On the Low-Temperature Onset of Molecular Flexibility in Lipid Bilayers Seen by Raman Scattering

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For dipalmitoylphosphatidylcholine (DPPC) lipid/water bilayers, a detailed temperature dependence of the Raman scattering spectra at the spectral range of the CH_2-stretching modes was investigated. Below 150 K the ratio of intensities of the 2880 cm^{-1} antisymmetric vibration line and the 2850 cm^{-1} symmetric one was found to be nearly temperature-independent. Between 150 and 230 K it decreases slightly as temperature increases; and above 230 K it decreases remarkably. This decrease is accompanied with broadening of the antisymmetric line, from 4.2 cm^{-1} at 100 K to 5.7 cm^{-1} at 296 K. According to literature, the decrease of the antisymmetric line may be interpreted in two ways: (i) the appearance of a static conformational disorder (or of a disorder fluctuating at the time scale larger than picoseconds) and (ii) relaxation at the ps time scale, which is induced by coupling with temperature-activated librational-torsional motion of the lipid chain. Both these interpretations imply that obtained data evidence the appearance of molecular flexibility of lipids around ~200 K. The observed effect is to be compared with low-temperature dynamical transition found in disordered media with neutron scattering, Mössbauer absorption, molecular dynamics simulations and other techniques. This transition implies that with temperature increase harmonic atomic motions are transformed to large-amplitude anharmonic (or stochastic) ones. The characteristic times of these motions lay at the ps time scale. The closeness of the temperature of the transition and of the time scale of motions with those found in this work by Raman scattering for lipid bilayers supports the dynamic nature of the 2880 cm^{-1} antisymmetric vibration line decrease (i.e., that it is induced by coupling with libration-torsion). To prove that the observed onset of flexibility is a property of a disordered state, Langmuir−Blodgett films of behenic acid were studied. These films contain, like lipids, long CH_2-tails, but, in opposite to bilayers, they have a well-ordered crystalline-like structure. The relative intensity of the antisymmetric/symmetric CH_2-stretching lines was found in these films to be temperature-independent in the whole temperature range studied, between 60 and 296 K.

Introduction

For proteins and other biological systems below ~200−230 K, the transition to a state without conformational flexibility normally takes place. According to numerical neutron scattering and Mössbauer absorption data,[1−8] atomic motion at low temperatures is harmonic, with mean squared amplitude varying linearly with temperature. Above 200−230 K, the rate of the amplitude increase with temperature becomes enhanced, which is attributed to dynamical transition to anharmonic (or stochastic) motion. The characteristic times of these motions lay at the ps time scale. This transition is accompanied with appearance of the functional activity.[7−10] The temperature where it occurs is called the dynamical transition temperature, T_d.

Dynamical transition is also detected in low-molecular glass formers, such as glycerol, o-terphenyl, alcohols, etc.,[11−14] and it does not occur in crystalline materials. Therefore, it is attributed to general properties of disordered state. The temperature T_d for molecular glasses depends on the nature of the glass.

Except for neutron scattering and Mössbauer absorption techniques, dynamical transition manifests itself in molecular dynamics simulations,[15,16] infrared (IR) spectroscopy,[17] pulsed EPR of spin probes and labels.[18,19]

Biological membranes, according to neutron scattering data,[5] also manifest dynamical transition around 200−230 K. Also, data on pulsed EPR of selectively labeled lipid chains in model bilayers[20] may be interpreted in this way. (However, in this paper the dynamical transition phenomenon[1−8] was not explicitly mentioned.)

As the dynamical transition is attributed to the onset of anharmonic or stochastic motions at the ps time scale, the study of atomic vibrations, which normally relax also at the same time scale, would allow a new insight into the nature of this transition. IR spectra for OH-stretching vibration mode for hydration water of the protein lysozyme[17] were found to be sensitive to dynamical transition near 220 K.

Lipid bilayers at low temperatures had been studied using Raman spectroscopy already quite a lot time ago.[21,22] For dipalmitoylphosphatidylcholine (DPPC) bilayers the Raman

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intensity of the antisymmetric CH_2-stretching line (2880 cm^{-1}) was found to decrease above 230 K.[21] For the 1130 cm^{-1} Raman line that is caused by skeletal vibrations a slight intensity decrease was observed above ~220 K.[22] In both these cases the intensity decrease was assigned to an increasing conformational disorder: all-trans conformations that are prevailing at low temperatures were assumed with increasing temperature to be replaced by conformations with growing number of gauche bonds. However, some time later, an alternative interpretation of the intensity changes with temperature for CH_2-stretching lines had appeared,[23,24] which explained these changes by relaxation of vibrations at the ps time scale. This relaxation was assumed to be induced by modulation of the vibration frequency arising from coupling with temperature-activated librational-torsional motions of the polymethylene chains.

The closeness of the time scale of the relaxation of CH_2-stretching vibrations[23,24] with the time scale of motions near dynamical transition from neutron scattering may drive us to a suggestion that the found 2880 cm^{-1} antisymmetric line decrease in lipids is induced by the phenomenon of dynamical transition. The early studies[21,22] were done when the phenomenon of dynamical transition had not yet been known. Therefore, the behavior of the Raman lines in the vicinity of ~200 K was not explored in detail. The purpose of the present work is a detailed study of the CH_2-stretching modes for DPPC bilayers in a wide temperature range including 200 K, comparison with data of other techniques that were interpreted within the framework of the dynamical transition in disordered media, and comparison with CH_2-stretching modes in Langmuir−Blodgett films composed of methylene chains which, in opposite to lipid bilayers, are packed into well-ordered structure.

It is known from neutron scattering that the environment is essential for the flexibility of proteins and lipids. Especially, the role of hydration water has been studied in detail.[25−30] In this work, we also study lipids hydrated at different level.

Experimental Section

Samples. Dipalmitoylphosphatidylcholine (DPPC) lipids were obtained from Avanti Polar Lipids. Three samples with different water concentration were prepared: sample "1", nominally dry DPPC; sample "2", 25 mg DPPC + 50 mg H_2O; sample "3", 25 mg DPPC + 100 mg H_2O. For the samples "2" and "3" lipids were dispersed in water by vortex mixing with the heating up to 55 °C, i.e., above the temperature of the reversible gel-to-liquid-crystal phase transition of DPPC (44 °C).

Structure of hydrated DPPC liquid bilayers has been studied with diffraction experiment in many works− see the literature for a review.[31]

The sample of Langmuir−Blodgett layers (LB-sample) of behenic acid $CH_3(CH_2)_{20}COOH$ was prepared in the following way. To form a monolayer, the acid was dissolved in hexane at a concentration of 10^{-4} M, and then the solution was spread onto the surface of deionized water (filtered through a Vladipore 0.2 μm membrane). Then the monolayers were transferred (Y-type deposition) onto a silicon substrate at a constant surface pressure of 25mN/m and at temperature of 21−22 °C. The sample contained 200 stacked layers.

Raman Experiment. Raman spectra of the opaque white samples were measured in nominally right scattering angle by a triple-grating TriVista 777 spectrometer in a multichannel mode. The image of the laser trace was projected onto the spectrometer entrance slit. The diaphragm selected only scattering light from the sample itself, excluding the scattering from the sample tube. A spectral slit of 3 cm^{-1} was used. Pumping

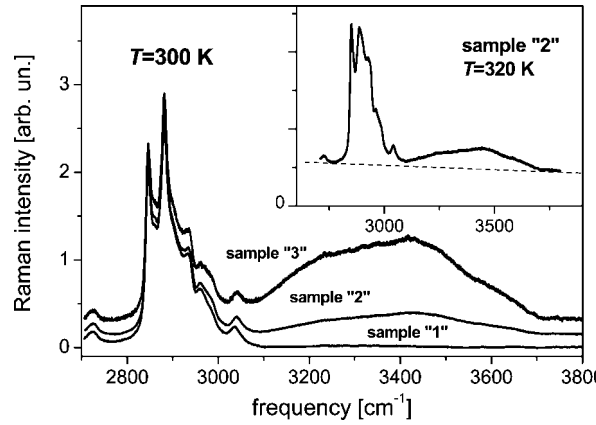

Figure 1. Raman spectra of three samples at room temperature. Spectra are shifted upward for convenience of presentation. The inset presents the example of the photoluminescence subtraction in the raw data.

was performed by a 514 nm argon laser line (power of 400 mW). It was found that this wavelength is optimal for the Raman-to-photoluminescence signal ratio at the range of CH_2-stretching lines (the other tested laser wavelengths were 488, 532, and 647 nm).

An optical closed-cycle helium cryostat was used, in which the sample tube was attached to a coldfinger through an indium layer. The cooling rate was 2 K/min, with a 1 h wait when crossing $T = 265$ K, which was accompanied with an *in situ* ice crystallization control by Raman spectra.

Raman scattering spectra of the LB-sample were measured in the near back-scattering configuration. The spherical-cylindrical lens was used in order to focus the laser beam onto a 10 × 0.2 mm rectangle on the sample surface that is being parallel to the spectrometer entrance slit. Such illumination of the sample results in a relatively low pump power density, reducing the sample heating.

Results

In the present research we focused on the study of the spectral range of 2800−3080 cm^{-1}, where the CH_2-stretching lines are seen. Representative Raman spectra of the three studied samples at $T = 300$ K are shown in Figure 1, where also the OH-vibrations range (3000−3700 cm^{-1}) is added. The photoluminescence background in these spectra was subtracted in the linear approximation as is shown in the inset to Figure 1. The manifestation of OH-vibrations is different for different water concentrations. The dry sample "1" manifests a negligible contribution from the OH-vibrations. The OH-band is 3.8 times more intense for the sample "3" as compared to the sample "2", instead of only 2 times as expected from the preparation. Probably, this difference is induced by a difference in Raman scattering for free water and water "adsorbed" on the bilayer polar surfaces. Indeed, for sample "3" a relative portion of free water must be higher than that for sample "2". However this issue needs a separate study.

The intensity of the 2850 cm^{-1} line that corresponds to symmetric CH_2-stretching vibration was found in our experiment to depend only slightly on temperature. To exclude the possible experimental uncertainty because of laser instability and of different sample positions in different measurements, all the data in this work were normalized by the intensity of this line. Note that intensity of the 2850 cm^{-1} line has been often used, since it was suggested,[32] as an internal standard by which to study the variation of the 2880 cm^{-1} line in different experiments.

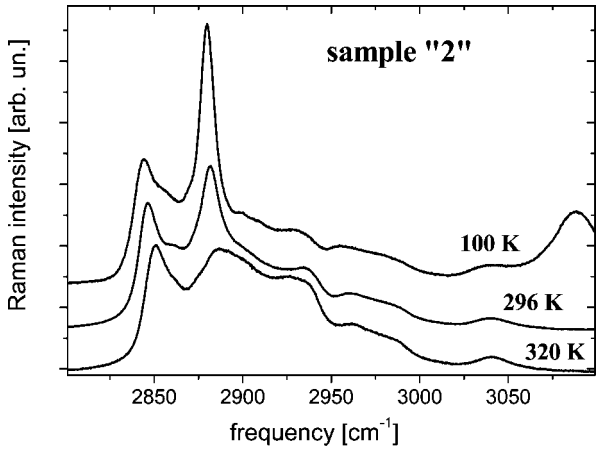

Figure 2. Raman spectra of the sample "2" at three selected temperatures: 100 K, 296 K, and 320 K. Spectra are shifted upward for convenience.

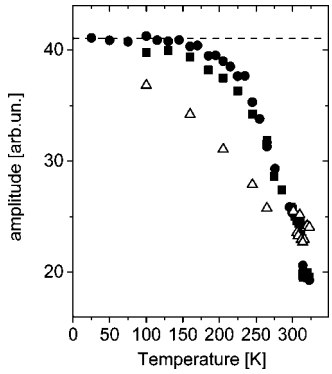

Figure 3. The temperature dependences of the amplitude of the Raman scattering line intensity near 2880 cm^{-1} for three samples: "1", triangles; "2", circles; "3", squares. For all temperatures, spectra are normalized by the intensity of the line near 2850 cm^{-1}. The dashed line shows the low-temperature limit.

One can see from Figure 1 that the Raman lines in the 2800−3080 cm^{-1} CH_2-stretching range look similar for the all 3 samples, and hydrated water does not provide a significant contribution at this spectral range.

Figure 2 shows representative Raman spectra at different temperatures (sample "2"). One can see that antisymmetric stretching line near 2880 cm^{-1} with increasing temperature is broadening and shifts to higher frequencies. The half-width at the half-maximum (which was estimated for the narrow peak excluding the broad background) increases from 4.2 cm^{-1} at 100 K to 5.7 cm^{-1} at 296 K. (Note that our spectral resolution of 3 cm^{-1} increases the natural line width approximately by this value. However, it does not affect the relative variation of the experimentally observed line width.)

The detailed temperature dependence of the 2880 cm^{-1} line intensity is shown in Figure 3. It is seen that for the lipid/water samples the intensity of this line at $T < 150$ K does not depend on temperature within the experimental uncertainty. At higher temperatures, the intensity decreases as temperature increases. At $T > 230$ K it decreases rapidly. At the temperature of the gel-to-liquid-crystal phase transition the intensity drops discontinuously, which agrees with the well-known results from early works (see, e.g., refs 33 and 34).

The behavior of the dry sample is somewhat different. The 2880 cm^{-1} line intensity decreases monotonously with temperature increase, in the whole temperature range studied (100−323 K for this sample).

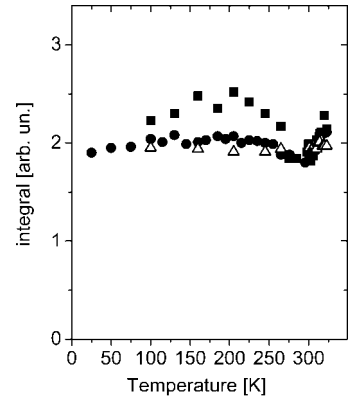

Figure 4. The temperature dependences of the integral intensity at the CH_2-stretching range: "1", triangles; "2", circles; "3", squares.

Figure 4 presents the integral intensity over the CH_2-stretching range (from 2800 cm^{-1} till 3080 cm^{-1}, see Figures 1 and 2), for different temperatures. One can see that this integral for samples "1" and "2" below 270 K is nearly temperature-independent (in the scale of the temperature dependence of the line amplitude seen in Figure 3). Some deviation from a constant below 270 K for the sample "3", containing a high water concentration, may be explained by the contribution of the tail of frozen water line that spreads from higher frequencies. We may conclude that Raman lines at the CH_2-stretching range with increasing temperature are only broadening, and/or the intensity redistribution may take place among different lines. In addition, the found temperature independence confirms the correctness of the used normalization by the 2850 cm^{-1} line intensity.

The integral intensity gradually increases (Figure 4) above 317 K (temperature of the gel-to-liquid-crystal phase transition).

Discussion

There are known two different mechanisms explaining temperature dependence of the ratio of intensities for antisymmetric and symmetric CH_2-stretching vibrations for alkane chains:

(i) The first one implies the influence of structural disorder, either of the lipid chains itself or of their packing (intramolecular and intermolecular disorder, respectively), which may be temperature-dependent. For solid alkanes[32] it was suggested that at different temperatures different packing may exist of the alkane chains, having different local symmetry. The latter would influence the vibrations of different symmetry in different ways. Also, a collective librational-torsional motion of the chain may result in a set of instantaneous distorted structures, each one generating its own stretching mode.[35] Then, Fermi resonance interaction involving binary contributions between methylene bending modes and the symmetric CH_2 stretching mode, may provide a contribution of symmetric CH_2-stretching mode at the spectral range of the antisymmetric line,[24,34] with this contribution depending on the disorder.

The ratio of line intensities for antisymmetric and symmetric CH_2-stretching vibrations was used as a criterion of disorder in a number of papers where lipids in bilayers were studied (see, e.g. refs 33, 34, 36, 37).

(ii) The other mechanism may be referred as a purely dynamical one. For alkanes in solids[24] and in alkane−urea clathrates[23] modulation of the CH_2-stretch frequency via anharmonic coupling with libration-torsion about the long axis of the lipid chain was proposed. This motion belongs to the same type of local symmetry as the antisymmetric stretching vibration.

Therefore, the latter is expected to be more influenced by librational and torsional motions than the symmetric one. It was specially emphasized[23] that in clathrates the alkane chains are uniformly packed in all-trans configuration and lack interchain interaction. This means that the interpretation based on the structural disorder is not applicable here. The quantitative model presented[23] explains, employing reasonable parameters, the line broadening of the antisymmetric line, which gradually varies in clathrates from ~4 cm^{-1} at low temperatures to ~10 cm^{-1} at room temperature. The librational-torsional relaxation time of about 1 ps obtained from the fitting the observed bandwidths were found to agree with the value obtained from quasielastic neutron scattering experiment for the same clathrate system.

The parameters found in ref 23 may be also invoked to explain our experimental data (Figure 2 and 3).

Anyway, both mechanisms, described above, imply the appearance of chain flexibility, which influences the line shape either in a static (i) or a dynamical (ii) way.

Note that the described above Fermi resonance for the symmetric CH_2-stretching mode is expected to reduce the 2850 cm^{-1} line intensity, as the resonance effect increases. In this case the normalization by the intensity of 2850 cm^{-1} line should influence the total integral, which is not the case. So we may propose that this mechanism does not contribute noticeably.

As both mechanisms (i) and (ii) imply the appearance of the chain flexibility, the data in Figure 3 reflect a suppressed chain flexibility of the lipid-water samples below 150 K, slightly increased flexibility above 150 K, and significantly increased chain flexibility above 230 K.

The observed effect is to be compared with the low-temperature dynamical transition observed in disordered media by neutron scattering, Mössbauer absorption, molecular dynamics simulations and other techniques.[1−19] This transition manifests itself as transformation, with temperature increase, of the atomic harmonic motions to anharmonic (or stochastic) large-amplitude motions. These motions occur at the ps time scale, which is close to the time scale of the motions discussed in this work. For membranes and other biological systems dynamical transition takes place in the temperature range of 170−230 K, which is also close to the temperature where the 2880 cm^{-1} antisymmetric vibration line decreases. These facts drive us to a suggestion that the found 2880 cm^{-1} antisymmetric line decrease in lipids is induced by the phenomenon of dynamical transition.

It is also known that in proteins an additional transition takes place near $T \sim 100$ K.[5,30,38−41] It appears in all samples regardless of hydration level and is ascribed usually to methyl group rotation. In our experiments this phenomenon is not expected to manifest itself because only the CH_2-stretching vibrations here are studied.

Raman spectra in Figure 3 for the dry lipid sample demonstrate the significant change of the 2880 cm^{-1} line intensity, as the temperature increases, even at temperature as low as 100 K. This effect may have however a trivial explanation. The hydrated water plays an essential role in organizing the bilayer supramolecular structure. In the dry lipid system this structure may be destroyed, and lipid chains may possess an increased static chain disorder depending on temperature, which would result in the broadening of the 2880 cm^{-1} line by the mechanism (i) described above. However, this suggestion needs further investigation. Anyway, Figure 3 shows for the dry sample a continuous drop of the intensity in the vicinity of 230 K, which is in contrast with the steep drop seen here for hydrated sample. This means that the dynamical transition does not take place for the dry sample, which is in agreement with numerical data on hydrated proteins.[30,38−41] Note in this respect that data for hydrated and dry samples cannot be directly compared, because the temperature dependence of the 2880 cm^{-1} line may be governed in these two cases by two different mechanisms (i.e., (i) and (ii)).

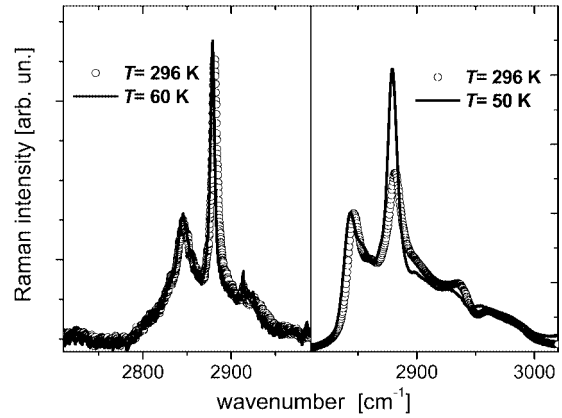

Figure 5. Raman spectra at the CH_2-stretching range for the LB-sample (left) and for the sample "2" (right), for different temperatures.

The lipid flexibility in a bilayer is expected to be higher when closer to the lipid terminuses (i.e., in the bilayer interior). In this respect, it is interesting to discuss results obtained by EPR for bilayers containing lipids spin-labeled at different chain positions.[20] For the labels located closer to the polar heads the onset of stochastic librational motion was found[20] to take place near 200 K, while that for the labels at the lipid terminuses was found to occur at much lower temperatures, starting from ~100 K, and becoming remarkable at ~140 K. The dependence of flexibility on the chain position explains the low-temperature decrease of the intensity at $T = 150$ K seen in Figure 3 as a result of mixture of Raman scattering from different chain positions along the lipid tail.

In this study we also compared CH_2-stretching vibrations with those of the CH_2-chains in LB-films of behenic acid. These films contain, like lipids, long CH_2-tails, but, in opposite to bilayers, they have a well-ordered crystalline-like structure.[42] The Raman scattering spectra of the LB-sample and of the sample "2" are shown in Figure 5 for two temperatures. It is seen from Figure 5 that the low-temperature (50−60 K) spectra of the LB and water/lipid samples are very similar. However, the relative intensity of the two lines for the LB-samples, in contrast to the lipid/water samples, does not change significantly with temperature. This result indicates that the observed effect of the decrease of the 2880 cm^{-1} line intensity is an exclusive property of the disordered state.

Conclusions

Temperature dependence was investigated in detail in a wide temperature range of Raman spectra of lipid/water samples of dipalmitoylphosphatidylcholine (DPPC) at the spectral range of CH_2-stretching modes. Below 150 K the ratio of intensity of the antisymmetric 2880 cm^{-1} line to intensity of the symmetric 2850 cm^{-1} one was found to be nearly temperature-independent. Between 150 and 230 K this ratio decreases slightly as temperature increases; and above 230 K it decreases remarkably. These data allow speaking on a transition in the vicinity of 200 K. This transition may be related with the appearance of static conformational disorder, or, alternatively, with relaxation at the ps time scale induced by coupling with librational-torsional

motion of the lipid chain. Both these interpretations mean the onset of molecular flexibility of lipids.

From the other hand, for disordered media numerous data on neutron scattering, Mössbauer absorption, molecular dynamics simulations and other techniques[1−19] point on a dynamical transition, which for biological systems occurs near 200 K. The dynamical crossover for atomic motions observed in these studies implies that harmonic motions with temperature increase are transformed to large-amplitude anharmonic (or stochastic) motions. As these motions occur at the ps time scale, these results provide a strong support to the interpretation of the found 2880 cm^{-1} antisymmetric CH_2 vibration line decrease as arising from the coupling with librational-torsional motions of the lipid chains.

The extended temperature of the observed transition in the Raman study may be explained by the dynamical heterogeneity, when in the interior of the bilayer the transition occurs at much lower temperature (∼100−140 K, as it follows from EPR of spin labels[20]) than in the vicinity of the polar heads of the lipids.

The dry lipid sample manifests the monotonous intensity decrease above 100 K. As water is an essential component for formation of the bilayer supramolecular structure, one may expect that hydrated and dry samples differ much in their physicochemical properties. Most probably, in the dry sample the decrease is related with the appearance of static conformational disorder monotonously increasing with temperature.

For the Langmuir−Blodgett behenic acid films, which are known to form a well-ordered crystalline-like state, the decrease of the antisymmetric CH_2-stretching line is found in this study to be temperature-independent. This evidence that the found transition is an exclusive property of the disordered state.

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