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NMR INVESTIGATIONS OF THE INTERACTION OF WATER WITH LECITHIN IN BENZENE SOLUTIONS

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SUMMARY

NMR investigations of ^{1}H (chemical shifts, line widths) and of ^{31}P (relaxation times, T_1) performed on the three-component system of lecithin–benzene–water show that there is an interaction of water with the phosphate group in two regions of different mobility and structure. A fast exchange of the water molecules takes place between both regions. The region of strong interaction involves about 2 and that of the weaker interaction about 5 water molecules per lecithin molecule. When the water concentration is increased a third region is formed which is assigned to the water molecules that are located beyond the two regions of interaction with the phosphate group, but within the micelle. This water has a different structure from that of the second region of interaction with the phosphate group and may also have a different mobility.

Addition of water increases the motion of the head groups of the lecithin molecules. This is due to a loosening of the packing of lecithin molecules.

INTRODUCTION

In recent years there has been a growing interest in investigations of simple lecithin systems as models of biological membranes. The aim of such studies is to provide information about the structure and function of biomembranes. Nuclear paramagnetic resonance spectroscopy is an efficient method for the study of some of the problems involved (see, e.g., refs 1 and 2).

Because of the importance of water in biological membranes its interaction with other components has been the subject of many investigations [1, 3–7]. For this purpose, systems of defined secondary structure and of variable water concentration, i.e. three-component systems, are preferred: lecithin, organic solvent, water. Such systems have been studied by Henrikson [4] (carbontetrachloride, water) and Walter et al. [5] (benzene, H_2O or $^{2}H_2O$) by line-width measurements of ^{1}H resonance and by Barett-Bee et al. [7] (chloroform, water) by line-width measurements of ^{14}N resonance.

The present work deals with the investigation of lecithin–benzene solutions with addition of specific amounts of water, not only by line width measurements but

also by measuring the chemical shifts of ^1H resonance and the relaxation time, T_1, of ^{31}P resonance.

EXPERIMENTAL

All measurements were performed with synthetic β, γ-dipalmito-L-α-lecithin obtained from Fluka, Switzerland, in different lots. Before the preparation of the samples lecithin was held under vacuum (some Torr) for some hours.

[^2H]Benzene (approx. 98 % ^2H) from Isocommerz, G.D.R., was used as solvent. It was dried additionally (for 12 h) by a mole sieve and treated with a dried N_2 gas stream to eliminate O_2.

Lecithin was added to the solvent by weight. Vigorous shaking of the sample provided a dim viscous solution that cleared at a concentration below 0.35 M* by heating at 45–50 °C. The addition of a small amount of water decreases the viscosity. Samples of different concentration of lecithin were used (I: 0.17 M; II: 0.20 M; III: 0.13 M; IV: 0.20 M).

The amount of residual water in the dried lecithin–benzene samples was determined from ^1H resonance. The minimum water content attainable, also after repeated treatment of the lecithin solution with a mole sieve, yielded about 1 water molecule per 1 lecithin molecule.

The addition of water (bidistilled and degassed) as well as the storage of the sample was done under N_2. Upon each addition of water the sample was exposed to ultrasonication for 15–30 s (ultrasonics device from Meinhard, G.D.R.; ultrasonic frequency 800 kHz, intermediate intensity) to homogenize the solution. At increasing water concentration (from about 35 %) an increased ultrasonication time is required and an increased dimness is visible.

The ^{31}P measurements were carried out at 36.4 MHz using the Fourier transform technique (HX90, Bruker AG, G.F.R.) with a pulse programm π–Δt–$\pi/2$–T–$\pi/2$ [8] for the determination of the longitudinal relaxation time, T_1. The ^1H measurements were performed at 100 MHz (HA-100, Varian, U.S.A.) using an external reference (capillary hexamethyldisiloxan). The measured chemical shifts have not been corrected for changes in susceptibility.

RESULTS

The ^{31}P relaxation time, T_1, of lecithin in benzene in relation to temperature (Fig. 1) does not show the characteristic change as observed in lecithin–water systems (ref. 9 and Frischleder, H., private communication). Its strong decrease below 40 °C in Sample II is due to the increased viscosity of the solution. The measurements of the temperature dependence of T_1 are reproducible within the error limits, as verified by repeated measurements of Sample I for 4 weeks after its preparation.

The ^{31}P-T_1 dependence on the amount of water added (Fig. 2) shows distinct characteristic changes such as ^1H shifts (Fig. 3) and the line widths of H_2O (Fig. 4).

Some difficulties arose with regard to ultrasonication required for the homogenisation at higher water concentrations (from 10 %). The ^1H lines of H_2O measured 12 h after homogenisation have widths approximately double those

* Molarity, number of diluted moles of lecithin per l solution.

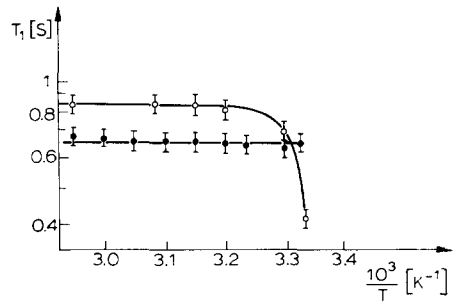

Fig. 1. Temperature dependence of the longitudinal ^{31}P relaxation time of Samples I (●) and II (○) without addition of water, with proton decoupling.

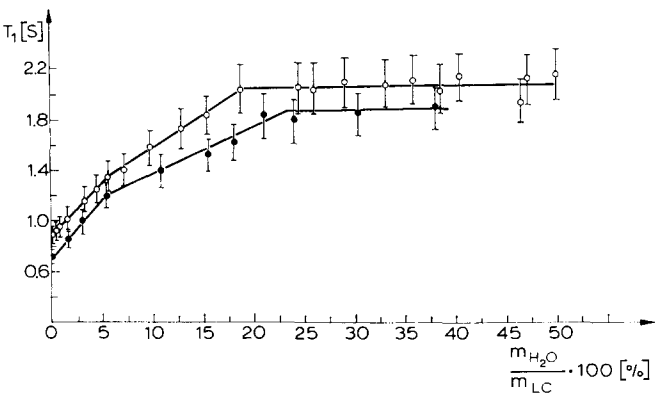

Fig. 2. Dependence of the longitudinal ^{31}P relaxation time on the amount of water added to Sample I (●) at 40 °C without proton decoupling, and to Sample II (○) at 50 °C with proton decoupling.

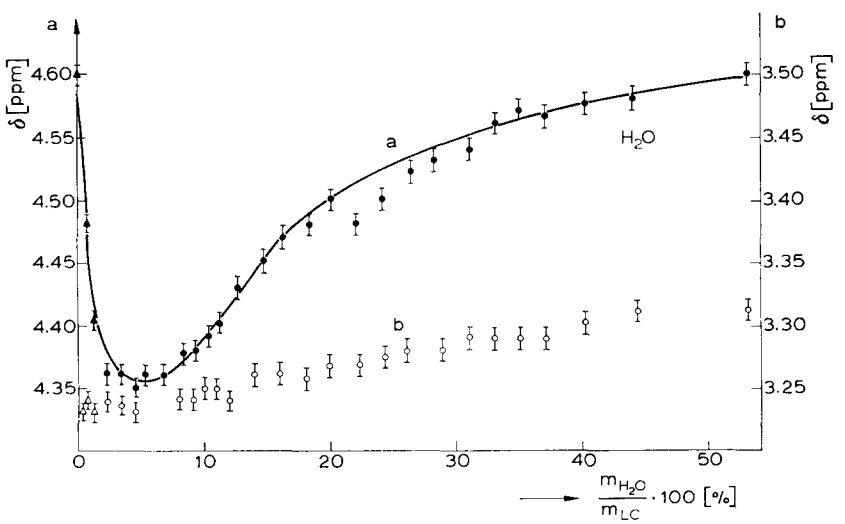

Fig. 3. Dependence of the ^{1}H shift of water (filled points) and of trimethylammonium (not-filled points) on the amount of added water at 50 °C. External reference: hexamethyldisiloxan. Sample III (▲, △). Sample IV (●, ○). Represented curve gives the theoretical dependence.

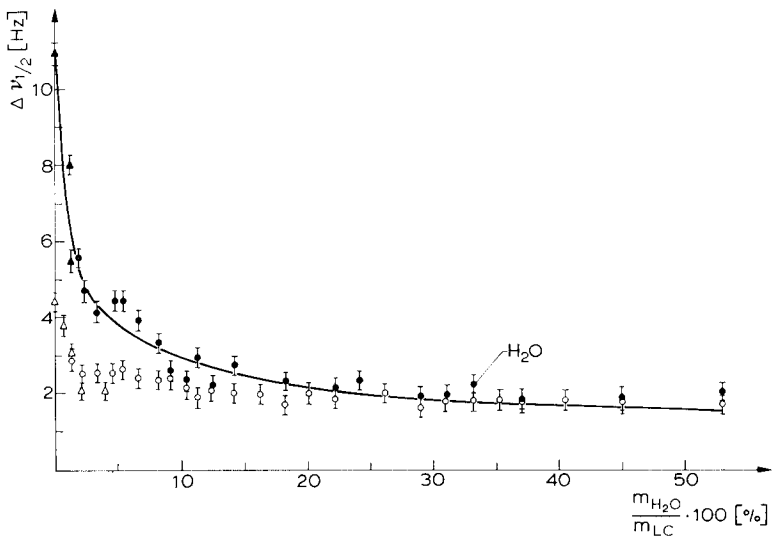

Fig. 4. Dependence of the ^{1}H line width of water (filled points) and trimethylammonium (not filled points) on the amount of water added. Sample III (▲, △). Sample IV (●, ○). Represented curve gives the theoretical dependence.

measured immediately afterwards (not indicated in Fig. 4). By renewed ultrasonication the original line width could be regained. The micelle aggregations are probably diminished by ultrasonication, which has also been observed for lecithin–water dispersions [10, 11].

Analysis of the ^1H resonance measurements of water

The chemical shifts were determined from the chemical bond and the chemical environment of the nuclei as well as from exchange processes.

An explanation of the dependence of the ^{1}H shift observed for water protons (Fig. 3) is possible by fast* exchange processes between 3 regions A, B, and C which have different chemical shifts δ_a, δ_b and δ_c:

$$\delta = p_a\delta_a + p_b\delta_b + p_c\delta_c$$

where p_a, p_b and p_c are the populations of the corresponding region with the relation $\Sigma_i p_i = 1$.

From Fig. 3 it follows that at water concentrations below 10 % the exchange process A $\rightleftharpoons$ B is predominant, whereas the process B $\rightleftharpoons$ C prevails at water concentrations above 10 %.

For the application of these relations, the chemical shifts δ_a, δ_b and δ_c, as well as one population, must be known. Linear extrapolation to water concentrations of 0 and 100 % in the solution yield $\delta_a = 465$ and $\delta_c = 470$ Hz**, respectively. For δ_b we obtained a value of 425 Hz** by fitting the theoretical δ dependence to the

* Mean life time of the water molecules in the regions smaller than the absolute values of the reciprocal differences in the chemical shifts.

** Relative to an external reference of hexamethyldisiloxan.

experimental values, taking into account a two-region exchange model A $\rightleftarrows$ B in the concentration range from 0 to 6 % of water (Fig. 3).

From the populations p_a, gained simultaneously in the concentration range, the number of water molecules per lecithin molecule $n_a^* = p_a n^*$ has been determined, which from the added water molecules per lecithin molecule

$$n^* = \frac{n_{H_2O}}{n_{LC}} = \frac{m_{H_2O}}{m_{LC}} \frac{M_{LC}}{M_{H_2O}}^{\star}$$

gets to the region A. As may be seen from the n_a^* dependence on water concentration in the range between 0 and 6 % (Fig. 5), the rising water concentration enables only a decreasing number of water molecules to reach region A, i.e. n_a^* shows typical saturation behaviour.

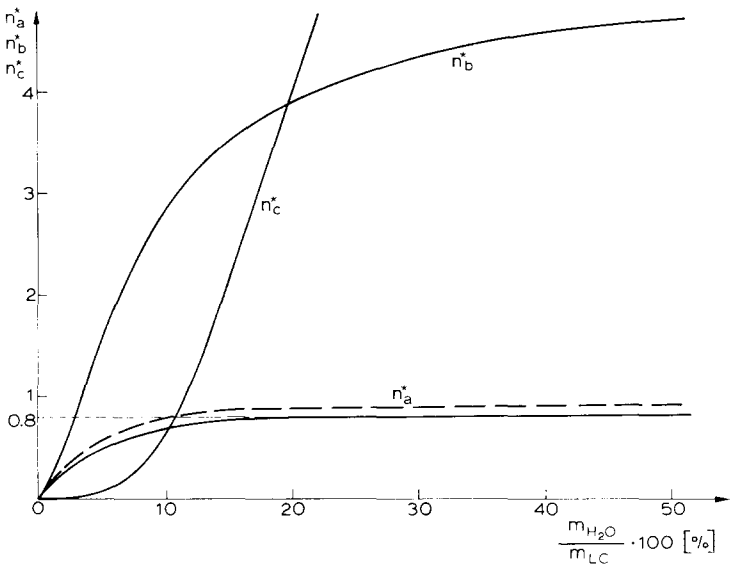

Fig. 5. Dependence of the water molecules per lecithin molecule on the amount of water added, directed to regions A, B and C. The solid curves follow from the analysis of the ^1H chemical shift and the dashed curve from the analysis of the ^1H line width of water.

Quadratic extrapolation gives a maximum value of 0.8 for n_a^*. From the n_a^* curve thus obtained, the populations p_a may be calculated for the total range of water concentration investigated. The formula

$$p_b = \frac{\delta - \delta_c - p_a(\delta_a - \delta_c)}{\delta_b - \delta_c}$$

provides the population of region B and according to $p_c = 1 - p_a - p_b$ we also get the population of region C. The results for the p_i thus obtained are given in Fig. 6. The theoretical dependence of the chemical shift of water on the concentration determined by these populations and the chemical shifts δ_a, δ_b and δ_c indicated above are shown

* $M_{H_2O} = 18$ and $M_{LC} = 751$ are the molecular weights of water and lecithin, respectively.

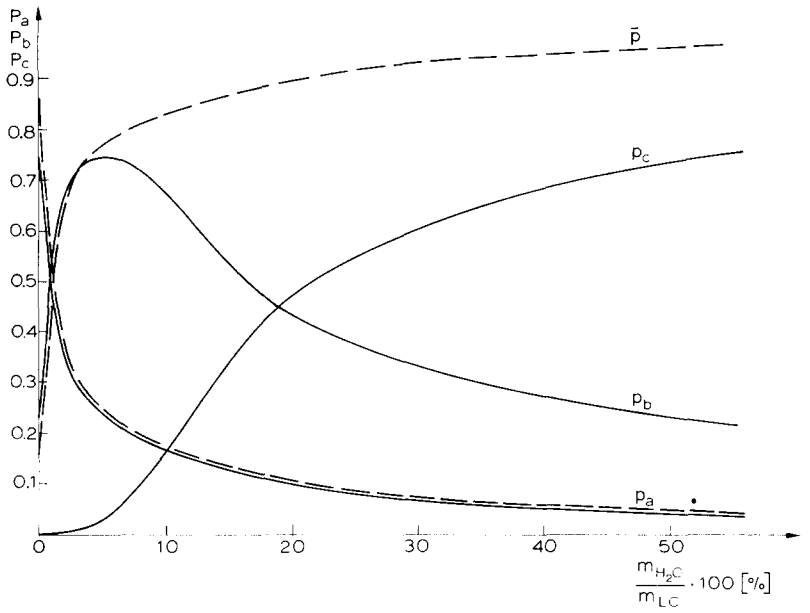

Fig. 6. Dependence of the populations of regions A, B, and C on the amount of water added. The dashed curves follow from the analysis of the ^{1}H line width of water based on a 2-site exchange model.

in Fig. 3. This is in good agreement with the experimental values. Curve n_b*, also showing saturation behaviour, gives a further test of the sensitivity of this procedure as well as the parameters obtained in this way.

An analysis of the ^{1}H line width of water in relation to the water concentration, (Fig. 4) taking into account a three-region exchange model, does not provide the desired information, however, when the results from the analysis of the chemical shift of water is considered because of the relatively large inaccuracy of the measurements and the small difference in the line widths of regions B and C. Therefore, for the evaluation a two-region exchange model has been used:

$$\Delta\nu_{\frac{1}{2}} = p_a\Delta\nu^a_{\frac{1}{2}} + p_b\Delta\nu^b_{\frac{1}{2}}.$$

$\Delta\nu^a_{\frac{1}{2}} = 12.5$ and $\Delta\nu^b_{\frac{1}{2}} = 1$ Hz give the theoretical curves which are also shown in Figs 5 and 6.

DISCUSSION

According to refs 12–16, lecithin in benzene forms micelles of the size of about 60–70 lecithin molecules at concentrations larger than 10^{-3} M. It may be assumed that the head groups are directed towards the interior of the micelles as in chloroform solutions [8, 17]. Addition of water does not break up the micelles and does not markedly change the number of lecithin molecules per micelle [16]. Upon addition of water a fast exchange of the water molecules generally between the three regions takes place as is unambiguously proved by the analysis of the ^{1}H measurements of

water. These regions are characterized by different chemical shifts and line widths.

The regions A and B may be saturated at about $n_a^* = 1.8$ (0.8 plus one water molecule per lecithin molecule not removable from the solution) and $n_b^* = 5$ water molecules per lecithin molecule, respectively. (Fig. 5). These obtained numbers are almost in agreement with those given by Walter et al. [5], which have been derived from changes in the ^1H line widths of the trimethylammonium group and of water supposing the change in mobilities to be the determining factor for changes in the line widths.

The ^1H shift of the water molecules in region A to a lower shielding with regard to the water molecules in region B and the change in mobility of the phosphate group, as follows from the relaxation times of ^{31}P resonance (see below), indicate that the water molecules of region A interact with the phosphate group. The mobility of these water molecules is slower than that of the water molecules in region B, as follows from the relatively large line width $\Delta\nu_{\frac{1}{2}}^a$, provided that the change of the line widths is mainly due to a change in the mobilities. The water molecules in region B form an additional weaker interaction range with the head group of the lecithin molecule.

The n_c^* dependence on the water concentration reveals no saturation behaviour in the concentration range investigated (Fig. 5). Since the solubility of water in pure benzene is rather low (0.07 % at 20 °C), region C must be formed by water molecules being beyond both interaction ranges with the head group, but within the micelle. According to [16] in egg lecithin–benzene solutions are thus soluble 25 % water by weight. The visible dimness of the solutions above about 35 % water concentration is due to an increasing dispersion of water in the benzene solvent.

High water concentrations dependent on ultrasonication favour possibly also the formation of other species of micelles. Some results [18] (decrease of ^{31}P-7T_1 values of Sample I at a water concentration of approx. 40 %) give rise to this assumption.

The information that can be obtained from the ^{31}P-T_1 measurements depends among others on the detailed knowledge of the interactions determining relaxation (cf. e.g. ref. 19). In lecithin–water dispersions the assumption has been made [9] that the dipole–dipole interactions with protons of adjacent methyl groups are dominating. Frischleder (private communication), however, shows that there may also be other significant contributions and the correlation times do not lie in the range of "extreme narrowing".

We assume [7, 9] that the ^{31}P-T_1 relaxation in our systems is determined by dipole–dipole interaction and that we are concerned with the "extreme narrowing" case, i.e. a prolongation of the ^{31}P-T_1 relaxation time means an increase of the mobility of the phosphate group. Increasing the water concentration up to about 20 %, the mobility of the phosphate group increases and then remains constant. This concentration is equivalent to 8.2 water molecules per lecithin molecule and agrees approximately with the filling of region B as follows from the analysis of the chemical shift of water (Fig. 5). The lines represented in Fig. 2 may not be overestimated because of the relatively large measuring errors and the uncertainty of the interpretation.

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REFERENCES

1 Penkett, S. A., Flook, A. G. and Chapman, D. (1968) Chem. Phys. Lipids 2, 273–290
2 Chapman, D. (1968) Biological Membranes, pp. 131–168 Academic Press, London
3 Veksli, Z., Salsbury, N. J. and Chapman, D. (1969) Biochim. Biophys. Acta 183, 434–446
4 Henrikson, K. P. (1970) Biochim. Biophys. Acta 203, 228–232
5 Walter, W. V. and Haynes, R. G. (1971) Biochim. Biophys. Acta 249, 528–538
6 Finer, E. G. and Phillips, M. C. (1973) Chem. Phys. Lipids 10, 237–252
7 Barrett-Bee, K., Radda, G. K. and Thomas, N. A. (1972) Proc. 8th FEBS Meet., pp. 231–252, Amsterdam
8 Lee, A. G., Birdsall, N. J. M., Levine, Y. K. and Metcalfe, J. C. (1972) Biochim. Biophys. Acta 255, 43–56
9 Barker, R. W., Bell, J. D., Radda, G. K. and Richards, R. E. (1972) Biochim. Biophys. Acta 260, 161–163
10 Finer, E. G., Flook, A. G. and Hauser, H. (1971) FEBS Lett. 18, 331–334
11 Finer, E. G., Flook, A. G. and Hauser, H. (1972) Biochim. Biophys. Acta 260, 49–58
12 Elworthy, P. H. (1959) J. Chem. Soc. 813–817
13 Elworthy, P. H. and McIntosh, D. S. (1961) J. Pharm. Pharmacol. 13, 633–637
14 Elworthy, P. H. and McIntosh, D. S. (1964) J. Phys. Chem. 68, 3448–3452
15 Haque, R., Tinsley, J. J. and Schmedding, D. (1972) J. Biol. Chem. 247, 157–161
16 Jendrasiak, G. J. (1971) Chem. Phys. Lipids 6, 215–224
17 Levine, Y. K., Birdsall, N. J. M., Lee, A. G. and Metcalfe, J. C. (1972) Biochemistry 11, 1416–1421
18 Stelzner, F. (1973) Diplomarbeit, Leipzig
19 Noack, F. (1971) in NMR-Basic Principles and Progress, Vol. 3, Springer-Verlag, Berlin-Heidelberg-New York