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High pressure infrared spectroscopy of lipid bilayers: new tests for interdigitation *

D.J. Siminovitch, P.T.T. Wong and H.H. Mantsch

Division of Chemistry, National Research Council of Canada, Ottawa, Ontario K1A 0R6 (Canada)

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High-pressure infrared spectroscopy is used to compare the barotropic behaviour of various interdigitated lipid bilayer systems (1,2-di-*O*-hexadecyl-*sn*-glycero-3-phosphocholine, 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine and 1-palmitoyl-*sn*-glycero-3-phosphocholine) with non-interdigitated bilayers of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine. In the pressure range between 0 and 20 kbar, we have monitored the pressure dependence of the relative peak height intensity ratio of the chain methylene scissoring band, δCH_2, and its correlation field component band, $\delta' CH_2$. We demonstrate that this parameter, in conjunction with a visual inspection of the pressure-induced correlation field splittings of the methylene scissoring and rocking mode bands, can provide reliable indications of chain interdigitation.

Recently, there has been considerable interest in phospholipids which, either spontaneously [1–10], or in the presence of inducing agents such as glycerol [11–13], protein [14] or ions [15], assemble into interdigitated bilayers. The degree of interdigitation, which may range from complete in 1-octadecyl-2-methyl-phosphatidylcholine [9], involving both head groups and hydrocarbon chains, to partial in mixed-chain phospholipids with substantially different chain lengths, such as sphingomyelin [4], will depend upon the packing geometry of the interdigitating lipids, a property easily modulated by conditions of temperature and/or hydration. Since many of the well-characterized interdigitators, particularly the lysophospholipids and some of their 2-acetyl analogs, play important roles in membrane associated events such as lysis, fusion and cell activation, some effort has been devoted to characterizing their physical properties, as well as detecting the presence and degree of interdigitation, when it exists. Until very recently, the only methods of inferring the presence of interdigitating bilayers have been the scattering techniques of X-ray [2,5,6,11,12] and neutron diffraction [16]. Although interdigitation can be reliably and easily detected by either of these diffraction methods, primarily through the significant reduction observed in bilayer thickness, the time-averaged picture of the lipid bilayer which they provide necessarily obscures details of lipid molecular dynamics and intermolecular interactions. Only through the use of other physical techniques, such as vibrational [4,13,17,18] or NMR [5,19,20] spectroscopy, can information on

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Abbreviations: 1,2-DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; 1,3-DPPC, 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine; 1,2-DHPC, 1,2-di-*O*-hexadecyl-*sn*-glycero-3-phosphocholine; lysoPC, 1-palmitoyl-*sn*-glycero-3-phosphocholine.

Correspondence: D.J. Siminovitch, Division of Chemistry, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6.

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hydrocarbon chain dynamics and interactions in the interdigitated bilayer be provided.

In our high-pressure infrared spectroscopic studies of various interdigitated and non-interdigitated lipid bilayer systems, we have sought to develop a spectroscopic parameter which would not only serve as an alternative diagnostic test for interdigitation, but would also reflect the differences in interchain interactions between interdigitated and non-interdigitated lipid bilayer systems. Since these interchain interactions are likely to be significantly enhanced in fully interdigitated lipid bilayers, we anticipate that this enhancement would be reflected in the bands of the chain methylene scissoring and rocking modes, whose pressure-induced correlation field splittings are interchain in origin [21]. In a previous study comparing the barotropic behaviour of interdigitated 1,2-di-*O*-hexadecyl-*sn*-glycero-3-phosphocholine (1,2-DHPC) bilayers and non-interdigitated 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (1,2-DPPC) bilayers [18], we have introduced such a parameter, the peak height intensity ratio $R = I\delta'/I\delta$ of the CH_2 scissoring mode components, where $I\delta$ and $I\delta'$ denote the peak height intensity of the scissoring band, δCH_2, and its correlation field component band $\delta' CH_2$, respectively. In the pressure range between 3 and 20 kbar, a comparison of the pressure dependence of this parameter in 1,2-DPPC and 1,2-DHPC clearly distinguished between these lipids [18], suggesting that the R parameter would be useful as an alternative method of determining chain interdigitation. In this report, we present additional spectroscopic evidence from other interdigitated systems which confirm that this parameter, in conjunction with a visual inspection of the pressure-induced correlation field splittings of the methylene scissoring and rocking mode bands, can provide reliable indications of chain interdigitation.

1,2-DPPC and 1-palmitoyl-*sn*-glycero-3-phosphocholine (lysoPC) were obtained from Sigma Chemical Co. (St. Louis, MO), while the positional isomer of 1,2-DPPC, 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine (1,3-DPPC) was obtained from Calbiochem-Behring Corp. (La Jolla, CA). The ether-linked analog of 1,2-DPPC, 1,2-DHPC, was purchased from Fluka Chemical Corp. (Hauppauge, NY). Fully hydrated ($\geqslant 40$ wt% ^2H_2O) lipid dispersions were prepared for the infrared experiments by heating lipid/^2H_2O mixtures in a closed vial to about 60 ° C, followed by vortexing. After immediate freezing of the samples in dry ice, the heat/vortex/freeze cycle was then repeated twice again. Homogeneous dispersions resulting from this freeze/thaw cycle were then placed at room temperature, together with powdered α-quartz and KRS-5, in a 0.34 mm diameter hole in a 0.23 mm thick stainless steel gasket mounted on a diamond anvil cell, as described previously [22]. Infrared spectra of the samples were measured at 28 ° C on a Bomem model DA3.02 Fourier transform spectrophotometer with a liquid nitrogen cooled mercury cadmium telluride detector. The infrared beam was condensed by a sodium chloride lens system onto the pinhole of the diamond anvil cell [23]. For each spectrum, typically 1000 scans were co-added, at a spectral resolution of 4 cm^{-1}. Pressures were determined from the 695 cm^{-1} infrared absorption band of quartz, using data reduction methods described previously [18,22]. Peak height intensities of the component bands of the CH_2 scissoring mode were obtained from bandshapes baseline-corrected between 1350 and 1550 cm^{-1}.

Fig. 1 compares the pressure dependence of the methylene scissoring mode δCH_2 in each of the four lipids investigated. As demonstrated previously for 1,2-DPPC [24] and 1,2-DHPC [18] bilayers, in each of these four saturated lipids, we observe in the spectra of Fig. 1 a pressure-induced correlation field splitting of the δCH_2 mode, which at low pressures (< 0.01 kbar) gives rise to a single band around 1470 cm^{-1}. The observation of only a single CH_2 scissoring band at atmospheric pressure in all of these lipids, regardless of whether their acyl chains are interdigitated or not, reflects the fact that under these conditions of temperature and pressure, there are significant reorientational fluctuations of the acyl chains. Increasing pressure leads to a damping of these reorientational fluctuations, and an increase in the interchain interactions which give rise to the observed correlation field splittings of the methylene scissoring and rocking (vide infra) mode bands. The inability to distinguish between interdigitated and non-interdigitated systems at atmospheric pressure emphasizes the fact that it is only under the perturbation of high pressure that differences in interchain packing and dynamical structure are clearly reflected in the infrared bandshapes. We note that, in comparison to the spectra of 1,2-DPPC bilayers, the splitting of the δCH_2 mode in the spectra of lysoPC, 1,3-DPPC or 1,2-DHPC into two well-defined bands at higher pressures is more abrupt, and immediately apparent in the stacked contour plots of this figure which highlight the pronounced 'valley' that appears between the two component bands of the correlation field splitting. On the other hand, the only initial manifestation of this splitting in the spectra of 1,2-DPPC as the pressure increases is a broad shoulder on the high frequency side of δCH_2, which steadily gains intensity until a well-defined correlation field component band $\delta' CH_2$ becomes apparent. However, even at this pressure, the corresponding 'valley' defined by the two component bands is comparatively shallow.

In thermotropic studies, X-ray diffraction measurements have established that at all temperatures below the pretransition at 35°C, fully hydrated 1,2-DHPC forms an interdigitated lamellar gel phase [5], while similar measurements of fully hydrated 1,3-DPPC show that at 28°C, this lipid also forms an interdigitated bilayer [2]. In a barotropic study of lysoPC [25], Raman spectroscopy experiments have shown that at 1.9 kbar, lysoPC converts from a cylindrical micellar phase to an interdigitated lamellar coagel phase. Therefore, since increasing pressure can only facilitate the conversion from a non-interdigitated to an interdigitated bilayer [16], and not vice versa, all of these lipids except 1,2-DPPC have in common the feature that under the conditions of temperature and pressure at which the correlation field splitting is observed, they form a lamellar phase with complete interdigitation of the hydrocarbon chains. The spectra shown in Fig. 1 demonstrate

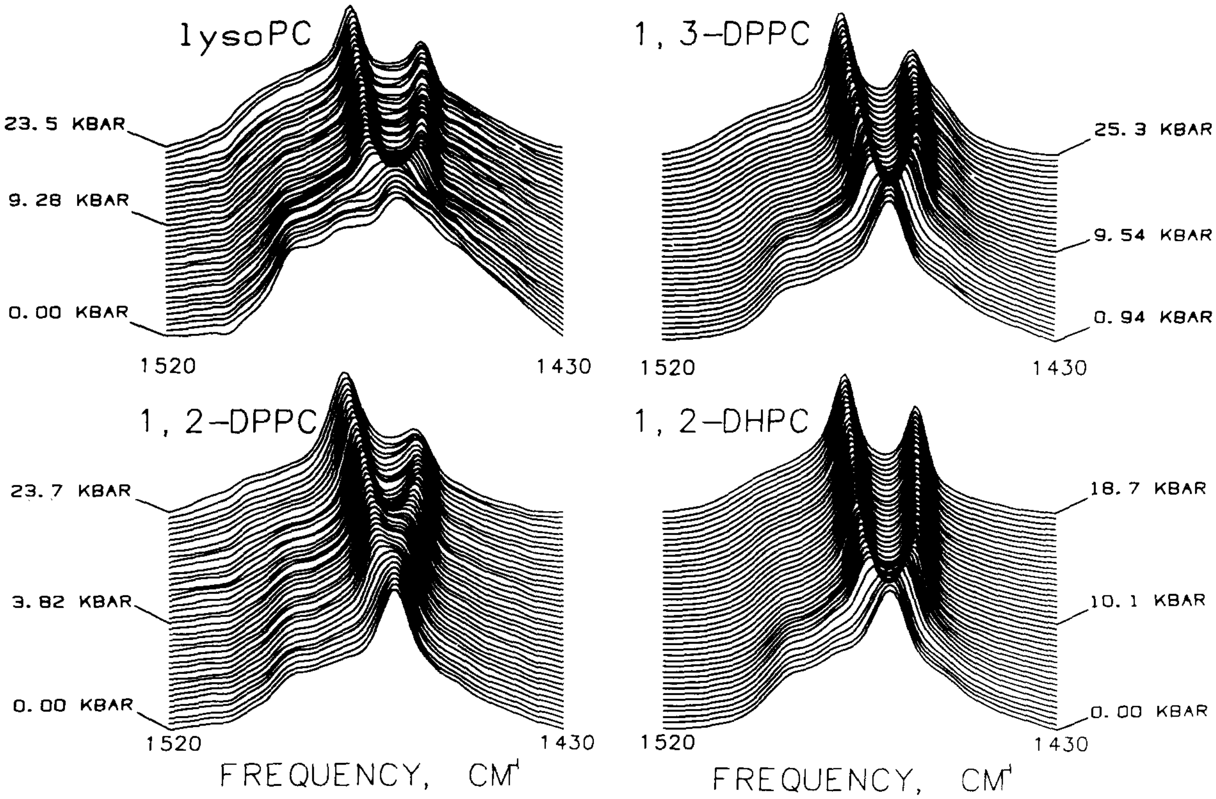

Fig. 1. Stacked contour plots of the infrared spectra of fully hydrated lysoPC (top left), 1,3-DPPC (top right), 1,2-DPPC (bottom left) and 1,2-DHPC (bottom right) in the CH_2 scissoring region. In this and the following figure, displayed spectra have been interpolated in the frequency domain.

that even qualitative features of the bandshapes, when displayed in stacked contour plots, can be used to distinguish between interdigitated (lysoPC, 1,2-DHPC, 1,3-DPPC) and non-interdigitated (1,2-DPPC) systems. Additional evidence to support this point is shown in Fig. 2, which displays the pressure dependence of the methylene rocking mode γCH_2 for three of the systems discussed above. Again, the 'valley' between the two component bands of the pressure-induced correlation field splitting of the CH_2 rocking mode is particularly evident in the stacked spectra of the interdigitated systems (1,2-DHPC and 1,3-DPPC), but much less evident in the non-interdigitated system (1,2-DPPC). The relatively strong (shaded) band in this figure is due to the phonon mode of the pressure calibrant quartz; at higher pressures, this band overlaps with the methylene rocking band γCH_2.

As suggested previously [18], a more quantitative method of distinguishing between non-interdigitated and interdigitated systems is to compare the pressure dependence of the R parameter. Fig. 3 compares the pressure dependence of the R parameter in non-interdigitated 1,2-DPPC bilayers with that in each of the three interdigitated systems considered in this report. To clarify the

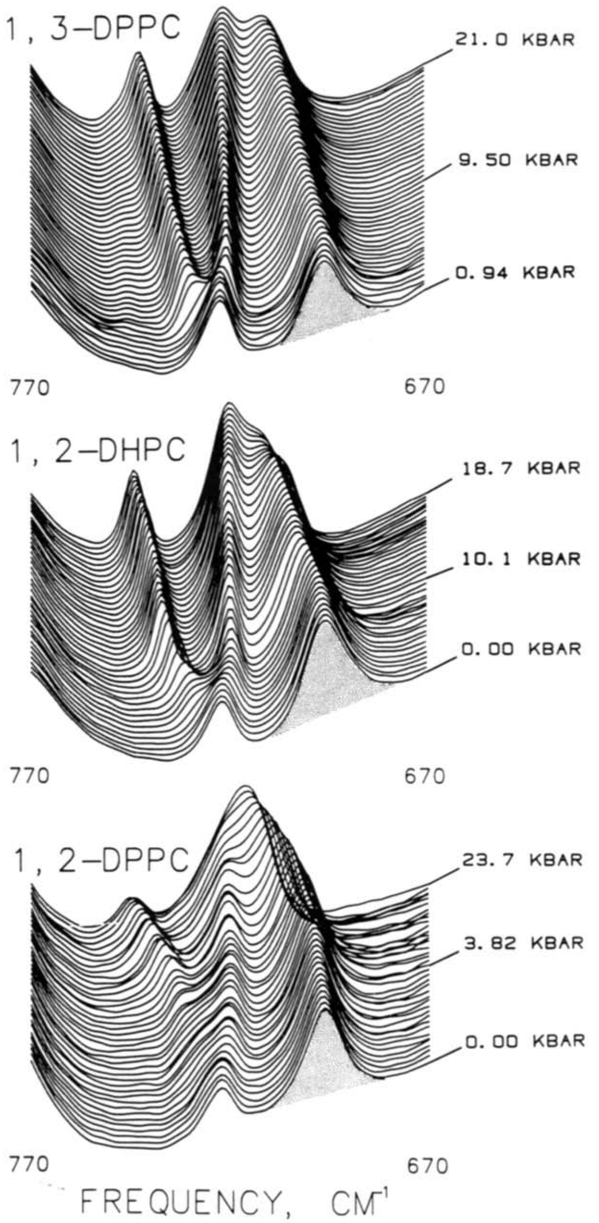

Fig. 2. Stacked contour plots of the infrared spectra of aqueous 1,3-DPPC (top), 1,2-DHPC (middle) and 1,2-DPPC (bottom) in the CH_2 rocking region. In each stacked contour plot, the shaded band is due to the phonon mode of the pressure calibrant quartz.

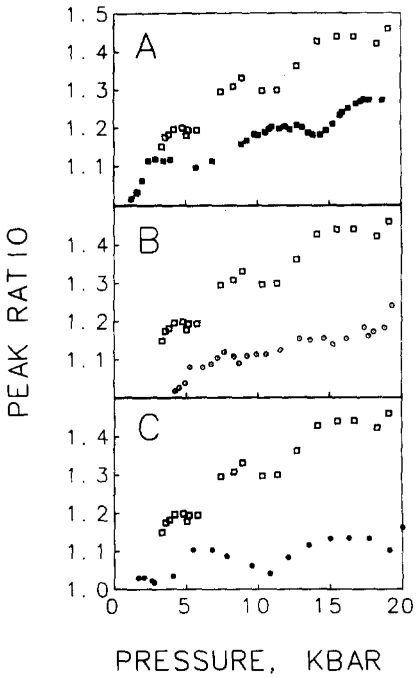

Fig. 3. Comparisons of the pressure dependence of the peak height intensity ratio $R = I\delta'/I\delta$ in 1,2-DPPC (open squares) with that in 1,2-DHPC (filled squares) (A), lysoPC (dotted circles) (B), and 1,3-DPPC (filled circles) (C).

central point of this figure, the following two comments are in order. First, in any of the pressure profiles of the R parameter, there are both large and small scale fluctuations. The small scale fluctuations (± 0.02) are inherent to the method of determining the R parameter, contain no information, and do not concern us here. The large scale fluctuations, on the other hand, which appear as sudden increases in R followed either by no change or a small decrease, can, in the case of 1,2-DPPC, 1,2-DHPC and lysoPC, be correlated with pressure-induced structural phase transitions previously identified in barotropic studies of these lipids [18,24,25]. Second, although the magnitude of the difference in R between 1,2-DPPC and each of the interdigitated systems above 10 kbar increases in the order 1,2-DHPC < lysoPC < 1,3-DPPC, possibly reflecting subtle differences in interdigitation, this variation in R among the interdigitated systems is small in comparison to the difference between non-interdigitated 1,2-DPPC and any of the interdigitated systems. In each case, at all pressures where the correlation field component bands $\delta' CH_2$ are clearly resolvable, the peak height ratio in non-interdigitated 1,2-DPPC is always greater than the corresponding ratio in the interdigitated system. A theoretical explanation of the success of this parameter in distinguishing between non-interdigitated and interdigitated systems is outside the scope of this report; in an extension of our phenomenological approach to partially interdigitated systems, for example, such a treatment would be justified and required. Here, we remark only that since the correlation field splittings of the CH_2 scissoring and rocking modes arise from interchain interactions [21], the enhancement of these interactions in interdigitated lamellar structures must alter the pressure dependence of the R parameter. We anticipate that the high-pressure spectroscopic methods for detecting interdigitation which we have described in this communication will not be limited to fully hydrated single component lipid systems, but will be generally applicable to a wide variety of lipid bilayer systems, under varying conditions of temperature, hydration and pressure. Interesting examples of such systems would include lipids in which interdigitation is induced by external agents such as ions [15], glycerol [11] or tetracaine [12], and 1,2-DPPC itself, in which interdigitation is induced by pressure at higher temperatures.

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