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Low-Temperature Dynamical and Structural Properties of Saturated and Monounsaturated Phospholipid Bilayers Revealed by Raman and Spin-Label EPR Spectroscopy

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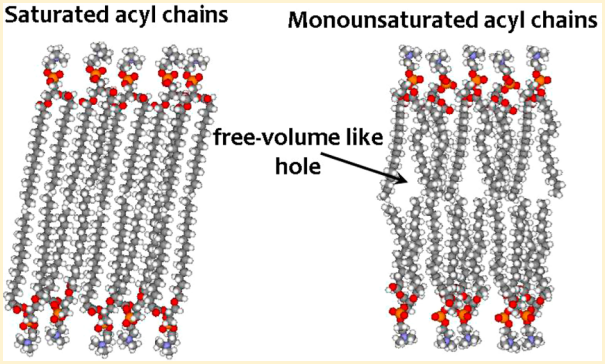
ABSTRACT: The Raman scattering and pulsed electron paramagnetic resonance (EPR) of spin-labeled saturated 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and monounsaturated 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) phospholipid bilayers in a wide temperature range were studied. Raman spectra in the frequency range of CH_2 and C−C stretching vibrations were obtained between 25 and 320 K. The modes sensitive to phospholipid interchain packing, interaction, and intrachain torsional motions (asymmetric CH_2 stretching mode at 2880 cm^{-1}) as well as conformational states (C−C stretching mode at 1130 cm^{-1}) were analyzed. The Raman intensities of these modes significantly depend on the temperature in the gel phase. In the saturated phospholipid DPPC, changes in the temperature dependence of Raman intensities occur near the same temperature for the CH_2 and C−C stretching modes, which is approximately 200−230 K. However, in monounsaturated POPC lipids, the temperature dependence for the C−C stretching mode at 1130 cm^{-1} reveals a transition near 170 K, and the temperature dependence for the asymmetric CH_2 stretching mode transition was near 120 K. For spin-labeled 5-DOXYL- and 16-DOXYL-stearic acids embedded into lipid bilayers, the anisotropic contribution to the electron spin−echo signal decays was interpreted as a result of nanosecond stochastic librations. The decay rates increased remarkably at temperatures above 200 K for DPPC and POPC, which is consistent with the Raman scattering data. A noticeable increase in the libration-induced relaxation rate was observed in POPC lipids above 120 K, and libration-induced relaxation was nearly temperature-independent in DPPC lipids up to 200 K. In the framework of the suggested interpretation, the bilayer structure of monounsaturated lipids contains defective, free volume-like places that provide freedom for phospholipid acyl-tail motions at low temperatures.



INTRODUCTION

Phospholipids are important constituents of all mammalian cell membranes. The presence of double bonds in the acyl chains of phospholipids was found to lead to dramatic changes in the properties of biological membranes. One double bond in the acyl chain of membrane forming phospholipids is enough to strongly affect the thermodynamic properties of membranes, such as "fluidity", and decrease the gel-phase transition temperature as well as increase the average area per lipid.[1,2] Despite extensive studies, the structural and dynamical characteristics of membranes composed of saturated and unsaturated phospholipids are still not fully understood. These properties have been insufficiently explored in the field, especially the influence of low temperatures.

It is commonly assumed that phospholipid bilayers are in a highly ordered state below the gel−fluid (gel−liquid crystalline) phase-transition temperature (T_m) and that the bilayer structure is the same at any temperature below the T_m. Indeed, structural and calorimetric techniques do not reveal significant changes below the T_m (except for transitions to ripple and subgel phases that are sometimes detected near the T_m). However, some spectroscopic techniques have observed changes in the sample when the lipid bilayer sample is heated from cryogenic temperatures to room temperature.[3,4] Usually, changes become significant at temperatures above 180−230 K. These changes may be associated with the phenomenon of "dynamical transition", which has been described for a number of biomolecules (proteins, DNA, biomembranes, and others).[5−8] This transition is viewed as a deviation of the mean square displacement of hydrogen atoms, which has been observed in neutron scattering from the linear temperature-dependence expected from the harmonic approximation.[5−8] In addition, the dynamical transition has been previously observed in other experimental techniques (e.g., Mössbauer absorption[9]

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as well as infrared and NMR spectroscopy[10]). Studies on the "dynamical transition" phenomenon of model membranes composed of saturated and unsaturated phospholipids at low temperatures may have important applications in cryobiology as well as the identification of "low barrier" motions and structural rearrangements that inevitably exist at higher temperatures, for example, the effect of saturated lipids on passive permeation across a lipid membrane.

Recently, we have shown that Raman spectroscopy can be successfully applied to study the properties of phospholipid bilayers in the gel state at cryogenic temperatures to reveal changes at the dynamical transition.[11,12] In studies using phospholipids with saturated acyl chains (1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)), it was shown that Raman bands corresponding to C−H and C−C vibrations of nonpolar chains are sensitive to the dynamical transition. It is known that the intensity of the 2880 cm^{-1} antisymmetric CH_2-stretching vibration line is sensitive to intermolecular packing interactions.[13] Therefore, the remarkable decrease in the intensity of this line relative to the intensity of the 2850 cm^{-1} symmetric CH_2-stretching vibration line above 150−230 K was interpreted as a consequence of the appearance of static conformation disorder and/or the appearance of temperature-activated librational-torsional motions in the lipid chain.[11] Both of these interpretations imply the appearance of molecular flexibility near 200 K. In contrast to the C−H vibrations, the C−C bands are expected to be mainly sensitive to chain conformations. It was observed that the intensity of the 1130 cm^{-1} line (C−C stretching vibration) decreases sharply at the dynamical transition.[12] The interpretation that only the all-trans state molecules contribute to the 1130 cm^{-1} line was used in this study. In addition, it was observed that the effects of the dynamical transition on CH_2 and C−C Raman bands are similar.[12] This result indicated that changes in intermolecular interactions and conformational states are related in the saturated DPPC lipids.

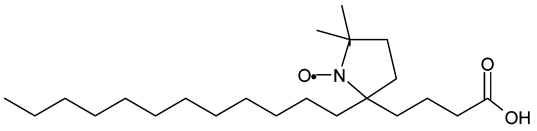
Pulsed electron paramagnetic resonance (EPR) allows for the study of the dynamic properties of spin-labeled molecules. As previously shown,[14] the electron spin−echo decay is sensitive to the presence of molecular stochastic librations that occur at the nanosecond time scale. Specific localization of spin labels at different positions along the lipid chain provides information on the dynamics of different parts of the phospholipid membrane.

Here, we studied model membranes composed of saturated (DPPC) and unsaturated (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)) phospholipids (common members of mammalian cell membranes[15]) in a wide temperature range using Raman and EPR spectroscopy. The combination of these two methods may provide better insight into the structural and dynamical transitions in membranes and avoid possible ambiguities in the interpretation of the experimental data.

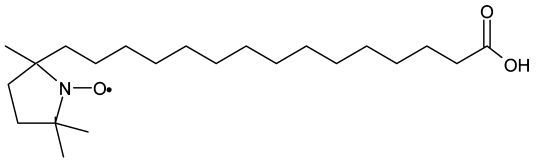
EXPERIMENT

Sample Preparation. For the Raman studies, DPPC and POPC (Avanti Polar Lipids) were hydrated for 48 h in distilled water at temperatures above the gel-to-fluid phase-transition temperature. Then samples were centrifuged for 1 h followed by removal of the excess water. The final lipid-to-water concentration was ∼1:1.5 by weight.

Spin-labeled 5-DOXYL-stearic acid (5-DSA) and 16-DOXYL-stearic acid (16-DSA) (Sigma-Aldrich) were used as spin probes for the EPR studies.



5-DOXYL-stearic acid



16-DOXYL-stearic acid

The nitroxide DOXYL (4,4-dimethyl-oxazolidine-1-oxyl) labels are specifically attached to either the fifth or sixteenth positions along the acyl chain of stearic acid. This allows for spin-label measurements located close to the surface and at the center of the bilayer. The acids were dissolved in chloroform with 30 mg of DPPC or 30 mg of POPC at a molar ratio of 1:100. The solvent was removed by nitrogen flow followed by 24 h under a vacuum. Membranes were hydrated according to the same procedure described above.

Raman Spectroscopy. Raman spectra of opaque white samples were measured at a nominally right scattering angle using a triple-grating TriVista 777 spectrometer and a 532 nm solid-state laser. Spectral ranges 675−1170 and 2750−3120 cm^{-1} between 25 and 320 K were studied. Other details of the experiment are the same as previously reported.[12]

Raman Data Treatment. Raw spectra of inelastic light scattering were corrected for detector dark counts and for photoluminescence background in linear approximation (an example is shown in ref 11). For the C−H stretching mode, the ratio of the intensities of the antisymmetric I_{aCH} (near 2880 cm^{-1}) and symmetric I_{sCH} (near 2850 cm^{-1}) modes was determined.

For C−C stretching vibrations, Raman mode near 722 cm^{-1} corresponding to C−N stretching vibrations was used as a reference and is temperature independent according to ref 13 (and references therein). The ratio of integral intensity of the 1130 cm^{-1} mode (Int_{CC}) and C−N stretching mode (Int_{CN}) was determined.

The interpretation used in ref 12 that only all-trans states contribute to the 1130 cm^{-1} line is not rigorous. It was determined using ab initio calculations[16] that only trans sequences longer than 10 trans bonds contribute to the 1130 cm^{-1} line intensity. Therefore, the conformational state of lipid chains containing 15 C−C bonds (DPPC) can also contribute to the 1130 cm^{-1} line intensity even if the molecule contains gauche conformations (beyond the 10 trans-bond sequence). The attribution of the 1130 cm^{-1} line intensity solely to the all-trans state overestimates the concentration of the all-trans state lipid chains. The magnitude of this overestimation is not clear; however, the agreement between the theoretical evaluation of the all-trans state concentration[17] and the estimation from previous Raman experiments (see Figure 5 in ref 12) indicates that the 1130 cm^{-1} line intensity is still a reliable measure of the all-trans states (see also remarks in ref 18) and allows one to obtain information about changes in the conformational state of the chains with temperature.

EPR Experiment. A Bruker ELEXSYS E580 X-band FT EPR spectrometer equipped with a dielectric cavity (Bruker ER

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4118 X MD-5) inside an Oxford Instruments CF 935 cryostat was used. The two-pulse electron spin−echo sequence ($\pi/2$-τ-π-τ-echo) was used in the experiments. The duration of the microwave pulses was 16 ns, and their amplitudes were adjusted to provide a $\pi/2$ turning angle (making the amplitude ∼6 G). The cryostat was cooled by cold nitrogen gas. The sample temperature was monitored with a calibrated Cu-constantan thermocouple and thermistor directly placed into the sample tube. The temperature was maintained with an accuracy of ±0.5 K.

RESULTS

Raman Spectroscopy Experiments. Typical Raman spectra in the spectral ranges of the C−C and CH_2 stretching vibrations can be found in previous studies.[11,12] Here, we will discuss the temperature dependencies of the ratios of lines that correspond to antisymmetric (2880 cm^{-1}, I_{aCH}) and symmetric (2850 cm^{-1}, I_{sCH}) CH_2-stretching lines, and the ratio of the integral intensities of the lines that correspond to C−C (1130 cm^{-1}, Int_{CC}) and C−N (720 cm^{-1}, Int_{CN}) stretching vibrations.

The ratio of I_{aCH}/I_{sCH} was found to exceed a value of 1 in the gel phase and to be ∼1 in the fluid phase.[11] Therefore, it is convenient to consider the parameter I_{aCH}/I_{sCH}-1 to visualize the evolution of the I_{aCH}/I_{sCH} ratio with temperature. In Figure 1a, the I_{aCH}/I_{sCH} -1 parameter versus temperature is shown for saturated (DPPC) and monounsaturated (POPC) phospholipid bilayers. Figure 1b presents the Int_{CC}/Int_{CN} ratio for the bilayers. The data for the DPPC samples are consistent with results from previous studies.[11,12] The discontinuous break of the temperature dependencies near 314 K for DPPC indicates the gel−fluid transition (see, e.g., ref 13). In Figure 1a, it is observed that the I_{aCH}/I_{sCH}-1 ratio is temperature independent below ∼150 K and sharply decreases as temperature increases above ∼230 K. The behavior of the I_{aCH}/I_{sCH}-1 ratio correlates with the dynamical transition[11] and can be interpreted as a change in interchain interactions with temperature. For the Int_{CC}/Int_{CN} ratio of the DPPC data (see Figure 1b), temperature dependence becomes noticeable only above ∼150 K, and a sharp decrease in temperature dependence is seen as the temperature is increased above ∼230 K. This is the manifestation of the change in conformational states of the nonpolar chains and can be described as a decrease in the all-trans states upon an increase in temperature.[12] In the saturated DPPC phospholipid bilayers, it is important that the indicators of interchain interactions (I_{aCH}/I_{sCH} ratio) and conformational states (Int_{CC}/Int_{CN} ratio) reveal the same temperature behavior (see Figure 4 in ref 12). This indicates that changes in the conformational states and interchain interaction are related in the saturated phospholipids.

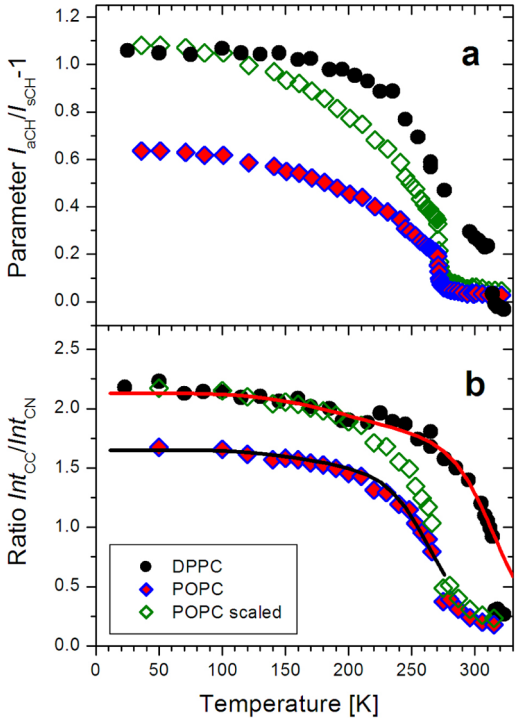

Figure 1. (a) I_{aCH}/I_{sCH}-1 versus temperature for DPPC (circles), POPC (solid diamonds), and the scaled parameter for POPC (open diamonds). (b) The ratio of the integral intensities of the C−C (1130 cm^{-1}) and C−N (730 cm^{-1}) stretching lines for DPPC (circles), POPC (solid diamonds), and the scaled ratio for POPC (open diamonds) versus temperature. Lines are fit by eq 1.

Figure 1 shows that the absolute values of the ratios in the POPC samples are lower in comparison with the DPPC sample. The lower values of the ratios are expected since the double bond in the tails causes less ordered states of POPC even in the low-temperature limit. The steplike peculiarity for the temperature dependencies of the ratios near 271 K corresponds to the gel−fluid transition in POPC. Scaled ratios for POPC are presented in Figure 1 for the comparison of the temperature behaviors in DPPC and POPC. The I_{aCH}/I_{sCH}-1 parameter deviates from a constant at ∼120 K in POPC, whereas the Int_{CC}/Int_{CN} ratio deviates from low-temperature behavior above ∼170 K. Therefore, in contrast to DPPC, there is no agreement between changes in conformational states and interchain interactions in the monounsaturated phospholipid POPC. Additional degrees of freedom observed for POPC, which are related to interchain interactions, are accessible even in low-temperature conformational states.

The question arises whether the changes in conformational states below T_m, which are associated with "dynamical transition" phenomenon, are related also to the "gel−fluid" transition. In our previous study,[12] it was shown that the temperature dependence of the all-trans concentration estimated from the Int_{CC}/Int_{CN} ratio is well described by Pink's model.[17] Pink's model treats 10 allowed conformational states of the acyl chain in a triangular lattice. In addition, it was shown that the simplified version of this model with only two excited states (two-excited-state model) describes the Raman data well.[12] In addition, Pink's model is known to describe the main phase transition of phospholipid bilayers.[19] Therefore, it is expected that the conformational states released with temperature in the acyl chains be related to the gel−fluid transition.

The ratio $R(T) = Int_{CC}/Int_{CN}$ can be described in the framework of the two-excited-state model (for details, see ref 12). In this version of the Pink model one of the excited state is the kink state (a state with two gauche conformations of the lipid hydrocarbon chain), and the second one is a highly disordered "melted" state. In the framework of the two-excited-state model the ratio $R(T) = Int_{CC}/Int_{CN}$ is described by

$$R(T) = \frac{R(T=0)}{g_k \exp\left(-\frac{U_k}{k_B T}\right) + g_m \exp\left(-\frac{U_m}{k_B T}\right) + 1} \quad (1)$$

where g_k and g_m are the degeneracy of "kink" and "excited melted" states, and U_k and U_m are their effective barriers,

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respectively. The results of curve fitting are shown in Figure 1b. In DPPC, the parameters were U_k/k_B = 650 K, g_k = 2.5, U_m/k_B = 6000 K and $g_m = 1.8 \times 10^8$. In POPC, the parameters were U_k/k_B = 650 K, g_k = 2.5, U_m/k_B = 4000 K and $g_m = 3 \times 10^6$. Estimated energy of the kink states U_k/k_B = 650 K corresponds well to the double value of internal energy needed to form a single gauche bond, as it would be expected. Estimated energy of the melted states is found to be less for the case of POPC that can be related, presumably, to conformational restrictions caused by the double bond. It appears that the two-excited-state model is able to describe the Raman data in the gel state of the DPPC and POPC lipid bilayers (Figure 1b).

Knowledge of the degeneracy and energy barriers of the states allows for the calculation of the temperature dependence of the internal energy $U(T)$ that is related to the excited states

$$U(T) = g_k U_k \exp\left(-\frac{U_k}{k_B T}\right) + g_m U_m \exp\left(-\frac{U_m}{k_B T}\right) \quad (2)$$

Therefore, we can calculate the contribution to the heat capacity of the phospholipid bilayers from conformational states within the two-excited-state model, $C_V(T) = \partial U/\partial T$. The calculated heat capacities are shown in Figure 2. The heat

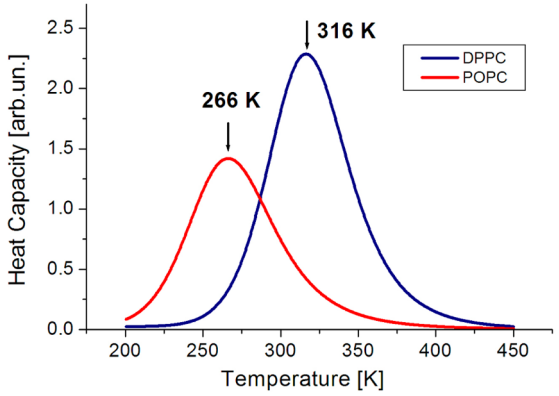

Figure 2. The heat capacity calculated from the two-excited-state model.

capacities reveal peaks, and the temperatures of the maxima are very close to the real phase transition temperatures of DPPC (T_m = 314 K) and POPC (T_m = 271 K), which are measured in calorimetric experiments. The heat capacity peaks are much broader than the real peaks because the real transition is cooperative phenomenon, which leads to a narrowing of the peaks. Nevertheless, the correct positions of the peak maxima for the two-excited-state model used in the calculations indicate that the conformational freedom states released above 150−230 K are related to the gel−fluid phase transition.

To obtain further insight into the relationship between the gel−fluid phase transitions and conformational degrees of freedom, it would be helpful consider the normalized Int_{CC}/Int_{CN} ratio taken at the low-temperature limit and plotted versus the temperature normalized by T_m. In Figure 3b, where these data are presented for DPPC and POPC, the temperature behavior analyzed in this manner is similar for the saturated and monounsaturated phospholipids. This result is evidence of a relationship between the main phase-transition and temperature behavior of the conformational states in the gel phase.

Figure 3a shows that the scaling approach does not work for the CH-modes, which are sensitive to intermolecular packing

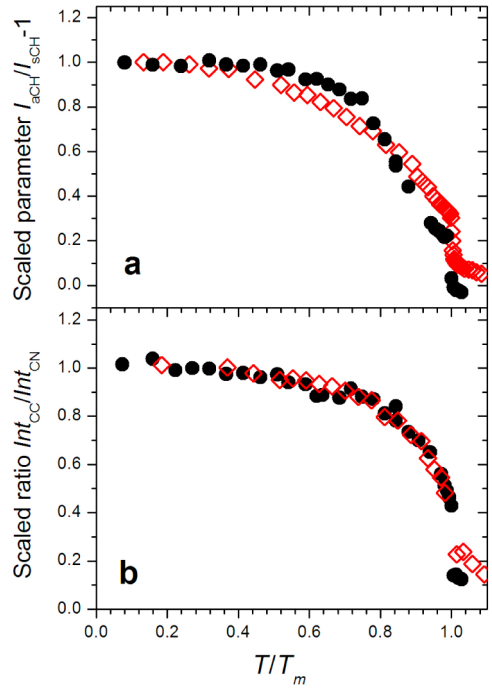

Figure 3. (a) Scaled I_{aCH}/I_{sCH}-1 versus T/T_m for DPPC (circles) and POPC (open diamonds). (b) Scaled ratio of integral intensities of C−C (1130 cm^{-1}) and C−N (730 cm^{-1}) stretching modes versus T/T_m for DPPC (circles) and POPC (open diamonds).

interactions in the lipid bilayer and to librational-torsional interactions.[13,20]

EPR Spectroscopy. The two-pulse electron spin−echo sequence ($\pi/2$-τ-π-τ-echo) allows for measuring the electron spin-phase memory T_2 because the spin−echo amplitude normally exponentially depends on the time delay τ between pulses, $V(2\tau) = \exp(-2\tau/T_2)$.

We recorded the electron spin−echo signal at different delays τ between pulses in two different magnetic fields, which represents two limiting cases of hyperfine interaction anisotropy (see insets in Figure 4) at the position of the maximum in the central component of the spectrum (that is nearly isotropic) and at the position in the right component (that possesses remarkable spectral anisotropy). Therefore, the spin−echo decay observed in the central component shows mostly motion-independent relaxation mechanisms, motions of nearby molecules, and partial motions of the spin label while the spin−echo decay observed in the right-field component reflects mostly motions of the spin label.

The electron spin−echo signal was observed to decay exponentially as a function of the time delay τ between two pulses (examples of experimental spin−echo decay time traces are given elsewhere[14]).

The temperature dependence of the transverse relaxation rate, $W = 2/T_2$, for spin labels located near the membrane surface (the fifth position along the acyl chain) and in the center of the bilayer (the sixteenth position) are shown in Figure 4 at the two different magnetic field positions mentioned above. Below 80 K, molecular motions do not significantly contribute to the transverse relaxation rate. For DPPC and POPC bilayers, W values are nearly the same and are determined by the temperature-independent mechanisms of spin−echo decay, such as "instantaneous diffusion".

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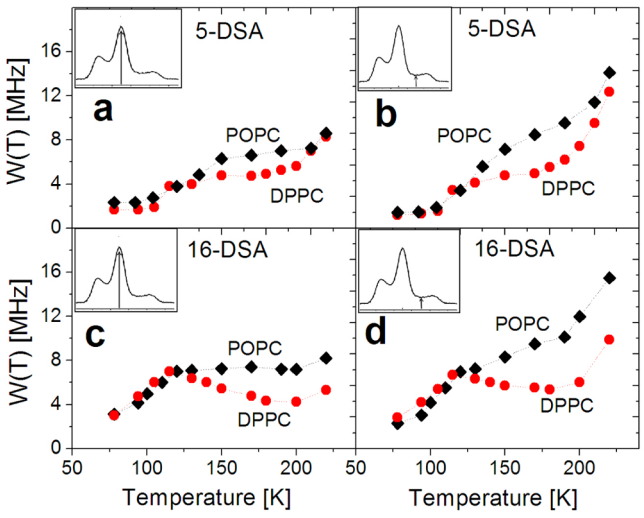

Figure 4. Transverse spin relaxation rates $W(T)$ obtained in the electron spin−echo experiment on spin-labeled stearic acids at the isotropic central spectral positions (a,c) and at the anisotropic high-field spectral positions (b,d) for 5-DSA (a,b) and 16-DSA (c,d) samples.

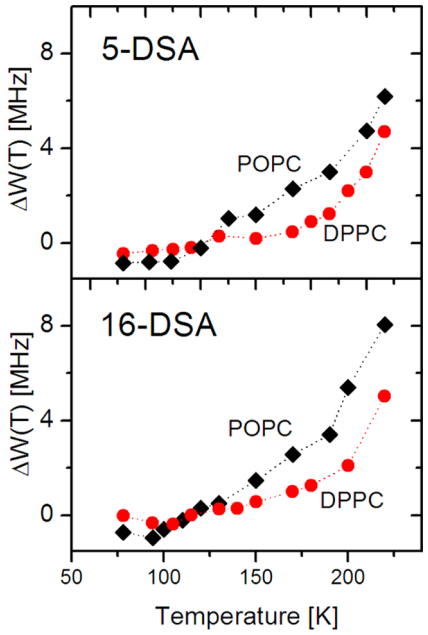

Figure 5. Results of the subtraction of the $W(T)$ relaxation rates collected at the central and high-field spectral positions (see Figure 4).

At temperatures above 100 K, an increase in the transverse relaxation rate is observed (see Figure 4). Therefore, molecular motions begin to contribute to relaxation above this temperature. The $W(T)$ temperature-dependence obtained in the central component (left panel in Figure 4) show low-temperature maxima near 120 K for the label at the sixteenth position and (less pronounced) near 140 K for the label at the fifth position. These maxima are likely related to the relaxation induced by the rotation of the methyl groups of nitroxides that is known to become effective near 130 K.[21]

At temperatures above 210 K in the DPPC bilayer, the W value for the label in the fifth position becomes slightly higher than that for the label at the sixteenth position (Figure 4a,c). This difference may be readily ascribed to contributions from the molecular motions of nearby surface water molecules.

In both cases, $W(T)$ increases remarkably above 200 K (that finally results in the disappearance of the spin echo above 220 K). This observation is in agreement with previous data on spin-labeled bilayers[22] and may be ascribed to the phenomenon of dynamical transitions.[23,24]

To determine the contribution of the motion of spin label to spin relaxation, one must compare the W values obtained in the central (isotropic) and high-field (anisotropic) spectral components (left and right panels in Figure 4). In Figure 5, the result of subtracting the two data sets (ΔW) is shown. As it was previously shown,[14] these data may be safely interpreted as the result of fast stochastic librations of the spin label with a correlation time in the nanosecond time scale. The temperature dependence of $\Delta W(T)$ is remarkably different for the DPPC and POPC phospholipid bilayers. For DPPC, $\Delta W(T)$ is nearly temperature-independent below 200 K, while a distinct increase of $\Delta W(T)$ is observed above 120 K for POPC. Therefore, POPC phospholipids participate in low-barrier relaxation-like motions more than DPPC.

DISCUSSION

Raman and EPR spectroscopy data show that the dynamical and structural properties of saturated and unsaturated lipid membranes are remarkably different at low temperatures. In model membranes composed of saturated phospholipids (DPPC) at temperatures above 200 K, the increase in phospholipid dynamics are accompanied with transitions of acyl tails from the trans-conformation to the gauche-conformation. In the model membranes composed of monounsaturated phospholipids (POPC), an increase in the transverse relaxation rate of the spin label and a deviation from constant of the I_{aCH}/I_{sCH} ratio in Raman scattering are observed at much lower temperatures than the transition of acyl tails from the trans-conformation to the gauche-conformation. This can be attributed to motions of the phospholipid acyl tails.

We suggest an interpretation for the experimental findings of the present work. The bilayers of a saturated phospholipid are in a very ordered state at the low-temperature limit. The lipid chains are mainly in the all-trans state with minimal defects due to chain packing. Activation of the degrees of freedom is possible with changes in the chain conformation. Therefore, the temperature dependencies of the Raman lines sensitive to conformational order and interchain interactions are similar, and the transverse relaxation rate is frozen at all spin label positions at temperatures below the dynamical transition.

The presence of a tilt in one of the chains of the monounsaturated phospholipids causes difficulty in POPC chain packing even at low temperatures. Under these circumstances, chain packing is expected to contain a number of defects, which may be similar to free volume holes. Thus, because the acyl chains cannot fill these holes, they have little effect on the temperature dependence of the conformational states. However, the chains at the boundary of free volume holes will have additional degrees of freedom for some limited motions, which are reflected in interchain interactions in Raman scattering and electron spin−echo decay for the spin labels. Therefore, the different behavior of the I_{aCH}/I_{sCH} ratio in comparison with Int_{CC}/Int_{CN} and an increase in electron spin−echo decay rates at temperatures significantly below 200 K can be due to the presence of defective, free volume holes in POPC bilayers.

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CONCLUSIONS

Raman spectroscopy and EPR with spin labels may provide complementary information, which boosts the conclusions outlined in each technique. The intensity, position, and width of Raman scattering lines indicate the structural and/or dynamical properties of the system. However, selection of the real source that induces spectral changes remains unclear. Pulsed EPR of spin-labeled probes provides information on the dynamics of spin-labeled molecules and their surroundings. However, questions arise on how the insertion of the label perturbs the system under study. Therefore, a combination of both techniques may allow for a solution to these questions.

Here, Raman and EPR spectroscopy were applied to study model membranes composed of saturated (DPPC) and unsaturated (POPC) phospholipids in a wide temperature range. It was observed that the intensities of the Raman lines sensitive to interchain packing and interactions (I_{aCH}, asymmetric CH_2 stretching, 2880 cm^{-1} line) and to conformational order (I_{CC}, C−C stretching of the all-trans conformational state, 1130 cm^{-1} line) reveal remarkable temperature dependence in the gel state. However, in the saturated lipid DPPC, the behavior of I_{aCH} and I_{CC} is similar with a significant temperature-dependence appearing above 200−230 K. In the monounsaturated lipid POPC, $I_{CC}(T)$ reveals a transition near 170 K, while the asymmetric CH_2 stretching mode displays a sharp temperature-dependence above 120 K.

For spin labels at the fifth and sixteenth carbon positions of stearic acids embedded into bilayers, the electron spin−echo signal decays were interpreted as nanosecond stochastic librations. The decay rates were observed to increase above 200 K for DPPC and POPC, which is consistent with Raman scattering data. However, spin relaxation of spin labels may arise because of molecular motions only. Therefore, either molecular motions may directly influence the intensities of the Raman scattering lines, or molecular motions may appear because of a loosening of the molecular structure that influences the Raman intensities. In both samples, dynamical and/or structural peculiarities in the bilayers appear at temperatures below the T_m.

A noticeable increase in the libration-induced relaxation rate of spin labels in POPC was observed also above 120 K; however, it was nearly temperature-independent in DPPC up to 200 K. The similarity of these results with the Raman scattering data, where the asymmetric CH_2 stretching mode was observed to depend on temperatures just above 120 K in POPC and to be temperature-independent below 200 K in DPPC, may be immediately observed.

In conclusion, we suggest that the gel-phase unsaturated lipids contain a number of defective free volume-like holes, which provide freedom for phospholipid acyl-tail motions at low temperatures, and these holes likely remain at physiological temperatures, thereby influencing the physical and chemical properties of membranes.

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Notes

The authors declare no competing financial interest.

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