Effects of Halothane on Phosphatidylcholine, -ethanolamine, -glycerol, and -serine Monolayer Order at a Liquid/Liquid Interface

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Vibrational sum-frequency spectroscopy has been employed to examine the vibrational spectra and determine the conformational order of diacylphosphatidyl lipid monolayers at a water−oil interface. These nonlinear vibrational spectra provide direct information about the orientation and the degree of order of the acyl chains of the lipid monolayers. Halothane was introduced into the lipid monolayers, and the resulting changes in acyl chain order were examined. Both neutral (zwitterionic) and charged lipid monolayers were studied in an effort to determine the likely sites of interaction between halothane and the lipid molecules. Two chain lengths (14 and 16 carbons) and four headgroups (choline, ethanolamine, glycerol, and serine) were studied. Each monolayer exhibits significant disorder, and the conformational order of each monolayer is perturbed after the introduction of halothane.

Introduction

For more than 100 years, the effects of inhaled anesthetics have been studied with the ultimate goal of understanding the mechanism, or action site, of general anesthesia.[1,2] Although tremendous effort has been expended in this endeavor and an overwhelming number of experiments have been completed, the controversy remains whether the primary site of action for inhaled anesthetics is the membrane lipid or the proteins embedded in the membrane.

Despite the continuing debate, there is much that is known about anesthetic action. It is clear that local anesthetics significantly change ion currents across neural membranes, that the ion channels through which they pass are composed of protein, and that these proteins are embedded in lipid.[1] However, there is little knowledge of the mechanism by which lipid-soluble anesthetics of such widely different structures affect the ion channels. Experimental studies during the last 20 years had shifted the focus from action in the lipid to direct binding to proteins.[3] More recently, several groups have suggested that the action of inhaled anesthetics may instead reside in function modulation of a number of transmembrane ligand-gated ion channels.[4−6] Very recently, interest in the role of membrane lipids was renewed when Cantor[7,8] hypothesized that perturbations in membrane structure induced by inhaled anesthetics induce changes in protein function. However, recent experiments examining this hypothesis utilizing relatively simple model lipid systems do not agree on the active sites occupied by inhaled anesthetics in the membrane. Experimentally suggested sites include the acyl chain domain,[9] various locations in the headgroup region,[10,11] and the lipid/water interface.[12,13]

In the present study, vibrational sum-frequency spectroscopy (VSFS) was employed to measure the vibrational spectra of a series of phospholipid monolayers at an organic/water interface and to examine the effect of a common inhaled anesthetic, halothane ($CF_3CHClBr$), on their conformation. Phospholipid monolayers at an organic/aqueous interface are a useful model system for the study of cell membranes because they provide a realistic model of the hydrophilic and hydrophobic environments commonly found in vivo. In addition, the understanding and thermodynamics of these monolayer systems have been extensively described and rigorous theoretical analyses have been reported.[14] In these studies, the CCl_4/D_2O interface is used for experimental convenience, and although this interface is nonbiological, it does mimic the hydrophobic/aqueous interface found in many biological systems.[15] While the halothane has direct access to the acyl chains in these studies, in bilayer membranes halothane must access the acyl chains by crossing the headgroup region from the aqueous phase. In these studies on monolayers and the molecular dynamics (MD) studies on bilayers,[2] the interactions of halothane with lipid headgroups and acyl chains are examined independently from the mechanism of halothane transport from the aqueous phase through the headgroup region.

The phospholipid monolayers examined in this study were all saturated, symmetric, 1,2-diacyl-*sn*-glycero-3-phosphate based lipids. Four different lipid headgroups

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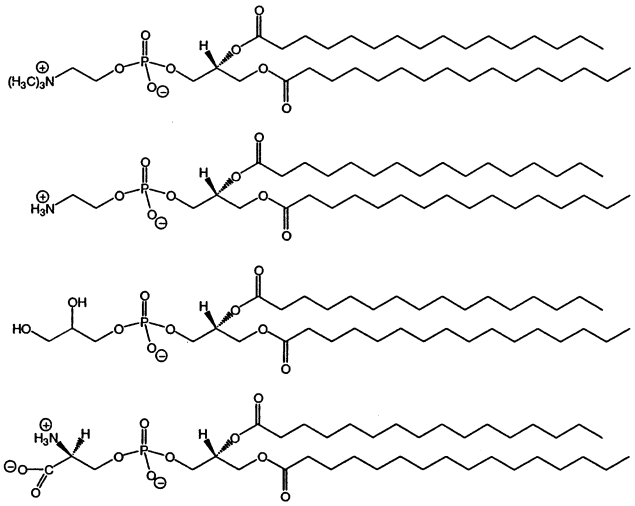

Figure 1. Chemical structures of the dipalmitoyl phospholipids employed in this study. From top to bottom, DPPC, DPPE, DPPG, and DPPS.

attached to the phosphate were examined: choline, ethanolamine, glycerol, and serine (see Figure 1 top to bottom, respectively). Each lipid features a glycerol backbone, two saturated fatty acid chains, and a phosphatidyl headgroup. The phosphatidylcholine and the phosphatidylethanolamine have neutral, zwitterionic headgroups, and the choline is more hydrophilic than the ethanolamine. The phosphatidylglycerol and the phosphatidylserine are negatively charged, and each has its charge localized in a different portion of the headgroup. The serine has two additional localized charges in the headgroup (the same zwitterion as in the ethanolamine). The choline headgroup was chosen for study because dipalmitoyl-*sn*-glycero-3-phosphocholine is the most prevalent component of membrane lipid and the main component of lung surfactant. The other headgroups were chosen to examine the significance of minor membrane lipid components (and the significance of charged lipids) on the effect of halothane on monolayer structure.

Spectroscopic Background

VSFS is a second-order nonlinear optical method that is interfacially specific and sensitive to the molecular species present at the interface. VSFS was first treated theoretically by Bloembergen and Pershan in 1962[16] and was first demonstrated experimentally by Shen et al. in 1987.[17] In VSFS, a laser beam with a fixed visible frequency is overlapped spatially and temporally with a tunable infrared beam to induce a nonlinear polarization in the medium under study. This nonlinear polarization generates a coherent beam at the sum of the two incident frequencies, and the intensity of this generated beam is measured as a function of the incident IR frequency. The sum-frequency intensity is dependent on the square of the second-order polarization in the following manner:

$$I(\omega_{sfg}) \propto |P^{(2)}|^2 \propto |\tilde{f}_{sfg} f_{vis} f_{IR} \chi^{(2)} : E_{vis} E_{IR}|^2 \qquad (1)$$

where $\tilde{f}_{sfg}$, f_{vis}, and f_{IR} are the Fresnel coefficients for each of the fields and $\chi^{(2)}$ is the macroscopic second-order susceptibility of the medium. The Fresnel coefficients are geometric factors that take into account the effective electric fields at the interface, their polarizations, and their propagation directions. The nonlinear susceptibility is the macroscopic response of the medium to the incident electric fields. The efficiency of sum-frequency conversion is enhanced if the infrared beam is tuned on resonance with a molecular vibration. The nonresonant contribution to the nonlinear susceptibility can be separated from the resonant contributions as follows:

$$\chi^{(2)} = \chi_{NR}{}^{(2)} + \sum_{\nu} \chi_{R}{}^{(2)}(\nu) \qquad (2)$$

where the sum is over all vibrational modes of all molecular species in the medium. The resonant macroscopic susceptibility for a particular vibrational mode is related to the molecular susceptibility for that mode β_ν as follows:

$$\chi_{R}{}^{(2)}(\nu) = \frac{N}{\epsilon_0}\langle\beta_\nu\rangle \qquad (3)$$

where the brackets indicate an orientational average and N is the number of molecules. Assuming that the fields interact with the medium solely through dipolar interactions (the dipole approximation), the molecular hyperpolarizability, β_ν, has the following form, which is derived from perturbation theory:

$$\beta_{lmn,\nu} \propto \frac{\langle g|\alpha_{lm}|\nu\rangle\langle\nu|\mu_n|g\rangle}{\omega_{IR} - \omega_\nu + i\Gamma_\nu} \qquad (4)$$

where the numerator is the Raman transition probability multiplied by the IR transition probability, ω_{IR} is the frequency of the incident IR electric field, and Γ_ν is the natural line width of the transition. Since the hyperpolarizability is zero if either the Raman transition probability or the IR transition probability is zero, VSFS is forbidden in media possessing an inversion center (conversely, it is naturally allowed at an interface). The ability to do spectroscopy arises from the denominator which shows a large enhancement in the hyperpolarizability, and subsequently the sum-frequency intensity, when the laser is tuned over a vibrational resonance of the molecule. After convolving eq 4 with a Gaussian to account for inhomogeneous broadening, eqs 2−4 can be combined to obtain the following expression:[18]

$$\chi^{(2)} = \chi_{NR}{}^{(2)} e^{i\phi} + \sum_{\nu} \int_{-\infty}^{\infty} \frac{A_\nu e^{i\varphi_n} e^{-[\omega_L - \omega_n/\Gamma_n]^2}}{\omega_{IR} - \omega_L + i\Gamma_L} d\omega_L \qquad (5)$$

where the complex exponentials represent the relative phases between the various contributions, A_ν is the numerator from eq 4, and the integral is over Lorentzian lines (centered at ω_L with a width Γ_L) having a Gaussian distribution. Equation 5 is utilized to fit the experimental data. In the experiments described below, the nonresonant contribution is negligible and is given zero amplitude. In addition, the Lorentzian widths Γ_L are set to 2 cm^{-1}, and Γ_ν is allowed to vary since we cannot separate the two contributions to the line width.[19]

Experimental Considerations

Sample Preparation. All lipids were purchased as lyophilized powders from Avanti Polar Lipids and used without further purification. Deuterated chloroform 99.8% and D_2O 99.9% were

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purchased from Cambridge Isotopes Labs and used as received. HPLC grade carbon tetrachloride 99.9+% was purchased from Sigma-Aldrich and tested for purity in the C−H stretching region by Fourier transform infrared (FTIR) spectroscopy. Halothane was purchased from Sigma and distilled prior to use to remove the 0.010% thymol stabilizer and any residual impurities. Anhydrous methanol was purchased from Mallinckrodt and used as received. Reagent grade sodium phosphate (monobasic) was purchased from Mallinckrodt and dried before use in preparing the buffer.

Stock lipid solutions were prepared by dissolving 0.5−1.0 mg/mL of lipid in deuterated chloroform (used to avoid the absorption of IR by C−H in chloroform). A drop of methanol and a drop of Nanopure water (17.8 MΩ resistivity) were added to the ethanolamines, glycerols, and serines, and these solutions were heated in a water bath to facilitate dissolution. Due to the instability of chloroform under ambient light and at ambient temperatures, the stock solutions were stored in a freezer at −20 °C and brought back to room temperature (and reheated if necessary) before use. Stock solutions were discarded after 2 weeks.

Interfaces were prepared by placing 25.0 mL of D_2O buffered to pD 7.0 (10 mM in total phosphate) over 38 mL of CCl_4 in a custom Teflon cell fitted with CaF_2 windows followed by the addition of the lipid monolayer using a microsyringe. This was accomplished with two additions of sufficient lipid to form a monolayer (10−20 μL) with 2 h between additions. An additional 2 h was allowed for equilibration of the monolayer before alignment of the experiment and the collection of spectra. Spectra acquired an hour apart confirmed that equilibrium had been reached. After the acquisition of spectra, 200 μL of halothane was injected directly into the CCl_4 phase using another microsyringe, and at least 12 h was allowed for equilibration before a second set of spectra were acquired. To ensure that the spectral changes observed were due to halothane and not due to aging of the monolayer, some monolayers were examined and then allowed to stand for 12 h and examined again. The resulting spectra were identical within the noise of the data.

Prior to their use, all sample cell parts and any glassware were cleaned using NoChromix reagent followed by rinsing with copious amounts of Nanopure water and allowed to dry.

Spectroscopic Measurements. The laser and tunable IR source used in these experiments has been described in detail elsewhere,[20] so only a brief overview will be given here. The 1064 nm output of a Nd:YAG laser operating at 20 Hz with a 3.5 ns pulse duration is spit into two beams. The first is frequency doubled to 532 nm, and the power of this beam is maintained at a constant value. The second is used to generate tunable IR (2600−4000 cm^{-1}) in an OPO−OPA assembly producing 1−4 mJ/pulse with a bandwidth of 1 cm^{-1}. The IR beam is focused at the interface and overlapped spatially and temporally with the ~1 mm diameter visible beam. A total internal reflection (TIR) geometry is utilized in which the visible beam is incident upon the interface near its critical angle, and the generated sum-frequency light is collected near its critical angle.[21] This geometry can result in an enhancement in the generated sum-frequency intensity by more than 2 orders of magnitude.[21−23]

The input and output polarizations can be selected to permit the sampling of selected elements of the macroscopic nonlinear susceptibility. It is a 27-element tensor that has only four independent nonzero elements in a system with $C_{\infty v}$ symmetry.[24] This is the symmetry of a system that is isotropic in the plane of the interface between two bulk phases that are centrosymmetric, which describes the interfaces studied here. The ssp polarization combination (where the short hand notation ssp corresponds to the polarizations of the sum-frequency, visible, and IR, respectively) probes only the element χ_{yyz} which is sensitive to IR transition dipoles that have a component normal to the interface. The sps polarization combination probes only χ_{yzy} which is sensitive to IR transitions that have a component in the plane of the interface.

The sum-frequency signal is separated physically from the reflected beams using apertures and optically through the use of appropriate filters. The resulting signal is collected by focusing the beam into a PMT and through the use of gated electronics. A power spectrum of the tunable IR is collected simultaneously with the VSFS spectrum. The spectra are corrected for IR power, for the wavelength dependence of the transmission of the collection optics, and for the wavelength dependence of the Fresnel factors that account for the effective electric fields at the interface. All spectra described below were acquired by averaging 200 shots per data point, and data were recorded for every wavenumber over the spectral region.

Table 1. Vibrational Assignments for VSFS Peaks (cm^{-1}) in the C−H Stretch Region (2800−3000 cm^{-1})

VSFS peak positions	vibrational mode	literature peak positions
2849	CH_2SS	2846−2850[a,b]
2875	CH_3SS	2870[a]
2904	CH_2FR	2895−2925[c]
2932	CH_3FR	2931[a]
2950	CH_3AS	2950−2960[a]

[a] From ref 26. [b] From ref 25. [c] From ref 27.

Results and Discussion

Figure 2a−d shows VSFS spectra of dipalmitoyl phosphatidylcholine (DPPC), dipalmitoyl phosphatidylethanolamine (DPPE), dipalmitoyl phosphatidylglycerol (DPPG), and dipalmitoyl phosphatidylserine (DPPS) adsorbed at the CCl_4/D_2O interface. Fits to the data are superimposed (solid line). The spectra cover the C−H stretch region. The IR and Raman spectral features of alkyl chains in the C−H stretching region of these phospholipids have been previously assigned.[25−27] Similar assignments have been made for the acyl chains of phospholipids in previous VSF studies.[28,29] The same general assignments are used here with peak positions determined from fits to the data employing eq 5 (see Table 1).

The spectra from each of the four monolayer systems exhibit similar relative peak intensities and similar peak positions. The peak at 2849 cm^{-1} is assigned to the methylene symmetric stretch (CH_2SS), the peak at 2875 cm^{-1} to the methyl symmetric stretch (CH_3SS), and the broad peak at ~2905 cm^{-1} to the methylene Fermi resonance (CH_2FR). The peak at 2932 cm^{-1} is assigned to the methyl Fermi resonance (CH_3FR) with the possibility of some contribution from methylene asymmetric stretch (CH_2AS). Both the CH_3FR and CH_2AS have been assigned to peaks in this region in previous VSF studies.[18,19,28−31] A peak at 2950 cm^{-1} is present as a small shoulder on the CH_3FR and is attributed to the methyl asymmetric stretch (CH_3AS).

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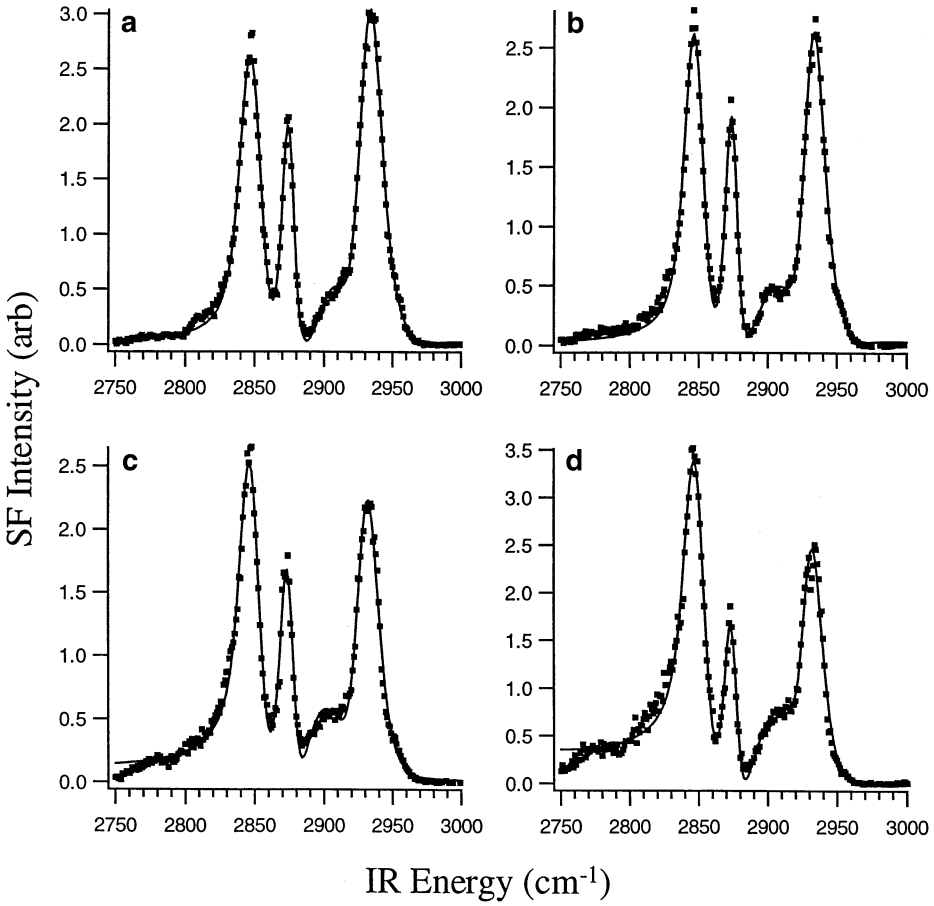

Figure 2. Representative spectra of the C−H stretching region of dipalmitoyl phospholipid monolayers at the CCl_4/D_2O interface under the ssp polarization scheme: (a) DPPC, (b) DPPE, (c) DPPG, and (d) DPPS. The solid lines are fits to the data.

When applying the fitting routine to the data, several parameters can be specified. Since it is not possible to separate the natural line widths of the transitions Γ_L from the inhomogeneous broadening Γ_ν in these experiments, Γ_L was set at 2 cm^{-1} and only Γ_ν was allowed to vary.[19] The methylene modes, CH_2SS and CH_2FR, are in phase due to symmetry considerations and were given the phase of zero. In addition, the relative phase difference between the CH_3SS and the CH_3AS is π due to symmetry. The CH_3FR is in phase with the methyl symmetric stretch also due to symmetry. The spectra were fit as a consistent set using the same peak positions and relative phases in every fit. However, the CH_2FR is a difficult feature to fit as it is somewhat broad, it can be composed of several overlapping features,[32] and its position can vary from molecule to molecule. In the present studies, the width of the CH_2FR was constrained to give consistent fits, and the position was allowed to vary from one headgroup to another (all positions fell within a 10 cm^{-1} window). The positions derived from the fits were consistent for all spectra of an individual phospholipid. For the DPPG and DPPS spectra, a contribution from the tail of the O−D stretch modes of D_2O was necessarily included to fit the data. For the uncharged phospholipids, DPPC and DPPE, it was not necessary. We attribute this O−D intensity for the charged phospholipids to the known enhancement of O−H or O−D stretch modes for interfacial water when adsorbed charges are present.[33]

A useful parameter for examining the relative ordering of alkyl chains in linear spectroscopy is the ratio of the integrated methyl symmetric stretch intensity to the integrated methylene symmetric stretch intensity.[25] In VSFS, a ratio of the square root of the integrated areas of these two peaks is employed since these values are proportional to the number of molecules contributing to the SF intensity. If the incoming IR beam is p-polarized, the IR transition moment for the methyl symmetric stretch in an all-trans chain is aligned with the electric field, resulting in a large SF signal. However, since there are local inversion centers at the center of each C−C bond in an all-trans chain, intensity in the methylene symmetric stretch is forbidden under the dipole approximation. Conversely, under this same polarization (ssp), highly disordered chains lead to a more isotropic orientation of the methyl groups (and a reduction in SF intensity), while the gauche defects in the acyl chains relax the symmetry constraints resulting in an increase in methylene symmetric stretch intensity. Thus, for an all-trans chain, the intensity ratio $(CH_3SS)^{1/2}/(CH_2SS)^{1/2}$ approaches infinity with the methyl symmetric stretch strong and the methylene symmetric stretch absent, while a much smaller ratio is expected for highly disordered chains as the methylene mode becomes dominant over the methyl mode.

The spectra in Figure 2 show considerable intensity in both the CH_2SS and the CH_3SS modes for each of the phospholipids examined, indicating that the monolayers are all relatively disordered. The spectra also indicate that the nature of the headgroup does not significantly impact the ordering of the acyl chains. Information extracted from spectral fits indicates that DPPC, DPPE,

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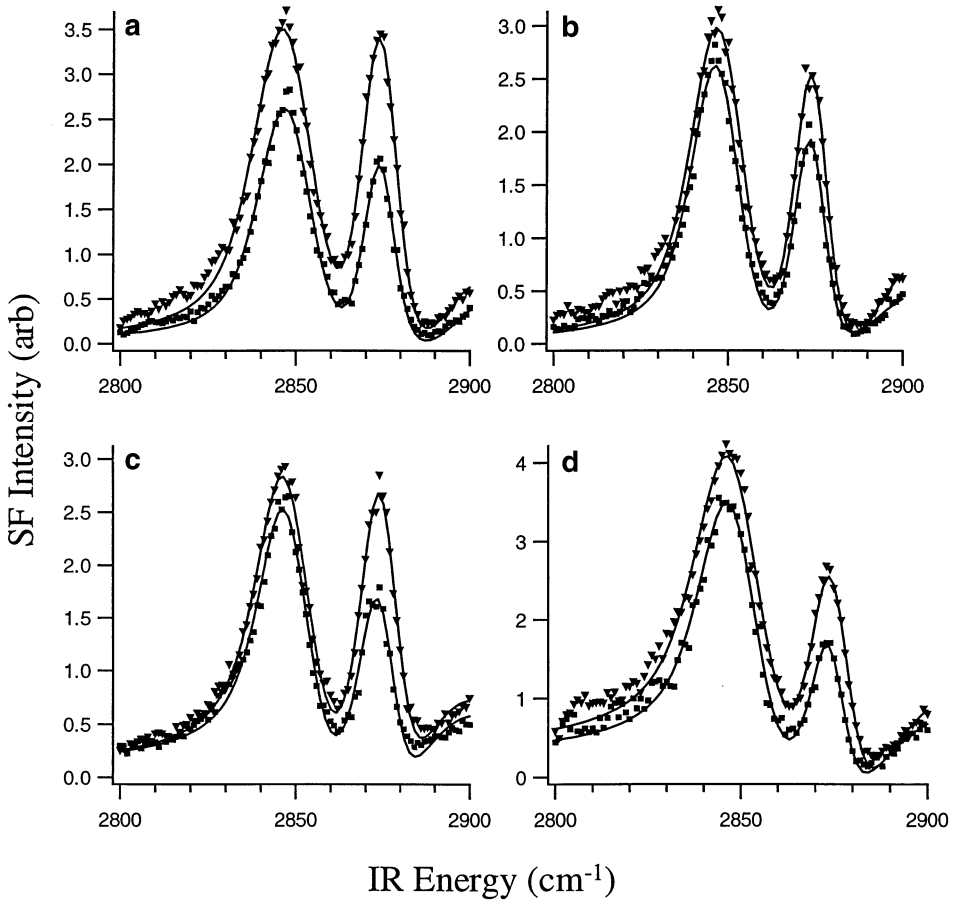

Figure 3. Representative spectra of dipalmitoyl phospholipid monolayers at the CCl_4/D_2O interface under the ssp polarization scheme showing just the CH_2SS and CH_3SS peaks: (a) DPPC, (b) DPPE, (c) DPPG, and (d) DPPS. Spectra of the monolayers are shown with solid squares. Spectra of the monolayers with halothane are shown with solid triangles. The solid lines are fits to the data.

and DPPG are all similarly ordered with parameters of 0.62, 0.67, and 0.65, respectively, and that DPPS is the most disordered with an order parameter of 0.55. (Uncertainties are on the order of 5%.) The degree of chain order for these monolayers agrees well with previous results from this laboratory for phosphatidylcholines.[28] The large degree of disorder of these monolayers compared to monolayers at the air/water interface can be attributed to the intercalation of CCl_4 into the chains which reduces van der Waals interactions between the chains. As a comparison, the order parameters for a series of diacyl phosphatidylcholines at the air/water interface (determined in this laboratory) were found to be 1.9 for 12 carbon chains, 2.1 for 14 carbon chains, 5.3 for 16 carbon chains (DPPC), and 11.9 for 18 carbon chains.[28]

An attempt was made to study changes in interfacial water structure in the presence of halothane in the monolayer; however, total absorption of the IR just above 3000 cm^{-1} by halothane in the CCl_4 prevents the acquisition of a complete, continuous spectrum.

Effect of Halothane on the Monolayers. Figure 3 shows the methylene and methyl symmetric stretch region of the spectrum for each headgroup before (solid squares) and after (solid triangles) the exposure of the monolayer to halothane. For each spectrum shown, there is a small increase in the overall intensity when halothane is present. This increase occurs across the entire spectrum for each of the phospholipids studied. Figure 4 shows an example of this effect for DPPC. We believe that the small change in intensity is due to the alteration in the index of refraction in the interfacial region due to the presence of halothane. In the TIR geometry used, the SF intensity is highly sensitive to the incident angles of the incoming beams that are in turn dependent on the index of refraction. The spectra are corrected for the indices of refraction of the two input beams and the outgoing SF beam based on the indices for the bulk liquids and cannot be corrected for the change in the index of the interface due to halothane because that change is unknown.

For all phospholipids shown in Figure 3, the CH_3SS and the CH_2SS peaks are affected by the introduction of halothane. Visual inspection of the spectra suggests that the order parameter increases in the presence of halothane with the CH_3SS intensity increasing more than that of the CH_2SS. However, verification of this increase in the order parameter requires fitting the data as previously described. When this is done, it is found that the order parameter does increase for each of the monolayers studied. For DPPC, the order parameter increases from 0.62 to 0.74 with uncertainties on the order of 5%. DPPE shows a change from 0.67 to 0.71, DPPG from 0.65 to 0.76, and DPPS from 0.55 to 0.60 (Table 2). The results from monolayers composed of lipids with dimyristoyl chains instead of dipalmitoyl chains are qualitatively similar, also showing a slight increase in chain order (see Table 2). These results demonstrate that halothane has an effect on the interactions between phospholipid molecules in the monolayer leading to changes in the average molecular conformation. The effect is small, suggesting that halothane is not strongly bound to the monolayer. The interaction could be local, a particular binding site, for example, or nonlocal, that is, the halothane just takes up

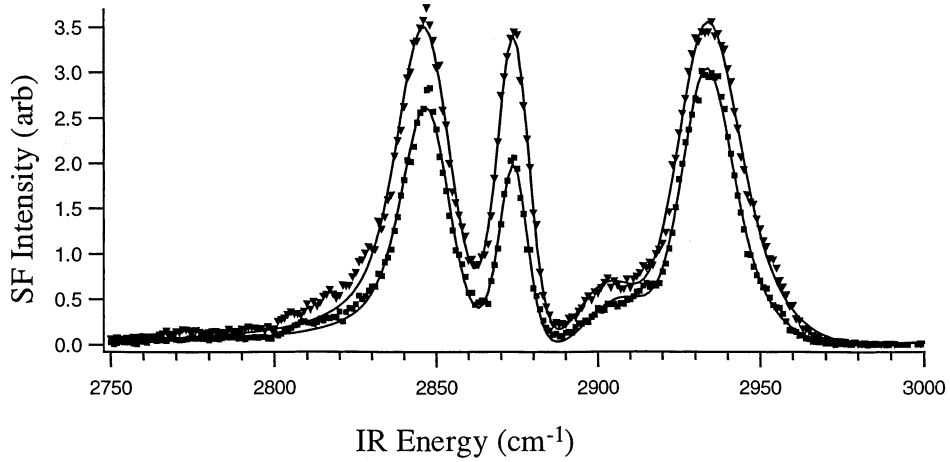

Figure 4. Representative spectra showing the increase in SF intensity across the entire spectrum after halothane (solid triangles) is added to the lipid monolayer (solid squares) as described in the text. The spectra shown are for DPPC, and the solid lines are fits to the data.

Table 2. Order Parameter for Acyl Chains in Monolayers[a]

	monolayer	monolayer with halothane
DPPC	0.62	0.74
DPPE	0.67	0.71
DPPG	0.65	0.76
DPPS	0.55	0.60
DMPE	0.60	0.77
DMPG	0.69	0.75

[a] Uncertainties on the order of 5%.

space forcing the chains to order due to steric effects. These ideas will be explored more in the next section.

The general trend of increased chain order in the presence of halothane observed here is different from the results of recent NMR experiments of and molecular dynamics calculations concerning DPPC bilayers (see ref 2 and references therein) where a net decrease in order was seen. However, one must be careful when making direct comparisons between this work and previous work in the literature based solely on changes in the order parameters. The order parameter utilized in the studies presented here is very sensitive to the average orientation of the terminal methyl group and does not distinguish between different parts of the acyl chain undergoing conformational changes (indeed, different parts of the chain may be affected in opposite ways). The NMR and MD studies did observe an increase in order in the chains near the carbonyls and a decrease in order toward the end of the chain. In the NMR and molecular dynamics work, the order parameter utilized is sensitive to the orientation of individual methylene units in the acyl chains with respect to the interface normal and completely ignores the methyl group orientation. In addition to the very different order parameters utilized in the studies, the lipid systems under study are quite different as well. The NMR and MD studies examined phosphocholine bilayers that were free to expand and contract in all three dimensions, whereas the monolayers studied here are confined to a fixed area. Expansion (which is seen in the MD simulation) is unlikely in the monolayer since there is a large barrier to desorption of monomers from the monolayer into the bulk aqueous solution of more than 60 kJ/mol.[28,34] There is also a large entropic barrier to desorption from the interface as well since considerable reorganization of water molecules would be required to accommodate the acyl chains. In these respects, the monolayer does not behave like half of a bilayer; however, in both the monolayer and the bilayer halothane perturbs acyl chain order.

Since chain order could be affected indirectly through halothane interactions with the phospholipid headgroup, the second objective of this study was to determine whether the headgroup has a significant effect on chain ordering induced by halothane. The distributions of charge in the headgroups of the lipids studied here are quite varied, suggesting that any interactions between halothane and the phospholipid headgroup would be quite varied as well (Figure 1). The ethanolamine and choline are zwitterions with the ethanolamine being less hydrophilic and having a more localized positive charge than the choline. The glycerol has a negative charge localized on the phosphate, and the serine exhibits the zwitterion of the ethanolamine in addition to a negative charge on the carboxyl group adjacent to the amine. Considering the significant differences in the polarities of these headgroups, the fact that each monolayer was affected in a qualitatively similar manner suggests that the mechanism of halothane action is not strongly associated with the headgroup or the headgroup/water interface. However, the present study does not preclude the glycerol backbone as the site of interaction, which has been suggested as the probable site in the molecular dynamics study of the DPPC bilayer referred to above.

Conclusions

These VSF studies demonstrate the feasibility of studying lipid monolayers at liquid/liquid interfaces to provide insight into the role played by anesthetics in the alteration of membrane structure. VSFS has been employed to measure the spectra of a series of saturated, symmetric diacyl phospholipids (DPPC, DPPE, DPPG, and DPPS) at the CCl_4/D_2O interface, to examine their acyl chain ordering and to examine the effect of halothane on that ordering. The four lipids are spectroscopically similar, and they display similar degrees of chain order. It was determined that halothane perturbs the acyl chains, leading to a slight increase in chain order for each of the lipid monolayers studied. Additionally, the effect of halothane on the magnitude of the ordering induced in the acyl chains does not appear to be affected by the constitution of the lipid headgroup. These findings suggest that the lipid could be the site of halothane action, and

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if it is, that the action is manifested in the phosphatidylglycerol backbone or in the acyl chains.

The subtle changes in monolayer conformation noted here suggest that the hypothesis presented by Cantor that small perturbations to membrane structure could be responsible for anesthetic action is a reasonable one.[7,8] In that work, it was shown that perturbations in the membrane would result in large changes in the lateral stresses in different parts of the membrane because the stresses themselves vary greatly in magnitude as a function of position along the bilayer normal. Although the changes in the stresses are small relative to the stresses themselves, the absolute magnitude of the change in the force felt by a transmembrane protein may be large enough to affect the function of the protein.

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