THE FLUIDITY OF WATER AT LIPID-WATER INTERFACES JUST BELOW 0 C

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ABSTRACT -Using several different electron paramagnetic probes we have studied the phase transitions of two lipids with transition temperatures both well above and well below 0 C in lipid-water mixtures: we show that about 20-25 moles of water per mole of lipid will remain in a fluid state just below 0 C only if the lipid is already in the gel state (i.e. below its phase transition temperature).

1.INTRODUCTION

Previous studies of hydrated lipid-systems using calorimetry [1,2], deuteron magnetic resonance [3-5] and electron spin resonance(ESR) [6] have revealed that as many as 21 moles of water per mole of lipid have their mobility restricted and can be distinguished from bulk water. Part of this water (usually less than 10 moles per mole of lipid) is strongly immobilized and is referred to as "bound" water [7]. It has been suggested [1] that this "bound" water forms a complex hydrate structure around the polar group of the lipid.

We have used electron paramagnetic probes to study the fluidity of lipid-water systems, using lipids with transition temperatures both above and below 0 C. The experimental evidence obtained suggests that the amount of non-freezable water at or just below the freezing point of the bulk water is strongly dependent on the fluidity state of the lipid phase.

2.MATERIALS AND METHODS

A Varian E-109 X-band electron spin resonance spectrometer with a Varian E-257 variable temperature controller (allowing the control of temperature at the sample within less than 0.5 C) was used. Temperatures were checked with a thermocouple inserted into similar micropipets as those used as sample holders and placed in the magnetic cavity; temperature changes were stabilized within 1-2 minutes. Dimyristoyl phosphatidylcholine (DMPC) and Dioleoyl phosphatidylcholine (DOPC) were the two lipids chosen since they had transition temperatures well above (+23 C for DMPC) and well below (-20 C for DOPC) the freezing temperature of bulk water. They were purchased from Sigma Chemical Co. (Poole, England). The neutral paramagnetic probes 2,2,6,6-tetramethylpiperidone-4-oxyl (Tempone), 2,2,6,6 - tetramethylpiperidine - N - oxyl (Tempo) and the cationic probe 4-nonyldimethyl ammonium -1 - oxyl -

(2,2,6,6-tetramethyl-piperidine) bromide (CAT_9) were provided by Dr. R.J. Mehlhorn.

Lipid-water mixtures were prepared under nitrogen using standard techniques [6] at an approximate molar ratio of 80 moles of water per mole of lipid and containing 0.125 mM paramagnetic probe (corresponding to roughly 1 mole of probe per 4000 moles of lipid). Samples of 50 microlitres were placed in micropipets (that had been thoroughly flushed with nitrogen) which were then sealed to prevent the subsequent entry of oxygen (this is particularly important if we wish to minimize lipid peroxidation). ESR spectra were recorded at temperatures in the range between +30 C and -60 C. Spectra of the same paramagnetic probes dissolved in water alone were also recorded across the same temperature profile. The viscosity of the medium in which the probe is located is proportional to the correlation time t given by:

$$t=6.5*10^{-10}\,\Delta H_0[(h_0/h_{-1})^{1/2}-1]$$

where h_{-1} and h_o are the heights of the high field and central lines of the ESR spectrum and ΔH_o is the line width of the central line in Gauss [6].

3. RESULTS AND DISCUSSION

Fig. 1 shows a series of spectra obtained from the system DMPC-water, using the paramagnetic probe Tempo at the indicated temperatures. At 25 C the lipid is still in the fluid state (as we are still above the transition temperature for this lipid) and most of the paramagnetic probe can be seen to be located in the lipid phase. As the temperature is lowered past 23 C (the transition temperature for this lipid) the probe is progressively excluded from the gel lipid phase such that most of it is now to be found in the liquid water phase. When this lipid-water

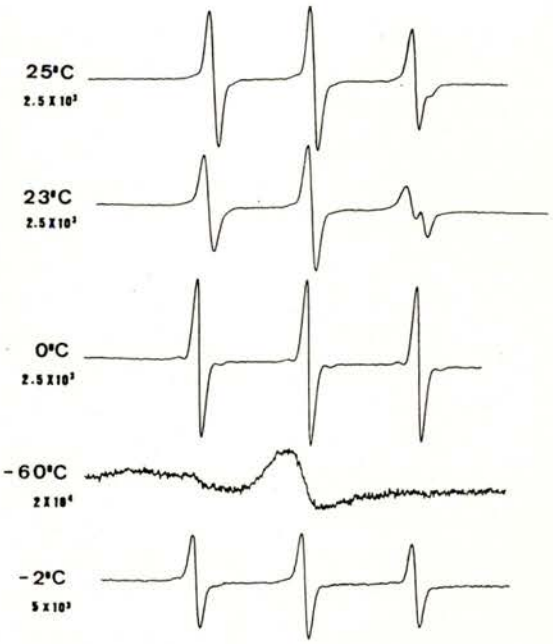

Figure 1 - ESR spectra of the nitroxide spin probe Tempo in DMPC-Water systems at the indicated temperatures and gains.

system is cooled to -60 C the observed signal is one that is characteristic of the strong immobilization of the probe that is to be expected from it being trapped in frozen water. In the DMPC-water system

the hyperfine splitting of spectral lines is approximately 17.40 Gauss for the probe in the liquid water, and 16.05 Gauss for the probe in the fluid lipid environment respectively. Upon heating from -60 C to -2 C and then further to 0 C (and waiting about 15 minutes for the spectra to stabilize for each temperature) we observe only one freely mobile population of probe with a hyperfine splitting constant of 17.35 Gauss characteristic of its presence in an aqueous environment. While accurate quantification is problematic, if one assumes that well below the phase transition of this lipid (23 C) most of the probe is to be found in the aqueous environment, then the spectrum taken at -2 C suggests that the amount of fluid water is approximately 25% of the total water; this means that 20-25 moles of water per mole of lipid remain unfrozen at -2 C. Data of Salsbury et al [4] have shown quite clearly that as many as 21 moles of water per mole of lipid have their motion restricted in some manner; more recent data indicate some solutes, like sucrose, will affect the amount of unfrozen water [8].

Fig. 2 displays representative spectra obtained for the DOPC-water system with the same paramagnetic probe Tempo at several temperatures. At 0 C DOPC is fluid and the high-field spectral line clearly exhibits a signal from the paramagnetic probe in both lipid and the aqueous phases. The hyperfine splitting for the probe in the lipid phase of the DOPC-water system is the same as in the DMPC-water system: approximately 16.05 Gauss. Both at -60 C and at -2 C the spectra show no evidence of the existence of a liquid aqueous phase.

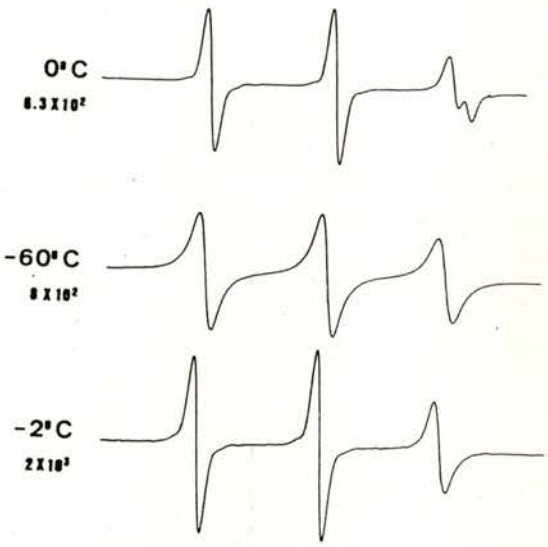

Figure 2 - ESR spectra of the nitroxide spin probe Tempo in DOPC-water systems at the indicated temperatures and gains.

The viscosity of the liquid aqueous phase, when present at -2 C (as is the case for the DMPC-water system), is greater than the viscosity of water at 0 C; $t_{water}^{(0)} = 3.6*10^{-12}$s while $t_{water}^{(-2)} = 7.5*10^{-11}$s. Similarly the viscosity of the lipid system increases as the temperature is lowered; while it is impossible to measure the viscosity of DMPC below its phase transition using these paramagnetic probes, because they are excluded from the lipid phase, the spectra in fig. 2 show that t_{lipid} increases from $4*10^{-10}$ s to $8.3*10^{-10}$ s as the temperature is lowered from -2 C to -60 C. Interestingly enough, it appears that once the water is frozen, lowering the temperature well below the freezing point of DOPC (i.e. below -20 C) increases the

viscosity of the lipid phase, but does not exclude the probe from the lipid environment. Such an observation is consistent with the idea that it is unlikely for the paramagnetic probe to be able to move in and out of the medium of frozen water; it is also consistent with our earlier observation that as the temperature is lowered below the transition temperature of DMPC (+23 C) the probe can be excluded from the lipid phase since it can now move into the liquid aqueous phase.

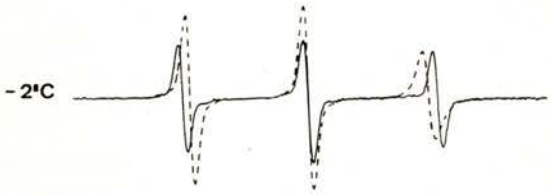

Figure 3 - Superimposed spectra of Tempo in DMPC (——) and DOPC-water (-------) systems at -2 C.

In Fig. 3 we have superimposed the spectra of Tempo in both the DMPC-water and the DOPC-water systems at -2 C; this figure leaves absolutely no doubt that at -2 C the only system that shows any fluid aqueous environment is the DMPC one. It could be argued that in the DOPC - water system at -2 C the solubility of the paramagnetic probe is much larger in the lipid environment than in this aqueous environment of "restricted" water (thereby explaining the absence of a probe signal from the latter environment); however additional experiments performed (data not shown) with other paramagnetic probes (Tempone and CAT_9), and at different concentrations, are in complete agreement with the results obtained with Tempo. Our results do not depend on the type or electrical charge of the paramagnetic probe used, and furthermore they show quite clearly that in lipid-water systems just below 0 C the presence or absence of a fluid aqueous environment depends on whether the lipid is already in the gel state or not. The nature (chemical structure) of this fluid (when compared to frozen water) aqueous environment, and its accurate quantification cannot be determined by our methodology; it seems, though, that our results indicate about 20-25 moles of water per mole of lipid, in agreement with other results [4]. It also appears that the term "restricted" might be appropriate when comparing it to fluid bulk water (above 0 C); in our case an equivalent amount of water remains fluid when compared to frozen bulk water (below 0 C) but only if the lipid is already in the gel state.

ACKNOWLEDGMENTS

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REFERENCES

[1] Chapman, D., Williams, R.M. and Ladbrooke, B.D., Chem. Phys. Lipids **1**, 445 (1967).
[2] Elworthy, P.H., J. Chem. Soc., 5385 (1961).

[3] Finer, E. G. and Darke, A., Chem. Phys. Lipids **12**, 1 (1974).
[4] Salsbury, N. J., Darke, A. and Chapman, D., Chem. Phys. Lipids **8**, 142 (1972).
[5] Atkinson, D., Hanser, H., Shipley, G.G. and Stubbs, M., Biochem. Biophys. Acta **339**, 10 (1974).
[6] Keith, A.D., Snipes, W. and Chapman, D., Biochem. **16**, 634 (1977).
[7] Ladbrooke, B.D., Williams, R.M. and Chapman, D., Biochem. Biophys. Acta **150**, 333 (1968).
[8] Strauss, G. and Hauser, H., Proc. Natl. Acad. Sci. USA **83**, 2422 (1986).