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Fourier Transform Infrared Studies on Phospholipid Hydration: Phosphate-Oriented Hydrogen Bonding and Its Attenuation by Volatile Anesthetics

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SUMMARY

Water-phospholipid (dimyristoylphosphatidylcholine) interaction was analyzed in a water-in-oil (benzene) reversed micellar system using Fourier transform infrared spectroscopy, and the effects of inhalation anesthetics (halothane, enflurane, chloroform, and carbon tetrachloride) on the interaction were studied. The O—H stretching frequency, representing water, increased from 3369 cm^{-1} to a steady 3430 cm^{-1} when the water/phospholipid mole ratio exceeded 18. The value did not quite reach the frequency of free water of 3490 cm^{-1} at the water/phospholipid mole ratio of 30. The O—H bending frequency of water did not appear until the water/phospholipid mole ratio exceeded 9. The P═O stretching frequency in the polar head group of unhydrated dimyristoylphosphatidylcholine was 1262 cm^{-1} and decreased with the addition of water, reaching a steady value of 1238 cm^{-1} at the water/phospholipid mole ratio of 9. However, the $(CH_3)_3N^+$ stretching of the choline head, as well as the C—H stretching of the hydrocarbon tail and the C═O stretching of the ester linkage, showed little change by the addition of water. The present results suggest that the primary hydration site of dimyristoylphosphatidylcholine is the phosphate moiety, and up to 18 water molecules are restricted at the polar head group. Apparently, the choline head has a minor role in the hydration of phospholipids despite the positive electrostatic charge. Among the water molecules interacting with the phospholipid head group, about 9 water molecules are strongly bound. The water content in the micelles correlated linearly with the ratio of the absorbance band area between O—H and C═O stretching. The addition of polar anesthetics (halothane, enflurane, and chloroform) increased the O—H stretching frequency and elevated the ratio of the absorbance band area between O—H and C═O stretching, implying that the anesthetics released the structured water molecules bound at the phospholipid-water interface. The anesthetics disrupted the hydrogen bond between the phosphate moiety of the phospholipid and water. Although apolar carbon tetrachloride also released bound water molecules, the magnitude was less than that of the polar anesthetics, as expected. The anesthetics did not affect the C—H stretching or C═O stretching bands, indicating that the disordering action upon the hydrocarbon core of phospholipid membranes is minimal at low water content. These results support our view that the primary site of action of inhalation anesthetics is the membrane-water interface, releasing bound water molecules.

The disordering effects of anesthetics upon phospholipid membranes are well established (see, for instance, a review in Ref. 1). Because the order-disorder transition of membranes is the property assigned to the state of hydrocarbon tails of phospholipids, anesthetic action sites are often assumed to be the lipid core of the membrane (1).

In contrast, based on the finding that inhalation anesthetics induced a large positive enthalpy change (endothermic effect) and entropy change (disordering effect) on a solubilized firefly luciferin-luciferase system (2), Eyring *et al.* (3) proposed that hydrogen bond breaking action at the macromolecular surface is the main cause of anesthesia. This theory postulates that anesthetics release the water clusters, structured at the surface of macromolecules. In the present communication, the term macromolecule is used to describe large structures, including proteins, lipid membranes, and micelles. The structured water cluster is ice-like, and the large increases in the enthalpy and entropy by anesthetics are attributed to melting of this ice-like structure (4); the action site of anesthetics is the water-macromolecule interface. In this connection it is noteworthy that all inhalation anesthetics currently in clinical use contain appreciable intramolecular dipole moments and are likely to associate with polar parts of macromolecular structures. This does not mean that apolar molecules lack anesthetic potency. Molecules without dipole moments, such as xenon and cyclopropane, are good anesthetics. However, it is known that anesthetic potency of apolar molecules is weaker than that of

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ABBREVIATION: MAC, minimum alveolar concentration of anesthetic that anesthetizes 50% of the population.

their polar counterparts (5, 6). As an example, halothane owes its high anesthetic potency to the acidic proton intentionally left at one end of its structure to incorporate dipole moment into the molecule (5, 6). Apolar molecules tend to penetrate into the core of lipid bilayers. We contend that the core-oriented binding of these molecules may be responsible for their relatively weak anesthetic potency. Their interfacial action is secondary to the membrane expansion caused by their interaction with the membrane core.

By the use of infrared spectroscopy, Di Paolo and Sandorfy (7, 8) have shown hydrogen bond breaking activity of halogenated hydrocarbon anesthetics in a nonaqueous system of diethylamine that self-associates by forming N—H- - -H hydrogen bonds. The addition of anesthetics into the diethylamine solution disrupted the hydrogen bond. Sandorfy and co-workers (9) established that the most potent inhalation anesthetics, such as halothane and methoxyflurane, are the most potent hydrogen bond breakers in various nonaqueous solvent systems.

Lipid bilayers cannot be formed without water. Can one make soap bubbles without water? Similarly, protein conformation is dictated by interaction with water. Assuming that a peptide bond can adopt two conformations, 2^{582} conformations are possible for bovine serum albumin (single strand, consisting of 582 amino acids). This figure translates into $1 \cdot 10^{157}$. Among these literally innumerable combinations, only one conformation stands out when the protein is immersed in water. Although this is an oversimplified description, biologically meaningful protein conformation is formed by interacting with water. Dry proteins are said to be dead proteins (10). Water is not a simple supporting matrix of macromolecules (membranes and proteins); rather, the water molecules are components of these structures. Our previous studies on lipid membranes, micelles, and proteins have shown that the effect of anesthetics is to perturb the association between water and macromolecules, irrespective of proteins, lipid membranes, or micelles (11–22). Anything that weakens the water-macromolecule interactions disorders the structure. We have postulated that the primary action site of these amphipathic anesthetics is the water-macromolecule (including lipid membranes and proteins) interface, and that the action of anesthetics is to release the structured (bound) water.

By differential scanning microcalorimetry of partially hydrated phospholipid multilayer systems, we demonstrated the presence of surface-bound water molecules that froze at subzero temperature; anesthetics released these bound water molecules (22). We also have shown in a water-in-oil reversed micellar system with proton nuclear magnetic resonance spectroscopy that the spin-spin and spin-lattice relaxation times of water molecules are shorter than those of free bulk water, indicating strongly limited motion of bound water molecules in the reversed micelles (19). Inhalation anesthetics released these bound water molecules from the interface, as evidenced by an increase in the relaxation times.

The present study is an extension of the NMR study to locate the particular sites(s) in phospholipid molecules where the bound water molecules are released by anesthetics. Fourier transform infrared spectroscopy was used to investigate the interaction between water and dimyristoylphosphatidylcholine in a reversed micellar system (phospholipids in benzene), and the effects of inhalation anesthetics on such interactions were studied. It will be shown that the main hydration site on the phospholipid membrane is the phosphate moiety and that anesthetics disrupt the hydrogen bond between the phosphate moiety and water.

Materials and Methods

Synthetic dimyristoylphosphatidylcholine was obtained from Sigma and was kept in a desiccator at reduced pressure. Spectroscopy grade benzene (water content less than 0.05%) was obtained from J. T. Baker. Anesthetics used for this study include: halothane (Ayerst), enflurane (Ohio Medical Products), and chloroform (Fisher, reagent grade), as well as carbon tetrachloride (Fisher, reagent grade). Anesthetics and benzene were dried by passage through aluminum oxide (Fluka) columns. The free radical scavenger (thymol) contained in the halothane preparations was removed by this procedure. Water was undetectable by infrared spectroscopy in these preparations. Triply distilled water was used throughout.

Reversed micelles were prepared by dispersing dimyristoylphosphatidylcholine in benzene (50 mg/ml). Benzene was chosen because the data can be compared with literature values on the lecithin reversed micelles in benzene (23–25). The anesthetic property of benzene is not expected to vitiate the present study because it is used as a supporting medium to represent the hydrophobic domain of the membrane, analogous to the use of the octanol (an anesthetic) as an organic phase for the oil/water partition coefficient to assess anesthetic hydrophobicity. The action of benzene is expected to be mainly confined in the hydrophobic lipid tail region.

The phospholipid suspension in benzene was sealed in a glass container, immersed into a Sonicor (New York, NY) model 3C-50T waterbath, and sonicated for 20 min. Water was added to the suspension with a microsyringe and again sonicated until the solution became clear. After the baseline infrared spectra were obtained, anesthetics were added to the solution with a microsyringe. All experiments were carried out under ambient conditions at 22°.

A Perkin-Elmer (Norwalk, CT) model 1750 FTIR spectrophotometer equipped with a Perkin-Elmer model 7300 computer was used for the sample analyses. The experimental conditions were as follows: Cell window: NaCl, detector: TGS, sample form: capillary film (approximately 20 μm), frequency range: 400–4000 cm^{-1}, and resolution: 4 cm^{-1}. The data were Fourier transformed and averaged after 10 scans. The difference spectrum was acquired by subtracting the spectrum of benzene from that of the reversed micelle. Since the difference in film thickness between samples was compensated by the computer software, the subtraction errors between samples with different pathlengths were minimized. For hydrated reversed micelles with an anesthetic, the difference spectrum was obtained by subtracting the spectrum of benzene and the anesthetic from that of the sample. The band area was computed electronically.

To compare the present results with clinical potencies, the anesthetic concentrations in the gas phase in equilibrium with benzene solution were estimated by gas chromatography. The anesthetic concentrations in the liquid phase were also determined. The reaction mixture was placed in a 5-ml glass bottle with an airtight, Teflon-lined rubber cap. The gas and liquid samples were collected by a glass microsyringe. Atmospheric pressure was measured by a mercury barometer, and the partial pressures of anesthetics in the system were estimated from the gas phase concentrations.

A Shimadzu (Columbia, MD) gas chromatograph with a flame ionization detector was used. The column for separation of chloroform, halothane, and enflurane was 1/8 in × 6 ft stainless steel tubing packed with Porapak Q 80/100 mesh. The operating conditions were isothermal at 160° and a flow rate of 20 ml/min of helium as the carrier gas. Because this column failed to separate carbon tetrachloride from benzene, a 1/8 in × 6 ft stainless steel tubing containing 10% AT-1000 of Chromosorb W-AW 80/100 mesh was used for carbon tetrachloride. The operating conditions for the Chromosorb column were isothermal at 55° and a flow rate of 5 ml/min of helium.

Results

Fig. 1 is a typical difference spectrum of dimyristoylphosphatidylcholine dispersed in water-free benzene without added water. The spectrum was obtained by subtracting the absorption bands of benzene from those of the reversed micelle. The assignments of the major infrared bands (23) in this spectrum are shown in Table 1 and are in general agreement with those of phospholipids in various reports (23–32). The bands at 2919 cm^{-1} and 2851 cm^{-1} are due to the C—H stretching modes in the hydrocarbon chain region of dimyristoylphosphatidylcholine. The peak at 1729 cm^{-1} arises from the C═O stretching in the ester group, and the frequencies at 1262 cm^{-1} and 967 cm^{-1} are assigned to the P═O stretching and $(CH_3)_3N^+$ stretching in the polar head region, respectively.

When water was added to the dimyristoylphosphatidylcholine dispersion, the O—H stretching band of water appeared at 3369 cm^{-1}, shifted to higher frequencies with the increase in water content, and reached a steady value of 3430 cm^{-1} after 18 water molecules were added per phospholipid molecule. The frequency did not quite approach the free water value of 3490 cm^{-1}, and stayed constant to the limit of this study, where the water/phospholipid mole ratio was increased to 30. The O—H bending band of water was not observable at low water content. At a water/phospholipid mole ratio of 9, the O—H bending band appeared at 1654 cm^{-1} and decreased to 1645 cm^{-1} with further hydration. The effect of water content on the vibrational frequencies of functional groups in dimyristoylphosphatidylcholine is also given in Table 1.

For comparison, benzene-water interaction was studied by observing the O—H stretching frequencies in the absence of the phospholipid. At a water content equivalent to a 1:1 water/phospholipid mole ratio, the O—H stretching frequency was 3401 cm^{-1}. At the mole ratio levels of 2, 3, and 6, the values were a steady 3407 cm^{-1}. At the 9:1 ratio level, water droplets were formed and the value was increased to 3451 cm^{-1}. Although water interacts with benzene, the magnitude was much smaller than with the phospholipid, and the change in the water signal by anesthetics may safely be attributed to their effect upon the phospholipid-water interaction.

Among the absorption bands of dimyristoylphosphatidylcholine, Figs. 2, 3, and 4 show the C—H stretching, the C═O stretching, and the P═O stretching, respectively. Each of these figures contains three vibrational bands of the same functional group obtained from the unhydrated phospholipid and two other hydrated samples in which water was added at mole ratios of 3.0 and 9.0 relative to the phospholipid.

The frequencies of C—H stretching and C═O stretching shifted to higher values upon the addition of initial water, but further hydration beyond one water molecule per phospholipid molecule had little effect on the frequencies of C—H stretching (2926 cm^{-1} and 2854 cm^{-1}), C═O stretching, (1739 cm^{-1}) and $(CH_3)_3N^+$ stretching (969 cm^{-1}). In contrast, the P═O stretching frequency (1262 cm^{-1}) decreased upon the addition of water, continued to decrease with further hydration, and eventually reached a steady value of 1239 cm^{-1} at nine water molecules per phospholipid molecule.

The ratio between the areas under the O—H stretching band and C═O stretching band was plotted as a function of water concentration in dimyristoylphosphatidylcholine and is shown in Fig. 5. The area under the C═O stretching band remained nearly unchanged after the addition of the first water molecule to the phospholipid, whereas the O—H stretching continued to increase with an increase in water content. A linear correlation between this ratio and water content in the system was demonstrated.

The effects of anesthetics, including halothane, enflurane, chloroform, and carbon tetrachloride, on hydrated dimyristoylphosphatidylcholine samples are presented in Table 2 and Fig. 6.

The anesthetic concentration used was one anesthetic molecule per two phospholipid molecules (anesthetic/phospholipid mole ratio 0.5). The concentrations were confirmed by gas chromatography. The bulk anesthetic concentration, however, has little meaning because it does not represent the anesthetic/phospholipid ratio at the membrane. The anesthetic concentration at the membrane depends upon the partition coefficient and the volume ratio between the membrane and the solvent. For this reason, the anesthetic partial pressure in the gas phase in equilibrium with the benzene micellar system was measured. The values were: halothane 1.08 kPa (8.1 mmHg), enflurane

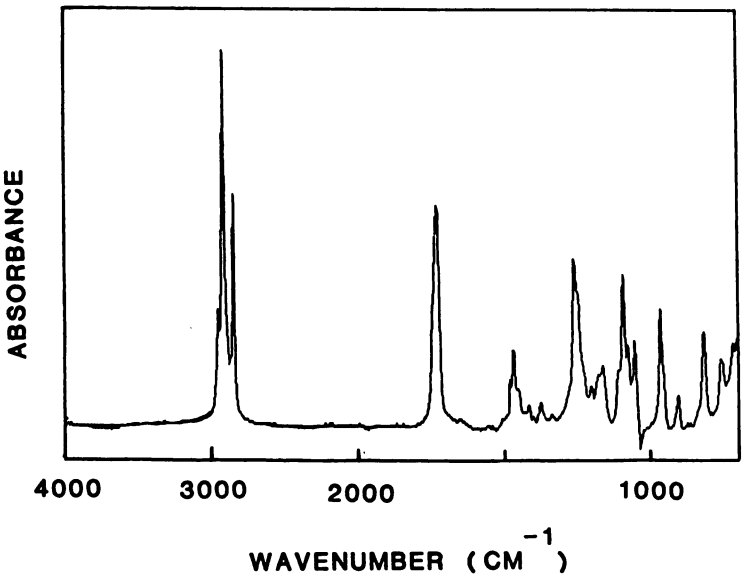

Fig. 1. Infrared difference spectrum of dimyristoylphosphatidylcholine without added water after subtraction of benzene absorption bands.

TABLE 1

Infrared frequencies (cm^{-1}) for dimyristoylphosphatidylcholine at various water contents

Band	Water/phospholipid mole ratio												
	0	1	2	3	6	9	12	15	18	21	24	27	30
O—H stretching		3369	3369	3371	3392	3401	3412	3413	3430	3430	3430	3430	3430
O—H bending						1654	1645	1645	1644	1644	1644	1644	1644
CH_2 asymmetric, stretching	2919	2926	2926	2926	2926	2926	2925	2925	2925	2925	2925	2925	2925
CH_2 symmetric, stretching	2851	2854	2854	2854	2854	2854	2854	2854	2854	2854	2854	2854	2854
C—O stretching	1729	1739	1739	1739	1739	1738	1738	1738	1738	1738	1738	1738	1738
P—O stretching	1262	1252	1250	1247	1240	1239	1237	1237	1239	1239	1239	1237	1238
$(CH_3)_3N^+$ stretching	967	969	970	970	971	971	971	971	971	971	971	970	970

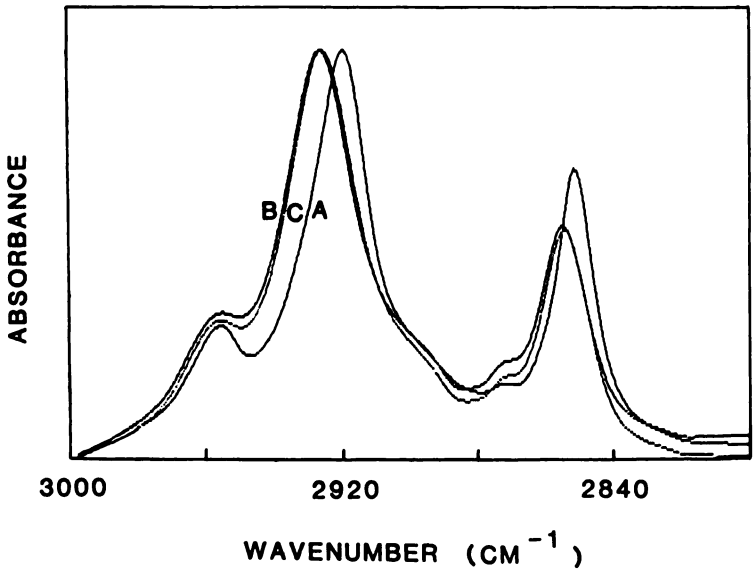

Fig. 2. The C—H stretching frequencies in the hydrocarbon chain region: A, dimyristoylphosphatidylcholine without added water; B, water/phospholipid mole ratio 3; and C, mole ratio 9.

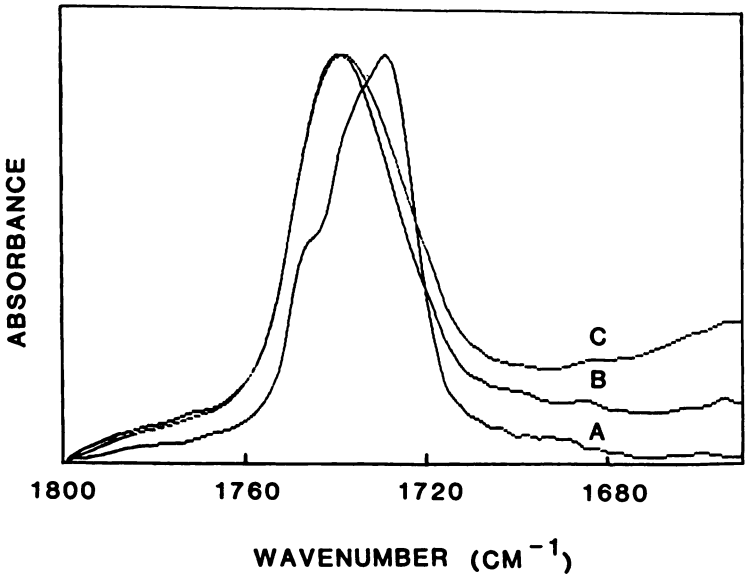

Fig. 3. The C—O stretching frequencies in the ester group: A, dimyristoylphosphatidylcholine without added water; B, water/phospholipid mole ratio 3; and C, mole ratio 9.

2.43 kPa (18.2 mmHg), chloroform 0.88 kPa (6.6 mmHg), and carbon tetrachloride 1.77 kPa (13.3 mmHg). The MAC values in dogs are (33): halothane 0.88 kPa (6.6 mmHg), enflurane 2.23 kPa (16.7 mmHg), and chloroform 0.78 kPa (5.9 mmHg). The dog values were used because the human value of chloroform is not available. When expressed by fractional MAC values, the anesthetic concentrations in the present study were: halothane 1.23 MAC, enflurane 1.09 MAC, and chloroform 1.12 MAC. These anesthetic concentrations were in the clinical range, despite the erroneous impression caused by the phospholipid/anesthetic mole ratio.

Because the anesthetic potency of carbon tetrachloride is not available, its data on infrared spectra are not readily comparable with those of other anesthetics. However, Ferguson (34) postulated the usefulness of thermodynamic activities over the concentrations in expressing drug actions, because the drug

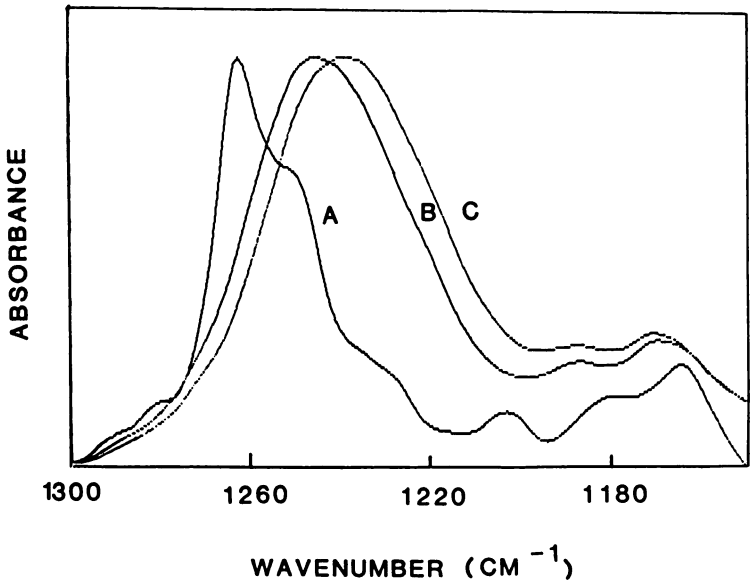

Fig. 4. The P—O stretching frequencies in the polar head region: A, dimyristoylphosphatidylcholine without added water; B, water/phospholipid mole ratio 3; and C, mole ratio 9.

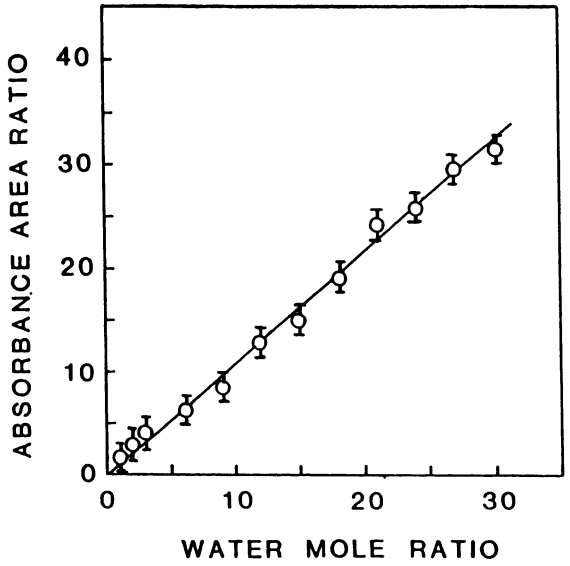

Fig. 5. The ratio between the area under O—H stretching signal and C=O stretching signal as a function of water content in dimyristoylphosphatidylcholine.

action is proportional to the thermodynamic activity and not to the bulk concentration. At equipotent concentrations, the thermodynamic activities should be approximately equal. With volatile anesthetics, the thermodynamic activity can conveniently be expressed by the ratio between the minimum partial pressure that induces anesthesia, P_{anes}, and the vapor pressure of the pure liquid, P°. Mullins (35) has shown that the wide scatter of anesthetic potency of various hydrophobic molecules spanning 5 orders of magnitude (nitrogen 30 atmos and methoxyflurane 0.0016 atmos) converges into almost a constant value of 0.03 when expressed by $P_{anes}/P°$. Of course, the value varies according to the animal species, method of evaluation of the anesthetized state, molecular structure, etc., but the scatter is decreased to about 1 order of magnitude. By assuming the validity of this rule, the present carbon tetrachloride concentration translates into 2.1 MAC.

Table 2 and Fig. 6 give the effect of halothane on dimyristoylphosphatidylcholine samples at eight levels of hydration with water/phospholipid mole ratios ranging from 1.0 to 2.70. The effects of enflurane and chloroform on some of the hydrated dimyristoylphosphatidylcholine samples are also presented in Table 2 and Fig. 6. These anesthetics increased the O—H stretching frequency and the band area of water, indicating release of bound water. At the low end of hydration, the anesthetics decreased the P=O stretching frequency of the hydrophilic interface. No measurable effect was observed on the C—H stretching frequency of the hydrocarbon region, the C=O stretching of the ester linkage, or the $(CH_3)_3N^+$ stretching of the choline head.

The effects of carbon tetrachloride on five hydrated dimyristoylphosphatidylcholine samples are presented in Table 2 and Fig. 6. The O—H stretching frequency also increased upon the addition of carbon tetrachloride, and the area under water signal increased slightly.

Discussion

The present result that the water peak at the O—H stretching frequency was not observable in the dimyristoylphosphatidylcholine dispersion in dry benzene may indicate that the preparation was essentially free of water contamination. But it is generally recognized that one water molecule is strongly bound to a phospholipid molecule and is difficult to eliminate. It may be that the water was so strongly bound to the phospholipid that it was invisible in infrared spectroscopy.

The O—H stretching frequency of water appeared at 3369 cm^{-1} upon the addition of one water molecule per phospholipid. Further addition of water increased the frequency of the O—H stretching band, but the increase leveled off at 3430 cm^{-1} when the water/phospholipid mole ratio exceeded about 18. Free water has a very strong and large absorbance band of O—H stretching at 3490 cm^{-1} (36). The shift of the O—H stretching frequency to a higher value is attributable to the lower degree of binding of less structured water molecules to the phospholipid. Nevertheless, the frequency did not reach the level of free water; hence, the motion of the water molecules added later was still restricted in comparison to that of free water molecules.

The absence of the O—H bending band of water when the

TABLE 2
Effects of anesthetics on the infrared spectra of hydrated dimyristoylphosphatidylcholine

	Water/phospholipid mole ratio										
	1	2	3	6	9	12	15	18	21	24	27
Halothane											
O—H stretching	3400	3401	3401	3413	3416		3424		3430		3435
O—H bending					1647		1646		1646		1646
P═O stretching	1247	1245	1236	1237	1237		1237		1238		1237
Enflurane											
O—H stretching	3392		3392	3401	3415	3412	3430	3430		3435	3435
O—H bending					1654	1643	1645	1644		1642	1644
P═O stretching	1248		1247	1240	1239	1238	1238	1239		1238	1238
Chloroform											
O—H stretching	3391		3390		3402		3413		3429		3435
O—H bending					1643		1643		1643		1643
P═O stretching	1248		1248		1239		1240		1239		1239
Carbon tetrachloride											
O—H stretching	3384		3385	3385	3418		3422				
O—H bending					1653		1653				
P═O stretching	1250		1248	1242	1238		1238				

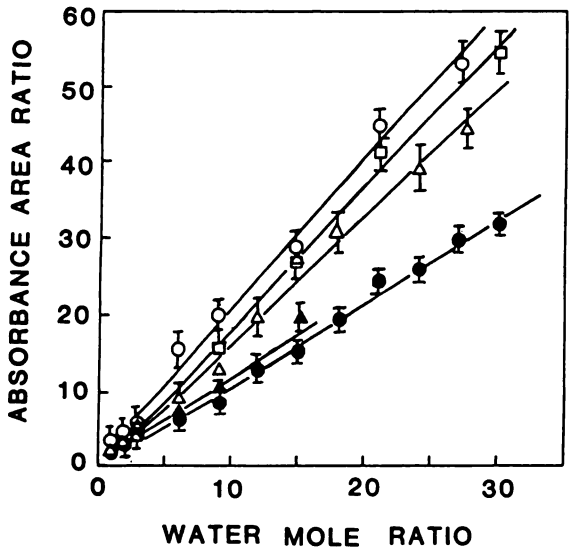

Fig. 6. Effects of anesthetics on the absorption band area ratios between O—H stretching and C═O stretching in hydrated dimyristoylphosphatidylcholine samples. The anesthetic/phospholipid mole ratio was 0.5. This is the overall ratio between the anesthetic and the phospholipid in the benzene-water system, and is not the ratio at the membrane surface. When expressed by fractional MAC, the values are: halothane 1.23 MAC, enflurane 1.09 MAC, chloroform 1.12 MAC, and carbon tetrachloride 2.1 MAC (estimated value, see the text). ●, controls without anesthetics; □, halothane; △, enflurane; ○, chloroform; and ▲, carbon tetrachloride. The *error bars* signify standard deviations ($n = 5$).

water content was less than 9 mole ratio may indicate that these water molecules are strongly bound to the phospholipid and the signal is invisible.

Among the functional groups of dimyristoylphosphatidylcholine, only the P═O stretching frequency decreased as water molecules were added. Other groups, such as the C—H, C═O, and $(CH_3)_3N^+$ stretching bands, increased their frequency upon the addition of water. This frequency shift of the P═O stretching as a result of hydration is attributed to hydrogen bonding between water and the phosphate group in the polar head region (23, 25, 26, 30).

The choline head appears to contribute little to hydration despite the presence of the positive electrostatic charge. Shielding of the charge with large hydrophobic CH_3 groups may be responsible for this weak role in hydration. The small increase in frequency of $(CH_3)_3N^+$ stretching due to hydration is possibly caused by the rearrangement of this group in response to the strong hydrogen bonding formed between water molecules and the phosphate groups.

The increase in the frequency of stretching bands signifies an increase in the movement of the bond and, therefore, an increase in disorder. The present result on the C—H stretching of the hydrocarbon region and the C═O stretching of the ester region indicates that the addition of water slightly disordered these parts. This result is in concert with our differential scanning microcalorimetry data (22) on partially hydrated dimyristoylphosphatidylcholine multilamellar systems, where at very low water content, the increase in hydration lowered the order-disorder phase-transition temperature.

The response of the phosphate group appears to be the only part that shows the ordering effect. The restriction of the movement of P═O moiety subsequently lowers the vibrational energy of P═O stretching during the hydration of dimyristoylphosphatidylcholine.

From the O—H stretching frequency data, it is estimated that about 18 water molecules are bound to each dimyristoylphosphatidylcholine molecule. Among these, 9 water molecules are strongly bound to the phosphate moiety according to the O—H bending data. This does not mean that only 18 water molecules are bound to the hydrophillic head group. The motion of water molecules outside of this hydration shell are also restricted to a minor degree, as shown by the lower O—H stretching frequency compared to the free water.

The present data show that halothane disrupts water binding to the phospholipid molecules, as evidenced by the increase in the O—H stretching frequencies. The change in the frequencies of the water signal is a weighted average of water molecules. For this reason, the frequency increase becomes less at higher water content. Apparently, the hydration shell consists of multiple layers of water molecules. The first layer is tightly bound,

and as the number of water molecules is increased, the interaction force is gradually decreased. Presumably, a low affinity water layer is first released when anesthetics interact with the membrane.

The addition of anesthetics to hydrated dimyristoylphosphatidylcholine either increased the O—H stretching frequency of water molecules or widened the O—H stretching absorbance band. In some of the samples, both of these effects were observed (e.g., 1.0 water/phospholipid mole ratio sample). For this reason, it is difficult to analyze anesthetic effect by the absorbance shift alone. The 4 cm^{-1} resolution, however, should not be confused with uncertainty. When the peaks of two neighboring bands are closer than 4 cm^{-1}, separation of the two bands is difficult. Nevertheless, a shift of less than 4 cm^{-1} of a single peak is still detectable. The absorbance area of the O—H stretching band may better depict the overall water activity. Hence, the increase in the area under the water O—H stretching signal by anesthetics is illustrated by taking the ratio to the hydrocarbon C=O stretching (which is constant throughout the hydration). The difference between the control and in the presence of anesthetics shows the increase in water activity by freeing the bound water molecules from the phospholipid molecules. It is apparent that anesthetics increased the water activity by disrupting the hydrogen bonds.

Among the phospholipid functional groups, only the P=O stretching frequency was affected by the interaction with halothane. The frequencies for other functional groups were not affected by the addition of halothane. Under the present experimental condition at low water content, disordering of the hydrocarbon chain by halothane was not observed. The anesthetic action site appears to be the hydrophilic phosphate group where bound water molecules are released by the anesthetic. Similar results were obtained with enflurane and chloroform.

The hydrogen bond breaking activity of anesthetics confirms our proton nuclear magnetic resonance data on the reversed micelles (19) and differential scanning microcalorimetry data on partially hydrated phospholipid multilamellar systems (22), and is consistent with our view that the primary action site of inhalation anesthetics is the membrane-water interface and the anesthetic action is to break the hydrogen bonds and release bound water molecules (4, 11–22). The state of anesthesia is presumably related to this increase in hydrophobicity of the membrane surface.

The effect of carbon tetrachloride was generally less than those of inhalation anesthetics. Although the effects upon O—H stretching and bending frequencies are not strikingly different from those of other anesthetics, the carbon tetrachloride concentration was estimated to be about 2.1 MAC, which was almost twice that of other anesthetics. Because of the apolar nature of carbon tetrachloride, the weaker interfacial activity was not unexpected. We contend that apolar molecules manifest interfacial actions by the lateral expansion of lipid membranes caused by their interaction with the hydrophobic core. Presumably, a lipid bilayer structure may be necessary for apolar molecules to show significant interfacial actions.

Acknowledgments

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