Hydration and Orientation of Carbonyl Groups in Oppositely Charged Lipid Monolayers on Water

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ABSTRACT: The carbonyl groups of glycerolipid monolayers on water play an important role in the formation of the interfacial hydrogen bond network, which in turn influences the interactions of lipids with, for example, metabolites. As the frequency of the carbonyl absorption band strongly depends on the hydration state of the lipid headgroups, the carbonyl band is a sensitive reporter of changes in the headgroup environment. Here, we use phase-resolved sum frequency generation spectroscopy to obtain information about the orientation and hydration of the carbonyl groups in lipid monolayers. We find that there are two distinct carbonyl moieties in the lipid monolayers, oppositely oriented relative to the surface plane, that experience substantially different hydrogen-bonding environments.

INTRODUCTION

In glycerolipids, the headgroups are connected to the lipid tails via a glycerol moiety. The carbonyl groups of those ester linkages not only terminate the interfacial hydrogen bond network of water, but also seem to dominate the signal in some surface potential measurement methods. The ester carbonyl groups present in lipid molecules have a strong absorption around 1700–1750 cm⁻¹. The exact frequency of the absorption band is strongly influenced by the packing of the lipid acyl chains and headgroups as well as the hydration state of the headgroups. It is thus a very useful band for characterization of changes in the environment and hydrogen bonding of lipid molecules and for identifying interactions with ligands.

There have been multiple studies using IR spectroscopy to investigate the absorption band of the carbonyl stretch vibration as well as some sum frequency generation (SFG) studies. The carbonyl signal has been reported to have an asymmetric appearance, which has been assigned to the existence of at least two overlapping bands. These bands have been predominantly assigned to hydrogen-bonded and non-hydrogen-bonded carbonyl groups. However, there are also some reports suggesting that the two signals originate from the two different carbonyl groups within one lipid molecule that experience a slightly different chemical environment.

Thus, the position and strength of the carbonyl band provide useful information about the hydration state of the lipid molecules in contact with water. The hydration changes upon changing the lipid density; as the lipid layer becomes more densely packed, the headgroups become less hydrated which results in a shift of the carbonyl vibration to higher wavenumbers. However, little is known so far about the absolute orientation of the lipid carbonyl groups in the monolayer, despite IR studies that characterize the relative orientation of carbonyls in lipid layers. The orientation of the molecular moieties might well influence their ability to form hydrogen bonds to adjacent water molecules and thus the hydration state of the lipid molecules. The absolute orientation is also highly relevant for the contribution of the CO groups to the surface potential. Therefore, investigating the orientation of the lipid carbonyl groups in the lipid monolayer is expected to yield valuable insights into the structure and properties of these biologically relevant interfacial systems. The absolute orientation of molecular moieties at the interface can be elucidated using phase-resolved SFG spectroscopy.

Here, we present intensity and phase-resolved SFG spectra of the lipid–water interface, using the positively charged lipid DPTAP and the negatively charged lipid DPPG as model systems for positively and negatively charged lipid layers, in the carbonyl vibration region at different lipid coverages. DPTAP was chosen for its relatively high surface tension at relatively low coverage. Interestingly, we observe two carbonyl signals with opposite sign. Furthermore, there are significant changes in the carbonyl signals upon changing the lipid coverage.

EXPERIMENTAL SECTION

Sample Preparation. 1,2-Dipalmitoyl-3-trimethylammonium-propane (chloride salt) (DPTAP) and 1,2-dipalmitoyl-

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**RESULTS AND DISCUSSION**

Figure 1a shows the molecular structure of the positively charged glycerolipid DPTAP. Intensity SFG spectra of DPTAP on D$_2$O as well as Im $\chi^{(2)}$ spectra of DPPG are shown in Figure 1b,c. We added 10 mM NaCl to the D$_2$O subphase to keep the ionic strength constant upon changing the lipid coverage. The Im $\chi^{(2)}$ spectra in panel c clearly show that there are two carbonyl signals with opposite sign at 1720 and 1750 cm$^{-1}$. It has previously been reported that the C═O moieties are oriented at a very large angle to the surface normal.$^{9,13}$ We thus expect that both C═O populations are positioned almost parallel to the water surface, albeit being oriented in opposite directions. A positive (negative) signal indicates that the C═O dipole is pointing toward air (water). Thus, the carbonyl groups contributing to the low-frequency signal at 1720 cm$^{-1}$ are oriented with their oxygen atoms pointing toward the water subphase. This orientation toward water enables the formation of hydrogen bonds to adjacent water molecules. As hydrogen bonding lowers the frequency of the carbonyl stretch vibration, we attribute the signal at 1720 cm$^{-1}$ to hydrogen-bonded carbonyl groups, which is consistent with previous IR studies.$^{5,7,15}$ The opposite sign of the signal at 1750 cm$^{-1}$ shows that there are also carbonyl moieties that are oriented with their oxygen atoms pointing up toward the air. The fact that they are oriented in this direction makes hydrogen bonding to water molecules underneath unlikely. The frequency of this signal is accordingly significantly higher, which indicates that it originates from carbonyl groups that do not participate in hydrogen bonding.$^{15}$ A schematic of the orientation of the two carbonyl groups on a lipid is shown in Figure 2. Note that the two oppositely oriented carbonyl groups do not necessarily have to reside on the same molecule. One molecule might have two carbonyl groups with both oxygen atoms pointing down while another molecule might have two carbonyl groups with both oxygen atoms pointing up. Experimentally, we cannot distinguish between the two
scenarios of the two opposite orientations appearing within one molecule or in separated molecules.

Upon comparing the spectra of the DPTAP–water interface at different coverages, one sees that the high-frequency signal increases in amplitude upon increasing the lipid coverage. Furthermore, the frequency of both carbonyl signals shifts to lower wavenumbers upon increasing the lipid coverage. To quantify those changes, the intensity and Im $\chi^{(2)}$ increase with increasing the lipid coverage. This shift is more prominent in the high-frequency than in the low-frequency signal. This interconversion would give rise to the average distance between “free” carbonyl groups decreasing more quickly than expected from the increase in density, resulting in increased coupling and larger frequency shift. As it is known that water is squeezed out from the headgroup region upon increasing the lipid density of the monolayer, it is likely that the relative number of hydrogen-bonded carbonyl groups decreases.

The absolute areas of the two carbonyl resonances obtained from the fits as a function of lipid coverage are shown in Figure 3b. Interestingly, the low-frequency signal remains constant while the high-frequency signal increases with increasing amount of lipid in the monolayer. Upon increasing the lipid coverage, the number of lipid molecules increases. As a result, a larger SFG signal is expected. The fact that the area of the low-frequency signal stays constant while the “free” carbonyl signal increases is thus consistent with the interpretation that there is interconversion from hydrogen-bonded to “free” carbonyl moieties. However, one has to keep in mind that the SFG signal intensity depends not only on the number of molecules but also on their orientation. It is therefore difficult to unambiguously interpret these changes in the data. The constant area of the low-frequency signal might also be explained by a change in the orientation of the hydrogen-bonded carbonyl groups that compensates an increase in the number of oscillators. The increase in the high-frequency signal might also result from a change in the orientation and not necessarily mainly from an increase in the number of groups is about 5 Å, because there are two carbonyl groups per lipid molecule. It is thus plausible that dipole–dipole coupling is responsible for the observed red shift with increasing density.

As mentioned above, the red shift is more pronounced in the high-frequency than in the low-frequency signal. This might be explained by the interconversion of hydrogen-bonded to “free” carbonyl population upon increasing the lipid coverage. This interconversion would give rise to the average distance between “free” carbonyl groups decreasing more quickly than expected from the increase in density, resulting in increased coupling and larger frequency shift. As it is known that water is squeezed out from the headgroup region upon increasing the lipid density of the monolayer, it is likely that the relative number of hydrogen-bonded carbonyl groups decreases.
oscillators. Nevertheless, the observed frequency shifts combined with the changes in the peak areas seem to support our interpretation. A decrease in headgroup hydration upon increasing the lipid coverage has also been reported by Ma and Allen, who investigated the phosphate vibration of the lipid headgroup.

To demonstrate the generality of the finding of different carbonyl groups that are differently oriented, we also measured SFG spectra of the negatively charged DPPG on D2O in the carbonyl vibration region. Figure 4a shows the molecular structure of DPPG. The resulting intensity and Im $\chi^{(2)}$ spectra of the DPPG–D2O interface at a lipid coverage of 2.2 molecules/nm$^2$. The dotted lines in the panels b and c represent the experimentally acquired spectra and the solid lines the fits.

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