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Influence of the Antileprosy Drug, DDS, on the Phase Transitions in Lyotropic, Mixed Lipid-Water Systems

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DSC investigations of the chain melting (CM), chain ordering (CO) and ice-water transitions in mixed lipid model membranes of DPPC-DPPE-water (weight ratio of DPPC to DPPE being 2:1) have been carried out and the effect of the drug, DDS, on these, have been studied. The water to lipid weight ratio, X, was in the range $0.5 \leqslant X < 3.0$ and the molar ratio, R_m of DDS to lipid was 0, 0.05 and 0.3. A mixture of phases (I and II) has been observed to exist in both the drug-free and drug-doped systems. The drug induces a decrease in the CM/CO transition temperatures of phase I, indicating that the DDS molecules enter the acyl chain region in this phase. From the X-dependences of the ice-water transition enthalpies, ΔH_{iw} and ΔH_{wi}, it has been deduced that the water of hydration for mixed lipids is significantly higher than that for either DPPC or DPPE. Our results have been explained in the light of a model picturing phase I to consist predominantly of DPPC molecules and phase II to be an admixture of DPPC and DPPE molecules, wherein the interaction between head groups is weaker and that between water and head group is stronger than the corresponding interactions in single lipid-water systems.

I. INTRODUCTION

Diamino Diphenyl Sulfone(DDS) has been used as an effective antileprosy drug for more than three decades. Fluorescence quenching

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studies have shown that DDS interacts with proteins through aromatic stacking.[1] Our earlier studies with model membranes using single lipids, DPPC-H_2O[2] and DPPE-H_2O[3], suggest that the drug is located predominantly near the interface and it perturbs the vicinal water structure. We have now extended our investigations to mixed lipid(DPPC-DPPE)-H_2O systems in which the weight ratio of DPPC to DPPE is 2:1 as is the case in some biological membranes.[4] These mixed lipid systems undergo chain melting transitions when heated. But the phases in which they exist prior to and following the chain melting transitions are not known. In this paper we describe the results of our calorimetric studies on mixed lipid systems, with and without DDS.

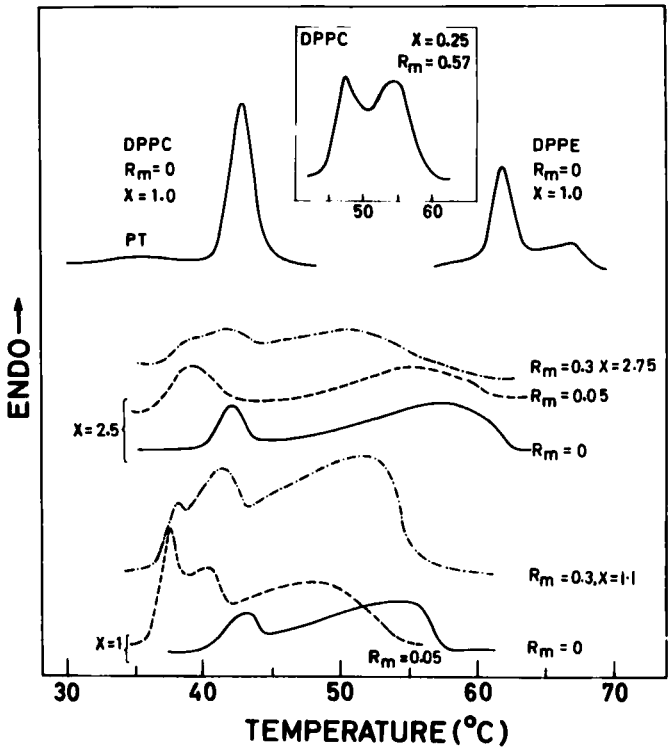

FIGURE 1 Typical DSC scans of chain melting in mixed lipids, DPPC-DPPE and the constituent single lipids. Solid lines refer to $R_m = 0$ and 0.57, the dashed lines to $R_m = 0.05$ and the dot-dashed lines to $R_m = 0.3$.

II. EXPERIMENTAL DETAILS

DDS was obtained from Burroughs Wellcome India Ltd., and the lipids were from Sigma Chemicals. Stock solutions of the lipid mixture and DDS were prepared in analar chloroform and methyl alcohol respectively and used for the sample preparation as detailed earlier.[5] However, for getting a thorough dispersion of water into mixed lipid-DDS samples, the sample tubes were heated between successive vorticising, in a water bath maintained at 75°C (instead of at 65°C as in the case of DPPC-H_2O[5]). Molar ratios, R_m, of DDS to lipid mixture used were 0.05 and 0.3, and the weight ratio, X, of water to mixed lipid was in the range $0.5 \leqslant X < 3.0$.

A Perkin-Elmer DSC-2C instrument was used for the experimental measurements. The scanning speeds for observing the chain melting(CM) and chain ordering(CO) transitions were 10°, 5° and 2.5°C/min. The transition temperatures $T_{CM/CO}$ were obtained by extrapolation to zero scanning speeds. For the ice-water (iw, wi and (wi)') transitions the scans were done at 10° and 5°C/min. The quoted values of transition temperatures and enthalpies for these transitions are those obtained for a scanning speed of 5°/min. The weight of the samples used ranged from 6–12 mg. Measurements for samples which had a weight loss (of water) of more than 0.1 mg during a scan were not considered.

III. RESULTS:

A. Chain melting(CM) and chain ordering(CO) transitions

Typical chain melting scans for mixed lipid systems are given in Figure 1. Given for comparison are the scans for single lipid (DPPC-H_2O and DPPE-H_2O) systems. The CM and CO scans of the mixed lipid systems consist of a low temperature CM1/CO1 peak and a relatively broad high temperature, CM2/CO2 hump(s). From a consideration of CM/CO transition temperatures in single and mixed lipid systems, one can say that these two transitions most probably result from the existence of DPPC-rich(I) and DPPE-rich(II) phases in the sample. A similar separation into two phases, has been observed in mixtures of DPPC and DPPE for other weight ratios also.[6,7] The pretransition(PT) observed earlier with DPPC-H_2O[2] is not present in the mixed lipid scans. This is in agreement with the results of Petrov and others.[6] If Scott Jr's theory[8] for the occurrence of PT is considered, its absence

would imply a slightly denser packing of the acyl chains in this system than in DPPC-H_2O[2].

(*i*) *CM1, CO1 transitions:*

The transition widths, $\Delta_{CM1/CO1}$ (full width at half maximum) of the CM1/CO1 transitions in the mixed lipid system, increase on addition of DDS, just as in the case of single lipid[2,3] systems (see Figure 1). However, for $X \leqslant X_m$ ($X_m = 1.0$ for $R_m = 0.05$ and $X_m = 1.5$ for $R_m = 0.3$) the CM1/CO1 transitions split into two. For $R_m = 0.05$, the low temperature peak is sharp and intense as compared to the high temperature one. For $R_m = 0.3$ the relative intensity of the sharp peak decreases. Such a splitting has also been observed in the case of DPPC-H_2O[5] for $R_m > 0.3$. (inset in Figure 1). This supports our conjecture about the CM1/CO1 transition being related to a DPPC-rich region. For $R_m = 0$ and 0.05, $\Delta_{CM1} \approx \Delta_{CO1}$, whereas for $R_m = 0.3$, $\Delta_{CM1} > \Delta_{CO1}$. The variation of Δ_{CM1} with X is shown in Figure 2. In the case of split peaks, $\Delta_{CM1/CO1}$ are taken as the total width at half height of the two peaks, since they could not be resolved. For $R_m = 0$ and 0.05, Δ_{CM1} decreases with increasing X. For $R_m = 0.3$, after an initial decrease upto $X \approx 1.5$, Δ_{CM1} increases with increasing X. Δ_{CO1} for $R_m = 0.3$ is also shown in Figure 2, and it seems to be more or less independent of X. For DPPC-H_2O[2], Δ_{CM1} is almost independent of X for $R_m = 0$, but decreases with increasing X for $R_m = 0.05$ and 0.3. In DPPE-H_2O[3], there is a very slight increase in $\Delta_{CM/CO}$ with X for $R_m = 0.05$ and 0.3. The behaviour of Δ_{CM1} resembles that of Δ_{CM} for DPPC-H_2O. This would be expected if the CM1 transition occurs in the DPPC-rich region. The broadening of the transitions can be related to varied environment for the lipid molecules caused by DDS, and the splitting to the occurrence of drug-rich and drug-poor regions.

The transition temperatures, $T_{CM1/CO1}$, are plotted as a function of X in Figure 2. It should be noted that for $R_m \neq 0$, where split peaks occur, the average of the two temperatures is plotted. The temperatures of split peaks are also shown for the case $X = 1.1$, $R_m = 0.3$. It is seen that in the absence of the drug, $T_{CM1/CO1}$ decrease with increasing X for $X < 1.5$ but are almost independent of X for $X \geqslant 1.5$. For $R_m \neq 0$, $T_{CM1/CO1}$ are almost independent of X, although an increase of $T_{CM1/CO1}$ is observed for $X > 2.0$ for the case of $R_m = 0.3$. There is a definite shift ($\delta T_{CM1/CO1}$) of the CM1/CO1 transitions to lower temperatures, in the presence of drug even at low concentration ($R_m = 0.05$). Increase of R_m from 0.05 to 0.3 leads to no further decrease of $T_{CM1/CO1}$. The values of δT(2.5°C to 4°C)

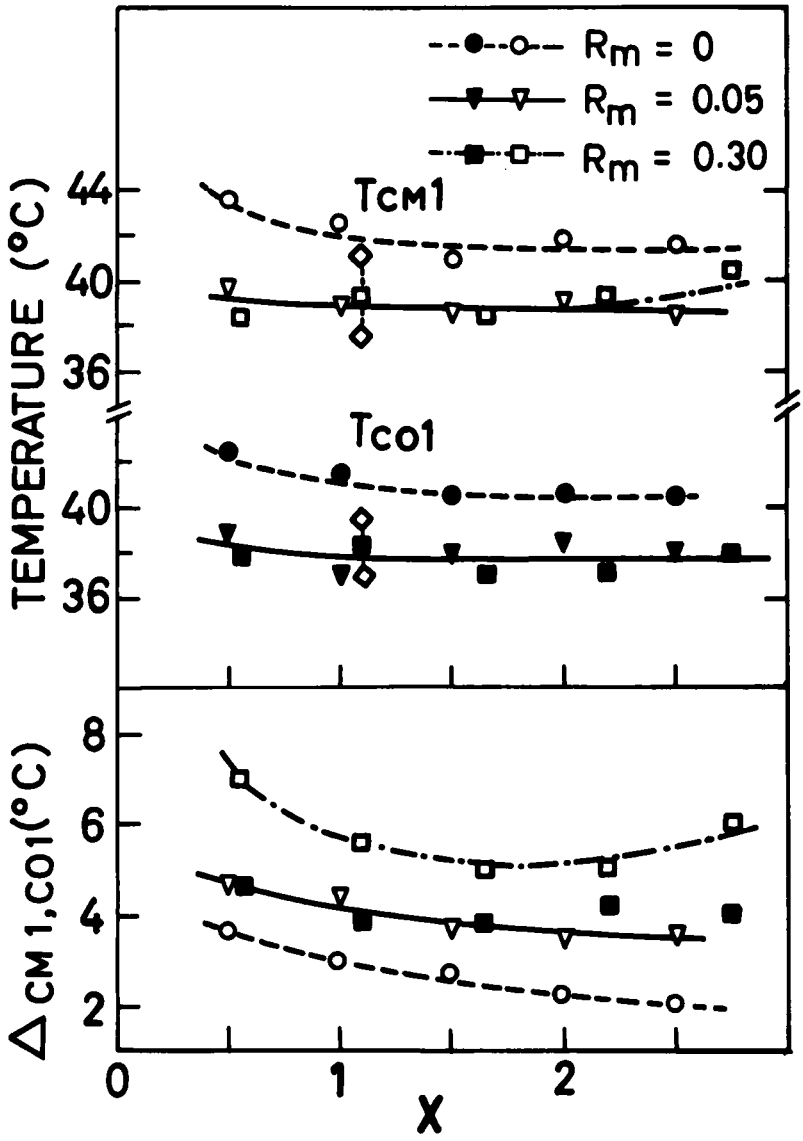

FIGURE 2 Variation of the chain melting (T_{CM1}) and the chain ordering (T_{CO1}) transition temperatures, and the corresponding full widths at half maximum, Δ_{CM1} and Δ_{CO1}, of the transition peaks, as a function of X. (○, ▽, □) correspond to CM and (●, ▼, ■) correspond to CO transitions. ◇ denotes the values for split peaks for R_m = 0.3. The lines are guides to the eye.

obtained here, are much larger than those observed for the single lipid systems.[2,3] These results indicate that although DDS molecules do not enter the acyl chain region in the case of single lipid systems, they certainly do so in the case of the DPPC rich region of the mixed lipid systems, although the number of molecules that can enter this region seems to be limited.

(ii) CM2, CO2 transitions:

The widths of the CM2/CO2 transitions are unusually large, even for $R_m = 0$ (Figure 1). Hence, it would seem that the DPPE-rich component is not a homogeneous one. It is also difficult in this case to draw any conclusions about the change of these widths on the addition of DDS. The CM2/CO2 transitions are similar for $R_m = 0$ and for $R_m = 0.3$. However, for $R_m = 0.05$ they seem to consist of two or more peaks in some cases. The reason for the latter is not understood. The values of the ratio, R_1, of the area of the CM1 peak to that of the CM2 peak, are given in Table I. The errors in the values of R_1 are 30% to 50%, due to the uncertainty in resolving the transition peaks. It is seen that there is a change in this ratio on addition of the drug. Compared to the value for $R_m = 0$, the ratio increases for $R_m = 0.05$ and decreases again for $R_m = 0.3$ (see Table I).

The values of $T_{CM2/CO2}$ are given as a function of X in Table II. The errors on these temperatures are large ($\approx$2.5°C) because of the broad nature of the peaks. Unlike $T_{CM1/CO1}$, the temperatures $T_{CM2/CO2}$ show no definite trend with respect to change in R_m or X. This could be due to the large errors in the $T_{CM2/CO2}$ values.

Taking into account, the observations regarding the relative intensities of the split CM1(CO1) peaks and those of CM1-CM2 peaks we can state that DDS changes (a) the lipid to water ratio in the DPPC-rich phase components and (b) lipid to lipid and the lipid to water ratios in phase II.

(iii) Transition enthalpies, $\Delta H_{CM/CO}$

The transition enthalpies $\Delta H_{CM/CO}$ (the added enthalpies of the components I and II) are given in Table III as a function of X for different values of R_m. With increasing X, $\Delta H_{CM/CO}$ decreases sharply for $X \leq 1$, but varies little for $X > 1$ for all values of R_m. Within experimental errors, the values of $\Delta H_{CM/CO}$ for doped and drug-free samples are the same. These values compare well with the values obtained for the single lipid systems wherein $\Delta H_{CM/CO}$ remain independent of R_m and of X in the range $0.5 \leq X \leq 2.5$ (see Table III).

TABLE I

Variation of R_1 (ratio of the area of the CM1 peak to that of the CM2 peak) with X.

	X	0.5	0.55	1.0	1.1	1.5	1.65	2.0	2.2	2.5	2.75
$R_m = 0$	R_1	0.21	-	0.44	-	0.43	-	0.85	-	0.28	-
$R_m = 0.05$	R_1	0.39	-	0.98	-	1.32	-	2.08	-	0.62	-
$R_m = 0.3$	R_1	-	0.25	-	0.63	-	0.33	-	0.59	-	0.55

TABLE II

$T_{CM2/CO2}$(°C) as a function of X. In the case of $R_m = 0.05$, although more than one transition peak was observed, the temperatures are given only for the strongest peak. However, when the peaks were of comparable intensity, the temperatures for both peaks are given (X = 1.5).

	X	0.5	0.55	1.0	1.1	1.5	1.65	2.0	2.2	2.5	2.75
$R_m = 0$	T_{CM2}	56.2	-	54.5	-	53.5	-	60.1	-	56.1	-
	T_{CO2}	55.2	-	51.6	-	52.5	-	58.0	-	56.3	-
$R_m = 0.05$	T_{CM2}	53.0	-	49.3	-	52.6 61.0	-	60.8	-	53.8	-
	T_{CO2}	52.0	-	48.5	-	51.0 59.5	-	60.2	-	54.4	-
$R_m = 0.3$	T_{CM2}	-	55.5	-	50.5	-	52.5	-	51.5	-	50.7
	T_{CO2}	-	53.2	-	49.1	-	52.5	-	50.5	-	50.2

TABLE III

Chain melting and chain ordering transition enthalpies as a function of X. Average values of these enthalpies have been given for mixed lipid-H_2O, DPPC-H_2O, and DPPE-H_2O.

	X	0.5	0.55	1.0	1.1	1.5	1.65	2.0	2.2	2.5	2.75
$R_m = 0$	ΔH_{CM}	17.10	-	8.74	-	11.62	-	8.82	-	8.53	-
	ΔH_{CO}	16.97	-	8.30	-	11.43	-	8.61	-	8.33	-
$R_m = 0.05$	ΔH_{CM}	16.94	-	10.54	-	9.14	-	9.30	-	8.90	-
	ΔH_{CO}	15.89	-	10.20	-	9.22	-	9.13	-	8.57	-
$R_m = 0.3$	ΔH_{CM}	-	15.70	-	9.90	-	9.93	-	8.74	-	8.46
	ΔH_{CO}	-	15.19	-	9.98	-	8.71	-	9.12	-	8.62

Average $\Delta H_{CM/CO}$ = 9.32 (Mixed lipid)[a]
Average $\Delta H_{CM/CO}$ = 8.45 (DPPC)[2]
Average $\Delta H_{CM/CO}$ = 9.25 (DPPE)[3]

Values of $\Delta H_{CM/CO}$ are given in KCal/Mole.
[a]The values for X = 0.5 and 0.55 are not included in the averaging.

B. Ice-water interactions:

(i) *Transition temperatures, T_{iw}, T_{wi} and $T_{(wi)'}$*

The ice-water(iw) and water-ice (wi and (wi)') transitions seen in single lipid systems[2,3] are also observed in mixed lipid systems at around the same temperatures. Typical scans of ice-water transitions are shown in Figure 3 for X = 0.5 and 1.5 and for R_m = 0 and 0.3. The iw transition occurs around 1.5°C. The wi transition occurs anywhere in the range −16°C to −24°C, while the (wi)' transition occurs at about −42°C. These temperatures are independent of both X and R_m. For small values of X, for both drug-containing and drug-free samples, a hump is observed in the heating scan at $T < T_{iw}$ as in the case of DPPE-H_2O (Figure 3). The hump approaches the iw transition as X increases, and for $X \geq 2$ the hump is no longer observed. In the case of DPPC-H_2O, such a hump was observed for small X, although only for the drug doped samples. For $R_m \neq O$, as in the

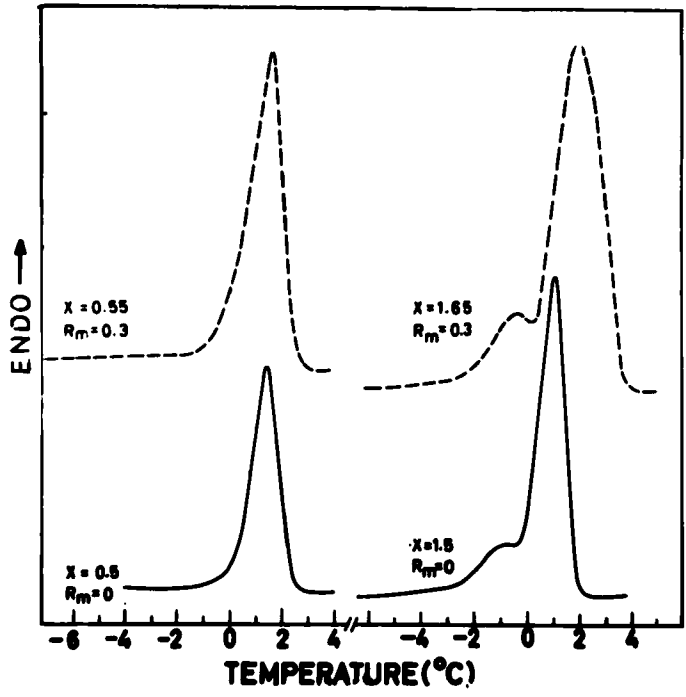

FIGURE 3 Typical DSC scans of the ice-water (iw) transition. Continuous curves correspond to R_m = 0 and the dashed curves correspond to R_m = 0.3.

case of single lipids, the iw transition is asymmetric, the width of the low temperature part being larger than that of the high temperature part. This asymmetry decreases with increasing X. This is indicative of the perturbation of the vicinal water by DDS.

As has been observed with single lipids, the cooling transition, wi is a large, narrow transition whereas the (wi)′ transition is a small, broad one. The intensity of the (wi)′ transition reduces drastically with the reduction of scanning speed, showing that this transition involves a metastable phase. For X = 0.5, these water to ice transitions were seen to be greatly affected on addition of drug in DPPC-H_2O. No such changes occur in mixed lipid-H_2O. The transition widths Δ_{iw} and Δ_{wi} increase with X for all R_m, due to the increased water content of the samples at higher X values. On the other hand, $\Delta_{(wi)'}$ remains almost independent of X and R_m possibly because this transition involves a metastable phase.

(ii) Ice-water transition enthalpies, ΔH_{iw}, ΔH_{wi} and $\Delta H_{(wi)'}$

Figure 4 gives the X-dependences of the enthalpies of the iw, wi and (wi)′ transitions. It should be noted that the values of $\Delta H_{(wi)'}$ which are given for a scanning rate of 5°/min, would change for other scanning rates. As in the case of single lipids, ΔH for all these transitions increases with increasng X. While ΔH_{wi} seems to become constant for $X \geqslant 1.5$, ΔH_{iw} and $\Delta H_{(wi)'}$ for ($R_m = 0$) continue to increase for $X > 1.5$. Extrapolating to $\Delta H = 0$ for iw and wi transitions for $R_m = 0$, we find that free water appears in mixed lipid-H_2O for X greater than about 0.44($= X_{hyd}$). This shows that the water of hydration for the mixed lipids is greater than that for DPPC($X_{hyd} = 0.25$)[2,9] and DPPE($X_{hyd} = 0.21$).[9]

The presence of drug, even in small concentration ($R_m = 0.05$) results in a very slight decrease of ΔH_{iw}, but no further decrease is observed when R_m is increased to 0.3. This behaviour is different from that observed with DPPC-H_2O.[2] In the latter case a change of R_m from 0 to 0.05 leads to hardly any change in ΔH_{iw} whereas for $R_m = 0.3$, ΔH_{iw} decreases by a factor that is larger than that found in the mixed lipid systems. By contrast ΔH_{iw} is independent of R_m in DPPE-H_2O.[3] Thus, in the case of the mixed lipid systems, X_{hyd} increases slightly on addition of drug, due to the decrease in ΔH_{iw}. $\Delta H_{(wi)'}$ also decreases when R_m is changed from 0 to 0.05 but changes no further on increasing R_m to 0.3. For $R_m \neq 0$, $\Delta H_{(wi)'}$ is independent of X. In the single lipid systems, $\Delta H_{(wi)'}$ decreases as R_m is changed from 0 to 0.3 and no saturation effects are seen for $R_m > 0.05$. ΔH_{wi} is independent of R_m in the mixed lipid system as in the single lipid systems.[2,3]

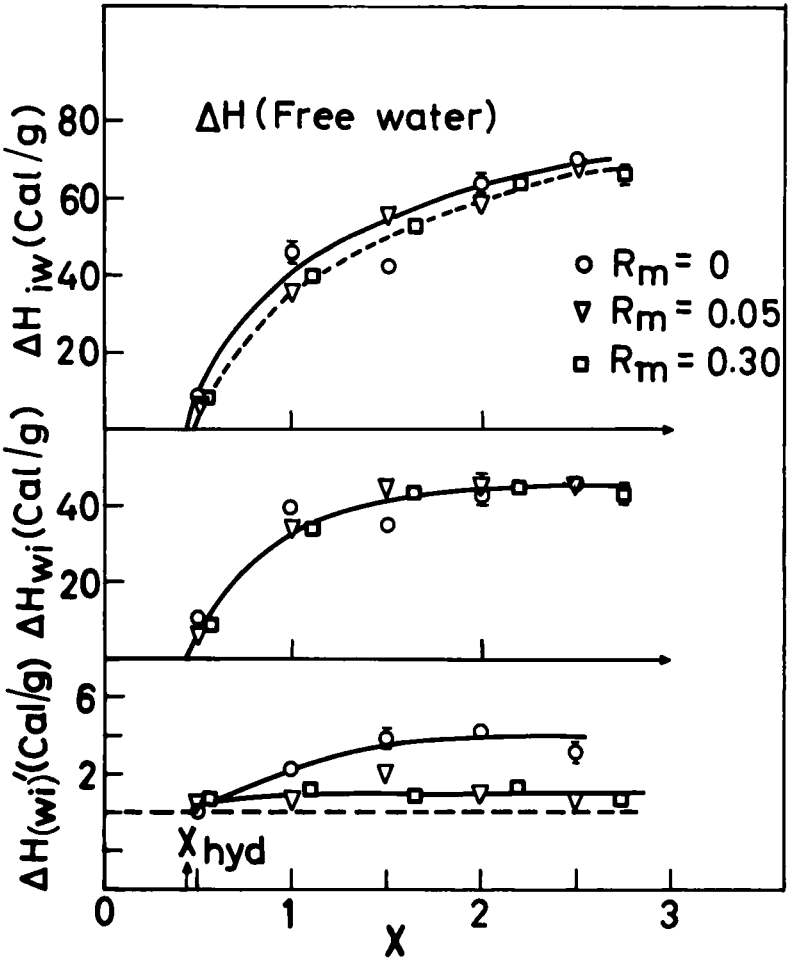

FIGURE 4 Enthalpies, ΔH, for the ice-water transitions as a function of X.

Variation of R_2 [$=(\Delta H_{wi} + \Delta H_{(wi)'})/\Delta H_{iw}$] with X, is given in Figure 5. R_2 is independent of R_m and decreases linearly with X, tending to the value for free water at large X. This is similar to its behaviour in the case of single lipids.[2,3]

IV. DISCUSSION

Our results show that the DPPC-DPPE-Water system consists of coexistent DPPC-rich(I) and DPPE-rich(II) phases. The observations

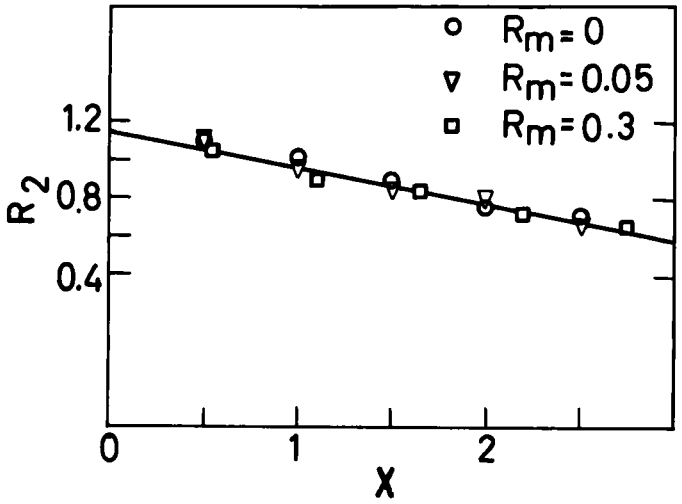

FIGURE 5 The ratio R_2 as a function of X.

that the chain melting transition temperature of Phase I(T_{CM1}) is comparable to that of the DPPC-H_2O system and that there is a splitting of the CM1 peak in the presence of drug as in the case of DPPC-H_2O, imply that phase I contains a large fraction of DPPC molecules. The broad nature of the chain melting transition of phase II and the lowering of the corresponding CM transition temperature, T_{CM2}, with respect to that of the DPPE-H_2O system, imply that there is an admixture of DPPE and DPPC molecules in phase II. We describe below a model for the mixed lipid-H_2O system which takes into account the observed coexistence of the two phases described above.

A. A model for the DPPC-DPPE-water system

In both the single lipid (DPPC and DPPE)-water systems the head groups lie parallel to the plane of the bilayer.[10] The packing of the PE group is known to be quite compact, due to the strong interaction of N^+ and P^- of neighbouring molecules. However, in DPPC, the shielding of P^- by the water of hydration and the shielding of N^+ by the methyl groups prevents such a strong interaction, causing a less dense packing of the head group than in DPPE.[11] This preference of PC and PE head groups for different packing arrangements leads to the existence of two separate phases, phase I and phase II in mixed lipids. Phase I, which is rich in DPPC, has a small percentage of DPPE molecules. Their presence perturbs the structure of the DPPC

bilayer, causing a looser packing of the moleules in their neighbourhood. In phase II, the mixture of DPPE and DPPC molecules leads to a loose packing of the molecules and hence to a greater separation of the head groups. This results in a reduced N^+-P^- interaction, which leads to an increased penetration of water into the polar region of the bilayer and hence to an increased interaction between water and head group. This causes an increase in the water of hydration. Due to the strong head group-water interaction in phase II, (1) the effective concentration of water as seen by phase I would be less than the actual concentration (that is, X_{eff}(phase I) $< X$), and (2) the DDS molecules interact less with the head groups in phase II, thus increasing the number of drug molecules available to phase I (that is, R_m^{eff} (phase I) $> R_m$). In the light of the above model, most of our results can be explained.

In phase I, the perturbing influence of the DPPE molecules on the DPPC bilayer, would result in a less compact packing of the acyl chains than that in DPPC-H_2O. Therefore, the observed absence of pretransition in the case of phase I cannot be related to a denser packing of the acyl chains, as predicted by Scott Jr.[8]

The relatively loose molecular packing in the vicinity of the DPPE molecules in phase I, facilitates the entry of DDS into the acyl chain region. This would explain the observed decrease in T_{CM1} in the presence of DDS even for $R_m = 0.05$. However, the number of DDS molecules that can enter this region would be limited by the concentration of DPPE in phase I, which is small. This is the reason why an increase in drug concentration from $R_m = 0.05$ to $R_m = 0.3$, leads to no further decrease in T_{CM1} (Sec. III, A(i)). It is assumed here that a decrease in the chain melting transition temperature can only be due to the entry of DDS moleules into the acyl chain region. This is a good assumption since, in the DPPC-H_2O system, where no change is observed in T_{CM} for values of R_m up to 0.3,[2] preliminary IR absorption experiments[12] have shown that DDS interacts mostly with the lipid head groups.

In the presence of DDS, a splitting of the CM transition peak occurs both in DPPC-H_2O and in phase I (Sec. III, A(i)). While in DPPC-H_2O, the splitting is observed for $R_m > 0.3$ and $X \leqslant 0.5$, in phase I the splitting is seen even for $R_m = 0.05$ and for $X = 1.0$. This difference can be explained by our model, since phase I would see an effective lower water concentration and an effective higher drug concentration, as explained earlier. The splitting occurs as a result of separation into drug-rich and drug-poor regions in phase I. In the doped systems, at sufficiently large values of X, the DDS molecules

predominantly interact both with the lipid head groups and vicinal water molecules. When the water concentration is decreased, there is a reduction in the number of water molecules bound to DDS. This might lead to self association of DDS molecules through hydrogen-bonding between the S = 0 and NH_2 groups, resulting in drug-rich and drug-poor zones in phase I. Such a separation has indeed been predicted by De Verteuil et al.[13] in lipid-drug systems, wherein the drug-drug interactions are strong as compared to drug-lipid interaction. Since this theory does not take into consideration the presence of water, no quantitative comparisons can be made between its predictions and our results.

The dependence of the ratio, R_1, on R_m (sec. IIIA(ii)), implies that when R_m is changed from 0 to 0.05, the population of DPPC in phase I increases but when R_m is further changed to 0.3, this population decreases. This would imply that as R_m is changed, the ratio of the number of DPPC to that of DPPE molecules in phase II would change, bringing about a change in the molecular packing in this phase. This would, in turn, change the population and structure of vicinal water. No such changes would occur in phase I, in which the DPPC concentration continues to be very high. The reason for the observed behaviour in R_1 is not understood. It is felt that the self association of DDS molecules is instrumental in bringing about these changes.

The value of 0.44 for X_{hyd} for the mixed lipid, is surprisingly large as compared to that of 0.25 for DPPC[2,9] and 0.21 for DPPE[9] (Sec. III, B(ii)). The number of water molecules, n_w, bound to a lipid molecule is equal to $[(M_l/M_w)X_{hyd}]$, where M_l and M_w are the molecular weights of lipid and water respectively (for the mixed lipid, $M_l = (2M_{DPPC} + M_{DPPE})/3$). Thus n_w (DPPC) $\approx$ 10, n_w (DPPE) $\approx$ 8 and n_w (mixed lipid) $\approx$ 18. As discussed above, our model for the mixed lipid system can account for a larger value of n_w than that for DPPC or DPPE, due to the strong head group-water interaction in phase II. However if this has to explain the large value of n_w, it would imply that in phase II, the number of water molecules bound to the lipid head group is more than double that in the case of either of the single lipids, which seems to be unlikely. It might be more likely that a certain number of water molecules enter the acyl chain region in phase II, increasing the number of water molecules bound to the lipid. This might explain the high X_{hyd} value in mixed lipid-water.

For the mixed lipid – H_2O system,

$$n_w(\text{mixed lipid}) = n_w^I f_I + n_w^{II} f_{II}, \qquad (1)$$

where n_w^I and n_w^{II} are the number of bound water molecules per lipid molecule in phases I and II and f_I and f_{II} are the fractions of lipid molecules ($f_I + f_{II} = 1$) in phases I and II. Since, in our model we assume that phase I is mostly populated by DPPC, we take,

$$n_w^I = n_w \text{ (DPPC), and } n_w \text{ (mixed lipid)}$$

$$= n_w \text{ (DPPC)} f_I + n_w^{II} f_{II} \quad (2)$$

When DDS is added, there is a small increase in n_w (mixed lipid) which is observed as a small increase in X_{hyd} (a small decrease of ΔH_{iw}). Since our model assumes that most of the DDS molecules bind to the DPPC head groups and vicinal water in phase I, the presence of DDS would hardly change n_w^{II} if f_I and f_{II} are not affected by the drug. Hence, the variation in n_w (mixed lipid) would be brought about by the change in n_w^I. In the presence of DDS,

$$n_w^I = n_{w,R_m=0}(\text{DPPC}) + \delta n_w(\text{DPPC}) \quad (3)$$

where, δn_w(DPPC) is the change in n_w observed in DPPC-H_2O on addition of DDS, for $R_m = R_m^{eff}$ (phase I). Thus,

$$n_w(\text{mixed lipid}) = n_{w,R_m=0}(\text{DPPC}) f_I + n_w^{II} f_{II} + \delta n_w(\text{DPPC}) f_I \quad (4)$$

If f_I, f_{II} and n_w^{II} remain constant when DDS is added to the mixed lipid-water system, then

$$[n_{w,R_m=0}(\text{DPPC}) f_I + n_w^{II} f_{II}] = \text{constant} \quad (5)$$

The observed increase in n_w(mixed lipid) would then be equal to δn_w(DPPC)f_I. Since $f_I < 1$, the increase in n_w and X_{hyd} and the decrease in ΔH_{iw} in mixed lipid-H_2O would be less than that observed in DPPC-H_2O. This explains our observations on the R_m-dependence of X_{hyd} and ΔH_{iw}. However, the assumption (5) on which this explanation is based is not valid, since we know that f_I and f_{II} vary with R_m. This is indicated by the large variation of $R_1 (\propto f_I/f_{II})$ with R_m. The observed variation in n_w(mixed lipid) with R_m would result from changes in f_I and f_{II}, and variations in n_w^I, and to a lesser extent, in n_w^{II}, caused by the presence of DDS.

Our model seems to explain most of our results. However other experimental techniques, such as X-ray diffraction and NMR will have to be used to examine its validity.

V. CONCLUSIONS:

From our results we have drawn the following important conclusions.

(1) In the DPPC-DPPE-H_2O system, the different packing arrangements preferred by the PC and PE head groups leads to a separation into two phases: Phase I which is rich in DPPC and phase II which is an admixture of DPPE and DPPC.

(2) The presence of DPPE in Phase I causes a local perturbation in the DPPC bilayer structure which allows the penetration of DDS molecules into the acyl chain region.

(3) The loose packing of head groups in phase II leads to a greater head group-water interaction and also perhaps to a penetration of H_2O into the acyl chain region. Hence one observes a very high value (0.44) for x_{hyd}.

(4) At low water concentrations the self association of DDS creates drug-rich and drug-poor environments in phase I.

(5) Addition of the drug DDS, alters the relative populations of lipids in phases I and II and changes the number of bound water molecules. The latter is due to (a) the interaction of DDS with water and lipid and (b) changes in f_I and f_{II}.

(6) Due to the differing local concentrations of DDS and water, a smaller concentration of DDS is needed to bring about an observable perturbation in the lipid bilayer and vicinal water structures in the mixed lipid systems than in the single lipid systems.

The present work indicates that very low concentrations of DDS would influence the functions of multicomponent biological membranes by perturbing (a) the vicinal water structure and (b) the composition of the phases in the lipid bilayer.

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