The Nature of Water Inside Phosphatidylcholine Micelles in Diethyl Ether†

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ABSTRACT: The properties of water bound to phosphatidylcholine micelles in diethyl ether solutions have been investigated by infrared and pmr spectroscopy, by measuring the fluorescence properties of *N*-dansylphosphatidylethanolamine (dansyl-PE) incorporated into the micelles, and by the spectral properties of $CoCl_2 \cdot 6H_2O$ incorporated into the micelles. Infrared studies of the OH stretching of HOD show the existence of two types of bound water. The first which occurs at low water concentrations is characterized by an absorption band at 3530 cm^{-1} and a narrow bandwidth. The second form, which coexists with the first, is found at higher concentrations of water, and has infrared properties similar to those of pure water. Pmr studies do not show two forms of bound water; however, the two states can be distinguished by the shorter relaxation times of the water protons bound in the first state. As the amount of water bound to the micelle increases there is a marked increase in the relaxation time of the polar head group of the lipid. In addition the depolarization of dansyl-PE fluorescence decreases. These results indicate increased molecular motion of the lipid–water interface as the extent of hydration increases. The fluorescence emission maximum of dansyl-PE shifts to longer wavelengths as the extent of hydration increases, suggesting an increased polarity of the lipid–water interface. At low water levels cobalt exists as a tetrahedral complex. As the water concentration increases, there is a gradual conversion to an octahedral complex. The relationship of the properties of bound water to the activity of *Crotalus adamanteus* phospholipase A_2 shows that the enzyme is only active when dissolved within a micelle, which contains bound water whose properties closely resemble those of pure water.

Recent reports from this laboratory have demonstrated that the amount of water bound to reversed micelles of phosphatidylcholine in diethyl ether solutions markedly influences the activity of phospholipase A_2 (Misiorowski and Wells, 1974) and the physical properties of the phosphatidylcholine micelles (Poon and Wells, 1974). In the latter report evidence was presented which suggested that water can exist in at least two states within the micelle. At low levels of hydration the water association follows an apparently simple equilibrium; however, phospholipase A_2 is inactive. At higher hydration levels, there is apparently cooperative binding of water and phospholipase A_2 activity can be observed.

Several reports have appeared which treat the hydration of phosphatidylcholine in a variety of different systems and by a variety of physical techniques (Demchenko, 1961; Elworthy 1961, 1962; Elworthy and McIntosh 1964a,b; Small, 1967; Ladbrooke and Chapman, 1969; Hendrikson, 1970; Jendrasiak, 1971; Rigaud *et al.*, 1972; Walter and Hayes, 1971; Salsbury *et al.*, 1972; Haque *et al.*, 1972; Gottlieb *et al.*, 1973; Finer and Drake, 1974; Jendrasiak and Hasty, 1974). It appears that the following generalizations can be made with regard to the hydration of phosphatidylcholine. (1) At low water levels there is a class of tightly bound water amounting to approximately 6 mol of water/mol of phospholipid. It appears that both the polar head groups and the water bound in this first hydration state do not have complete freedom of motion. There is some evidence that this first hydration state may be comprised of two separate states (Elworthy, 1961, 1962; Jendrasiak and Hasty, 1974; Finer and Drake, 1974). (2) Above water levels which saturate the first hydration state additional water can be bound, the amount being dependent on the system. In this second state both the polar head group of the lipid and the water exhibit greatly increased freedom of motion. The water bound in this second state appears to be closely related to solvent water.

It was of interest to investigate the hydration of phosphatidylcholine micelles in diethyl ether with particular reference to the nature of the bound water and to correlating the properties of the bound water with the activity of phospholipase A_2.

Materials and Methods

Except as noted below, these have been described in the preceding papers (Misiorowski and Wells, 1974; Poon and Wells, 1974).

N-Dansylphosphatidylethanolamine (dansyl-PE)[1] was prepared by the method of Waggoner and Stryer (1970) using phosphatidylethanolamine, which had been prepared by transphosphatidylation (Yang *et al.*, 1967) of egg yolk phosphatidylcholine in the presence of ethanolamine and cabbage phospholipase D (Calbiochem, La Jolla, Calif.).

D_2O (99.8% D) was purchased from Bio-Rad Laboratories (Richmond, Calif.), and deuterated diethyl ether (D_{10}, 99% D) from Merck, Sharpe and Dohme of Canada Ltd. (Montreal).

Fluorescence measurements were made with a Perkin-Elmer fluorescence spectrophotometer, Model MPF-2A. Excitation slits were 2.5 nm and emission slits were 5.0 nm. Fluorescence polarization measurements were made in the

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[1] Abbreviation used is: dansyl-PE, *N*-dansylphosphatidylethanolamine.

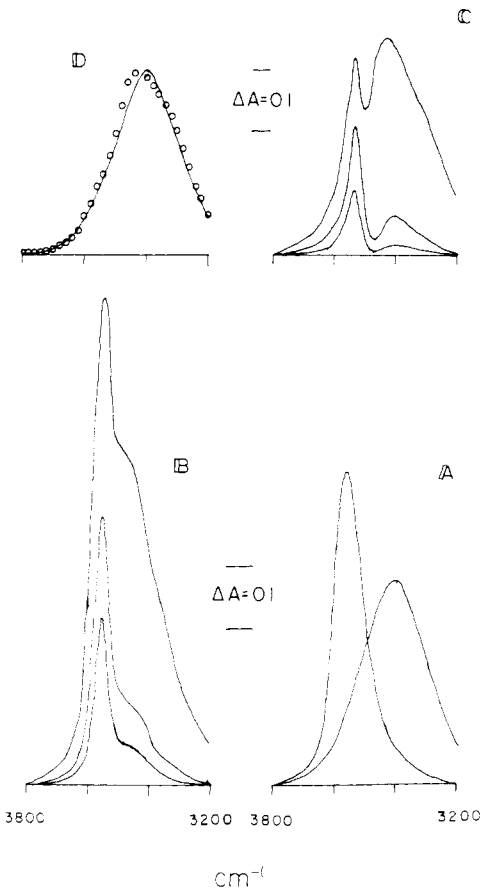

FIGURE 1: Infrared spectra of HOD. (A) The curve with a maximum near 3400 cm^{-1} represents HOD in D_2O, the curve with a maximum near 3500 cm^{-1} represents HOD in diethyl ether. (B) Difference spectra between phosphatidylcholine in dry ether (reference cell) and phosphatidylcholine (10 mg/ml) plus water in ether (sample cell). (Lower curve) Total water, 2.6 mg/ml; (middle curve) total water, 5.0 mg/ml; (upper curve) total water, 9.8 mg/ml. (C) Difference spectra of water in ether (reference cell) and phosphatidylcholine (10 mg/ml) plus water in ether (sample cell). (Lower curve) Reference cell contained 2.0 mg/ml, sample cell, 2.6 mg/ml of water; (middle curve) reference cell contained 4.0 mg/ml, sample cell, 5.0 mg/ml of water; (upper curve) reference cell contained 7.5 mg/ml, sample cell, 9.8 mg/ml of water. (D) Comparison of the spectrum of HOD in D_2O (—) and the calculated spectrum of water in the second hydration state in phosphatidylcholine micelles in ether (○). The open circles represent the difference between the upper and middle curves from Figure 1C.

usual way. Proton magnetic resonance spectra were recorded using a Varian HA-100 operating at a probe temperature of 34–35°. Tetramethylsilane was used as an internal lock standard. Nuclear magnetic resonance (nmr) tubes were flushed with N_2 just prior to sample preparation. Temperature equilibration was judged to be complete when the chemical shift of the water protons remained constant with time (approximately 30 min).

Infrared spectra were recorded on a Beckman IR-12 using the absorbance mode and by use of variable path length cells with IR-TRAN windows (Precision Cells, Hicksville, N.Y.). The path length of the sample cell was set to 0.075 mm, and the path length of the reference cell was adjusted such that the base line was smooth (without sharp peaks) and constant to within ±0.02 absorbance unit. Ethereal solutions containing phosphatidylcholine or water were used to balance the cells, depending on the composition of the reference cell in each experiment. After balancing the cells, the sample cell was drained and washed several times with ether, and dried by passing nitrogen through the cell. The sample cell was filled with solution and the spectrum recorded. The cell was drained and cleaned and filled with reference solution and another base line recorded. If this base line did not agree with the first, the experiment was repeated.

Results

Infrared Spectroscopy. Diethyl ether is transparent to infrared radiation in the region between 3200 and 3800 cm^{-1}. It was, therefore, possible to study the infrared spectrum of water bound to phosphatidylcholine micelles in diethyl ether. In order to simplify the interpretation of the spectra, all measurements were made of the uncoupled OH stretching vibration of HOD by using solutions which were 5.5 M H_2O in D_2O. The advantage of this approach is that each species of water gives a single, nearly gaussian, absorption band (Eisenberg and Kauzmann, 1969). As seen in Figure 1A HOD in D_2O gives a broad band centered at 3400 cm^{-1} with a width at half-maximal intensity of 260 cm^{-1}. In diethyl ether the band is shifted to 3560 cm^{-1} and is sharper with a width at half-maximal intensity of 120 cm^{-1}.

Two experimental procedures were used in these studies. In the first, the reference solution contained phosphatidylcholine in dry ether and the sample solution contained phosphatidylcholine and water in ether. In the second procedure the reference cell contained water in ether and the sample cell contained phosphatidylcholine and water in ether. The amount of water in the reference cell was chosen to be equal to the unbound water in the phosphatidylcholine–water solution (Poon and Wells, 1974). In the first protocol lipid in dry ether was used in both cells for balancing, in the second case water in ether was used. The spectra presented in Figure 1 represent absorbance *vs.* wave number data which have been corrected for the base line at 10-cm^{-1} intervals.

Figure 1B presents data using phosphatidylcholine in ether as the reference solution. In the lower curve the total water was 2.6 mg/ml, in the middle 5.0 mg/ml, and in the upper 9.8 mg/ml. At the lipid concentration used (10 mg/ml) the amount of bound water is 0.6, 1.0, and 2.3 mg, respectively. These curves represent the spectra of the free water (Figure 1A) plus the spectra of the bound water. At low water the sharpness of the band near 3530 cm^{-1} suggests that a band in this region may be superimposed on the free water absorption band. As the water concentration is increased there is a prominent shoulder near 3450 cm^{-1}.

A clearer picture of the spectrum of the bound water emerges when water in ether is used as the reference solution in order to subtract the spectrum of the free water. In Figure 1C the lower curve represents the difference between lipid with 2.6 mg/ml of water and the reference with 2.0 mg/ml of water; the middle curve the difference between lipid with 5.0 mg/ml of water and the reference with 4.0 mg/ml of water; the upper curve the difference between lipid with 9.8 mg/ml of water and the reference with 7.5 mg/ml of water. At low water the most prominent peak is at 3530 cm^{-1} and is rather narrow with a width at half-maximal intensity of 55 cm^{-1}. At higher water there is a broad peak at 3410 cm^{-1} in addition to the peak at 3530 cm^{-1}. The calculated difference spectrum between the upper and middle curves of Figure 1C is shown in Figure 1D as the points. The solid line represents the spectrum of HOD in D_2O. The half-width of the calculated difference spectrum is 280 cm^{-1}. The integrated intensities of the spectra shown in Figure 1C are in the ratio 1.0:2.0:12.8

TABLE I: Infrared Properties of Water Bound to Phosphatidylcholine Micelles.

Sample	λ_{max} (cm^{-1})	$\nu_{1/2}$ (cm^{-1})[a]
(A) Uncoupled OH stretching frequencies		
HOD (water)	3400	260
HOD (ether)	3560	120
Bound water-I	3530	55
Bound water-II	3410	280

Free Water (mg/ml)	δ[b]	Rel Intensity	Rel Amount of Bound Water
(B) Intensity of OH Stretching Band			
2.0	0.06	1.0	1.0
4.0	0.10	2.0	1.7
7.5	0.23	12.8	3.8

[a] Width at half-maximal intensity. [b] g of H_2O/g of phosphatidylcholine (taken from Poon and Wells, 1974).

(lower:middle:upper) whereas the ratio of water content is 1.0:1.7:3.8.

These data suggest that two types of water are present inside the phosphatidylcholine micelles. The first and most tightly bound gives a sharp OH stretching band at 3530 cm^{-1} which is less intense than the band of the second type of water which occurs at 3410 cm^{-1} and is broad. The properties of these two types of water are summarized in Table I. The intensity of the 3530-cm^{-1} band increases when the water content is raised from 2.6 to 5.0 mg/ml. However, its intensity does not increase when the water content increases from 5.0 to 9.8 mg/ml, as evidenced by the absence of this peak in the calculated difference spectrum shown in Figure 1D. This result suggests that there is a limited amount of water, characterized by the 3530-cm^{-1} band, which can be bound to the micelle.

Pmr Studies. Figure 2 shows a portion of the pmr spectra of H_2O in deuterated ether, phosphatidylcholine in dry deuterated ether, and phosphatidylcholine and H_2O in deuterated ether. Curve A represents H_2O in ether. The sharp resonance at τ 7.56 is due to water protons, the peaks near τ 6.6 are resonances of residual protons in the deuterated ether. Curve B represents the spectra of phosphatidylcholine in dry ether. The broad resonance at τ 6.63 is due to the protons of the trimethylamino group of the choline. This resonance is superimposed on the resonance of the residual protons of the solvent. Curve C represents the spectra when the free water content is 2.0 mg/ml, and curve D when the free water content is 4.0 mg/ml. In going from curve B to C to D note the increased sharpness of the $N(CH_3)_3$ resonance at τ 6.60 which is evident even though there are also solvent protons in this peak. The H_2O proton resonances are near τ 6.0 in the presence of lipid. The peak is broad at low water (curve C) and sharper at higher water (curve D). As the water content increases the water peak continues to become sharper as does the $N(CH_3)_3$ peak. The chemical shift and $1/T_2$, calculated from the width at half-peak height for the water and the $N(CH_3)_3$ protons, are summarized in Table II. Also shown in Table II are experiments in which 0.01 M $CaCl_2$ was substituted for water.

The apparent differences in the chemical shift of the water protons at various hydration levels can be explained

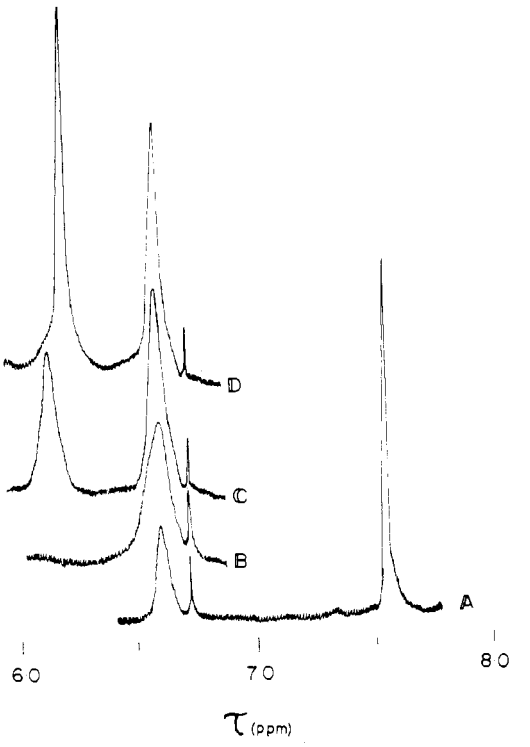

FIGURE 2: Pmr spectra of water and phosphatidylcholine in deuterated diethyl ether. Lower scale is ppm from the internal tertramethylsilane standard. (A) H_2O in ether; (B) phosphatidylcholine (40 mg/ml) in dry ether; (C) phosphatidylcholine (40 mg/ml) and H_2O (4.3 mg/ml) in ether. (D) phosphatidylcholine (40 mg/ml) and H_2O (8.0 mg/ml) in ether.

by assuming that the observed chemical shift is the average of the chemical shift of the free water and the bound water, the average depending on the fraction of bound water. The chemical shift of the bound water can be estimated by

$$\tau_{\text{bound water}} = \tau_{\text{free water}} - (\Delta\tau/Q) \qquad (1)$$

where $\tau_{\text{free water}} = 7.56$, $\Delta\tau$ is the observed difference between the chemical shift of water in ether and water in the lipid solution, and Q is the fraction of the water in the lipid solution bound to the lipid. The values of $\Delta\tau/Q$ for a total water concentration of 4.3, 8.0, 12.1, and 16.7 mg/ml are 2.58, 2.76, 2.76, and 2.63 respectively. The average is 2.68 and $\tau_{\text{bound water}} = 4.88$. These results show that the bound water is characterized by a single chemical shift value, regardless of the extent of hydration of the micelles. On the other hand, the relaxation time of the bound water protons shows a marked increase as the extent of hydration increases, which is paralleled by an increase in the relaxation time of the $-N^+(CH_3)_3$ protons of the phosphatidylcholine. At low water, inclusion of $CaCl_2$ has no effect on either the chemical shift or relaxation time of either the water or $N(CH_3)_3$ protons. At higher water and $CaCl_2$ concentrations there is no effect on the $N(CH_3)_3$ protons, but the chemical shift of the water protons is moved downfield, without a change in T_2. This downfield shift may represent an increased fraction of the total water bound to the micelle in the presence of higher concentrations of $CaCl_2$, or rapid exchange of water with the solvation shell of the Ca^{2+}, thereby affecting the average water proton shielding constant.

Fluorescence Measurements. The fluorescence emission maximum of dansyl-PE in dry either is 475 nm. As the water content of the solution is increased the maximum

TABLE II: Summary of Pmr Studies on the Hydration of Phosphatidylcholine Micelles in Diethyl Ether.[a]

Total H_2O (mg/ml)	δ^b	H_2O Proton		$N(CH_3)_3$ Protons	
		τ^c	$1/T_2$ (sec^{-1})^d	τ^c	$1/T_2$ (sec^{-1})^d
0	0			6.63	0.245
4.3	0.06	6.12	0.220	6.60	0.172
8.0	0.10	6.20	0.088	6.59	0.138
12.6	0.15	6.21	0.063	6.61	0.126
16.7	0.23	6.11	0.063	6.60	0.126
4.3	(0.01 M $CaCl_2$)	6.12	0.213	6.60	0.176
16.7	(0.01 M $CaCl_2$)	6.00	0.069	6.61	0.124
Water in ether		7.56	0.044		

[a] Lipid concentration, 40 mg/ml in d_{10}-ether. [b] g of H_2O/lipid (Poon and Wells, 1974). [c] Ppm downfield from Me_4Si. [d] Calculated from peak width ($\nu_{1/2}$) at half-peak height. $1/T_2 = \pi\nu_{1/2}$.

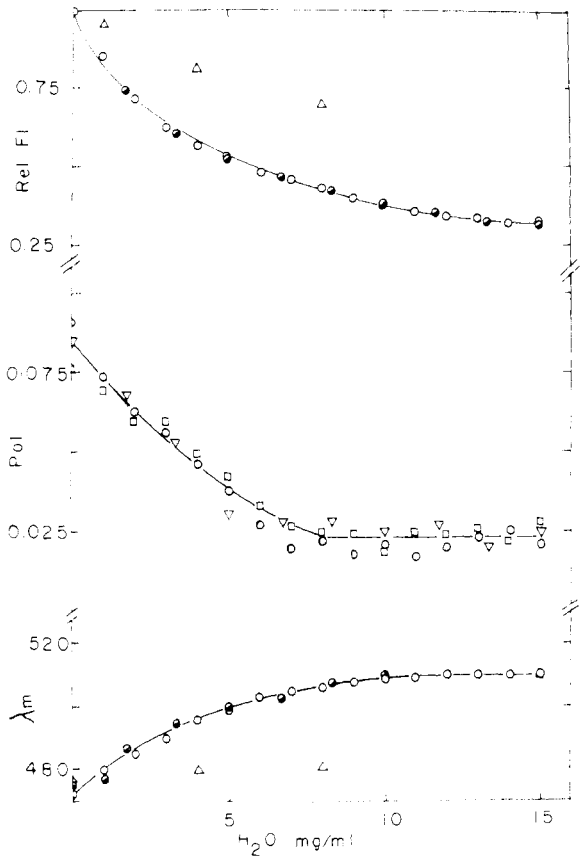

FIGURE 3: Effect of the total water concentration on the fluorescence properties of dansyl-PE in ether. (Lower curve) Fluorescence emission maximum (activation at 340 nm): (Δ) 10 μM dansyl-PE in ether; (○,●) two separate experiments with 10 μM dansyl-PE and 12.5 mM phosphatidylcholine in ether. (Middle curve) Polarization of dansyl-PE fluorescence. The different symbols refer to three separate experiments using 10 μM dansyl-PE and 12.5 mM phosphatidylcholine in ether. (Upper curve) Relative fluorescence of dansyl-PE at the wavelength of maximal fluorescence (activation 340 nm): (Δ) 10 μM dansyl-PE in ether; (○,●) two separate experiments using 10 μM dansyl-PE and 12.5 mM phosphatidylcholine in ether.

shifts to 488 nm and the relative fluorescence decreases from 1.0 to 0.69 (see Figure 3). The polarization of the fluorescence was ±0.005 in the presence or absence of water. When 10 μM dansyl-PE is mixed with 12.5 mM phosphatidylcholine markedly different results are obtained as seen in Figure 3. The emission maximum shifts from 472 nm (in the absence of water) to a limiting value of 510 nm at high water. Waggoner and Stryer (1970) report that the emission maximum of dansyl-PE in phosphatidylcholine vesicles in water is 515 nm. The relative fluorescence decreases from 1.0 to 0.32 as the water content increases. In the absence of water the polarization of fluorescence is 0.085. This decreases to a limiting value of 0.025 at high water.

TABLE III: Molar Absorptivity of $CoCl_2 \cdot 6H_2O$ in Phosphatidylcholine Micelles (4.2×10^{-3} M $CoCl_2 \cdot 6H_2O$ in 12.5 mM Phosphatidylcholine).

Total Water (mg/ml)	a_m^{650} (M^{-1} cm^{-1})	Total Water (mg/ml)	a_m^{650} (M^{-1} cm^{-1})
0	190	6.0	57
1.0	183	7.0	30
2.0	171	8.0	1.2
3.0	144	9.0	0.8
4.0	128	10.0	0.3
5.0	85		

Visible Spectrum of Co^{2+}. In aqueous solution $CoCl_2 \cdot 6H_2O$ is octahedrally complexed and its solutions are weakly colored and pink. Tetrahedral complexes of Co^{2+} are intensely blue and give rather complex visible spectra (Phillips and Williams, 1966). $CoCl_2 \cdot 6H_2O$ is insoluble in diethyl ether; however, when the salt is added to a solution of phosphatidylcholine in diethyl ether it readily dissolved to give an intensely blue solution, which is characteristic of a tetrahedral complex. As water is added to the solution the blue color fades as reflected by a decrease in molar absorbancy at 650 nm (Table III).

Discussion

The methods used in this investigation were chosen such that various aspects of the nature of the water within the phosphatidylcholine micelles could be assessed. Infrared and pmr spectroscopy measure the properties of the water directly. Infrared spectroscopy has the advantage of being able to detect the simultaneous existence of more than one hydration state, if the exchange rate between the two states is slower than the time for a vibrational mode (10^{-13}–10^{-14} sec). In order to detect two states by pmr the exchange rate must be slower than 10^{-4} sec. Both pmr, through T_2 measurements, and fluorescence, through polarization measure-

ments, provide information about the mobility of the lipid–water interface. In addition, the fluorescence emission maximum also provides information about the polarity of the lipid–water interface. Cobalt is anticipated to reside within the core of the micelle and provide information about this region.

In agreement with previous data (Poon and Wells, 1974), the hydration of phosphatidylcholine micelles in diethyl ether occurs in a stepwise fashion with two distinguishable hydration states. This conclusion agrees with the results of other studies on the hydration of phosphatidylcholine (see introduction). The first hydration state, which involves the uptake of 6–7 molecules of water per lipid molecule, has the following characteristics. (1) The bound water has a sharp infrared band at 3530 cm^{-1}. The sharpness of the band suggests that the water exists in a narrow range of structural forms, whereas the wavelength of the absorption band and its weak intensity suggest that the water is weakly hydrogen bonded, or that its oxygen neighbors are farther removed than in water or ice (Eisenberg and Kauzmann, 1969). (2) The water in the first hydration state is characterized by a short T_2, indicative of restricted molecular motion. This restricted motion seems to apply to the entire lipid–water interface as evidenced by the short T_2 of the polar head group of the lipid and the degree of polarization of the fluorescence of dansyl-PE. (3) The peculiar properties of the interface in the first hydration state are also indicated by the short wavelength of the fluorescence emission maximum of dansyl-PE, and by the presence of tetrahedrally coordinated cobalt.

The water added in the second hydration state seems to be very similar to pure water on the basis of the following observations. (1) The infrared spectrum is very similar, if not identical, to that of pure water. (2) Cobalt is present as an octahedral complex, presumably the aquo ion. (3) The wavelength maximum of dansyl-PE fluorescence is very similar to that found in phosphatidylcholine vesicles in water. In the second hydration state the interface becomes more mobile, as reflected by the increased T_2 of the bound water and the polar head groups of the lipid. The decrease in polarization of the dansyl-PE fluorescence also supports this conclusion, although the observed change in polarization must be taken as a minimal indication of the change in mobility since the decreased quantum yield and the increasing size of the micelle (Poon and Wells, 1974) would both tend to increase the polarization.

We assume that the first hydration state represents water bound to the polar head group and the second hydration state represents a central core of water. Infrared studies show that both states coexist; however, the single pmr signal for bound water shows that there is fairly rapid exchange of water between the two states. All the data support the conclusion that there is a gradual change in the properties of the bound water and the lipid–water interface as the first hydration state is filled. This change in properties is nearly complete at a hydration level of 6–7 water molecules/molecule of lipid (δ 0.15). This coincides with the hydration level at which phospholipase A_2 activity can first be detected (Poon and Wells, 1974).

Beyond the important questions raised as to the physical chemistry of micelle formation and the nature of water bound to micelles, the data presented in this and the preceding papers (Misiorowski and Wells, 1974; Poon and Wells, 1974) provide a striking example of how interfacial properties can markedly influence the activity of phospholipase A_2. We can define two micellar states, one of which is a substrate for the enzyme and the other of which is not. In the latter case the lack of activity cannot be ascribed to a lack of interaction, since the protein is dissolved within the micelle, but must reflect an interaction which is inappropriate to support activity. The nature of this inappropriate interaction cannot be defined explicitly at the present time, but may arise from any or all of the following considerations. (1) The fairly rigid nature of the interface may not allow the enzyme to bind to the polar head group in the proper manner. This would be in accord with the observation that monomolecular films are not attacked at high surface pressures (Hughes, 1935, Colacicco and Rapport, 1966). (2) The limited amount of water available within the micelle may alter the hydration state of the protein. Such a proposal would be consistent with the presence of tetrahedrally complexed cobalt in the micelle at low water concentrations. (3) Other possible effects could arise from the interactions of the interface with cations, conformational differences in the polar head group at low water compared to high water, or reduced stability of the active, dimeric, form of the enzyme.

It appears that the reactive micelle can be described somewhat more completely. It seems to represent a small water droplet, containing the enzyme and cations, which is coated by a monolayer of lipid. This situation allows free movement of the protein and favors productive interaction of the enzyme with the substrate molecules. Although this situation requires a distinct interface between the polar head groups of the lipid and the central water core, this seems to be a favorable arrangement for phospholipase A_2 (Wells, 1974). It is possible that this distinct interface is lacking in the unreactive micelle, and that the enzyme and polar head groups are present in some ill-defined homogeneous phase, from which the enzyme cannot readily move.

A more precise understanding of the nature of the protein–interface interactions in the two types of micelles must await data on the environment experienced by the protein. Such studies are complicated by the low solubility of the protein in the lipid solution in ether, and by the necessity to use cations which inhibit the enzyme. If these technical problems can be solved, a more complete understanding of these interactions will be possible.

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Nuclear Magnetic Resonance Studies of the Interaction of Alamethicin with Lecithin Bilayers†

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ABSTRACT: The interaction of alamethicin with both unsonicated lecithin multilayers and sonicated bilayer vesicles has been investigated by nuclear magnetic resonance (nmr) spectroscopy and electron microscopy. It is shown that alamethicin is a surface active agent, which interacts primarily with the polar choline head groups of the lecithins. Alamethicin also induces aggregation and subsequent fusion of small bilayer vesicles ~300 Å in diameter, a process which was found to have a profound influence on the nmr spectral properties of these bilayers.

Alamethicin (Payne *et al.*, 1970), an extracellular macrocyclic polypeptide from the fungus *Trichoderma viride* (Meyer and Reusser, 1967), has been shown to induce ion movements across certain biological membranes (Pressman, 1968). It has also been shown that this antibiotic interacts with black lipid membranes to form voltage gateable ion channels with discrete conductance states (Muller and Rudin, 1968; Gordon and Haydon, 1972). Since alamethicin itself is not known to be translocated across a black lipid membrane, it has been proposed that voltage gateable ion pores are induced by alamethicin (Eisenberg *et al.*, 1973).

A knowledge of the mode of interaction of alamethicin with phospholipid bilayer membranes is essential toward the understanding of the action of this antibiotic. Various methods may be used to elucidate this interaction, including electrical measurements (Eisenberg *et al.*, 1973; Lau and Hall, 1974), X-ray diffraction and differential scanning calorimetry (Chapman *et al.*, 1969), electron spin resonance (esr) spin labeling (Finer *et al.*, 1969; Levine *et al.*, 1973), circular dichroism (McMullen *et al.*, 1971), and fluorescence techniques (Case *et al.*, 1974). Nuclear magnetic resonance (nmr) spectroscopy, however, offers unique advantages by virtue of its sensitivity toward unravelling structural details and environmental changes. This sensitivity, for example, has been exploited in many of the nmr investigations of the structural and dynamic properties of lecithin bilayers (Penkett *et al.*, 1968; Chan *et al.*, 1971; Lee *et al.*, 1972, 1973; Levine *et al.*, 1972; Horwitz *et al.*, 1972; Seiter and Chan, 1973; Michaelson *et al.*, 1973; Feigenson and Chan, 1974; Seelig and Seelig, 1974). More recently, proton magnetic resonance (pmr) spectroscopy has also been used to monitor the interaction of various oligopeptides with lipid multilayers and phospholipid bilayers (Hsu and Chan, 1973; Chang and Chan, 1974). This present work is concerned with a similar pmr study of the interaction of alamethicin with dipalmitoyllecithin bilayers in both the sonicated and unsonicated states.

Unsonicated liposomes and sonicated bilayer vesicles offer two different, although interrelated, model membrane systems. In terms of bilayer structure these two model membrane systems differ primarily in their surface curvatures (Sheetz and Chan, 1972). It has been pointed out that vesicle curvature can have a profound influence on the molecular packing of the phospholipid molecules in a bilayer (Seiter and Chan, 1973; Chan *et al.*, 1973). More specifically, it has been argued that the packing arrangements of these molecules in flat bilayers are more regular than in bilayer vesicles ~300 Å in diameter. This difference in the molecular packing of the lipid molecules is reflected most dramatically in the segmental motions of the fatty acid chains. Whereas the segmental motion of the hydrocarbon chains is relatively restricted in the case of flat bilayers, this motion is much freer in small bilayer vesicles and the motional state of the hydrocarbon region in the sonicated vesicles is thought to very nearly resemble that of a

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