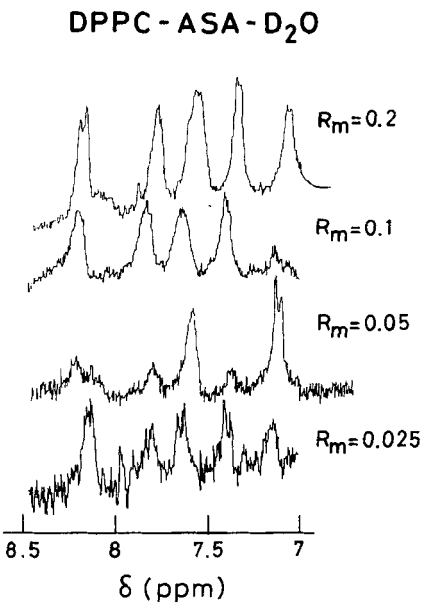

FIGURE 8 Aromatic proton resonances of ASA in DPPC-D_2O for different values of R_m.

protons and DPPC.

The NMR results show that aspirin interacts with DPPC in the model membrane and the aromatic group of the drug is involved in this interaction. However, this group neither interacts with the methylene groups of the acyl chains nor with the choline group of DPPC, since these resonances are not affected by aspirin. Hence, the aromatic group of

ASA must be interacting with the glycerol moiety of DPPC. This is supported by the results of ^1H NMR studies of ASA-DPPC interaction in reverse micelles of DPPC in $CDCl_3$[8]. Hence the aromatic group of aspirin is expected to be located near the the DPPC glycerol moiety with its carboxylic group interacting with (a) the vicinal water or (b) the P=O(DPPC) group or (c) the C=O(DPPC) group, through H-bonding. Of these interactions, (a) and (b) are very much more likely than (c), considering that the acetyl CH_3 is located in D_2O.

CONCLUSIONS

(1) The structure of the gel phase of DPPC-H_2O/D_2O membrane is changed in the presence of aspirin even at low concentration, inhibiting the pretransition, $L_{\beta'} \rightarrow P_{\beta'}$.
(2) Aspirin interacts with DPPC in the model membrane for temperatures both above and below T_{CM}.
(3) The aromatic group of aspirin participates in the ASA-DPPC interaction. This group is located near the glycerol moiety of DPPC with the acetyl CH_3(ASA) group being located in the interlamellar water. The carboxylic group of ASA is most probably H-bonded either with the vicinal water or P=O(DPPC).
(4) The conformation of the aspirin molecule relative to the DPPC headgroup in the membrane, is dependent on the drug concentration.
(5) The enhancement of the membrane permeability which is related to the decrease in T_{CM}, would be of physiological interest.

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