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Membrane fluidity response to odorants as seen by ^{2}H-NMR and infrared spectroscopy

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Abstract

Fourier transform infrared spectroscopy (FTIR) and deuterium nuclear magnetic resonance spectroscopy (^{2}H-NMR) have been used to study the location of two odorants, β-ionone and menthone, in a model membrane of dimyristoylphosphatidylcholine, as well as the effect of the odorants on the structure and dynamics of the phospholipids. The interaction has been investigated for two lipid-to-odorant molar ratios, 10:1 and 1:1. The two odorants were found to affect the fluidity of the membrane. More specifically, the ^{2}H-NMR results indicate that at a lipid-to-odorant molar ratio of 10:1, both β-ionone and menthone increase the order of the deuterons in the interfacial and headgroup regions of the lipid while the incorporation of the odorants at a lipid-to-odorant molar ratio of 1:1 decreases the order of both the lipid headgroup and acyl chains. On the other hand, the infrared results show that the incorporation of β-ionone and menthone decreases the phase transition temperature and cooperativity of the lipid acyl chains. The results suggest that the site of incorporation of β-ionone and menthone is very similar in DMPC membranes.

Keywords: Model membrane; Membrane fluidity; Odorant; β-Ionone; Menthone; FTIR; NMR, ^{2}H-

1. Introduction

Although it is well known that olfactory systems respond selectively to numerous odorant substances, the fundamental mechanisms of olfactory reception are still unknown. A number of theories have been suggested to explain odor discrimination but none of them have been so far fully accepted. The existence of specific protein receptors have been for a long time predicted to play an important role in the odorant reception mechanisms [1–3]. For example, Fesenko and co-workers [4] have isolated protein receptors for decanal and camphor from the rat olfactory epithelium and they have demonstrated that antibodies to these proteins blocked the olfactory response to the two odorants. Moreover, the binding protein of 2-isobutyl-3-methoxypyrazine was also isolated from the bovine and rat olfactory epithelium [1,2]. However, it was shown in that case that the protein does not play a direct role on the odorant reception but plays a role in the transport and concentration of the odorant.

On the other hand, it is now well established that odorants induce response not only in olfactory systems but in non-olfactory systems such as the mouse neuroblastoma cell [5,6], the vomeronasal organ [7], the turtle trigeminal nerve [7] and the frog taste cell [8]. Since the olfactory systems are unrelated to the non-olfactory systems, it is unlikely that the later can provide any specific protein receptors for odorants. The responses to odorants in mouse neuroblastoma cell (N-18 clone) was studied by fluorescence spectroscopy [5,6] which was used to monitor both the changes in membrane potential and membrane fluidity. The membrane fluidity was changed in concentration ranges of odorants similar to those where the membrane potential changes occurred. In addition, the changes in membrane fluidity in response to various odorants using five different fluorescence dyes showed that different species of odorants induce changes in the membrane fluidity at different regions of the membranes. It has been found that the profiles of the membrane fluidity changes are quite similar to those observed with a suspension of porcine olfactory cells [9], suggesting that the receptor mechanism of odorants in olfactory cell suspensions is similar to that in the N-18 cell. This indicates that specific receptor proteins unique to olfactory cells are not involved in odorant reception in the porcine olfactory system.

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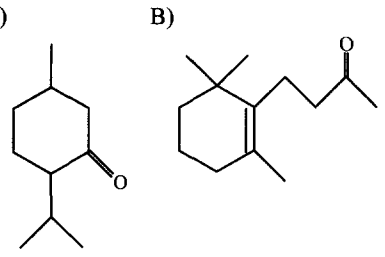


Fig. 1. Structure of menthone (A) and β-ionone (B).

Recently, it has been reported that different odorants also depolarize various liposomes and that there is a good correlation between the order of the threshold depolarization concentrations for various odorants in azolectin liposomes and that in the frog and the porcine olfactory systems [10,11]. In addition, the effect of different odorants on the fluidity of lipid liposomes has been investigated by fluorescence spectroscopy. These studies have shown that the lipid composition of liposomes is one of the factors that control the sensitivity to odorant responses [10–12]. As was observed in the N-18 cell, it has been shown that the patterns of membrane fluidity at different depths and regions of the membranes are similar for odorants having a similar odor and different in response to odorants having different odor responses [12]. The differences were not as pronounced in liposomes of simple composition, such as phosphatidylcholine liposomes, but differences where still observed between some odorants.

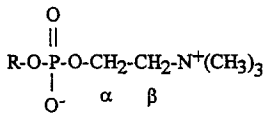
In the present study, we have used solid-state ^{2}H-NMR and infrared spectroscopy to study the interaction between two odorants having similar structures but very different smells, menthone and β-ionone (Fig. 1), with pure dimyristoylphosphatidylcholine (DMPC) membranes. Fluorescence studies have shown that these odorants both decrease the fluidity of the lipid headgroup while the fluidity of the lipid acyl chains is decreased in the presence of β-ionone and increased in the presence of menthone [12]. The lipid-to-odorant molar ratios used in these studies were 1:100 for menthone and 1:10 for β-ionone. However, it was shown that smaller concentrations of odorant still induce changes in membrane potentials. Two different lipid-to-odorant molar ratios were investigated in the present study, 1:1, a concentration at which both odorants are known to depolarize the olfactory cells and at a lipid-to-odorant molar ratio of 10:1.

Deuterium-NMR has proven to be a sensitive tool to study the structure and dynamics of lipid membranes [13,14]. We have used this method to study the effect of the incorporation of the two odorants at different depths in the membrane using lipid molecules specifically deuterated on the polar head and on one of the lipid acyl chains. In addition, Fourier transform infrared spectroscopy (FTIR) has been used to study the effects of the incorporation of the odorants on the gel-to-liquid-crystalline phase transition and conformational order of the lipid acyl chains. The results suggest that the odorant molecules are incorporated into the lipid membrane near the interfacial region, therefore acting as spacers between the lipid molecules. The effects of β-ionone and menthone on the membrane fluidity are very similar, which suggests that different odorants with different odors are incorporated in a similar location in phosphatidylcholine membranes.

2. Materials and methods

2.1. Materials

Dimyristoylphosphatidylcholines deuterated on the *sn*-2 acyl chain (DMPC-d_{27}) and on the headgroup (DMPC-d_4) were purchased from Avanti Polar Lipids (Alabaster, Al) and used without any purification. The following nomenclature is used to indicate deuteron positions in the phosphocholine headgroup:



Deuterium-depleted water, menthone and β-ionone were purchased from Aldrich (Milwaukee, WI). Odorants used were of the best grade available. The salt used in the preparation of the buffer was of analytical grade.

2.2. Preparation of samples

Samples of DMPC/odorant were prepared in two molar ratios, 10:1 and 1:1. Appropriate amounts of lipid and odorant were dispersed in a Hepes (*N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid)) buffer (100 mM) at pH 7.5. The samples for ^{2}H-NMR experiments were prepared using deuterium-depleted water. In order to ensure the complete incorporation of the odorants, the samples were incubated at 52°C for several minutes and were submitted to five cycles of heating (52°C)-vortex shaking-cooling (0°C). For the NMR measurements, the samples were immediately transferred in a sealed tube to prevent evaporation of the odorants. For the FTIR measurements, the samples were transferred between BaF_2 windows (Wilmad Glass, Buena, NJ) and the spectra were recorded immediately.

2.3. FTIR measurements

Infrared spectra were recorded with a Nicolet Magna 550 Fourier Transform spectrometer equipped with a liquid nitrogen-cooled mercury cadmium telluride detector. Samples were inserted between BaF_2 windows using a 12-μm Mylar spacer. 250 interferograms were recorded

with a resolution of 2 cm^{-1}. Each spectrum was corrected for the contribution of water by subtracting appropriate polynomial functions.

2.4. NMR measurements

^{2}H-NMR spectra were recorded at 50°C on a Bruker ASX-300 spectrometer (Bruker Spectrospin, Milton, ON) operating at a ^{2}H frequency of 46.1 MHz. The spectra were recorded using a quadrupolar echo sequence [15] with a delay of 16 μs between pulses and a recycle delay of 500 ms. The 90° pulse length was 5 μs and the sweep width was set to 250 kHz for experiment using DMPC-d_{27} and 125 kHz for experiments using DMPC-d_4.

2.5. Analysis of the acyl chain NMR spectra

The powder spectrum obtained for a dispersion of DMPC-d_{27} in the fluid phase involves the contribution of every deuterons along the myristoyl chain. Each pair of deuterons for every carbons has a distinct quadrupolar splitting so that the powder spectrum is a superposition of 13 powder spectra. Therefore, it becomes difficult to assign specific splittings to specific deuterons in the acyl chain. However, it is possible to convert the spectrum into one that has the characteristics of an oriented sample using the dePaking technique [16,17], resulting in a better resolved spectrum. The quadrupolar splittings have been measured according to the methods of Lafleur and coworkers [18] in order to obtain the smoothed orientational order profile of the lipid acyl chains. First, the well resolved innermost doublet is assigned directly to the terminal methyl of the myristoyl chain of the lipid. The remaining area of the dePaked spectrum is normalized to the 24 deuterium nuclei of the methylene groups contributing to the signal. In the original method of Lafleur and co-workers, the spectrum was divided into equal areas and the quadrupolar splittings were associated to the remaining 12 carbon positions of the myristoyl chain. In the present study, a slight modification of the method was used (Prosser R.S., personal communication). More specifically, the area of each resolved peak in the spectrum was calculated and assigned to a carbon position. Moreover, the integrated area of the most intense peak, which is associated to the plateau region, was divided into small equal areas according to the area of a pair of deuterium nuclei in order to obtain a monotonically decreasing variation of the quadrupolar splittings with the carbon position.

3. Results and discussion

3.1. ^{2}H-NMR results on the lipid acyl chains

The use of DMPC fully deuterated on the *sn*-2 acyl chain allows the simultaneous investigation of the effects

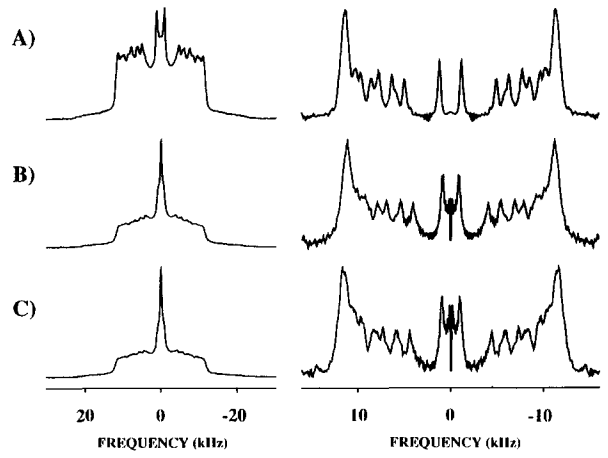


Fig. 2. Original (left) and dePaked (right) ^{2}H-NMR spectra at 50°C of DMPC-d_{27} in the absence (A) and in the presence of β-ionone (B) and menthone (C) at a lipid-to-odorant molar ratio of 1:1.

of the odorants on the plateau and the tail regions of the lipid chain. The quadrupolar splitting ($\Delta\nu_Q$), directly measurable between the peaks of the powder spectra, is related to the orientational order parameter by:

$$\Delta\nu_Q = \tfrac{3}{4}A_Q S$$

where A_Q is the quadrupolar coupling constant ($\approx$ 167 kHz) and S is the orientational order parameter [19–22].

The original and dePaked ^{2}H-NMR spectra obtained at 50°C are shown in Fig. 2 for pure DMPC-d_{27} (Fig. 2A) and for DMPC-d_{27} in the presence of β-ionone and menthone (Fig. 2B and 2C, respectively) at a lipid-to-odorant molar ratio of 1:1. These spectra are a superposition of the powder spectra of all deuterons along the myristoyl chain. The order parameter is high and similar for the first deuterons of the myristoyl chain and decreases rapidly with increasing distance from the carbonyl group to low values near the terminal methyl group [19–22]. The intense shoulder at the edge of the ^{2}H spectrum is due to the deuterons in the interfacial region of the lipid bilayer.

It should first be noted that a small isotropic peak, centered at 0 kHz, is present in the spectra for the lipid systems in the presence of odorants. An isotropic peak has also been observed in the ^{31}P-NMR spectra recorded for these systems (results not shown), indicating that lipids in small structures give rise to the isotropic contribution in both the ^{31}P- and ^{2}H-NMR spectra. The proportion of isotropic spectrum has been estimated from the ^{31}P-NMR spectra to be about 20% for DMPC in the presence of both β-ionone and menthone. For the lipid-to-odorant molar ratio of 10:1, the amount of isotropic structure is negligible.

The spectra presented in Fig. 2 indicate that the presence of odorants at a lipid-to-odorant molar ratio of 1:1 induces conformational disorder in the lipid acyl chains since the change in spectral lineshape observed in the presence of odorants is very similar to that observed with

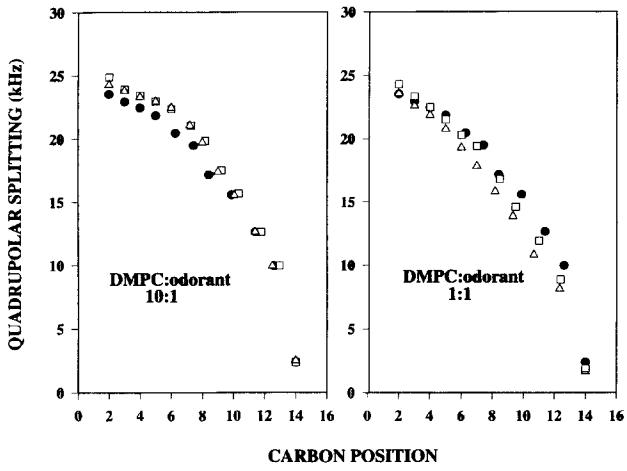


Fig. 3. Quadrupolar splittings of the deuterons of DMPC-d_{27} in the absence (●) and in the presence of β-ionone (△) and menthone (□) at 50°C as a function of the carbon position.

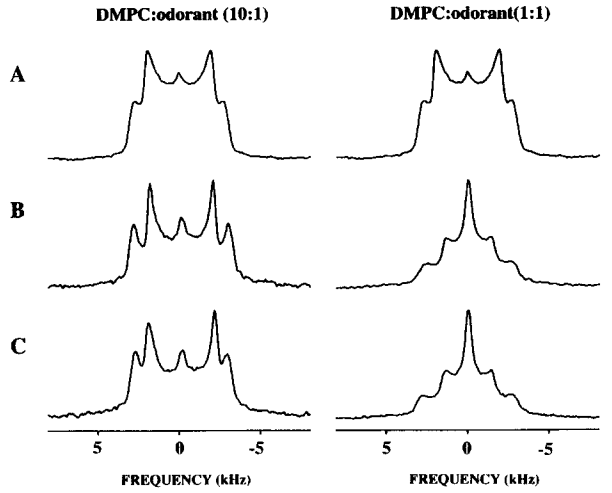


Fig. 4. ^{2}H-NMR spectra of headgroup deuterated DMPC at the α- and β-positions, either pure (A) and in the presence of β-ionone (B) and menthone (C).

increasing temperature [20,23]. In addition, the dePaked spectra indicate that the quadrupolar splitting is decreased for several spectral peaks. In order to emphasize these effects, the smoothed order parameter profiles have been calculated, as described in Section 2, for the lipids in the absence and presence of both odorants for the lipid-to-odorant molar ratios of 10:1 and 1:1. The results are presented in Fig. 3.

For the lipid-to-odorant molar ratio of 1:1, the presence of odorants results in a decrease of the quadrupolar splittings of the deuterons in the center and tail regions of the lipid acyl chains while the order of the deuterons in the plateau region is not significantly affected. These effects are slightly more pronounced in the presence of β-ionone.

On the other hand, for a lipid-to-odorant molar ratio of 10:1, a small but non-negligible increase in the quadrupolar splittings is observed for the peak doublets associated with the deuterons located near the interfacial region of the lipid molecules, which is indicative of an increase of the order in that region. This ordering effect is limited to the deuterons located near the interfacial region and decreases as the distance of the deuterons from the carbonyl group increases. The effect of the odorants for the carbons near the center of the bilayer is negligible. These results can be compared with those obtained for lipid bilayers in the presence of cholesterol [23–28]. It has been shown that the cholesterol ordering effect is similar for every deuterons of the lipid molecule as opposed to the ordering effect of the odorants which is more pronounced for the deuterons located near the interfacial region of the lipid. It is well established that the ordering effect of cholesterol is associated to its rigid frame. However, the odorants used in this study have rigid ring structures substantially smaller than the rigid frame of the cholesterol molecule and cannot cover the whole length of the lipid acyl chains. This is in agreement with the fact that the ordering effect of the odorants is localized near the carbonyl groups of the lipid and not all along the lipid acyl chains.

3.2. ^{2}H-NMR results on the lipid headgroup

We have also used ^{2}H-NMR to study the effects of the odorants on the conformation and order of the lipid headgroup. Fig. 4 shows the ^{2}H-NMR spectra at 50°C of headgroup-deuterated DMPC at the α and β position, either pure (A) or in the presence of β-ionone (B) and menthone (C) for two lipid-to-odorant molar ratios, 10:1 and 1:1. Previous experiments [29,30] have shown that the larger and the smaller quadrupolar splittings are respectively attributed to the β-CD_2 and α-CD_2 methylene of the choline moiety. ^{2}H-NMR of the phosphatidylcholine headgroup has been widely used to examine electrostatic interactions between charged species and a membrane surface [31]. In such cases, counterdirectional changes in the magnitudes of the α- and β-deuteron quadrupolar splittings have been observed in response to changes in membrane surface charge density. In this respect, phosphatidylcholine is said to behave like a 'molecular voltmeter'.

The quadrupolar splittings for the two methylene groups, obtained from the dePaked spectra, are given in Table 1

Table 1
Quadrupolar splittings measured at 50°C for headgroup deuterated DMPC in the absence and presence of β-ionone and menthone

System	Quadrupolar splitting (kHz)	
	α-CD_2	β-CD_2
Pure DMPC	5.830	4.182
DMPC + β-ionone (10:1)	6.380	4.365
DMPC + menthone (10:1)	6.258	4.487
DMPC + β-ionone (1:1)	5.984	3.113
DMPC + menthone (1:1)	5.952	3.085

for DMPC in the absence and presence of β-ionone and menthone. The addition of odorants at a lipid-to-odorant molar ratio of 1:1 results in a large decrease of the β-CD_2 quadrupolar splitting while that of the α-CD_2 is not significantly affected.

These results are similar to those reported by Brown and Seelig [32] in their study of the influence of cholesterol on the polar region of phosphatidylcholine bilayers. They have shown that in the presence of cholesterol, the quadrupolar splitting of the β-CD_2 is reduced by almost a factor of two while that of the α-CD_2 headgroup methylene is almost unchanged. However, unlike cholesterol, the presence of both odorants (at high concentration) disorders the hydrocarbon chains of the lipid.

In addition, very close analogy can be made with the results on the study of the effect of the local anesthetic tetracaine on model membranes. Hence, it has been shown that uncharged tetracaine decreases the quadrupolar splitting of the β-CD_2 moiety of headgroup deuterated dipalmitoylphosphatidycholine whereas the quadrupolar splitting of the α-CD_2 was unchanged [33]. Moreover, the anesthetic, like β-ionone and menthone, disorders the hydrocarbon chains of the lipid molecules.

It has also been observed that the β-deuteron splitting decreases with increasing temperature while the α splitting is effectively unchanged [34]. It has been suggested that increasing temperature increases torsional motion around the C_α-C_β bond. However, a large change in the quadrupolar splitting of the β-CD_2 splitting does not necessarily imply an extensive reorganization of the phosphocholine headgroup since the quadrupolar splittings are rather sensitive to small variation in torsion angle. Therefore, the effect of temperature and both cholesterol and tetracaine is to increase the separation between the lipid headgroups. The same effect is observed for DMPC bilayers in the presence of both odorants at a lipid-to-odorant molar ratio of 1:1.

On the other hand, the results presented in Table 1 indicate that the addition of menthone and β-ionone to phospholipid membranes at a lipid-to-odorant molar ratio of 10:1 results in an increase of the quadrupolar splittings for both α-CD_2 and β-CD_2, the effect being more pronounced for the α deuteron. This effect can be associated with an increase in the order of the lipid headgroup [35] in the presence of a smaller concentration of odorants. These results are in agreement with those obtained for the chain deuterated lipids which had shown that at smaller concentrations, the odorants have an ordering effect in the interfacial region.

3.3. Infrared spectroscopy results

We have used Fourier transform infrared (FTIR) spectroscopy to investigate the effects of the incorporation of both β-ionone and menthone on the acyl chain order and the gel-to-liquid-crystalline phase transition of the lipid acyl chains of DMPC. The two lipid-to-odorant molar ratios have been investigated, 10:1 and 1:1. FTIR spectroscopy is particularly useful to study the conformational properties and thermotropic behavior of phospholipids in odorant/lipid systems since the use of deuterated *sn*-2 acyl chain lipid (DMPC-d_{27}) allows the investigation of the thermotropic behavior of DMPC without spectral contributions of the odorants in the spectra. The thermotropic behavior of DMPC-d_{27} was followed from the frequency of the 2090 cm^{-1} band which is assigned to the CD_2 symmetric stretching mode [36]. This band becomes broader and shifts to higher frequencies as the temperature is increased. The increase in bandwidth can be assigned to the increase of the rotational mobility of the acyl chains while the frequency shift is due to the introduction of *gauche* conformers in the lipid acyl chains [37].

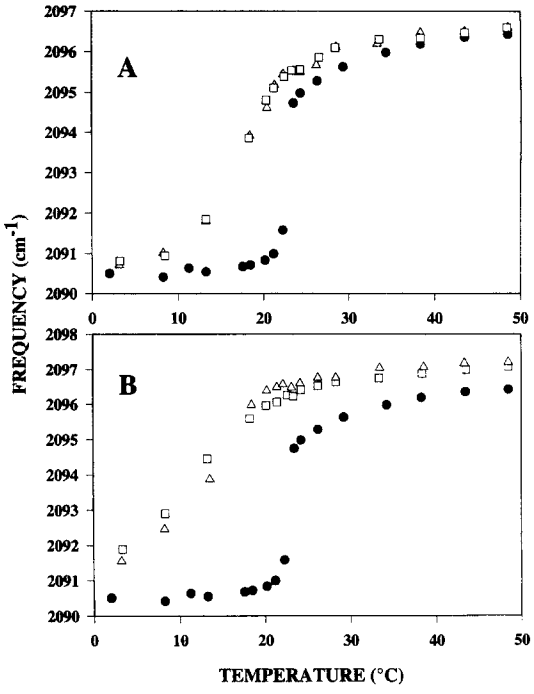


Fig. 5. Temperature dependence of the CD_2 symmetric stretching vibration frequency for pure DMPC-d_{27} (●) and for DMPC-d_{27} in the presence of β-ionone (△) or menthone (□) at lipid-to-odorant molar ratios of 10:1 (A) and 1:1 (B).

Fig. 5 shows the temperature profiles derived from the frequency of the symmetric CD_2 stretching mode band near 2090 cm^{-1} of DMPC-d_{27} in the absence and presence of β-ionone and menthone at molar ratios of 10:1 (Fig. 5A) and 1:1 (Fig. 5B). The interaction of β-ionone and menthone with lipid membranes results in an increase of the frequency of the 2090 cm^{-1} band, both below and above the gel to liquid-crystalline phase transition temperature of the lipid. This effect is much more pronounced for the lipid-to-odorant molar ratio of 1:1. In addition, the incorporation of β-ionone and menthone decreases the gel to liquid-crystalline phase transition temperature of the lipid and the phase transition cooperativity. Again, these

effects are much more pronounced for the lipid-to-odorant molar ratio of 1:1. These results indicate that the interaction of the odorants with DMPC bilayers leads to an increase in *gauche* conformers in the lipid acyl chains [38,39].

3.4. Comparison between the infrared and NMR results

For the samples with a lipid-to-odorant molar ratio of 1:1, the increase in *gauche* conformers observed in the infrared spectra is in agreement with the results obtained by ^{2}H-NMR. The ^{2}H-NMR results show a decrease of the quadrupolar splittings in the presence of β-ionone and menthone for the deuterons located in the center and tail regions of the lipid acyl chains, therefore indicating an increase in disorder. However, the increase in *gauche* conformers reported by FTIR reflects the entire sample including the contribution of the isotropic component (vide supra). This makes the comparison between the NMR and FTIR results more complex since the order profiles do not take into account the portion of the sample responsible for the isotropic signal. The NMR order profiles only reflect the conformational behavior and dynamics of the lipid molecules in the lamellar phase. Therefore some care should be taken in the interpretation of the data.

When the lipid molecules are organized as small vesicles, the high degree of curvature at the surface will affect the molecular packing of the lipid molecules by disrupting the molecular area available for the acyl chains and the polar headgroups. In small vesicles, the molecular area available for the lipid acyl chains will be increased, therefore increasing the probability of observing *gauche* bonds along the hydrocarbon chains. Nevertheless, the NMR results obtained for DMPC in the presence of β-ionone and menthone show a net increase in disorder of the lipid molecules in the lamellar phase. This increased disorder will therefore contribute to the increase of the frequency of the CD_2 vibration band observed in the temperature profiles shown in Fig. 5B.

For the samples with a lipid-to-odorant molar ratio of 10:1, the increase in *gauche* conformers observed by FTIR spectroscopy contradicts the NMR results at 50°C which show an increase in order for the deuterons located near the interfacial region of the lipid, as opposed to the small increase in disorder observed by FTIR at that same temperature.

Infrared and ^{2}H-NMR measurements can be affected by several motions. Whereas infrared is thought to be mostly affected by *trans-gauche* isomerizations, ^{2}H-NMR is sensitive to *trans-gauche* isomerizations and changes of the lipid director axis orientation caused by tilting of the molecules or by surface undulations [20]. Kodati et al. [40,41] have shown in a study of the contribution of the intermolecular coupling and librotorsional mobility in the methylene stretching modes in the FTIR spectra that considerable shift of the methylene stretching bands (hydrogenated and deuterated species) can be induced without variation of the conformational order. More specifically, they have shown that in addition to *trans-gauche* isomerization, intermolecular vibrational coupling and twisting motion along the long axis of the acyl chains of the lipids may influence the frequencies of the methylene stretching modes. Moreover, they have shown that isotopic dilution will shift both the symmetric and anti-symmetric CH_2 bands toward high frequencies due to a restriction in the intermolecular vibrational coupling. A perturbation of the Fermi resonance caused by the restriction of the interchain vibrational coupling is proposed to be at the origin of the frequency shift observed for the CH_2 vibration [40,42]. The effect on the CD_2 stretching modes is less pronounced but still significant. Therefore, for the two systems investigated in this study at a lipid-to-odorant molar ratio of 10:1, the small increase in the frequency of the CD_2 methylene stretching vibration observed in the temperature profiles derived from the FTIR spectra could be the result of restricted intermolecular vibrational coupling due to isotopic dilution. The incorporation of β-ionone and menthone in the membrane, acting as spacers between the lipid molecules, could therefore break or decrease the intensity of the intermolecular vibrational coupling.

On the other hand, as mentioned before, ^{2}H-NMR can be affected by different motions. The deuterium orientational order profiles may also reflect motions like whole chain libration (i.e. reorientation within a cone). The presence of odorants in small amount located near the interfacial region of the lipid bilayer may restrict the amplitude of that motion, therefore explaining the increase of order. Moreover, the observation of ordering at low concentration of both odorants and disordering at high concentration may reflect saturation of the site which promotes ordering. After complete saturation of that site, disruption of the membrane could occur, giving rise to the formation of the isotropic structure, possibly small vesicles.

4. Conclusions

FTIR and NMR have been used in the present study to investigate the effects of two odorants, β-ionone and menthone, on the order of phosphatidylcholine bilayers. The combined used of these two techniques has allowed a detailed investigation of the order of the bilayer in the headgroup, the interfacial region and the acyl chains of the lipid molecules. The results indicate that the changes in membrane order induced by the presence of both odorants are very similar, which suggests that different odorants with different odors are incorporated in a similar location in lipid membranes of simple composition. More specifically, ordering of the deuterons located near the interfacial region of the lipid acyl chains and deuterons of the polar head have been observed at low concentration of odorants as opposed to a disordering effect at high concentration.

Acknowledgements

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