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Domain Formation in Lipid Bilayers Probed by Two-Dimensional Infrared Spectroscopy

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Two-dimensional infrared (2D-IR) spectroscopy has been used to probe structure and dynamics in binary sphingomyelin/phospholipid liposomes. The liposomes consist of 1-palmitoyl-2-linoleyl phosphatidylcholine (PLPC) and sphingomyelin (SPM) in the ratio 1:1. The diagonal part of the 2D-IR spectra shows two bands which are due to amide I of SPM and to the carbonyl moieties of PLPC. The diagonal components of the 2D-IR spectra reveal a difference in the molecular dynamics. The presence of off-diagonal cross-peaks indicates the occurrence of intermolecular structural correlation. The intensity of the cross-peaks is consistent with segregation of two lipid components into PLPC and SPM molecular domains.

There is a hypothesis that structural segregation and microdomain formation in a native membrane defines an efficient network of molecular trafficking. This would affect molecular transport, recognition, and signal transduction in a living cell.[1] The hypothesis is based on the results of a comparative analysis of observations in artificial membrane composites and in chemical fractions extracted from cellular membranes. In fact, both the complexity of native membrane and the expected size of an active raft in it (∼50 nm) do not allow direct verification of the hypothesis with the methods of optical spectroscopy, particle, and electron scattering techniques. Recently, two-dimensional infrared (2D-IR) spectroscopy has been demonstrated to provide a powerful and unique tool for probing molecular dynamics and intra- and intermolecular structural correlations in a liquid membrane environment.[2,3] Following this, we use 2D-IR spectroscopy to probe molecular dynamic properties in sphingomyelin/phospholipid binary membranes, which are known to be present in some native systems.[4]

L-α-Phosphatidylcholine (Soy − 95%) extract was obtained from AVANTI-POLAR-LIPIDS, Inc., Alabaster, U.S. 1-Palmitoyl-2-linoleyl phosphatidylcholine (PLPC) is the main molecular component (97.2%) of the extract. Chicken egg yolk sphingomyelin (SPM) was purchased from Fluka. Figure 1 shows the molecular structures. The preparation of a suspension of phospholipid liposomes was done with a miniextruder from AVANTI-POLAR-LIPIDS using 100 nm pore polycarbonate membranes and following the methodology according to the description and references provided at www.avantilipids.com/Extruder.html. Preparation was repeated three times (10 extrusions with a set of three fresh filters each time) until the suspension demonstrated perfect optical quality. According to the preparation protocol from AVANTI-POLAR-LIPIDS, the sample should contain liposomes with an average size distribution of about 100 nm. About 2 μL of sample was needed for the infrared experiments. The liposomes were composed of PLPC and SPM lipids in the molar ratio 1:1. We recorded the steady state IR spectra using a BioRad 175C Fourier transform spectrometer. The time-resolved 2D-IR experiments were accomplished using an ultrafast IR spectrometer, the details of which are provided elsewhere.[5]

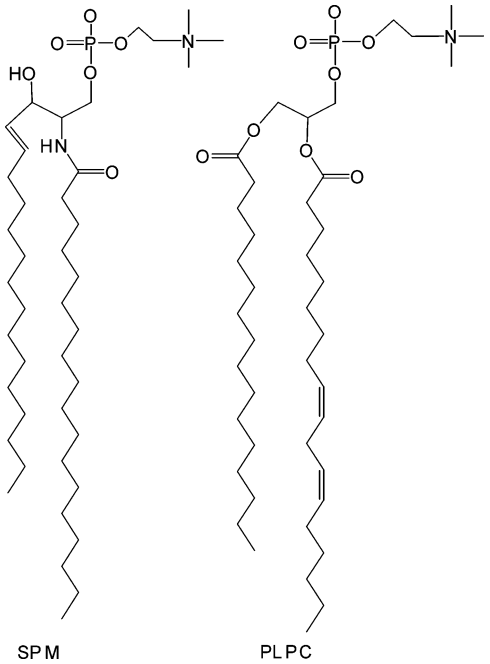

Figure 1. Structure of SPM and PLPC phospholipids.

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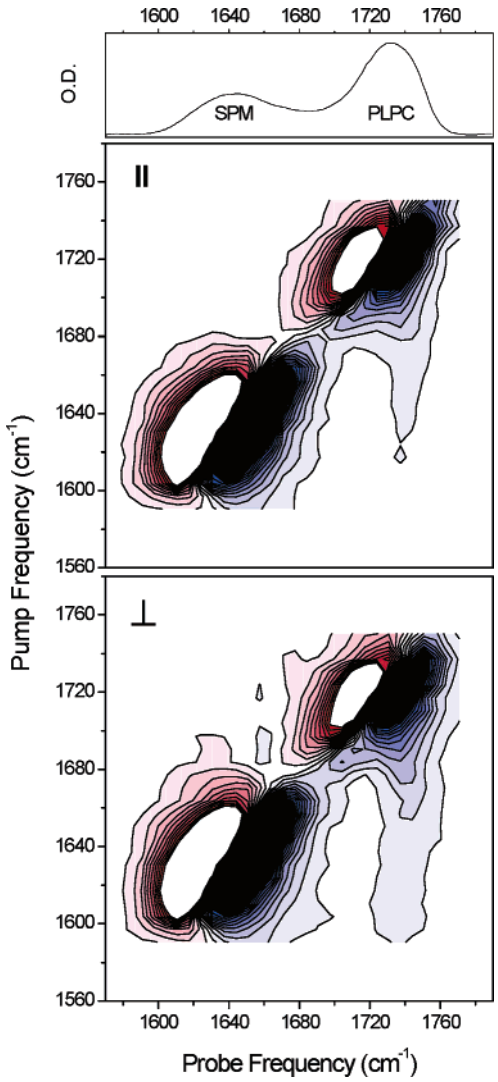

Figure 2. FTIR and time-resolved 2D-IR spectra of SPM/PLPC liposomes with parallel and perpendicular polarization of pump and probe pulses.

In Figure 2, we show the linear Fourier transform infrared (FTIR) spectrum and time-resolved 2D-IR responses from the prepared SPM/PLPC liposomes recorded at a 0.7 ps delay time between pump and probe pulses. The linear FTIR spectra and the diagonal part of the 2D-IR spectra reveals two transitions at about 1645 and 1730 cm^{-1} which are due to amide I of SPM and the two carbonyl groups of PLPC, respectively. Fitting of the linear spectra suggests a single spectral component to contribute to SPM resonance and a pair of substates to the response of PLPC carbonyls (Supporting Information Figure 1S). The nature of the two substates in the PLPC response is the subject of current investigation. Following the approach of ref 6, we fit the 2D line shape of PLPC and SPM transitions (Supporting Information Figures 1S and 2S, respectively) using a Kubo line shape correlation function, $\xi(t) = \delta(t)/T_2^* + \Delta_0$. The first term in the correlation function introduces the inertial component contribution $\delta(t)$ over T_2^*, which is pure dephasing. Since we did not observe any obvious spectral dynamics on the time scale of population relaxation, we set the second term to be governed by quasistatic inhomogeneity Δ_0. The fitted values for T_2^* and Δ_0 are 0.61 ± 0.01 ps and 13.1 ± 0.1 cm^{-1} for SPM and 0.96 ± 0.01 ps and 8.5 ± 0.5 cm^{-1} for each substate of PLPC. These results indicate significantly different molecular dynamics of SPM and PLPC molecules.

The off-diagonal components of the 2D-IR spectra (Figure 2 and Supporting Information Figure 3S) are the signatures of intermolecular structural correlations between the carbonyl moieties of the two molecular components in the liposomes. The mean anisotropy of the cross-peaks[7] is about 0.15 ± 0.04. This corresponds to two possible angular distributions between the transition dipole moments centered at 40 ± 4 or 140 ± 4 degrees. The numerical values indicate that carbonyl moieties of SPM and PLPC are not randomly oriented with respect to each other. The relative orientation of the transition dipole moments of the two molecular components is under current investigation in a series of molecular dynamics simulations.

Following the procedure given in ref 7, we use the bandwidth of the probed transition ($\Delta\omega_l$) and the relative intensity of the diagonal (I_{kk} and I_{ll}) and off-diagonal (I_{kl}) resonances to calculate the off-diagonal anharmonicity, $\Delta\epsilon_{kl} = \Delta\omega_l I_{kl}(I_{kk}I_{ll})^{-1/2}$. The off-diagonal anharmonicity is proportional to the square of the intermolecular coupling constant as $\beta^2 = \Delta\epsilon_{kl}(\epsilon_k - \epsilon_l)^2/4\Delta$. Since the value of diagonal anharmonicity (Δ) can be directly appreciated from the transient spectra, and the $\epsilon_k - \epsilon_l$ value in the SPM/PLPC systems is known to be about 1−3 cm^{-1},[4] we calculate the intermolecular coupling constants to be 0.20 ± 0.06 cm^{-1} under SPM excitation and 0.18 ± 0.04 cm^{-1} under PLPC excitation. To rationalize the coupling constants, we calculate the average coupling of a pair of carbonyl oscillators using the transition dipole coupling (TDC) model.[8] The following conditions are applied: (1) according to our experimental finding, the angle between the transition dipole moments (TDMs) is set to either 140 or 40 degrees; (2) we assume the same value of 2.73 D $Å^{-1}$ $amu^{-1/2}$ for the TDMs of SPM and PLPC carbonyls;[2] and (3) we model the relative structural distribution using a right circular cone geometry, where one TDM is fixed in the vertex point, while the position of another is varied in the basal plane of the cone. The opening angle and the height of the cone are 60 degrees and 0.7 nm, respectively. A height of 0.7 nm gives a safely overestimated measure of the average distance between the carbonyl moieties.[9] This geometry (Supporting Information Figure 4S) brings the base diameter of the cone to the width of the carbonyl distribution along the bilayer normal in a phospholipid membrane.[10] According to this tentative model, we find an average coupling constant of 64 cm^{-1}. Although this value is consistent with those obtained by Torii and Tasumi for di- and tripeptides,[11] it is 2 orders of magnitude higher than the values observed in our experiment. This is an approximate evaluation which might suffer from two possible sources of error. The first is due to insufficiency of the TDC approach on a short distance range. Specifically, the effect of higher order multipoles was demonstrated by Moran and Mukamel[12] to contribute on a distance of about 3−5 Å. However, the distance we adopt in our model makes TDC a reasonable approximation. The second contribution is due to the uncertain value of the dielectric constant in the region of the carbonyl moieties. Assuming the value of ϵ to be in the range from 1 to 20 (20, by large, is an overestimated value), we obtain a coupling constant more than 1 order of magnitude larger than that observed in the experiment, that is, in the range going from 64 cm^{-1} ($\epsilon = 1$) to 15 cm^{-1} ($\epsilon = 20$). The explanation evokes the fact that the cross-correlation is due to the coupling between the carbonyl moieties located at the domain interface. Molecular segregation in sphingolipid phosphatidylcholine composite membrane is well documented[13−15] and attributed to a difference of gel-to-fluid transition temperatures of involved molecular components.[16] Assuming complete segregation of the molecular species in a 100 nm diameter SPM/

PLPC liposome (with a 1:1 approximate composition), the ratio of molecules in bulk to those at the interface is in the range of 100:1. This provides a reasonable explanation for the difference between the experimental and theoretical values. In a forthcoming paper, we will provide an extended discussion on the intermolecular arrangement, correlation, and dynamics at the domain interface of SPM/PLPC membranes in comparison with the results of molecular dynamics simulations.

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Supporting Information Available: Experimental linear FTIR absorption and nonlinear 2D-IR time-resolved spectra of carbonyl moieties in composite phospholipid membranes and the results of their fitting. Two selected horizontal slices from 2D-IR spectra help the reader to better see the off-diagonal peaks. Finally, we report a schematic representation of the model we used for estimating the coupling constant. This material is available free of charge via the Internet at http://pubs.acs.org.

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