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OBSERVATIONS BY NUCLEAR MAGNETIC RESONANCE OF THE INTERACTIONS OF WATER WITH LECITHIN MICELLES IN CARBON TETRACHLORIDE SOLUTION

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SUMMARY

The state of the water contained within lecithin micelles in carbon tetrachloride solution has been studied by NMR. The proton relaxation rate for both the water and certain groups on the lecithin molecule has been determined as a function of water concentration. About 12 water molecules per lecithin molecule are required to form a hydration shell around the phosphorylcholine part of the lecithin. Anaesthetic agents influence the relaxation rate of the water protons but there was no correlation with anaesthetic potency.

INTRODUCTION

The physical relationships of lipid molecules to each other and to water are of fundamental importance in membrane structure. Model micellar and lamellar systems of various types have been studied by X-ray diffraction[1], light scattering[2], and NMR[3,4]. In addition, characteristics of phospholipids in monolayers have been studied extensively[5,6]. NMR has also been applied in studies of erythrocyte membranes[7] and frog nerve[8].

In the studies reported here, NMR relaxation techniques were applied to a study of the relationship of lecithin (phosphatidylcholine) with small amounts of water. The actual system used consisted of egg yolk lecithin micelles in carbon tetrachloride solution, with small amounts of water added. Their interactions produced readily measurable changes in the NMR spectra. The relaxation rates of the water protons and of certain protons of the lecithin molecules were studied as a function of the water concentration. In addition, attempts were made to determine whether certain anaesthetics had an effect on those parameters.

If the chemical group containing the proton is unrestrained and has relatively free motion, its proton relaxation rate is low and its NMR line is narrow. If the molecule is restrained and its motion is restricted in the vicinity of the group containing the proton then the relaxation rate increases and the line broadens. In general the relaxation rate is given by $\pi\Delta\nu_{1/2}$ where $\Delta\nu_{1/2}$ is the width of the line at half height, in cycles/sec (ref. 9).

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MATERIALS AND METHODS

Egg yolk lecithin monohydrate was prepared by standard methods. Its purity was confirmed by thin-layer chromatography. The lecithin monohydrate was stored in chloroform–methanol (2:1, v/v) at 4°.

Phosphorus was determined by the method of FISKE AND SUBBAROW[10]. The alkaline hydroxylamine procedure of SNYDER AND STEPHENS[11] was used to determine esters. All the lecithin preparations that were used in these studies had ester–phosphorus ratios of 1.97–2.05.

For each NMR sample, the solvent was evaporated from 330 μmoles of lecithin. The lecithin was then dissolved in 1.0 ml of carbon tetrachloride and the appropriate amount of water was added. The NMR spectra were obtained on a Varian A 60 spectrometer at 60000 Mcycles. The probe temperature was 33°. All the samples contained tetramethylsilane as an internal standard. Relaxation rates for the water and trimethylamine peaks were found by measuring the width of the peak at half height. Those two peaks were recorded four times and the average line widths were used.

RESULTS

Two typical lecithin spectra are given in Fig. 1. Spectrum A was obtained from a sample to which 70 mg of water had been added; spectrum B was recorded from the same sample before the water was added. The chemical shift assignments were made from the values published by CHAPMAN AND MORRISON[12]. The spectrum in the absence of added water (lecithin monohydrate) was very broad and diffuse with only the

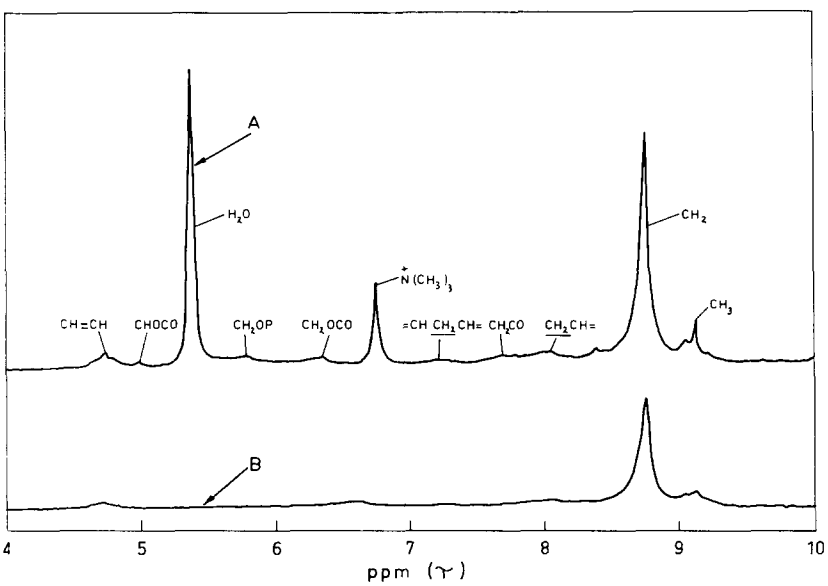

Fig. 1. NMR spectra of lecithin samples prepared as described in MATERIALS AND METHODS. For line A, the sample contained 70 mg of water; line B was recorded for the same sample before the water was added. The reference peak for tetramethylsilane appeared at 10τ.

major peaks discernible, but a dramatic improvement in the resolution of the whole spectrum occurred after the addition of 70 mg of water to the sample.

The great change in the relaxation rate which brought about the improved resolution is attributed in part to a drop in the viscosity that accompanies the addition of water to the lecithin solution, since there is a direct relationship between relaxation rate and viscosity[9]. A change in the relaxation rate caused by a drop in the viscosity should affect similarly all the peaks in the spectrum. Although no measurements of viscosity were made, a dramatic drop in viscosity was observed when 10 mg of water were added to a lecithin sample. When the amount of water was increased further, only the water peak and the trimethylamine peak were affected. There were no further changes in the peaks for the aliphatic protons. Thus some or all of the changes in the spectrum resulting from the addition of 10 mg of water were attributable to changes in viscosity. The effect of further additions of water on specific peaks in the spectrum had some other cause.

As can be seen from Fig. 2 the relaxation rate of the water protons decreased rapidly with increasing water content of the system at low water levels, and asymptotically approached a limit at high water levels. A similar observation was made for the trimethylamine peak. The position of the trimethylamine peak moved steadily upfield as the water content increased until it reached a maximum value at 70–75 mg of water.

The experimental system described here presented an opportunity to test the clathrate theory of anaesthetic action. According to this theory[13,14], anaesthetics might form clathrates with the water molecules within membranes, thereby reducing the mobility of the water and the activity of various cell processes. About 20 anaesthetic agents were tested for their ability to affect the relaxation rate of the water protons within the lecithin–carbon tetrachloride system. Complex effects were observed; no correlation of anaesthetic potency and changing relaxation rate could be made.

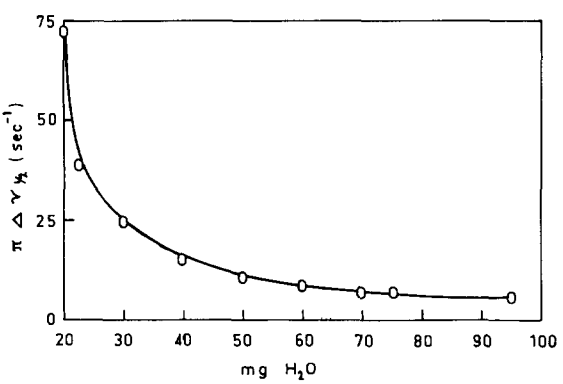

Fig. 2. Relaxation rate of the water protons as a function of the water level.

DISCUSSION

When lecithin dissolves in an organic solvent it forms micelles in which the polar phosphorylcholine groups strongly associate together on the inside and the

nonpolar fatty acid chains remain in solution on the outside[2]. Because they are zwitterions, a very strong electrostatic attraction exists between the phosphorylcholine groups, and they are held closely together. This close association results in severely restricted molecular motion which is reflected both in the high viscosity of the solution and in the broad lines and low resolution of the NMR spectrum of the monohydrated lecithin. Any water that is added to the system must be incorporated into the micelle, since the solubility of water in carbon tetrachloride is very low (not enough would dissolve to give an NMR signal) and since lecithin is very hygroscopic. By dissolving among the phosphorylcholine groups, the water molecules relieve the tight packing and allow greater freedom for molecular motion. This increased motion is reflected in a greatly reduced microscopic viscosity and in lower relaxation rates in the NMR spectra.

The viscosity of the system drops on the addition of water, but only part of the change in the NMR spectrum was due to that. CERBON[4] has shown that the relaxation rate of the water protons increased more rapidly than the increase in the bulk viscosity of a lipid dispersion in water. In that system (egg yolk lipid ultrasonically dispersed in water) mechanisms in addition to changing viscosity were responsible for the changes in the relaxation rate of the water protons. CERBON concluded that the mobility of the water was restricted by the lipid itself. Similar conclusions can be drawn from the data presented here. A change in viscosity could be expected to affect the relaxation rate of all the protons in the spectrum and the addition of the first 10 mg of water did result in a lowered relaxation rate for all the peaks. Further additions of water affected only the water and trimethylamine peaks. The effect of viscosity on relaxation rate as reflected in the total spectrum was limited to the first 10 mg of water added. The changes in the water and trimethylamine were due to some other mechanism.

If one considers the progressive addition of water to the system described here, it appears that small amounts of water must be tightly bound within the micelle. The first molecules of water added would form hydrogen bonds with the charged groups on the phosphorylcholine part of the molecule. With the addition of progressively larger amounts of water a hydration shell would be formed around the whole polar end of the lecithin molecule. The first few water molecules would be very tightly bound, though the remainder (even though they were in the hydration shell) would be more free in their motion. The relaxation rate would be very high when a small amount of water was present, and would drop to a minimum as more water was added. When a complete hydration shell was formed, the addition of more water should not produce further changes in the relaxation rate, since in general only closest neighbors affect relaxation rates. As shown in Fig. 2, the relaxation rate approached a limit after about 75 mg of water were added. The behavior of the trimethylamine peak parallelled that of the water peak. This group was the only hydrophilic part of the lecithin molecule that gave a distinct signal. Both the position of the peak and the proton relaxation rate changed with the water content of the system. Both parameters reached a limit after about 75 mg of water were added.

The 75 mg of water that represented the maximum amount which produced an observable effect corresponds to about 12 molecules of water per lecithin monohydrate molecule. Presumably this is the amount of water that is to be found in the hydration shell around the phosphorylcholine group. A study of space filling molecular

models showed that a hydrogen bonded hydration shell could be constructed around the phosphorylcholine end of the lecithin molecule with a minimum of 11–12 water molecules. This value corresponds to the value of 10 found by CHAPMAN *et al.*[7] by calorimetry.

Anaesthetic agents would be expected to have an effect only within the hydration shell around the lecithin, if they did have an effect on water structure. For the 20 anaesthetics that were examined, no consistent effect on the relaxation rate of the water protons was observed.

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