Pressure-Induced Correlation Field Splitting of Vibrational Modes: Structural and Dynamic Properties in Lipid Bilayers and Biomembranes[1]

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ABSTRACT Correlation field splittings of the vibrational modes of methylene chains in lipid bilayers, isolated lipid molecules in perdeuterated lipid bilayers, crystalline lipid, and interdigitated lipid bilayers have been investigated by pressure-tuning Fourier-transform infrared spectroscopy. The correlation field splittings of these modes are originating from the vibrational coupling interactions between the fully extended methylene chains with different site symmetry along each bilayer leaflet. The interchain-interactions of the methylene chains with the same site symmetry only contribute to frequency shift of the vibrational modes. The magnitude of the correlation field splitting is a measure of the strength of the interchain-interactions, and the relative intensities of the correlation field component bands provide information concerning the relative orientation of the zig-zag planes of the interacting methylene chains. It has been demonstrated in the present work that the correlation field splitting of the CH_2 bending and rocking modes commonly observed in the vibrational spectra of lipid bilayers is the result of the intermolecular interchain-interactions among the methylene chains of the neighboring molecules. The intramolecular interchain-interactions between the sn-1 and sn-2 methylene chains within each molecule are weak. The correlation field splitting resulting from the intramolecular interchain-interactions exhibits a much smaller magnitude than that from the intermolecular interchain-interactions and is observed only at very high pressure. Interdigitation of the opposing bilayer leaflets disturbs significantly the intermolecular interchain-interactions and results in dramatic changes in the pressure profiles of the correlation field component bands of both the CH_2 bending and rocking modes. The relative intensities of the correlation field component bands of these modes and the magnitude of the splitting are also altered significantly. These results provide further evidence that the correlation field splitting of the CH_2 bending and rocking modes in the vibrational spectra of lipid bilayers is due to the intermolecular interchain-interactions. The present work has also demonstrated that the correlation field splitting of the vibrational modes in lipid bilayers is mainly contributed by the intermolecular interchain-interactions among the nearest neighboring molecules and that the long-range correlation interactions beyond the second neighboring molecules are insignificant.

INTRODUCTION

Pressure-tuning vibrational spectroscopy was first introduced to the study of structural and dynamic properties in biological systems from our laboratory about one decade ago (Wong, 1982). We have studied various biological systems ranging from aqueous biomolecular assemblies to intact tissues. One of our efforts has been the search for spectral features and their pressure dependences, which are related to the structural and dynamic properties in biological systems. Pressure-induced correlation field splitting of the vibrational modes of the methylene chains in lipid bilayers and biomembranes is one of these parameters. This spectral parameter has been used widely to monitor various structural and dynamic properties of a wide range of aqueous lipid bilayers and biomembranes. These structural and dynamic properties are: (i) large angle reorientational fluctuations (Auger, 1988; Choma, 1992; Tupper, 1992; Wong, 1988); (ii) interchain packing (Siminovitch, 1987a; Wong, 1982, 1984a, 1989a); (iii) interchain and intrachain configuration distortion (Wong, 1985a); (iv) mechanism for the formation of the lamellar subgel phase (Wong, 1986); (v) interdigitation (Auger, 1988; Siminovitch, 1987b, 1987c; Wong 1989b); (vi) interchain interactions (Hubner, 1990; Siminovitch, 1987b; Tupper, 1992); (vii) unsaturation induced changes in molecular configurations in lipid bilayers (Siminovitch, 1987a, 1987d, 1988; Wong, 1988); (viii) the effects of anesthetics (Auger, 1988, 1990), toxins (Ahmed, 1992; Zakim, 1990), drugs (Popovic, 1992; Taylor, 1992), alkanes (Wong, 1990), cholesterol (Wong, 1989a, 1989c), polypeptides (Carrier, 1990), proteins (Gicquaud, 1992; Philp, 1990), fluorescent probes (Chong, 1989, 1992), and other exogenous molecules (unpublished work from this laboratory) on the structural and dynamic properties in the bilayer interior of various lipids, and vice versa; (ix) the location and binding sites of these exogenous molecules in lipid bilayers (Ahmed, 1992; Auger, 1988, 1990; Carrier, 1990; Chong, 1989, 1992; Gicquaud, 1992; Philp, 1990; Popovic, 1992; Taylor, 1990; Wong, 1989a, 1989c, 1990; Zakim, 1990); and (x) changes in the structural and dynamic properties in biomembranes of whole cells and tissues with various diseases and conditions (Rigas, 1990; Wong, 1991a, 1991b, 1993a). Results from these studies have been summarized in several review articles (Wong, 1984b, 1987a, 1987b, 1987c, 1993b).

Correlation field splitting of the vibrational modes in solid n-alkanes and polyethylene has been studied in great detail in the literature (Snyder, 1961, 1979; Tasumi and Shimanouchi, 1965; Boerio and Koenig, 1970; Wu, 1974; Kobayashi

Received for publication 5 November 1993 and in final form 13 January 1994.

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[1]Issued as NRCC No. 37211.

0006-3495/94/05/1505/10 $2.00

et al., 1980; Snyder et al., 1992). According to the oriented gas model with the assumption that the transition moment of each of the coupled rocking modes among neighboring methylene chains in n-alkanes can be considered as a linear combination of the unperturbed transition moments of the neighboring chains, Snyder (1961) has suggested that the integrated intensities of the two CH_2 rocking correlation field component bands $\tau'CH_2$ and τCH_2 can be related to the angle θ between the methylene zig-zag plane and the a axis of the unit cell by $I_{\tau'}/I_{\tau} = \tan^2\theta$, where $I_{\tau'}$ and I_{τ} represent the integrated intensity of the high-frequency $\tau'CH_2$ component band and that of the low-frequency τCH_2 component band, respectively.

In our laboratory, we have also systematically studied pressure effects on correlation field splitting of the vibrational modes in solid n-alkanes and isolated n-alkanes (Wong et al., 1987; 1991c; Wong and Zakim, 1990). We have found that the correlation field interactions of the methylene chain in solid n-alkanes were considerably different from those in aqueous lipid bilayers due to the presence of a large head group and two methylene chains in each lipid molecule. For single chain lipids, they formed interdigitated structure (Siminovitch et al., 1987c) and, thus, their correlation field interactions were also different from those in solid n-alkanes. The main difference is that the correlation field splitting in solid n-alkanes is usually associated with a first order phase transition and exhibits a discontinuity, whereas that in aqueous lipid bilayers is increased gradually with pressure and is not associated with a phase transition.

When lipid molecules are dispersed into water, they form two-dimensional ordered lamellar bilayers. According to the vibrational spectra of inorganic crystals and crystalline n-alkanes with long methylene chains (Mitra, 1964; Snyder, 1961), it is certain that the correlation field splitting of the vibrational modes of methylene chains in the spectra of the two-dimensional ordered lipid bilayers is the result of the vibrational coupling interactions among the ordered methylene chains with different site symmetry in the two-dimensional matrix. However, the basic theory and the characteristics of these interchain-interactions in lipid bilayers still need to be established. In the common membrane lipids, there are two hydrocarbon chains in each lipid molecule. The vibrational modes of these two intramolecular chains would certainly interact with each other. It is unknown whether the interchain-interactions that result in the correlation field splitting take place within each lipid molecule (intramolecular interchain-interactions) or between neighboring molecules in the lamellar bilayers (intermolecular interchain-interactions). The relative contributions between the intramolecular and the intermolecular interchain-interactions to the correlation field splitting, and the effects of the long-range interchain-interactions and interdigitation on the correlation field splitting, are also unknown.

To address these problems, in the present work, the pressure-induced correlation field splitting in the infrared spectra of the following lipid bilayer systems are compared and analyzed: (i) aqueous bilayer dispersion of dimyristoylphosphatidylcholine (DMPC); (ii) isolated DMPC molecules in the bilayer matrix of perdeuterated DMPC; (iii) crystalline DMPC; and (iv) interdigitated phase of DMPC.

DMPC was chosen for this study because its crystal structure and the precise orientations of the sn-1 and sn-2 acyl chains in the molecule are well established (Hauser, 1981; Pearson, 1979). Because the conformation of the glycerol moiety, and thus the orientations of the sn-1 and sn-2 acyl chains in aqueous bilayers, is about the same as that in the solid state (Hauser, 1981), the magnitude of the intramolecular and intermolecular correlation field interactions in the crystalline DMPC can be used to estimate those in the aqueous bilayers. Moreover, the interdigitated phase of DMPC can be readily prepared by inserting exogenous molecules such as Tetracaine into the bilayer matrix (Auger, 1988).

EXPERIMENTAL

DMPC and perdeuterated DMPC-d_{54} were purchased from Avanti Polar Lipids (Birmingham, AL). Tetracaine hydrochloride was obtained from Sigma Chemical Co. (St. Louis, MO). All other materials were of analytical grade.

The solid DMPC/DMPC-d_{54} mixtures (1:1 and 1:2 molar ratio) were prepared by co-dissolving the solid components in chloroform and drying the solutions with nitrogen gas. The solid mixture samples were then dispersed into water and lyophilizing for 48 h. The dispersing and lyophilizing procedure was repeated twice. Fully hydrated DMPC bilayers and DMPC/DMPC-d_{54} mixed bilayers were prepared by dispersing about 50 mg of solid lipid in a Tris buffer made with D_2O 24 h before analysis. The hydrated DMPC was then concentrated by centrifugation to approximately 40% (w/w). For interdigitated DMPC dispersion, the lipids were hydrated with a buffer made with D_2O and containing 5 mg of tetracaine. The lipid dispersions were subjected to at least five freeze-thaw cycles. Anhydrous crystalline DMPC samples were prepared by loading the optical cell with crystalline DMPC, inserting the optical cell into the sample compartment of the infrared spectrophotometer, and purging with dry nitrogen gas for about 72 h. The optical cell was then sealed by closing the space between the diamond anvils and the gasket. The relative intensity of the H_2O stretching band to the lipid CH_2 symmetric stretching band was used to monitor the water content of the samples.

Small amounts (typically 0.1 mg) of the lipid samples were placed at room temperature, together with powdered α-quartz, into a 0.45 mm diameter hole in a 0.23 mm thick stainless steel gasket mounted on a diamond anvil optical cell, as described previously (Wong, 1985).

Infrared spectra were collected on a Digilab FTS-40A spectrophotometer with a mercury cadmium telluride detector. The infrared beam was condensed by a sodium chloride lens system onto the diamond anvil cell. For each spectrum, 512 scans were coadded, at a spectral resolution of 4 cm^{-1}. Pressures on the sample were determined from the 695 cm^{-1} phonon band of α-quartz (Auger, 1988; Wong, 1985). The vibrational frequencies were determined from the third-power derivative spectra (Cameron, 1984). All of the data analysis, including the determination of the correlation field splitting pressure, was made with the software developed by the Molecular Spectroscopy Laboratory at the National Research Council of Canada.

THEORY

In lipid bilayers, the vibrational interchain-interactions between the opposing bilayer leaflets are extremely weak. Therefore, only the correlation field interchain-interactions among the methylene chains within each bilayer leaflet are considered here. In an ordered zig-zag methylene chain, the translational repeat unit along the chain is C_2H_4, and each repeat unit contains two CH_2 chemical groups. Most of the observed bands in the infrared and Raman spectra of methylene chains are arising from the in-phase normal modes of

the C_2H_4 groups along each chain. Therefore, the in-phase vibrational modes of the C_2H_4 groups will be treated in this theory.

The potential function of each internal vibration of C_2H_4 groups in an isolated methylene chain j is

$$V_j = f_j q_j^2 \tag{1}$$

where q_j is the C_2H_4 normal coordinate of the jth methylene chain and f_j is the force constant of the normal mode in an isolated chain. In terms of the normal coordinate q_j, the potential energy of an internal vibration of all the methylene chains in each lipid bilayer leaflet is given by:

$$U = \sum_{j,j'} F_{jj'} q_j q_{j'} = \sum_j F_j q_j^2 + \sum_{j,j' \neq j} F_{jj'} q_j q_{j'} \tag{2}$$

F's are the force constants of each methylene normal mode for the entire bilayer leaflet and thus $F_j \neq f_j$. Combining Eqs. 1 and 2, one has

$$U = \sum_j V_j + \sum_j (F_j - f_j) q_j^2 + \sum_{j,j' \neq j} F_{jj'} q_j q_{j'} \tag{3}$$

The second and third terms in this equation represent the correlation field perturbation on the intrachain vibration. The second term in Eq. 3 expresses that a frequency shift of $1/2\pi(F_j - f_j)^{1/2}$ is resulted from the lowering of the molecular symmetry of the chains to the sites symmetry. The third term in Eq. 3 represents the coupling of the normal mode among different chains. The interchain correlation forces are the repulsion forces, hydrogen bonding, London forces, and Coulomb forces.

According to Eq. 3, the internal potential energy of the total bilayer leaflet is that of all the isolated chains plus a perturbation $\tilde{U}$. Namely,

$$U = \sum_j V_j + \tilde{U} \tag{4}$$

$$\tilde{U} = \sum_j (F_j - f_j) q_j^2 + \sum_{j,j' \neq j} F_{jj'} q_j q_{j'} \tag{5}$$

When the perturbation potential $\tilde{U}$ is expressed as a sum over pairs of chains,

$$\tilde{U} = \tfrac{1}{2} \sum_{jm} U_{jm} \tag{6}$$

it can be shown by the first order perturbation theory (Davydov, 1962; Hexter, 1960) that the energy levels of the excited states split into n branches, where n is the number of nonequivalent chains in the repeat unit along the bilayer leaflet. The energy difference between the spectrally excited state (E) and the ground state (E^0) is given by

$$E - E^0 = \epsilon - \epsilon^0 + \frac{h}{8\pi^2 c v_0} \sum_j^{nN} \left(\frac{\partial^2 U_{jm}}{\partial q_m^2} \right)_0 + \frac{nh}{8\pi^2 c v_0} \sum_{x_{j_x}}^{nN} B^*_{\alpha a} B_{\alpha x} \left(\frac{\partial^2 U_{j_x m}}{\partial q_{j_x} \partial q_m} \right)_0 \tag{7}$$

where ν_0 is the harmonic frequency of a C_2H_4 normal mode, $\epsilon - \epsilon^0 = h\nu_0$, and N is the total number of repeat units in each bilayer leaflet. Chain m is on site a, and chain j_x is on site x; x runs through $a, b, \ldots, n$ number of nonequivalent sites in a repeat unit. B is the transformation matrix between the symmetric coordinates of the chain repeat unit and the C_2H_4 normal coordinates of the isolated chains at the nonequivalent sites of the repeat unit along the bilayer leaflet. For a lipid bilayer leaflet with two nonequivalent chains per repeat unit ($n = 2$), the B matrix is

$$B = \begin{bmatrix} B_{\alpha a} & B_{\alpha b} \\ B_{\beta a} & B_{\beta b} \end{bmatrix} = \begin{bmatrix} 1 & 1 \\ 1 & -1 \end{bmatrix} \tag{8}$$

For $n = 2$, the exciton level splits into two branches (α and β), and thus there are two repeat unit modes for each intrachain vibrational mode. By substituting Eq. 8 into Eq. 7, one may obtain

$$v_\alpha = v_0 + \frac{1}{8\pi^2 c v_0} \left[\sum_j^{2N} \left(\frac{\partial^2 U_{jm}}{\partial q_m} \right)_0 + \sum_{j_a}^{N} \left(\frac{\partial^2 U_{j_a m}}{\partial q_{j_a} \partial q_m} \right)_0 + \sum_{j_b}^{N} \left(\frac{\partial^2 U_{j_b m}}{\partial q_{j_b} \partial q_m} \right)_0 \right] \tag{9}$$

$$v_\beta = v_0 + \frac{1}{8\pi^2 c v_0} \left[\sum_j^{2N} \left(\frac{\partial^2 U_{jm}}{\partial q_m^2} \right)_0 + \sum_{j_a}^{N} \left(\frac{\partial^2 U_{j_a m}}{\partial q_{j_a} \partial q_m} \right)_0 - \sum_{j_b}^{N} \left(\frac{\partial^2 U_{j_b m}}{\partial q_{j_b} \partial q_m} \right)_0 \right] \tag{10}$$

where

$$v_{\alpha,\beta} = \frac{1}{h} (E_{\alpha,\beta} - E^0)$$

Consequently, the exciton level splits into two branches (α, β). The splitting of each normal vibrational mode into α and β components in the bilayers is the so called correlation field splitting, which is

$$v_\alpha - v_\beta = \frac{1}{4\pi^2 c v_0} \sum_{j_b}^{N} \left(\frac{\partial^2 U_{j_b m}}{\partial q_{j_b} \partial q_m} \right)_0 \tag{11}$$

The frequency shift of each normal mode of the isolated chain in the bilayers is

$$1/2(v_\alpha + v_\beta) - v_0 = \frac{1}{8\pi^2 c v_0} \left[\sum_j^{2N} \left(\frac{\partial^2 U_{jm}}{\partial q_m^2} \right)_0 + \sum_{j_a}^{N} \left(\frac{\partial^2 U_{j_a m}}{\partial q_{j_a} \partial q_m} \right)_0 \right] \tag{12}$$

U_{jm} is a function of the interchain distance and the relative orientation of the methylene chains in lipid bilayers, which are obviously varied with pressure. Therefore, both frequency shift and correlation field splitting are expected to be pressure-dependent. By following their modification induced by pressure, one can monitor the structural changes in lipid bilayers. Any discontinuity of the relative orientation of the chains and of the interchain distances across the liquid crystal/gel and the gel/gel phase transitions may be detected

from the discontinuity in the frequency-pressure plot. Thus, the pressure dependence of frequency will provide useful information about the existence and the nature of a pressure-induced phase transition.

If the orientation of all the methylene chains in a bilayer system is parallel to each other, the fourth term in Eqs. 10 and 11 and also the $\nu_\alpha - \nu_\beta$ value in Eq. 12 will be equal to zero. Consequently, there will be no correlation field splitting in the methylene vibrational modes. Under the following two circumstances, the correlation field splitting will be also absent in the spectra: (i) the conformation of the methylene chains is highly disordered due to the presence of a large number of gauche bonds, and thus the coupling of the vibrational modes between neighboring chains is random and weak; (ii) the methylene chains are conformationally highly ordered and fully extended, but the orientation of these fully extended chains is disordered due to reorientational fluctuations and the torsion/twisting motions of the chains, which is usually observed at low-pressure or high-temperature. In these cases, only broadening rather than correlation field splitting in the vibrational bands of methylene chains will be observed.

It has been demonstrated that the conformational disorder, the reorientational fluctuations, and the torsion/twisting motions of the methylene chains in lipid bilayers can be ordered and dampened by an external pressure (Wong, 1984b, 1987a, 1987b, 1987c, 1993b). At high enough pressure, these disordered structures can be removed, and thus the correlation field splitting in the vibrational modes of the methylene chains would appear in the spectra provided that the equilibrium orientations of neighboring chains are nonequivalent. For orientationally more disordered chains, a higher pressure is required to stop these fluctuations and motions, and thus the correlation field splitting pressure at which the splitting appears is higher. Consequently, the order/disorder dynamics of the methylene chains in lipid bilayers can be determined by the magnitude of the correlation field splitting pressure. Moreover, the magnitude of the correlation field splitting is a measure of the degree of interchain-interactions in lipid bilayers.

RESULTS AND DISCUSSION

Pressure-induced correlation field splitting of the CH_2 bending mode (δCH_2) and the CH_2 rocking mode (τCH_2) of the methylene chains has been used most commonly to monitor the structural and dynamic properties in lipid bilayers and biomembranes (Wong, 1984b, 1987a, 1987b, 1987c, 1993b). Fig. 1 *A* shows the pressure contour of the infrared spectra of the CH_2 bending mode of aqueous DMPC bilayers. At atmospheric pressure, the infrared spectrum in this region consists of a central band at 1467 cm^{-1}, two shoulders at 1479 and 1490 cm^{-1} on the high-frequency side and one shoulder at 1457 cm^{-1} on the low-frequency side of this central band. The assignment of the infrared bands in this region is well established (Cameron, 1982; Fringeli, 1981). The central band at 1467 cm^{-1} is the out-of-phase δCH_2

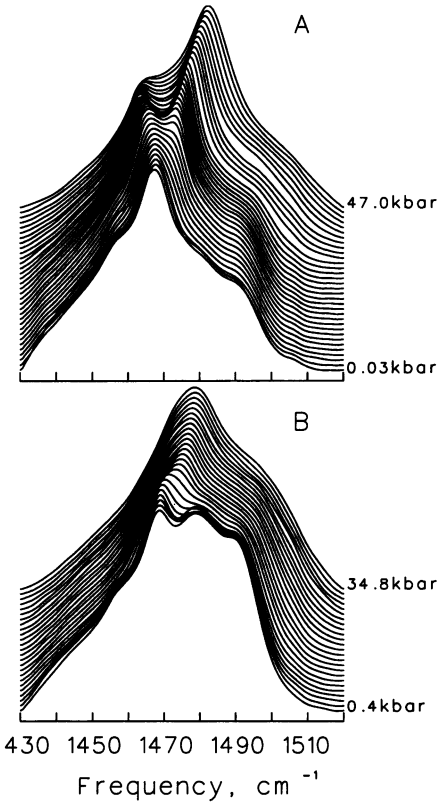

FIGURE 1 Stacked contour plots of the infrared spectra in the δCH_2 region of the aqueous DMPC bilayers (*A*) and the isolated DMPC in perdeuterated DMPC-d_{54} bilayers (*B*).

mode of the CH_2 groups in the methylene chains, the shoulders at 1490 and 1479 cm^{-1} are due to the symmetric and asymmetric CH_3 bending modes (δCH_3) of the choline methyl groups, respectively, and the shoulder at 1457 cm^{-1} is mainly due to the bending mode of the gauche CH_2 groups in the methylene chains. The band at 1457 cm^{-1} is also contributed by the asymmetric bending mode of the end methyl group. However, this contribution is extremely small as evident from the extremely week intensity of this band in the spectra of fully extended hydrocarbon chains of DMPC at high pressure (see Fig. 1 *A*). It is clear from Fig. 1 *A* that the δCH_2 mode of the methylene chains splits into two at high pressure, and the intensity of the correlation field component band is increased with increasing pressure. A phase transition in aqueous DMPC bilayers from the liquid crystalline phase to the gel phase is induced by a pressure of 0.2 kb at ambient temperature (28°C) (Wong, 1982). The first spectrum in Fig. 1 *A* was obtained at 0.03 kb and 28°C. Therefore, this is a spectrum of the liquid crystalline phase. In this spectrum, all the bands and shoulders are broader and the intensity of the gauche CH_2 bending band is stronger than those of the gel phase (second spectrum and up in Fig. 1 *A*). Band broadening is also observed in the other regions of the infrared spectrum of the liquid crystalline phase. These results are due to the conformational disorder of the methylene chains in the liquid crystalline phase with a large number of C-C gauche bonds (Wong, 1987).

Intermolecular and intramolecular correlation

To determine the contribution of the correlation field interactions between the neighboring methylene chains within each lipid molecule to the correlation field splitting (intramolecular correlation), the pressure profile of the infrared spectra in the CH_2 bending region of the isolated DMPC molecules in the bilayer matrix of perdeuterated DMPC-d_{54} molecules was measured (see Fig. 1 *B*). The relative intensities of the shoulder bands from the choline methyl groups at 1479 and 1490 cm^{-1} in these spectra are much higher than those of the pure DMPC bilayers in Fig. 1 *A* because the choline methyl groups are not deuterated in the perdeuterated DMPC-d_{54} samples. The CD_2 bending mode of the methylene chains of DMPC-d_{54} is shifted to lower than 1430 cm^{-1} by the mass effect, which is outside the frequency region in Fig. 1 *B*. Therefore, the 1467 cm^{-1} band in Fig. 1 *B* is due to the δCH_2 mode of the isolated DMPC. It is clear from Fig. 1 that the pressure behavior of the δCH_2 band of the isolated DMPC molecules (Fig. 1 *B*) is considerably different from that of the nonisolated DMPC molecules (Fig. 1 *A*). In the spectra of the isolated DMPC, the δCH_2 band exhibits as a singlet up to 35.7 kb and only slight broadening of the δCH_2 band is induced at high pressure.

The frequencies of the δCH_2 bands of the nonisolated DMPC and the isolated DMPC are plotted as a function of pressure in Fig. 2. In the nonisolated DMPC bilayers, the δCH_2 band splits into two at 3.5 kb, and the magnitude of the splitting increases gradually with increasing pressure. The frequency of the low-frequency component band decreases gradually and starts to increase slightly above 9.2 kb with increasing pressure. The frequency of the high-frequency component band increases linearly with increasing pressure above 9.2 kb. The frequency of the gauche δCH_2 band at 1457 cm^{-1} decreases with increasing pressure above 3.5 kb. This band disappears at 9.2 kb and is undetectable even in the third power derivative spectra. These results indicate that the methylene chains in DMPC bilayers become fully extended and the gauche C-C bonds are completely removed at 9.2 kb. Therefore, the smaller magnitude of the correlation field splitting below 9.2 kb is due to the presence of gauche bonds in the methylene chains. As pressure increases, these disordered gauche bonds are gradually removed. Thus, the magnitude of the correlation field splitting increases gradually. Above 9.2 kb these gauche bonds are completely removed, and the pressure-induced frequency shifts of the correlation field component bands become linear. The increase in the magnitude of the correlation field splitting above 9.2 kb is the result of the pressure-enhanced interchain-interactions. As shown in Eq. 12, the interchain-interactions among the orientationally equivalent chains will contribute to an increase in the mode frequency. Pressure will enhance these interchain-interactions and, thus, increase the mode frequency. This increase in frequency with increasing pressure is observed for both the correlation field component bands in DMPC bilayers above 9.2 kb.

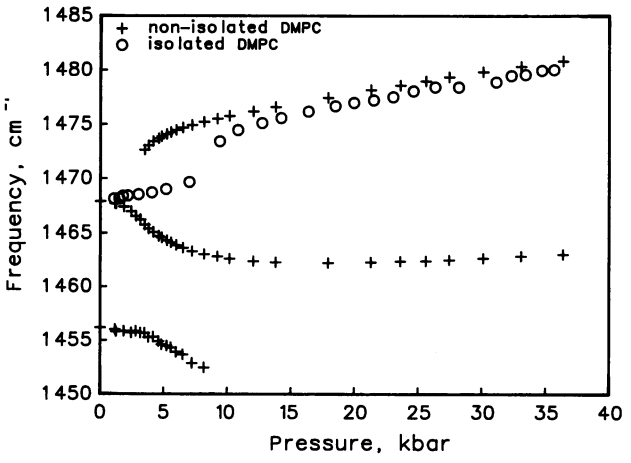

FIGURE 2 Pressure dependences of the frequencies of the δCH_2 modes for aqueous DMPC bilayers and the isolated DMPC in perdeuterated DMPC-d_{54} bilayers.

The frequencies in Fig. 2 were measured from the third power derivative spectra with a break point of 0.9 (Cameron, 1984). Even in the third power derivative spectra, the correlation field component band of the isolated DMPC is not detectable at all pressures up to 40 kb. However, a discontinuity in the pressure range near 9.4 kb is observed in the pressure dependence of the δCH_2 frequency of the isolated DMPC (Fig. 2). This discontinuity pressure almost coincides with the gauche/trans transformation pressure (9.2 kb) observed in the nonisolated DMPC bilayers. At this pressure the gauche bonds are completely removed from the methylene chains, and the conformation and orientation of the methylene chains become highly ordered. Therefore, the discontinuity in the pressure dependence of the δCH_2 frequency in the isolated DMPC at 9.4 kb is the result of disorder/order transition of the orientational and the conformational structure of the methylene chains in the isolated DMPC molecules.

It is evident from Figs. 1 and 2 that the correlation field interactions are insignificant among the isolated DMPC molecules and that the correlation field splitting observed in the infrared spectra of the nonisolated DMPC bilayers is mainly contributed by the intermolecular interchain-interactions among neighboring lipid molecules rather than the intramolecular interchain-interactions within individual lipid molecules.

It has been shown recently (Snyder et al., 1992) that a microphase segregation occurred in solid solutions of certain binary isotope mixtures of n-alkanes. As evident from Fig. 1 *B*, the scissors triplet and the correlation field component bands corresponding to those in the spectra of solid n-alkane mixtures (Snyder et al., 1992) and of the pure DMPC dispersions (Fig. 1 *A*) are not observed in the spectra of the DMPC/DMPC-d_{54} mixture up to 34.8 kb. The spectra in Fig. 1 *B* are reproducible and are comparable with the spectra of the DMPC/DMPC-d_{54} mixture with 1:2 molar ratio. Moreover, as shown by the recent study on microdomains in binary n-alkane solid solutions (Snyder et al., 1992), the segregation rate of the crystalline n-alkane mixtures decreased

dramatically as the chain-length difference between the components was decreased. In the C_{30}^{H}/C_{36}^{D} mixture, the observable changes in the spectra due to the segregation only took place 21 h after the mixture sample had been prepared. In our pressure experiments, each pressure run was completed within 4 h. Therefore, it is certain that there is no demixing domain in our samples of the DMPC isotope mixture. Our results, in turn, demonstrate that the phase behavior of aqueous dispersions of lipid isotope mixture is significantly different from that of binary n-alkane solid solutions.

Correlation interactions in crystalline DMPC

X-ray single crystal studies of DMPC (Hauser, 1981; Pearson, 1979) have shown that the unit cell contains four DMPC molecules arranged tail-to-tail in pair in a bilayer configuration. Each pair consists of two crystallographically independent molecules. The lateral arrangement of the four hydrocarbon chain planes of the two DMPC molecules in each monolayer resembles the hybrid chain packing mode (Abrahamsson, 1978). Consequently, the orientation of the chain planes of the methylene chains within each DMPC molecule is nearly perpendicular to each other, whereas the one between molecules is nearly parallel to each other.

The pressure contour of the infrared spectra of the δCH_2 mode in the crystalline DMPC is shown in Fig. 3 *A*. The pressure profile of the correlation field splitting of the δCH_2 mode in the crystalline DMPC is comparable with that in aqueous bilayers (Fig. 1 *A*) except for a slight difference in the relative intensities of the component bands at the corresponding pressures. The pressure dependences of the frequencies of the δCH_2 component bands in the crystalline DMPC are compared with those in the aqueous DMPC bilayers in Fig. 4. The correlation field splitting pressure at which the splitting of the δCH_2 band starts is about the same between these two DMPC samples. However, the magnitude of the splitting is much larger in the aqueous bilayers than in the crystal. It has been demonstrated that the molecular conformation of the glycerol backbone in DMPC crystal is essentially retained in aqueous DMPC bilayers and the intramolecular orientation of the hydrocarbon chain planes are largely determined by the conformation of the glycerol backbone (Hauser, 1981). Therefore, the intramolecular orientational structure in DMPC crystal may also be retained in aqueous DMPC bilayers. In this case, the intramolecular correlation field interactions within each DMPC molecule are expected to be comparable between the crystalline state and the aqueous bilayer state. The difference in the magnitude of the correlation field splitting between these two states of DMPC in Fig. 4 also indicates that this splitting is the result of the intermolecular interchain-interactions among the neighboring DMPC molecules.

Fig. 5 compares the pressure contours of the τCH_2 bands between the crystalline DMPC and the aqueous DMPC bilayers. The low-frequency correlation field band of the τCH_2 mode in the crystalline DMPC is extremely weak (see Fig. 5 *A*). This component band shifts from 729.3 cm^{-1} at 3.9 kb to 751.7 cm^{-1} at 44.4 kb. According to the oriented gas model (Snyder, 1961), the intensity ratio between the correlation field component bands of the τCH_2 mode is a measure of the relative orientations of the planes of the interacting methylene chains. If the correlation field splitting in DMPC is from the interactions between the methylene chains within each molecule, this intensity ratio would be close to 1 because of the nearly perpendicular orientation between the chain planes in each molecule. The peak intensity ratio of

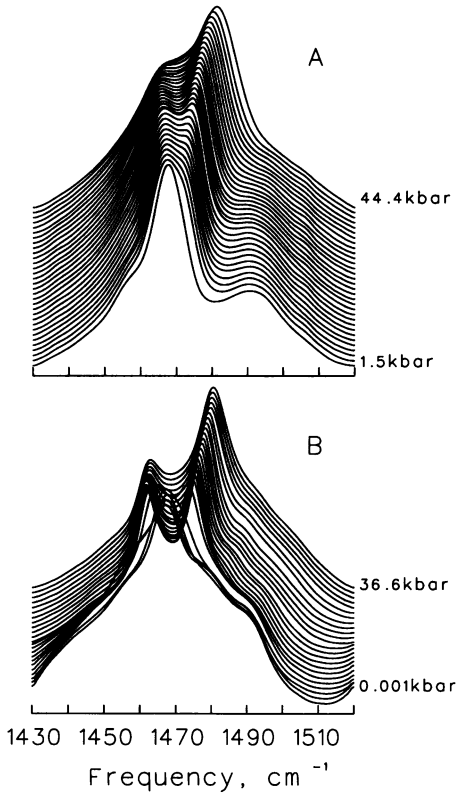

FIGURE 3 Stacked contour plots of the infrared spectra in the δCH_2 region of the crystalline DMPC (*A*) and the interdigitated DMPC (*B*).

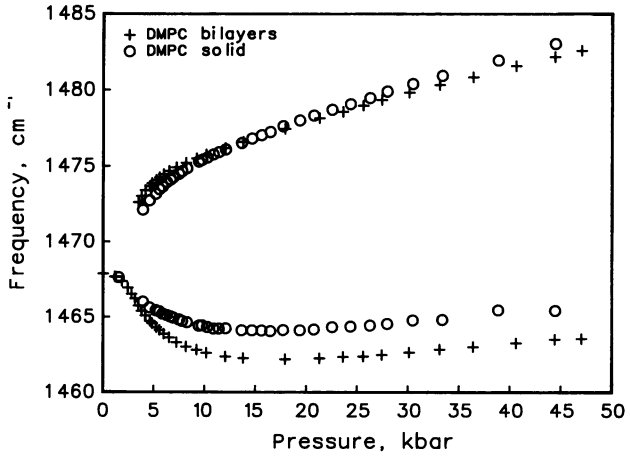

FIGURE 4 Pressure dependences of the frequencies of the δCH_2 modes for the crystalline DMPC and aqueous DMPC bilayers.

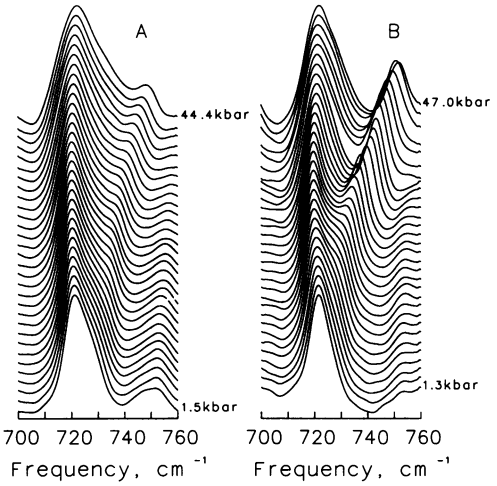

FIGURE 5 Comparison of the stacked contour plots of the infrared spectra in the τCH_2 region between the crystalline DMPC (*A*) and the aqueous DMPC bilayers (*B*).

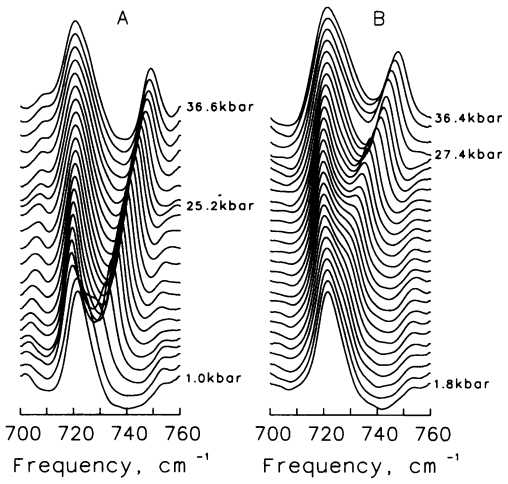

FIGURE 6 Comparison of the stacked contour plots of the infrared spectra in the τCH_2 region between the interdigitated DMPC bilayers (*A*) and the aqueous DMPC bilayers (*B*).

these component bands in the crystalline DMPC is extremely small. The maximum ratio, which is less than 0.2, is observed at 44.4 kb. This result further confirms that the correlation field splitting in the spectra of the crystal is from the intermolecular, rather than the intramolecular, interchain-interactions. The relatively low intensity ratio of the τCH_2 component bands of the aqueous DMPC bilayer shown in Fig. 5 *B* suggests that this correlation field splitting in aqueous DMPC bilayers is also from the intermolecular interchain-interactions.

The intensity ratio of the τCH_2 correlation field bands in the aqueous DMPC bilayers is 0.27 at 47 kb, which is slightly larger than that in the crystalline DMPC. Therefore, the intermolecular arrangement in the bilayer state is not exactly the same as that in the solid state.

Correlation interactions in the interdigitated bilayers

It has been demonstrated that in the presence of charged tetracaine DMPC molecules assemble into interdigitated bilayers (Auger, 1988; McIntosh, 1983). If the correlation field splitting is the result of the interchain-interactions among neighboring DMPC molecules, then the pressure profiles of the correlation field splitting of the δCH_2 and the τCH_2 modes in the interdigitated DMPC would be dramatically different from those of the noninterdigitated DMPC bilayers. The pressure profiles of the δCH_2 and the τCH_2 bands of the interdigitated DMPC are shown in Figs. 3 *B* and 6 *A*, respectively. The pressure profiles of the corresponding δCH_2 and τCH_2 bands of the noninterdigitated DMPC bilayers are given in Figs. 1 *A* and 6 *B*, respectively. It is evident from these figures that they are significantly different between the interdigitated and the noninterdigitated DMPC bilayers. The splitting of both the δCH_2 and τCH_2 bands in the spectra of the interdigitated DMPC into two well defined bands is more abrupt, and a pronounced "valley" between the correlation field component bands is observed. On the other hand, the correlation field component bands ($\delta' CH_2$ and $\tau' CH_2$) of the noninterdigitated DMPC bilayers appear as a broad shoulder on the high frequency side of the δCH_2 and τCH_2 bands and then they steadily gain intensity with increasing pressure. Moreover, the corresponding "valley" between the two component bands in the noninterdigitated DMPC bilayers is comparatively shallow. The ratios between the peak height of the correlation field component band ($\tau' CH_2$ and $\delta' CH_2$) and the height of the "valley" in the spectra of the τCH_2 and the δCH_2 bands are compared between the interdigitated and the noninterdigitated DMPC bilayers at various pressures in Fig. 7.

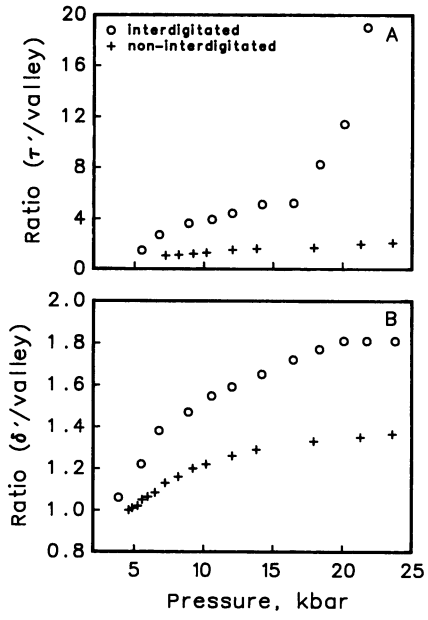

FIGURE 7 Pressure dependences of the ratio between the peak height of the $\tau' CH_2$ band and the "valley" (*A*) and the ratio between the peak height of the $\delta' CH_2$ band and the "valley" (*B*) for the interdigitated and noninterdigitated DMPC bilayers.

These ratios of both the τCH_2 and δCH_2 modes are larger at all pressures in the interdigitated DMPC. Moreover, the $\tau' CH_2/\tau CH_2$ and $\delta' CH_2/\delta CH_2$ intensity ratios of the interdigitated DMPC are higher than those of the noninterdigitated DMPC at all pressures (Fig. 8). Therefore, these ratios can be considered as parameters for the determination of the presence of interdigitation in lipid bilayers.

Fig. 9 shows the pressure dependences of the frequencies of the δCH_2 correlation field component bands of the interdigitated DMPC. It is evident from this figure that the correlation field splitting is more abrupt and that the magnitude of the splitting is larger in the interdigitated DMPC at all pressures. The larger splitting indicates that the packing of the neighboring methylene chains is tighter. The methylene chain packing within each DMPC molecule is mainly governed by the conformation of the glycerol moiety of the head group (Hauser, 1981; Pearson, 1979) and would not be significantly affected by interdigitation. On the other hand, the packing between neighboring molecules would certainly be strongly affected by the interdigitation. All of these results in the interdigitated DMPC bilayers also provide evidence that the correlation field interactions in lipid bilayers, which give rise to the splitting of the τCH_2 and δCH_2 modes, are predominated by the vibrational interactions among the methylene chains of the neighboring molecules.

Short-range and long-range correlation

In Fig. 1, the δCH_2 band of the isolated DMPC is broadened and becomes asymmetric at high pressure. This indicates that the δCH_2 band of the isolated DMPC consists of closely overlapping bands at high pressure. In the third power derivative spectra with an extremely high break point (0.98), this asymmetric δCH_2 band can be resolved into two bands at pressure above 30 kb (see Fig. 10). However, this splitting is much smaller than the intermolecular correlation field splitting in pure DMPC solid (Fig. 4), in aqueous DMPC dispersions (Fig. 10), or in the solid n-alkane domain (Snyder et al., 1992). Moreover, the component band near 1470 cm^{-1} in the isolated DMPC is also observed in the third power derivative spectra of the nonisolated DMPC bilayers with a break point of 0.98 at pressures above 25 kb (Fig. 10). Therefore, this small splitting in the δCH_2 band in the isolated DMPC is most likely the result of the short-range correlation field interactions between the two methylene chains in each DMPC molecule. The fact that the magnitude of this splitting is much smaller than that of the intermolecular correlation field interactions observed in the nonisolated DMPC bilayers suggests that the intramolecular correlation field interactions are much weaker than the intermolecular correlation field

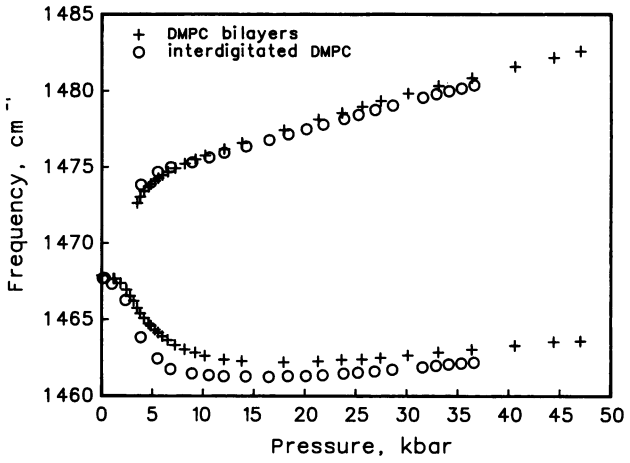

FIGURE 9 Pressure dependences of the δCH_2 modes for the interdigitated and noninterdigitated DMPC bilayers.

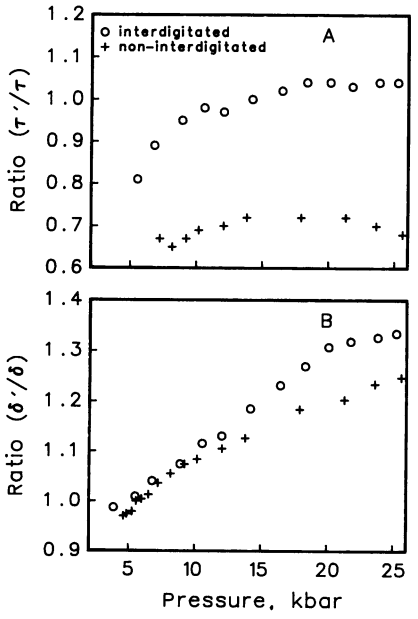

FIGURE 8 Pressure dependences of the peak height ratio between the $\tau' CH_2$ and the τCH_2 bands (A) and the peak height ratio between the $\delta' CH_2$ and the δCH_2 bands (B) for the interdigitated and noninterdigitated DMPC bilayers.

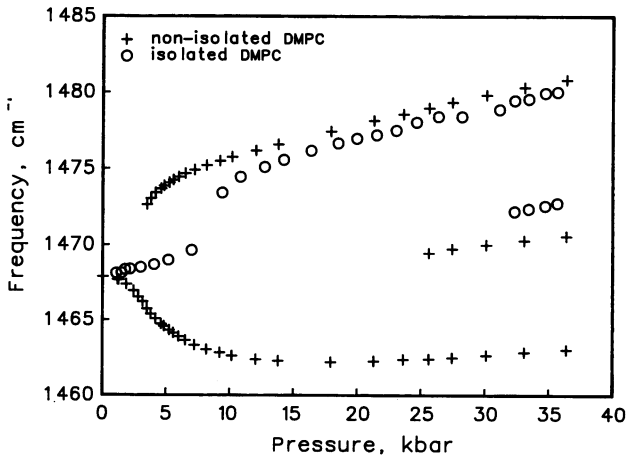

FIGURE 10 Pressure dependencies of the frequencies of the δCH_2 modes for aqueous DMPC bilayers and the isolated DMPC in perdeuterated DMPC-d_{54} bilayers obtained from the third power derivative spectra with a break point of 0.98.

interactions. The smaller magnitude in the intramolecular correlation field interactions is consistent with the molecular structure of the DMPC molecules (Hauser, 1981; Pearson, 1979). First, due to the restriction of the head group and the glycerol moiety in the DMPC molecules, the methylene groups of the two methylene chains in the fully extended DMPC molecules locate at different level with respect to the head group. Each methylene group of the sn-1 chain is at the position between two methylene groups of the sn-2 chain (Hauser, 1981; Pearson, 1979). Consequently, the distance between the correlated methylene groups in the two methylene chains within each DMPC molecule is large and the correlation field interactions among them are expected to be small. Second, in the isolated DMPC, only half of the methylene groups take place in the correlation field interactions between the two methylene chains in each DMPC molecule. The methylene groups on the far end of each zig-zag methylene chain in DMPC are too far to take part in the correlation field interactions. This certainly results in a smaller magnitude of intramolecular correlation field interactions. In the presence of neighboring DMPC molecules in the nonisolated DMPC bilayers, the above restrictions are removed, and all of the methylene groups in the methylene chains will take place in the intermolecular correlation field interactions.

The fourth term in Eqs. 9 and 10 includes the long-range correlation field interactions with the nth neighboring molecules. In the isolated DMPC dispersions in the present study, the molar ratio between DMPC and perdeuterated DMPC-d_{54} is 1:1. Therefore, in this isolated DMPC system, the first neighbors of each DMPC molecule are perdeuterated DMPC-d_{54} molecules, whereas the second neighbors are nonperdeuterated DMPC molecules. If the correlation field interactions are significant among the second or higher neighbors, there would be intermolecular correlation field splitting in the spectra of the isolated DMPC similar to that observed in the nonisolated DMPC bilayers shown in Fig. 2. The absence of this intermolecular correlation field splitting in the spectra of the isolated DMPC strongly suggests that the intermolecular correlation field interactions only take place among the nearest neighbors and that the long-range interactions with the second or higher neighboring molecules are insignificant.

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