Nuclear Magnetic Resonance Studies of Phospholipid Micelles*

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SUMMARY

The proton magnetic resonance spectrum of DL-α-lecithin at 100 MHz in CHCl₃ solvent is reported. An analysis of the spectrum is made. The chemical shifts of the N-(CH₃)₃ protons and the resonance peak associated with the condensed water molecule showed concentration dependence with the latter showing the more pronounced response. These chemical shift changes are interpreted in terms of micelle formation through intermolecular hydrogen bond formation between lecithin monomers. The chemical shift data are used to calculate the critical micelle concentration of lecithin as well as the number of monomer lecithin units required to form a micelle. The temperature-dependent studies showed a small negative enthalpy change associated with the micelle. The micellar behavior in CHCl₃ is compared with those in benzene and carbon tetrachloride.

Nuclear magnetic resonance spectroscopy has only recently been applied in the study of various complex lipids. Chapman and Salsbury (1) have used this technique in the study of molecular motion of lipids as well as their interaction with a number of organic compounds.

Although this technique has been successfully employed to investigate the structure of micelles in surfactant systems (2-8), it has not been used to any extent in the study of lipid micelles. Further clarification of the micellar properties of lipids would contribute to an understanding of the function of lipids in biological membranes. Chapman and Morrison (9) noticed that the line width of the protons of the polar head of phosphatidylcholine varied with the nature of the solvent molecule and suggested that it was related to the micellar properties of the compound.

Pronounced changes in the electronic environment of either the hydrophobic or hydrophilic region of the molecule can result from micelle formation and as a consequence one should expect changes in the chemical shift of the resonance peak of the nucleus of an atom as it moves from a monomeric phase to a micellar phase. Such a response is noticed with fluorokylate surfactants with a pronounced change in the ¹⁹F chemical shift being detected in the region of the critical micelle concentration. Changes in the proton magnetic resonance chemical shift with micelle formation are quite small (10).

The chemical shift of several of the resonance peaks especially the protons of associated water molecules and the choline -N(CH₃)₃ in the proton magnetic resonance spectrum of dipalmitoylphosphatidylcholine in CHCl₃ showed concentration and temperature-dependence. An interpretation of these changes in the chemical shift of the lecithin in terms of micelle formation is reviewed in this paper. Structural and thermodynamic properties of phospholipid micelles in CHCl₃ solution are defined.

EXPERIMENTAL PROCEDURE

Materials—DL-α-Lecithin (β,γ-dipalmitoyl-α-phosphatidylcholine) was obtained from Sigma Chemical Company and was used without further purification. All other chemicals used were of spectral grade.

Measurement of Spectra—Nuclear magnetic resonance spectra were recorded on a Varian HA 100 instrument operating at 100 MHz and using tetramethylsilane as internal reference. CHCl₃ was also used as an internal lock since the resonance peak of the CHCl₃ proton did not show any change in chemical shift in the presence of lecithin. A Varian variable temperature assembly was used to obtain temperature-dependence spectra.

RESULTS AND DISCUSSION

Proton Magnetic Resonance Spectrum of Lecithin

From the molecular formula of the lecithin under investigation (Formula I) we should expect a spectrum (Fig. 1) consisting of many peaks representing the protons a to j. The terminal methyl protons (j) show a triplet (J = 5.5 Hz) at 0.855 ppm. The methylene protons can be divided into three groups: g, h, and i. The protons i, although geometrically nonequivalent, have the same chemical shift and show a large single peak at 1.26

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The protons \( h \) appear at 1.6 ppm as a broad shoulder. The triplet \( J = 7.0 \text{ Hz} \) at 0.88 ppm is assigned to protons \( g \). The confirmation of the triplet for proton \( g \) was made by a spin-decoupling experiment. On irradiating with a frequency corresponding to the chemical shift of \( h \) the triplet collapsed to a singlet.

The \(-N(CH_3)_3\) protons show a single peak at 3.26 ppm, whereas the broad peak at 3.8 ppm may be assigned to the \( N(CH_2)_n \) protons \( 9 \). The spin-spin splittings due to the \(^{14}N\) nucleus are not observable, suggesting either a coupling constant of smaller magnitude or rapid nuclear spin relaxation of the \(^{14}N\) nucleus. The overlapping resonance peaks at 4.26 and 4.21 ppm may be due to protons \( c, d, \) and \( f \). The long range coupling \( ^{13}P-O-CH_2 \) is not observable. The \(-CH\) protons which should appear as a multiplet are not observable. A better analysis of the spectrum can be made at 220 MHz.

The spectrum of the lecithin (Fig. 1) always showed a peak corresponding to the 2 protons associated with the condensed water, which is probably oriented near the zwitter ions of the lecithin. This peak showed strong concentration and temperature-dependence of chemical shift.

**Concentration Dependence of Chemical Shift and Micellar Properties**

The proton magnetic resonance spectra of lecithin showed changes in the chemical shift of various protons when either its concentration was changed or the temperature of the sample was varied. These changes were more pronounced for the resonance peaks of the \(-N(CH_3)_3\) protons as well as the peak associated with the condensed water molecule. The \(-N(CH_3)_3\) proton peak showed a high field chemical shift of about 6.2 Hz at 44° when the concentration of the lecithin was increased from 1 to 100 mg per ml in CHCl₃ (Table I). However, in the same concentration range the water peak showed a chemical shift of 243 Hz to low field (Table III).

These changes in chemical shift strongly suggest some interaction of lecithin molecules, which is producing a dramatic influence in the electronic environment of the condensed water molecule. The lecithin molecule can be regarded as a non-ionic surface-active agent and undoubtedly there will be intermolecular and intramolecular hydrogen bond formation in the lecithin solution involving the protons of the water molecule. In addition, the presence of large aliphatic groups on the \(-CH_2\) and \(-CH\) carbons of glycerides will facilitate the formation of a micelle. Most probably the micelle will involve a network of intermolecular hydrogen bonds between monomeric lecithin units. Such micelle formation will produce large changes in the
chemical shift of the water protons which are more sensitive to the electronic environment and small changes in the chemical shift of less susceptible $-\text{N(}$CH$_2\text{)}_3$ protons (10).

The chemical exchange of the protons in the micellar phase and the monomeric phase of the lecithin is very fast and only one averaged peak is observable on the nuclear magnetic resonance time scale. There was no significant change in line width associated with changes in the concentration of the lecithin. This indicates that there is free movement of the molecules in the solution and that the formation of micelles does not hinder the molecular motion to any degree.

Critical Micelle Concentration—The micellar process can be expressed by the following equilibrium (i)

$$nL = L_n$$  \hspace{1cm} (i)

where $L$ is the free lecithin concentration, $n$ the number of monomers required to form a micelle, and $L_n$ and $L$ are related by Expression ii, where $C$ is the total concentration of the lecithin.

$$L + nL_n = C$$  \hspace{1cm} (ii)

If $\delta n$ and $\delta a$ are the chemical shifts of the lecithin molecule in the micellar phase and as the monomer, then the observed chemical shift $\delta o$ at a particular concentration can be expressed in terms of critical micelle concentration (cmc) as

$$\delta o = \delta n + \frac{cmc}{C} (\delta n - \delta a)$$  \hspace{1cm} (iii)

A plot of the observed chemical shift, $\delta o$ of a particular resonance peak as a function of $1/C$ should result in two straight lines intersecting at the critical micelle concentration of the lecithin. The chemical shift of the water protons shows the more pronounced response to changes in the lecithin concentration and thus is used to derive the critical micelle concentration using Expression iii (Fig. 2). It is interesting to note that although the changes in chemical shift are much larger, the general shape of the graph is very similar to that for changes in $^{19}$F chemical shift reported for fluorinated surfactants reported earlier (4–8). The values of $\delta a$, $\delta n$, and critical micelle concentration derived from Fig. 2 are given in Table II.

**Table II**

<table>
<thead>
<tr>
<th>Characteristics of lecithin micelles in CHCl$_3$ solution</th>
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<tr>
<td>Micellar shift, $\delta n$ (ppm)</td>
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<td>Monomer shift, $\delta a$ (ppm)</td>
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<td>Critical micelle concentration (mg/ml)</td>
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<td>Critical micelle concentration (mole fraction X 10$^{-3}$)</td>
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<td>$\Delta H^\circ$ (kcal/mole)</td>
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<td>$\Delta O^\circ$ (kcal/mole)</td>
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<td>$T\Delta S^\circ$ (kcal/mole)</td>
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Micelle Size—The chemical shift data at different concentrations can be used to calculate the number of monomer units, $n$, required to form a micelle (5). The equilibrium constant, $K$, for the process (Expression i) can be written as

$$K = \frac{[L_n]}{[L]^n}$$  \hspace{1cm} (iv)

On the basis of a simple statistical average the value of $[L]$ can be expressed in terms of chemical shifts $\delta o$ and $\delta n$ and concentration $C$ as

$$[L] = C \frac{\delta_n - \delta_o}{\delta_n - \delta_a}$$  \hspace{1cm} (v)

and using Expressions ii and v we can rearrange Expression iv in terms of chemical shifts as

$$\log C \left[ \frac{\delta_n - \delta_o}{\delta_n - \delta_a} \right] = n \log C \left[ \frac{\delta_n - \delta_o}{\delta_n - \delta_a} \right] + \log n K$$  \hspace{1cm} (vi)

Hence a plot of $\log C [(\delta_n - \delta_o)/(\delta_n - \delta_a)]$ against $\log C [(\delta_n - \delta_o)/(\delta_n - \delta_a)]$ should be a straight line. For most of the concentration range a straight line is obtained (Fig. 3), and the value of $n$ was found to be in the neighborhood of 3. At very high concentrations $n$ may be more than 3. The value of $n$
appears to be independent of temperature and the dominant species in the solution is a timer.

Thermodynamic Analysis—Assuming that the deviations from ideal behavior is not significant, the enthalpy of micelle formation can be estimated from the following equation proposed by Stainsby and Alexander (11);

$$
\Delta H^0 = RT \frac{d \ln (cmc)}{d \frac{1}{T}}
$$

where $R$ and $T$ are gas constant and absolute temperature, respectively. Such a plot of $\ln c_m c$ against $1/T$ (Fig. 4) is not strictly linear, indicating that $\Delta H^0$ depends on temperature. $\Delta H^0$ values obtained by drawing tangents at a particular temperature are plotted as a function of temperature in Fig. 5.

Micelle formation thus appears to involve a small negative enthalpy change (3.9 to 0.6 kcal per mole). The $\Delta H^0$ tends to become less negative as the temperature is lowered. This enthalpy change with the temperature is associated with a heat capacity change $\Delta C_p$ of approximately 100 cal per degree. Such behavior is not uncommon in micellar systems and has been noticed with many anionic surfactants in aqueous solution (12).

The changes in $\Delta H^0$ with temperature can be explained in terms of structural effects in CHC1$_3$ solution. The OH groups in solution are surrounded by CHC1$_3$ which exist at a comparatively low energy state, however, the motional characteristics result in aggregation. As the temperature is increased there is a breakdown in the structure of CHC1 network causing an increase in the energy. This causes the value of $\Delta H^0$ to fall with increasing temperature.

The magnitude of $\Delta H^0$ obtained in the present study can be compared favorably with a value of 0.4 kcal per mole for egg yolk lecithin micelles in benzene reported by Elworthy (13).

![Fig. 3. A plot between log C ($80 - 8a$)/($8n - 8a$) and log C ($8n - 8o$)/($8n - 8a$).](image)

![Fig. 4. A plot of 1/T against critical micelle concentration (cmc).](image)

![Fig. 5. Graph showing changes in $\Delta H$ and T.](image)

<table>
<thead>
<tr>
<th>TABLE III</th>
<th>Chemical shift of condensed water protons of lecithin in CCl$_4$, benzene and CHC1$_3$ at 34°</th>
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<tbody>
<tr>
<td><strong>Carbon tetrachloride</strong></td>
<td><strong>Chloroform</strong></td>
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<tr>
<td>Concentration</td>
<td>Chemical shift</td>
</tr>
<tr>
<td>mg/ml</td>
<td>Hz</td>
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<tr>
<td>100</td>
<td>454.6</td>
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<td>75</td>
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<td>9</td>
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<td>0</td>
<td>399.0</td>
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The standard free energy $\Delta G^0$ can be expressed by the following equation

$$\Delta G^0 = RT \ln mc$$  \hspace{1cm} (viii)

By the well known expression

$$\Delta G^0 = \Delta H^0 - TAS^0$$  \hspace{1cm} (ix)

one can calculate the enthalpy changes for the system. The calculated value of $\Delta G^0$, $TAS^0$, and $\Delta H^0$ are given in Table II.

**Structural Considerations**—The changes in the chemical shift of the condensed water proton of lecithin can be related to the observations of Clifford and Pethica (2) of the change in chemical shift of water protons produced by the addition of long alkyl chain salts. A high field shift of the water resonance peak was noted when either the salt concentration was increased or the temperature of the sample was raised. In explaining the chemical shift changes caused by reduction in the electrostatic field at protons in water near the water dipoles were small. The chemical shift changes were explained on the basis of increased covalent character of the bond in water. They assume that the effects on chemical shifts caused by the addition of the alkyl chain, the orientation of water dipoles, and the increased polarization of the O-H bond in water. They also observed a high field shift of the water resonance peak when the salt concentration was increased or the temperature of the sample was raised.

However, as the concentration of lecithin increases, the water molecules associate within the lecithin micelle and assume a more liquid-like water structure, producing a large low field shift. In a very dilute solution of lecithin in chloroform where it exists predominantly in a monomeric form the associated water molecule will experience a strong hydrophobic environment. However, as the concentration of lecithin increases, the water molecules associate within the lecithin micelle and assume a more liquid-like water structure, producing a large low field shift.

In chloroform saturated with water (~0.1 m) the proton magnetic resonance chemical shift of the water protons is about 1.25 ppm. This chemical shift corresponds to that observed for the water protons in the condensed water in a very dilute solution of lecithin, but is significantly different from that observed in a concentrated solution of lecithin (~4 ppm). In addition, changes in the chemical shift of the $\text{-N(CH}_3\text{)}_3$ protons with concentration suggest association of the lecithin molecules. Thus the possibility that the chemical shift may arise merely from the independent association of added water molecules without the involvement of lecithin molecules would seem remote. Furthermore, the infrared spectrum of a water-saturated CHCl₃ solution showed a O-H bond stretching frequency at 3620 cm⁻¹. In chloroform saturated with water (~0.1 m) the proton magnetic resonance chemical shift of the water protons is about 1.25 ppm. This chemical shift corresponds to that observed for the water protons in the condensed water in a very dilute solution of lecithin, but is significantly different from that observed in a concentrated solution of lecithin (~4 ppm). In addition, changes in the chemical shift of the $\text{-N(CH}_3\text{)}_3$ protons with concentration suggest association of the lecithin molecules. Thus the possibility that the chemical shift may arise merely from the independent association of added water molecules without the involvement of lecithin molecules would seem remote. Furthermore, the infrared spectrum of a water-saturated CHCl₃ solution showed a O-H bond stretching frequency at 3620 cm⁻¹. However, in a solution of lecithin in CHCl₃ this vibration frequency shifted to 3390 cm⁻¹, accompanied by a line broadening. This again substantiates the hypothesis that the associated water molecules in the lecithin are in the lecithin micelle in a hydrogen-bonded network (15).

With the a-monoglyceride micelles Debye and Prins (16) have shown that $n$ depends on the nature of the solvent. Using light scattering techniques they noted that the values of $n$ for a-monoglycerides are always lower in chloroform (3 to 109) than in benzene (11 to 100). Recent work of Elworthy and McIntosh (17) on egg yolk lecithin has suggested the formation of two kinds of micelles in benzene solution. The value of $n$ for the smaller micelle was around 55 whereas the larger micelle gave a value of 73. By contrast, lecithin formed much smaller micelles in alcohols. The value of $n$ increased from 3 in methanol to 24 in butanol and 28 in hexanol. These data illustrate the influence of solvent polarity on micellar size which is decreasing with increasing polarity. It appears that the nature of the micelle in CH₃OH solution is similar to that in CHCl₃ solution as indicated by the small value of $n = 3$.

We have carried out preliminary studies in CCl₄ and benzene solvents. A chemical shift change of 0.05 ppm was noticed for solutions in the concentration range 0.1375 to 0.082 m in CCl₄ solutions. In benzene the chemical shift change was 1.22 ppm for concentration range 0.104 to 0.0178 m. The chemical shift data for these two solvents are compared with CHCl₃ solvent in Table III. If we assume that the monomeric shift for the lecithin molecule in CCl₄ or benzene solvents does not differ considerably than in CHCl₃, then we must conclude that in dilute solutions the lecithin molecules are in the micellar phase. Even in the concentration range of 0.02 m there are enough hydrogen bonds in CCl₄ and benzene solution so that the resonance peak appears in the region of liquid water. One might anticipate that the value of $n$ in these two solvents is higher and the critical micelle concentration value is lower compared to CHCl₃.

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**REFERENCES**


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