THE EFFECT OF HYDRATION OF LECITHIN-WATER LAMELLAR PHASES: A COMPARATIVE STUDY OF SOLUTE DIFFUSION AND ESR SPECTROSCOPIC MEASUREMENTS

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SUMMARY

An analysis of the paramagnetic resonance spectra of spin labels in the lipidic region of lecithin-water lamellar phases as a function of phase water content has been carried out. The observed variation of the local organization and mobility of the lipids is consonant with previous results obtained from solute diffusion measurements. The previously observed sudden changes of solute diffusion for hydration of 9 and 18 molecules water per lecithin molecule are compared with the concomitant sudden changes as seen by ESR spectroscopy. The results also indicate that there is a gradient of fluidity across the lipid leaflets which are therefore not homogeneous to diffusing molecules.

INTRODUCTION

The diffusion rates of hydrophilic as well as of lipophilic molecules in lecithin-water lamellar phases exhibit a stepwise increase with increasing phase water content (1). An attempt was made to correlate this behaviour both to hydration effects and to the known structural parameters of the phases as deduced from X-ray analysis. Neither the diffusion of water, which occurs in the aqueous region of the phase, nor that of benzene, which occurs in the hydrocarbon region, could be accounted for on the basis of X-ray analysis data only. Much more promising correlations may be made between our solute diffusion measurements and the conclusions concerning local molecular movements reached by Hubbell and McConnell and others (2), (3), (4), on the basis of their studies of lipid-water systems by ESR spectroscopy. We carried out an ESR spectroscopy study of lecithin-water phases as a function of water content, in order to relate the local organization and the mobility of the lipidic constituents to their properties as a diffusional medium. The advantage of lamellar lipid-water phases is that the water content and hence the fluidity of the hydrocarbon region may be systematically varied over a considerable range, offering the possibility of overlap with the degree of fluidity observed in biological membranes.

MATERIALS AND METHODS

The lamellar phases were prepared by manual mixing of a weighed amount of lecithin containing the spin label at a concentration of $\leqslant 10^{-3}$ molar with a weighed amount of distilled water, as described in a previous paper (1). The phases were introduced into quartz tubes 4 mm in diameter. No significant preferential ordering of the lamellae was obtained in this way. Reproducibility of splitting and line-width measurements on the ESR spectra indicated that the samples were homogeneous. The spectra were obtained at a regulated temperature of (25 ± 0.25)°C, using a Varian V 4502 ESR spectrometer.

Four spin labelled molecules were used:

Probe I: The 2.2.6.6 - tetramethyl-1-Oxyl-4-Bromopiperidine (see Fig. 1B), synthetized according to Rosanstev (5).

Probe II (12,3), II (5,10), II (1,14): three stearic spin labelled fatty acids purchased from Synvar Associates (see Fig. 2).

RESULTS

Probe I is exclusively hydrophobic, and is dissolved in the hydrocarbon core of the lamellae. As such, the behaviour of this molecule may be compared to that of benzene diffusing within the lipidic region of the phase. The ESR spectra obtained show a rapid isotropic motion of the probe. The rotational correlation time, τ_c, for this molecule, obtained directly from the measured linewidth, is given as a function of lecithin hydration in moles of water per mole lecithin, in Fig. 1B. The abscissa is also given in g water per g phase. As expected from previous studies of lecithin-water systems, the rotational freedom of the probe molecule increases with increasing disorder of the hydrocarbon chains promoted by increasing the water content.

The variations of τ_c for this molecule may be compared with the variations of the diffusion coefficient D of benzene measured in the same system at different water contents (Fig. 1A). It can be seen that the increasing disorder of the hydrocarbon chains, reflected by a decrease in τ_c, is accompanied by an increase in the diffusion coefficient of benzene. It is striking that both the τ_c curve and the D curve exhibit slope changes for the same lecithin hydration, that is, about 9 and 18 water molecules per lecithin molecule (20% and 30% water in the lecithin-water phase). This suggests that the diffusion coefficient measured on a macroscopic scale by radiotracer is related, at least qualitatively, to the organization of the lipids at the molecular level and not more trivially to its macroscopic

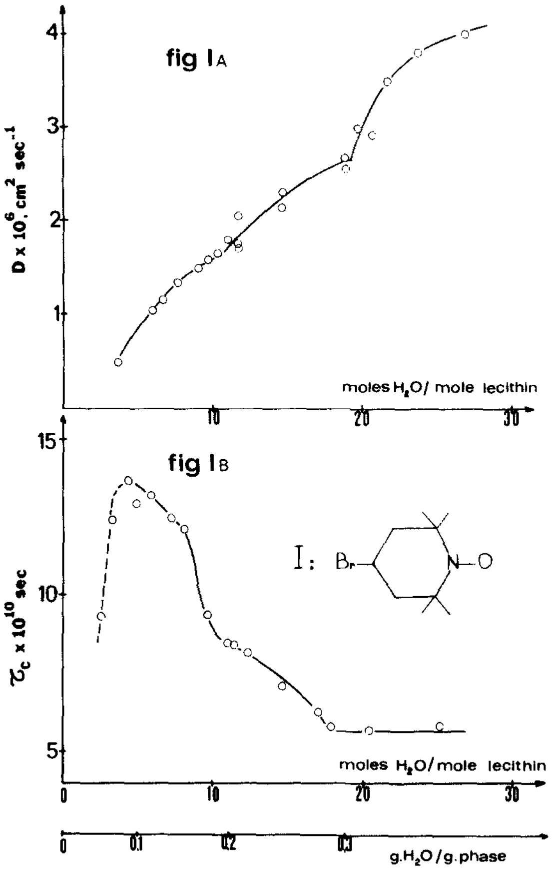

Fig. 1 A: Diffusion coefficient D of Benzene in lecithin-water lamellar phases as a function of lecithin hydration.

Fig. 1 B: Rotational correlation time τ_c of probe I in lecithin-water lamellar phases as a function of lecithin hydration.

organization, that is to the tortuosity of the diffusion path or the monocrystal size. The slope changes observed lend support to the idea that hydration of the polar head is important in determining the structure of both the aqueous and lipidic regions of the phases. The values of 9 and 18 water molecules per lecithin molecule agree reasonably well with the values obtained by microcalorimetry (6) and DMR (7).

On the other hand, a closer examination of the two curves in Figs. 1A and 1B shows two important differences between the two types of measurement. The slope changes at 9 moles H_2O/mole lecithin is more marked for τ_c than for D. Beyond 18 mole H_2O, τ_c remains constant while D increases with increasing water content. These differences could be

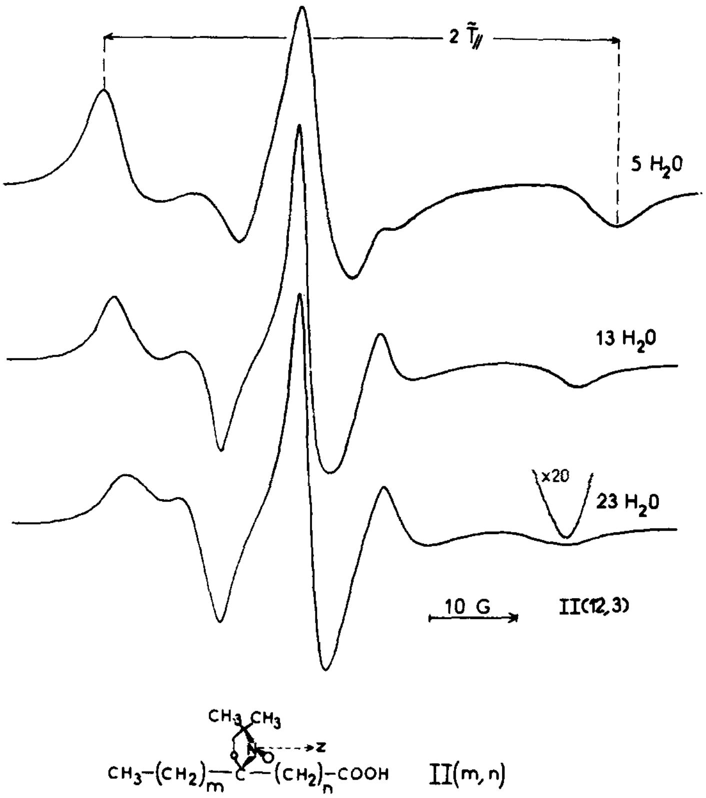

Fig. 2: E.S.R. spectra of probe II (12, 3) in lecithin-water lamellar phases containing 5, 13 and 23 moles H_2O/mole lecithin.

explained by the fact that τ_c is a measure of rotational freedom only, whereas diffusion reflects, in addition, freedom for translational motion. Other important factors could be the difference between the probe and benzene in size and in local interaction with lecithin. It is known that the fluidity of the hydrocarbon chains depends upon the distance from the polar edge of the lamellae and that a bigger molecule may be more sensitive to the disordering effect of water than a smaller one.

Probes II (m,n) are spin labelled stearic acids. In contradistinction to probe I, they are anchored by their polar group to the surface of the lamellae, in a position comparable to that of the lecithin molecules constituting the lipidic leaflets. Their spectra may therefore be considered as reflecting the behaviour of lecithin molecules. The nitroxide conformation of these probes is such that the 2 pz orbital of the nitrogen (z axis) is practically parallel to the axis of the paraffin chain. The

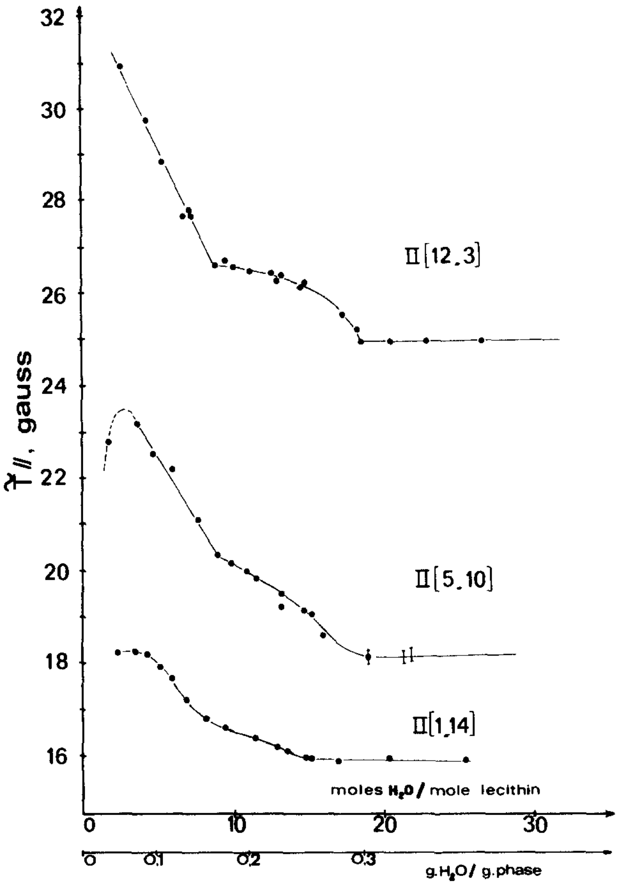

Fig. 3: Hyperfine splitting $\tilde{T}_{//}$ of probes II (m, n) in lecithin-water lamellar phases as a function of lecithin hydration.

distance 2 $\tilde{T}_{//}$ between the external maxima was measured as a function of the water content (Fig. 2). As shown by McConnell (2), this parameter can be related to the freedom of motion of the nitroxide radical and to the order parameter of the probe.

The $\tilde{T}_{//}$ values are plotted as a function of lecithin hydration in Fig. 3. $\tilde{T}_{//}$ decreases with increasing hydration, and here again, three regions may be distinguished. Up to 9 molecules water per lecithin molecule, $\tilde{T}_{//}$ decreases very rapidly, which indicates a very fast increase in the motion around the z axis of the probe. From 9 to 18 water molecules per lecithin molecule, $\tilde{T}_{//}$ continues to decrease, but more slowly. The spectra are characteristic of a liquid crystalline environment with perfect axial symmetry and rapid motion around the z axis and only rather slow motion in a direction perpendicular to the z axis. In the third region, beyond 18 molecules water per lecithin molecule, the spectra are the same but the value of $\tilde{T}_{//}$ no longer depends upon the water content. In agreement with the observations of several authors (2),(3),(4) it can be seen from Fig. 3 that the splitting depends upon the position of the nitroxide radical on the fatty acid chain, decreasing as the spin label is moved away from the polar head. The disorder and fluidity of the hydrocarbon chain increases with both the water content and the depth in the lamellae. Accordingly, the slope change of the curve at 9 molecules water per lecithin molecule is more marked for probe II (12, 3) than for probe II (5, 10), which in turn is more marked than for probe II (1, 14).

A detailed investigation, including the effect of cholesterol, will be described in a further publication.

CONCLUSIONS

The information concerning the dependence of the state of the lipidic regions of the phase on the water content, obtained from the diffusion of benzene, is corroborated by the ESR measurements. However, these measurements give additional information not obtainable by diffusion measurements, which reflect average properties of the diffusive medium. The ESR results indicate that there is a gradient of fluidity across the bilayer,and benzene must diffuse more rapidly in the fluid core than in the border region, where a more rigid organization of the chains is maintained. Similarly, benzene is probably not uniformly distributed across the bilayer, its solubility depending on the fluidity of the hydrocarbon chains. The change with hydration in this transversal distribution of probe I is demonstrated by the increase of its isotropic splitting due to change in the polarity of the environment. The bilayer is therefore not homogeneous to a molecule diffusing transversely across it (in a direction parallel to the hydrocarbon chains) and it could be expected that diffusion occurs much more rapidly along the plane of the bilayer, in the fluid core. This phenomenon could be of importance in carrier transport mechanisms. Large lipophilic carriers, in addition to shuttling back and forth across the membrane, could diffuse considerable distances along the plane, in the most fluid interior of the membrane.

It can furthermore be concluded from our results that the two regions of the lamellar phase, the aqueous and the hydrophilic, are in close interaction. The measurements of ^{3}HHO diffusion as a function of phase hydration exhibit behaviour similar to that observed for benzene diffusion, namely a stepwise increase with hydration, with slope changes at

9 and 18 water molecules per lecithin molecule. This result suggests that factors influencing the state of the hydrated lecithin polar head will simultaneously influence the organization of the lipidic core of the lamellae. Conversely, any factor determining the organization of the hydrocarbon chains, such as their length, the number of double bonds or the introduction of an additional lipid, for example cholesterol, will have an effect on the structure of the aqueous region and will modify its properties as a diffusional medium. This observation could be of relevance to membrane physiology in that the permeability of either the aqueous or the lipidic pathway could be controlled by the other or by a factor influencing either one.

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