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Alcohols dehydrate lipid membranes: an infrared study on hydrogen bonding

Jang-Shing Chiou, P.R. Krishna, Hiroshi Kamaya and Issaku Ueda

Department of Anesthesia, University of Utah School of Medicine, and Anesthesia Service 112A, VA Medical Center, Salt Lake City, UT (USA)

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The effects of alcohols (methanol, ethanol, and n-butanol) on the hydrogen bonding of dipalmitoylphosphatidylcholine (DPPC) were studied by Fourier-transform infrared spectroscopy (FTIR) in water-in-oil (carbon tetrachloride) reversed micelles. The bound O-H stretching mode of water, bonded to DPPC, appeared as a broad band at around 3400 cm^{-1}. The O-H bending mode of this complex appeared as a weak broad band at 1644 cm^{-1}. No free O-H signal was observed. When alcohols were added, a part of DPPC-bound water was replaced by the alcohols. The released 'free' water appeared at 3680 cm^{-1}. This free O-H stretching band represents water-alcohol complex. A new broad band of O-H stretching appeared at 3235 cm^{-1}, which represents the alcohol molecules bound to the phosphate moiety of DPPC. When the alcohol concentration was increased, the intensities of the free O-H stretching and bending bands increased. The P=O$^-$ antisymmetric stretching band at 1238 cm^{-1} became broader and shifted to lower frequencies. This means that alcohols interacted with the phosphate moiety and replaced the bound water. In the deconvoluted spectra of the C=O stretching mode, the ratio between the free *sn*-2 and the hydrogen-bonded *sn*-2 bands increased; a part of the bound water at the *sn*-2 carbon in the glycerol skeleton is also released and the free *sn*-2 signal increased. From the change in the intensity of the P=O$^-$ stretching band, the partition coefficients of alcohols between the phosphate region of DPPC and water were estimated: methanol 7.8, ethanol 16.7 at 22.0°C in mole fraction bases. In molality, these values translates into methanol 0.21 and ethanol 0.45. These results indicate that short-chain alcohols interact with lipid membranes at the phosphate moiety at the hydrophilic head, weaken the membrane–water interaction, and destabilize membranes.

Introduction

A number of action sites have been proposed for anesthetics and alcohols. These include $GABA_A$ receptor, *N*-methyl-D-aspartate (NMDA) excitatory receptor, glutamate non-NMDA receptor, nicotinic acetylcholine receptor, Na^+ channel, K^+ channel, Cl^- channel, Ca^{2+} channel, trans-membrane signaling, protein kinase C, phospholipase C, phosphatidylinositol turnover, etc., to name a few [1]. Apparently, anesthetics modify all of these protein functions. Although saturable binding of anesthetics with some receptors has been reported, it remains to be seen if there is a identical common binding site for anesthetics in all of these proteins. Our intention is to probe the mechanisms that generate such a broad spectrum of action.

Correspondence to: I. Ueda, Anesthesia 112A, VA Medical Center, 500 Foothill Boulevard, Salt Lake City, UT 84148, USA.

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; CCl_4, carbon tetrachloride; FTIR, Fourier-transform infrared spectroscopy.

An anonymous reviewer of this article commented that the general trend in anesthesia research is to locate the specific binding site for anesthetics in proteins such as the acetylcholine receptor, hence, studies on model systems have little value in elucidating the mechanism of anesthesia. Nevertheless, the specific antagonists of acetylcholine nicotinic receptor, such as curare, have no anesthetic potency whatsoever.

In the channel proteins, the membrane penetrating domains are identified by the hydropathy plot in the primary structure. This indicates that receptor proteins are formed into biologically meaningful structure by the hydrophobic effect: without water and lipid membranes, channels do not exist. Membrane excitation is an integral property of the excitation machinery, composed of proteins and lipids. The fundamental force that forms this system is the hydrophobic effect. These structures are supported by water molecules. Anything that attenuates the hydrophobic force destabilizes macromolecular structures, induces disorder, and expands the volume. We propose that anesthetics and

alcohols compete with water molecules in maintaining the macromolecular structures. The basic mechanisms of alcohol actions on proteins and lipid membranes are similar.

The alcohol interaction with lipid membranes has received strong research interests in elucidating the action mechanisms. There are ample reports on the membrane fluidizing action and reduction in the main phase-transition temperature of phospholipid membranes by alcohols and anesthetics. The effect is generally referred to fluidization. By applying the van't Hoff model on freezing point depression, membrane–water partition coefficients of additives can be estimated [2–6]. Because the main transition is characterized by the *trans-gauche* transformation of the hydrocarbon tails, it is generally believed that anesthetics interact with the lipid core of the bilayer and disorder the structure. Penetration into the membrane core and the presence of small molecules between the palisade of hydrocarbon tails are assumed to sterically disturb the all-*trans* structure and fluidize the membrane [7–10]. Nevertheless, short-chain alcohols are strongly amphipathic compounds. These molecules are likely to stay at the membrane–water interface. Despite the abundance of reports of the alcohol effects on the membrane core, those on the membrane surface are few.

In our previous study [11] on the alcohol–DPPC interaction in a non-aqueous medium, we have shown that the hydrogen bonding site of alcohols on DPPC was the phosphate moiety. The formation constants were estimated to be 19.0 M^{-1} for ethanol and 7.1 M^{-1} for n-butanol. In the present study, we measured interaction between alcohols (methanol, ethanol, and n-butanol) and DPPC dispersed in an H_2O-in-CCl_4 reversed micellar system. The study is aimed at elucidation of alcohol effects on the bound water at the water/membrane interface.

Materials and Methods

Synthetic dipalmitoylphosphatidylcholine (1,2-dihexadecanoyl-*sn*-glycero-3-phosphocholine, DPPC), spectroscopic grade methanol, and n-butanol were obtained from Sigma, spectroscopic grade carbon tetrachloride from EM Science (Cherry Hill, NJ), and high purity ethanol from U.S. Industrial Chemicals (Anaheim, CA). CCl_4 was passed through activated aluminum oxide columns (Fluka) several times to remove water. DPPC was kept in a desiccator at reduced pressure until use. Water was triply distilled.

The water-in-oil reversed micelles were prepared by dispersing DPPC and water in CCl_4 by sonication in the cup-horn of a Branson ultrasonic disrupter (Danbury, CT). Unless otherwise specified, the concentrations were DPPC 6 mM and water 36 mM. Alcohols were added to the DPPC/H_2O/CCl_4 mixture with a microsyringe under nitrogen gas. The sample was sealed and then vortex mixed.

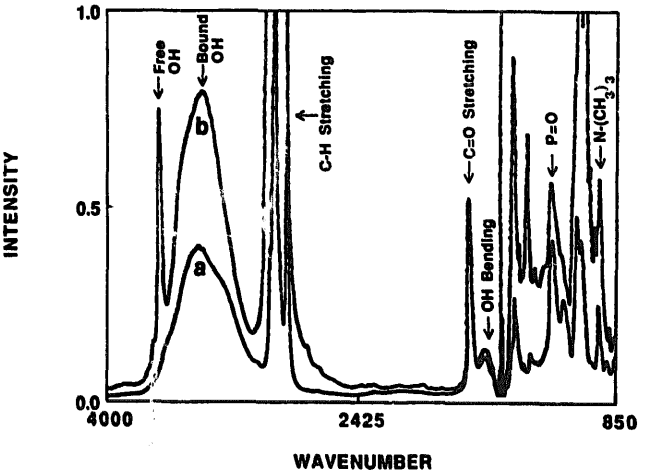

Fig. 1. IR difference spectra of DPPC-H_2O-CCl_4 reversed micellar system at 25°C after subtraction of the CCl_4 absorption bands. (a) Control without ethanol, and (b) with 0.10 M of ethanol (ethanol bands were not subtracted). The peak assignments are shown in the figure. The bound O-H stretching peak in (a) is for water at 3400 cm^{-1}, and in (b) is mainly for ethanol at about 3343 cm^{-1} which consists of ethanol multimers.

A Perkin-Elmer (Norwalk, CT) Model 1750 FTIR spectrophotometer interfaced with a Perkin-Elmer model 7300 computer was used for the analysis. The cell (FT 04–794) was a fixed 1.18 mm path-length with zinc selenide windows (Spectra-Tech, Stamford, CT). The cell was calibrated against the benzene band at 845 cm^{-1}. A TGS detector was used for all experiments. Each sample was scanned 40 times through the frequency range of 400–4000 cm^{-1} and averaged. The resolution of FTIR was 4 cm^{-1}. The spectra of the solvents were subtracted from the spectra of the solutions. The differential spectra were obtained by further subtracting the component spectra from the sample spectra. All spectra were acquired at 22°C. Perkin-Elmer software was used to deconvolute the C=O stretching band, with a half-width smoothing function of 18 cm^{-1} and a narrowing factor of 2.25.

Results

The differential spectra shown in Fig. 1 were obtained after subtraction of the absorption bands of CCl_4 from those of the reversed micelles. Fig. 1(a) shows the spectrum of DPPC in the H_2O-in-CCl_4 reversed micellar system without alcohols. The frequencies of the major functional groups are: bound O-H stretching band of bonded water at 3400 cm^{-1}, bound O-H bending band of bonded water at 1644 cm^{-1}, C=O stretching band of the ester group at the glycerol skeleton at 1734 cm^{-1}, $P{=}O^-$ stretching band of the phosphate moiety of the head group at 1238 cm^{-1}, and $(CH_3)_3$-N^+ stretching band of the choline

head at 970 cm^{-1}. No free O-H band was observed. These assignments agree with the corresponding bands in the phospholipid spectrum reported in the literature [12–14]. Fig. 1(b) shows a similar spectrum in the presence of 0.10 M ethanol. A new band appeared at 3680 cm^{-1}, which has been assigned [15–17] as the free O-H stretching band of water molecules in CCl_4. Because this system contains ethanol, the band is contaminated by the alcohol-water complex. The term 'free' is used in a liberal way to indicate that they are not bound to DPPC (see Discussion).

The intensity of the free O-H stretching band of water increased according to the increase in the ethanol concentration (Fig. 2). Because the O-H stretching bands of bound water and ethanol overlap at 3400 cm^{-1} region, the control spectrum of the reversed micelles are subtracted to reveal the alcohol effects. Similar effects were observed with n-butanol and methanol. These alcohols increased the free water molecules by releasing them from the membrane.

Fig. 3 is the effect of 0.15 M ethanol on DPPC in the H_2O-in-CCl_4 reversed micelles. This differential spectrum is obtained by subtracting the control spectrum of 0.15 M ethanol in CCl_4 from the spectrum of 0.15 M ethanol in the reversed micelles. The intensity of the ethanol free O-H stretching band at 3635 cm^{-1} decreased when DPPC and water were added, concomitant with the appearance of a new broad-band at 3235 cm^{-1}. The negative value of the peak at 3635 cm^{-1} indicates loss of the free ethanol by DPPC binding. The 'free' water peak at 3680 cm^{-1} is clear in this spectrum. The broad band at 3235 cm^{-1} is the bonded O-H stretching mode of ethanol caused by hydrogen bonding between ethanol and DPPC (i.e., an alcohol-DPPC complex).

P=O$^-$ site

Fig. 4 shows the effect of ethanol on the P=O$^-$ stretching band truncated between 1200 and 1280 cm^{-1}. The P=O$^-$ band of the partially hydrated reversed

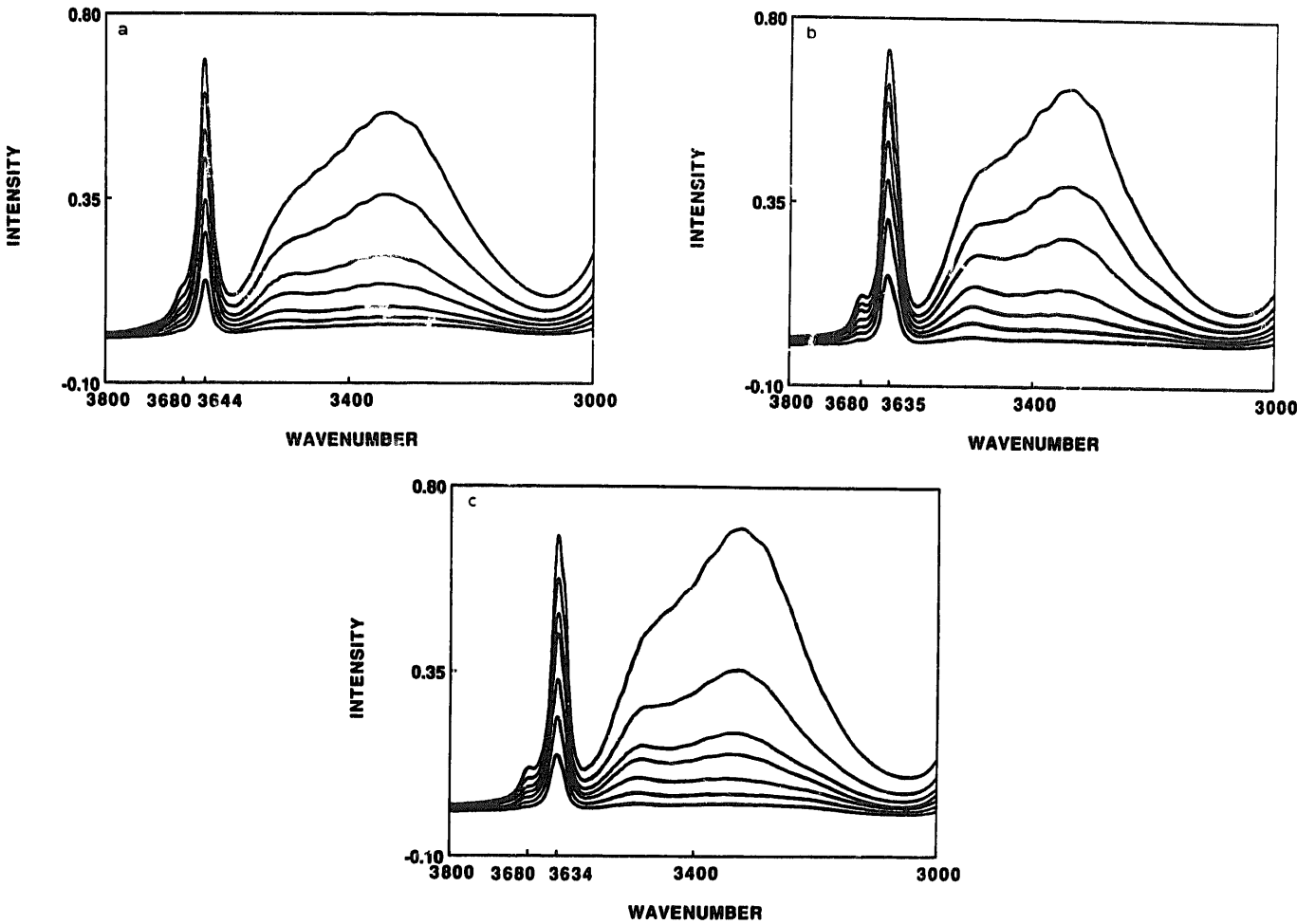

Fig. 2 (a,b,c). The bound and free O-H stretching bands as a function of the alcohol concentrations. (a) Methanol, (b) ethanol, and (c) n-butanol. The alcohol concentrations are from the top downward: (a) methanol 0.25, 0.20, 0.15, 0.125, 0.10, 0.075, and 0.050 M, (b) ethanol 0.25, 0.15, 0.125, 0.10, 0.075, 0.050, and 0.025 M, and (c) n-butanol 0.20, 0.15, 0.125, 0.10, 0.075, 0.050, and 0.025 M. The free alcohol O-H peak for methanol is at 3644 cm^{-1}, ethanol at 3635 cm^{-1}, and n-butanol at 3634 cm^{-1}. The bound alcohol O-H signal appears at about 3340–3350 cm^{-1} depending upon the alcohol concentration The 3680 cm^{-1} notch is the released free water peak.

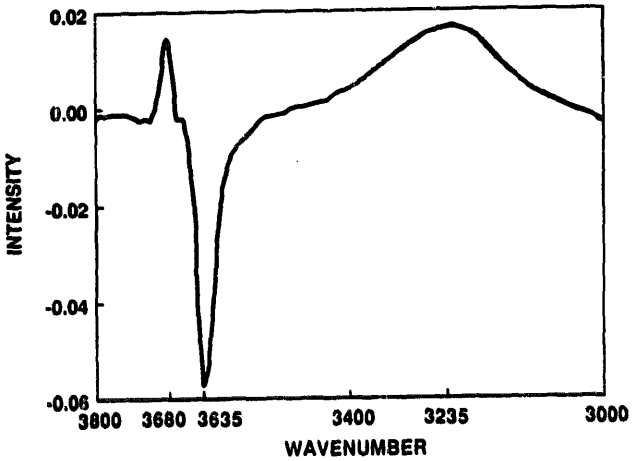

Fig. 3. The effects of ethanol in 0.006 M DPPC and 0.036 M H_2O reversed micelles. This is the difference spectrum between 0.15 M ethanol interacted with the reversed micelle and ethanol in CCl_4. The O-H signal of free ethanol at 3635 cm^{-1} shows negative value because of its loss by binding to DPPC. The bound ethanol O-H peak appears at 3235 cm^{-1}, and the free water peak appears at 3680 cm^{-1}.

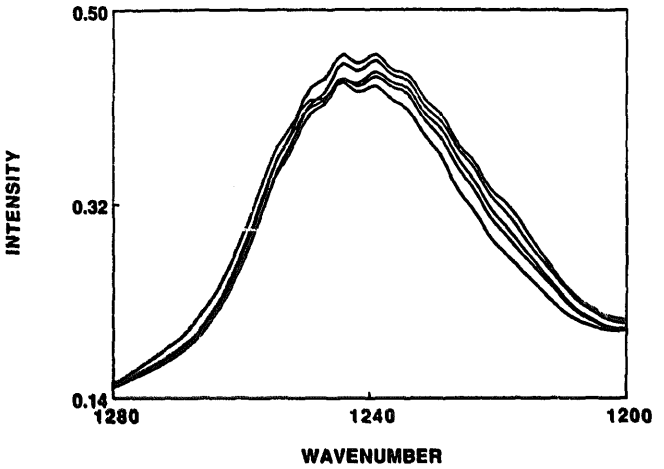

Fig. 4. The effect of ethanol concentrations on the IR difference spectrum of the P=O stretching band of DPPC. The baseline was adjusted for the total P=O symmetric and anti-symmetric region, 1130 to 1280 cm^{-1}, and truncated between 1200 and 1280 cm^{-1}. Ethanol concentrations are from the bottom upward: zero control, 0.025, 0.050, 0.075, and 0.10 M. The DPPC/water ratio was 6:36.

micelles is located at 1238 cm^{-1}. When alcohols were added to this system, this band became broader and shifted to lower frequencies. Apparently, the water molecules bound to the phosphate moiety is replaced by the alcohol molecules. The red shift was intensified by the increase of the alcohol concentrations. Similar results were obtained with other alcohols.

Fig. 5 is the effect of ethanol concentrations on the $P=O^-$ stretching band intensity at various hydration levels of DPPC. Ethanol shifted the $P=O^-$ band to lower frequencies and increased the intensity dose-dependently. The increase was greater with a higher lipid-to-water ratio. The increase of the $P=O^-$ band intensity was approximately linear to the ethanol concentrations under the present experimental conditions.

C=O site

A DPPC molecule contains two ester groups that link the glycerol skeleton to the two lipid tails. These two C=O bands were overlapped each other, and only one band was observed at about 1734 cm^{-1} (Fig. 6). In the absence of water, the unhydrated C=O stretching band was located at 1729 cm^{-1} which shifted to 1734 cm^{-1} when water was added [11]. The intensity of the 1734 cm^{-1} band decreased when alcohols were added to the system, and the band was shifted slightly to the high-frequency region. This indicates that the hydrogen-bonded water molecules are released. To further analyze the alcohol effects on the ester groups, the overlapped spectra were deconvoluted.

Deconvoluted C=O

Wong et al. [18] reported that the C=O stretching band of partially hydrated DPPC can be deconvoluted into three components: the free *sn*-1 C=O band, which is located closer to the lipid core; the free *sn*-2 C=O band, which is located closer to the polar head; and the hydrogen-bonded *sn*-2 C=O band, which is the hydrated *sn*-2 C=O. Only the C=O moiety closer to the hydrophilic head is partially hydrated. Fig. 7 shows the deconvoluted C=O spectrum with three bands at 1746 cm^{-1}, 1738 cm^{-1}, and 1729 cm^{-1}. The positions of these bands agree with the assigned values [18] of free *sn*-1, free *sn*-2, and hydrogen-bonded *sn*-2, respectively. Alcohols increased the intensity ratio between the free and the hydrogen-bonded *sn*-2 C=O bands in the deconvoluted spectra. Alcohols released hydrogen-bonded water molecules. Fig. 8 shows the effect of the

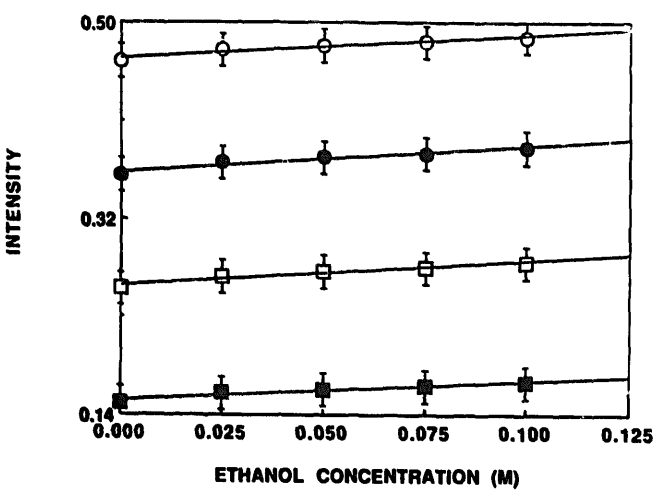

Fig. 5. The effect of ethanol concentrations on the $P=O^-$ stretching band intensity at various hydration levels of DPPC. Ordinate is the spectral intensity and abscissa is the ethanol concentration. DPPC/water ratios: open circles 8:36, filled circles 6:36, open squares 4:36, and filled squares 2:36.

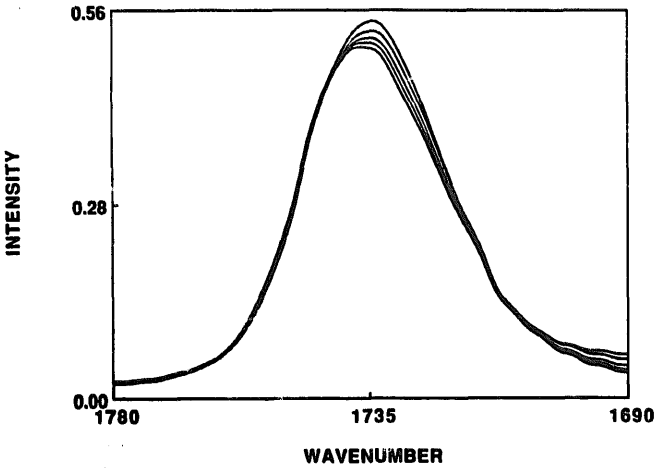

Fig. 6. The effect of ethanol concentrations on the C=O stretching band intensity. Ethanol concentrations are from the top downward: zero control, 0.025, 0.050, 0.075, and 0.10 M. The DPPC/water ratio was 6:36.

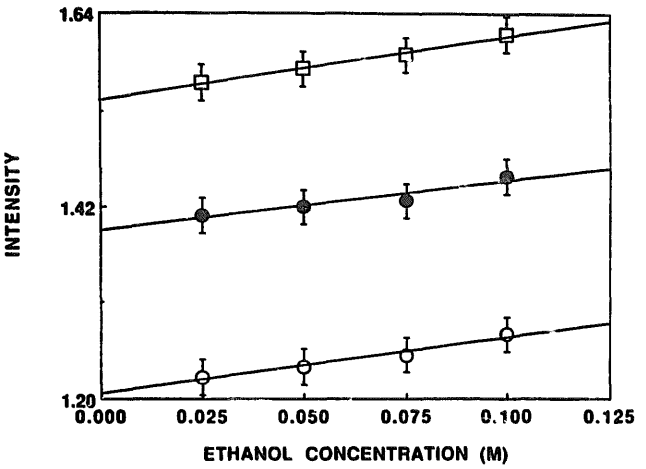

Fig. 8. The effect of ethanol concentrations on the ratio between the free and hydrogen-bonded *sn*-2 C=O stretching band intensities in the deconvoluted spectra at various hydration levels of DPPC. The DPPC/water ratios: open squares 6:36, filled circles 4:36, and open circles 2:36.

lipid-to-water ratio on the deconvoluted spectra. The ratio of the intensities between the free and the hydrogen-bonded *sn*-2 C=O stretching bands increased more when the lipid-to-water ratio was higher.

$(CH_3)_3$-N^+ site

In agreement with our previous studies with volatile anesthetics [11,19,20], the $(CH_3)_3$-N^+ stretching band in the reversed micellar system did not show appreciable changes when alcohols were added (not shown).

Discussion

The present result showed that short-chain alcohols released the hydrogen-bonded water molecules from the phosphate moiety and the C=O moiety near the

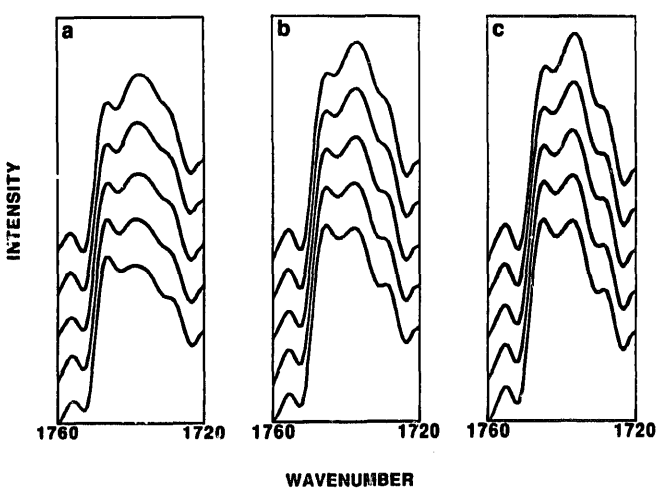

Fig. 7. Deconvoluted spectrum of the C=O stretching band of Fig. 8. DPPC/water ratios: (a) 2:36, (b) 4:36, and (c) 6:36. Ethanol concentrations from the bottom upward are: zero control, 0.025, 0.050, 0.075, and 0.10 M. The peaks are from the left: free *sn*-1 (1746 cm^{-1}), free *sn*-2 (1738 cm^{-1}), hydrogen-bonded *sn*-2 (1729 cm^{-1}).

head group, forming free water. The notion of the 'free' O-H stretching band for the 3680 cm^{-1} peak, which appeared when alcohols were added, is used to designate water molecules unbound to lipid molecules and does not mean rotating free monomer. Because of the extensive hydrogen-bond-forming capability of water molecules, no general agreement has been reached about the real state of water represented by this peak [15–17]. In a Raman study, Scherer et al. [15] attributed a weak broad band near 3650 cm^{-1} to a 'nearly free' O-H stretching vibration of a complex which closely resembles a 1:1 water-DMSO (dimethyl sulfoxide) complex. Fox and Martin [16] demonstrated by an infrared study on water in nonpolar solvents that there are three distinct bands of isolated water molecules; the symmetrical stretching vibrations at 3756 cm^{-1} and 3654 cm^{-1}, and the bending band at 1595 cm^{-1}. Fox and Martin [16] showed that the symmetrical bands of water, at low concentration in carbon tetrachloride, were 3705 cm^{-1} and 3614 cm^{-1} which shifted 40–50 cm^{-1} below the vapor values. In the ice spectrum, on the other hand, a broad weak band appeared at 1644 cm^{-1} [16]. Schiffer et al. [17] reported that the O-H stretching mode for H_2O, symmetrically hydrogen-bonded in CCl_4, was 3660 cm^{-1}. The present 'free' O-H stretching band at 3680 cm^{-1} represents an alcohol-H_2O complex.

The O-H bending band of water in the DPPC monohydrate was assigned at 1645 cm^{-1} [14]. Tsai et al. [21] reported that the O-H bending band of water appeared at 1644 cm^{-1} in dimyristoylphosphatidylcholine (DMPC) dispersed in a water-in-benzene reversed micellar system. In the present study, the intensity of the O-H bending band at 1644 cm^{-1} decreased when alcohols were added. This band is a bending mode of water multimers bound to DPPC.

The main hydrogen bonding site of the DPPC membrane is the phosphate moiety. Although the choline head $(CH_3)_3$-N^+ has a positive charge, it cannot form a hydrogen bond because there is no proton to donate and the positive charge prevents the acceptance of protons. The hydration is caused by the charge-dipole interaction and is weak, especially because the charge is covered by the hydrophobic methoxy moieties. We [11,19–21] have reported that the IR signal of $(CH_3)_3$-N^+ is insensitive to anesthetics.

In the absence of water, the $P{=}O^-$ stretching band appears at 1262 cm^{-1}. The frequency of this band decreased upon addition of water and was gradually shifted to 1238 cm^{-1}. Alcohols further shifted the peak to lower frequencies dose-dependently. The present data indicate that ethanol binds to the phosphate moiety in competition with water. The larger mass of alcohols compared with water may have suppressed the vibrational frequency. Alcohols weakened the water–lipid interactions and dehydrated the phosphate surface by competitive binding.

In contrast to the low-frequency shift of the $P{=}O^-$ signal, the C=O band shifted to the higher frequency. This suggests that the hydrogen-bonded water molecules were released from the glycerol skeleton by alcohols, but alcohols did not directly form hydrogen bonds with glycerol C=O moieties. The interactions between the glycerol C=O group and alcohols may be weaker than water probably because of steric hindrance in penetrating into this level and a weaker hydrogen-bonding capability of alcohols to the C=O group.

The decreased intensity of the C=O band and the increased ratio of the intensities of the free versus bonded *sn*-2 bands in the deconvoluted spectra also demonstrate the dehydration effect of alcohols. The constancy of the free *sn*-1 signal indicates that neither water nor alcohol molecules appreciably interact with this moiety which is closer to the hydrophobic region of the membrane. Alcohols interacted with the *sn*-2 carbonyl group which is closer to the hydrophilic region of the membrane.

We depict the overall effect of alcohols by the following reaction: (1) DPPC-water complex is partially replaced by DPPC-alcohol complex, and (2) released water forms an alcohol-water complex. In DPPC molecules, the main hydrogen bonding site is the phosphate group. Only the surface-side C=O group (*sn*-2) is partially hydrated. When alcohols are added, a part of the bound water is released from the phosphate and the *sn*-2 C=O moieties. The alcohols bind only to the phosphate group, releasing the bound water. The bound alcohols create a new broad band.

Interaction of alcohols with membranes is expressed by membrane–water partition coefficients. Partition implies distribution of solutes between two homogeneous phases, whereas membranes are highly structured pseudophase. One of the outstanding characteristics of vesicle membrane or micellar 'phases' is the enormously increased water/lipid interfacial region. The membrane–water partition coefficients treat lipid membranes as a homogeneous solvent and do not identify the solute location in the anisotropic membrane structure. In the partition studies of amphipathic nonelectrolytes such as alcohols between dimyristoylphosphatidylcholine (DMPC) and water, Diamond and Katz [22] estimated the submolecular location of solute in the bilayer by comparing the membrane–water partition coefficients with the partition coefficients between a series of organic solvents and water. They found that the solvent properties of this region resemble those of isoamyl alcohol, and proposed that short-chain alcohols were located towards the membrane surface rather than interior.

To compare the present results with literature values, the DPPC-alcohol partition coefficients are estimated from the change in IR spectra. The apparent DPPC-water partition coefficient of alcohols is expressed as,

$$X_A^L / X_A^W = P \tag{1}$$

where X is the mole fraction, subscript A is alcohol, and superscript L and W are lipid and water, respectively. The number of alcohol molecules in DPPC and water is

$$\begin{aligned} n_A - n_A^{oil} &= X_A^L \cdot n_L + X_A^W \cdot n_W \\ &= X_A^L \cdot n_L + \frac{1}{P} \cdot X_A^L \cdot n_W \\ &= X_A^L \left(n_L + \frac{1}{P} \cdot n_W \right) \end{aligned} \tag{2}$$

where n_A is the total number of alcohol molecules, n_A^{Oil} is the number of alcohol molecules in the oil (CCl_4) phase, and n_L and n_W are the total number of DPPC and water molecules. Because the CCl_4 size is very large compared to DPPC and water, n_A^{Oil} is assumed to be approximately constant after interacting with DPPC and water. By designating $n'_A = n_A - n_A^{oil}$, X_A^L is expressed as

$$X_A^L = \frac{n'_A}{n_L + \frac{1}{P} \cdot n_W} = \frac{P \cdot n'_A}{n_W + P \cdot n_L} \tag{3}$$

Because alcohols formed hydrogen bonds only with the phosphate moiety [11], the $P{=}O^-$ stretching band was used for the estimation. When the number of bound alcohol molecules is much less than the number

of lipid molecules, the difference in the intensity, ΔI, is written as

$$I_L - I_{LA} = \varepsilon_L \cdot n_L - [\varepsilon_{LA} \cdot X_A^L \cdot n_L + \varepsilon_L \cdot (1 - X_A^L) \cdot n_L]$$

$$= -\varepsilon_{LA} \cdot X_A^L \cdot n_L + \varepsilon_L \cdot X_A^L \cdot n_L$$

$$= X_A^L \cdot n_L (\varepsilon_L - \varepsilon_{LA}) \quad (4)$$

where I_{LA} and I_L are, respectively, the P=O$^-$ intensities in the presence and absence of alcohols, ε is the molar absorptivity, and subscripts L and LA indicate lipid alone and lipid-alcohol complex. Eqn. 4 is rearranged as,

$$\frac{I_L - I_{LA}}{\varepsilon_L \cdot n_L} = \left(\frac{\varepsilon_L - \varepsilon_{LA}}{\varepsilon_L}\right) X_A^L$$

or

$$1 - \frac{I_{LA}}{I_L} = \left(\frac{\varepsilon_L - \varepsilon_{LA}}{\varepsilon_L}\right) X_A^L \quad (5)$$

By combining Eqns. 3 and 5,

$$1 - \frac{I_{LA}}{I_L} = \left(\frac{\varepsilon_L - \varepsilon_{LA}}{\varepsilon_L}\right) \cdot P \cdot \frac{n'_A}{n_W + P \cdot n_L} \quad (6)$$

By taking reciprocal of each side

$$n'_A \left(\frac{I_L}{I_L - I_{LA}}\right) = \frac{\varepsilon_L}{\varepsilon_L - \varepsilon_{LA}} \cdot \left(\frac{1}{P} n_W + n_L\right) \quad (7)$$

or

$$\frac{I_L}{\Delta I} = \frac{n_W}{n'_A} \left(\frac{\varepsilon_L}{\varepsilon_L - \varepsilon_{LA}}\right) \left(\frac{1}{P} + \frac{n_L}{n_W}\right) \quad (8)$$

By plotting $I_L/\Delta I$ against the lipid concentration, a straight line should be obtained. From the slope and the Y intercept of the straight line, P and the constant expressed by $(n_W/n'_A)\{\varepsilon_L/(\varepsilon_L - \varepsilon_{LA})\}$ are obtained.

Fig. 9 shows the above plot with ethanol. The estimated apparent partition coefficients for ethanol were 16.03, 17.10, 16.94, and 16.85 for ethanol 0.1, 0.075, 0.05, and 0.025 M, respectively. The values for methanol were 7.89, 7.81, 7.76, and 7.66 for 0.1, 0.075, 0.05, 0.025 M, respectively. These values are estimated on the mole fraction concentration. Diamond and Katz [23] used dimyristoylphosphatidylcholine (DMPC) vesicles, and reported that the DMPC–water partition coefficients for ethanol and methanol were 0.441 and 0.206, respectively, at 25°C. These values are expressed in the molality concentrations and differ from the values estimated on mole fraction or molarity [24]. We reported [24] the conversion factors for the lecithin–water partition coefficients expressed by mole fraction, molarity,

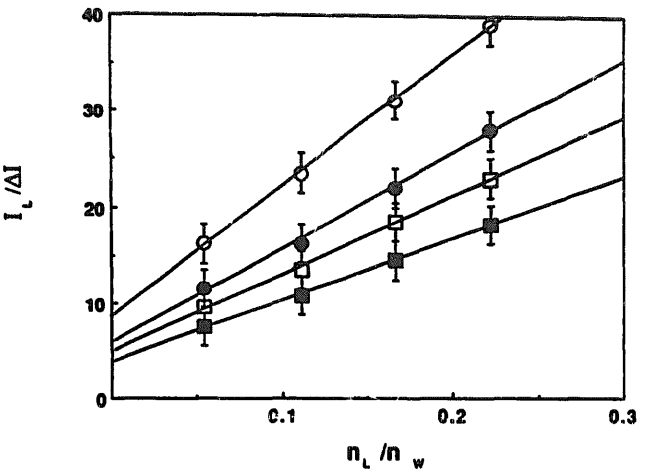

Fig. 9. The effect of DPPC/water ratio on the P=O stretching band intensity at various ethanol concentrations. Ethanol concentrations: open circles 0.1 M, filled circles 0.075 M, open squares 0.05 M, and filled squares 0.025 M. The ordinate is the reciprocal $\Delta I/I_L$, where ΔI is the P=O stretching signal intensity difference between the presence and absence of alcohol and I_L is the control intensity in the absence of alcohol. See Eqn. 8.

and molality: the conversion factor for molality to mole fraction in DMPC membrane is 37.5 and that for molarity to mole fraction is 36.6. Conversion of the molality data of Diamond and Katz [23] to mole fraction yields the value of 16.54 for ethanol and 7.73 for methanol. Considering the difference in the phospholipid species, these values agree reasonably well. The present result that the phosphate moiety is the hydrogen-bonding site for alcohols compares well with their estimated loci of alcohol interaction in the DMPC membrane.

The localization of amphipathic molecules at the water/lipid interface has also been demonstrated by analyzing the chemical shift in the frequency domain of ^{1}H- and ^{19}F-NMR spectra of inhalational anesthetic molecules in surfactant micellar solution [25,26]. They showed that the hydrophobic end of anesthetic molecules penetrated into the hydrophobic core of the micelles, yet, the hydrophilic end did not lose contact with the aqueous phase. By two-dimensional nuclear Overhauser effect ^{1}H-NMR, Yokono et al. [27] demonstrated that the protons of the hydrophobic end (CH_3-) of an inhalation anesthetic methoxyflurane (CH_3-O-CF_2-CCl_2H) formed a cross-peak with the protons of the hydrophilic choline head of DPPC. The proton on the hydrophilic end (-CCl_2H) did not interact with the membrane. No other cross-peaks were observed, indicating that these molecules stayed near the interface.

We [28–30] proposed that the main action of alcohols and anesthetics are breaking the hydrogen bonds of the water cluster in the solvation shell of macromolecules. Using differential scanning calorimetry (DSC), we [31] have shown that the free water peak, which freezes at 0°C, disappeared in a DPPC/water

system when the water content was decreased to less than 17 wt%. On the other hand, multiple subzero (−3 to −25°C) freezing peaks of the bound water remained. Addition of halothane and 1-hexanol to the above preparation restored the 0°C freezing peak at the expense of subzero peaks, indicating the formation of free water by releasing the surface-bound water that freezes at subzero temperatures. A Fourier-transform infrared (FTIR) study [11,19,20] on water–phospholipid interaction in a water-in-oil reversed micellar system showed that anesthetics released the structured water molecules bound to the phospholipid at the interface. These findings support the view that the primary action site of amphipathic solutes is near the membrane/water interface, and that they release bound water molecules.

The hydrogen-bond breaking activity of anesthetics was first demonstrated by Di Paolo and Sandorfy [32]. They found that in a brominated fluorocarbon solvent, the intensity of the bound O-H stretching band of secondary amines decreased when the temperature was decreased, whereas the free O-H stretching band increased. When the temperature was decreased to −190°C, the bound O-H stretching band disappeared. Surprisingly, decreasing temperature melted ice. They demonstrated similar ice-melting effects with volatile anesthetics. The hydrogen-bond breaking activity correlates well to anesthetic potency. The hydrogen-bond breaking action is not limited to halogenated hydrocarbon anesthetics. It was shown [33,34] that anesthetics (including cyclopropane and alcohols) can break hydrogen bonds between X-H_2O by competitively forming X-anesthetic bonds. The significance of the hydrogen-bond breaking activity on the anesthesia mechanisms has also been postulated by Sax and Pletcher [35] for local anesthetic actions and by Klemm [36] for alcohol intoxications.

By using core-binding and surface binding fluorophores, Hitzemann [37] reported that alcohols disordered the phospholipid membrane core (increased fluidity), but ordered the membrane surface (decreased fluidity). This opposing effect of ethanol at the core and surface of the membrane structure is intriguing. The decrease in the membrane 'fluidity' indicates hindered movement of the membrane molecule rather than the increase in viscosity (flow resistance). The decreased vibrational movement of phosphate moiety probably explains the decreased fluidity of the membrane surface found by the fluorophore study.

Membranes cannot be formed without water. Although lipid membranes are formed by the hydrophobic effect (antipathy), the precise mechanism of this effect is not clearly understood. The antipathy between hydrophobic molecule and water rests on the strong self-association of water. However, the surface free energy data show that the forces between hydrocarbons and water in contact with each other is attractive rather than repulsive [38].

Lipid bilayers maintain their structure by the balance between the lipid-lipid cohesive force and the lipid-water adhesive force. Anything that weakens the lipid–water interaction may disorder and fluidize the membrane structure [29,30]. The loss of the support from the structured interfacial water matrix by short-chain alcohols would destabilize the membrane and increase the 'fluidity' of the membrane core. The discrepancy between the surface and core fluidities may be explained by the above mechanism. When the interaction is overtly weakened, the apposing monolayers penetrate each other and the membrane transforms into the interdigitated state [39–43].

The water structure around macromolecules may be the determinant factor for the conformational stability. Phospholipids, dispersed in a water-in-oil reversed micellar system, provide a convenient model for the biological membranes with the advantage that the behavior of water molecules is easily identified.

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