Weakly hydrogen-bonded water at ionic lipid interfaces observed with heterodyne-detected sum frequency generation spectroscopy

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Many biochemical reactions proceed at biomembrane interfaces. Since the biomembrane is surrounded by water, the progress of the biochemical reaction is strongly influenced by the water. Although water inside biomembranes is considered to have a significant effect on the biochemical reaction, its molecular structure has not been revealed. In this study, we investigate the structure and the orientation of water at ionic lipid monolayer interfaces with heterodyne-detected sum frequency generation (HD-SFG) spectroscopy.

SFG is a second-order nonlinear optical process. Since the second-order nonlinear optical susceptibility $\chi^{(2)}$ is nonzero only at an interface where the centrosymmetry is broken, SFG is surface-selective. The relation between the real and the imaginary parts of $\chi^{(2)}$ is the same as that of the linear optical susceptibility. Therefore, the Im$\chi^{(2)}$ (imaginary part of $\chi^{(2)}$) spectra can be regarded as the “absorption” spectra of the interface and more informative than conventional $|\chi^{(2)}|^2$ spectra. Recently we developed the single-channel HD-SFG spectrometer that can directly provide the Im$\chi^{(2)}$. The spectrometer has a spectral resolution of 4 cm$^{-1}$, which is high enough to investigate the precise band shape of the OH stretching band of the weakly hydrogen-bonded water within the lipid monolayer.

Widely-used ionic lipids, dipalmitoyl-phosphoglycerol (DPPG), dipalmitoyltrimethylammonium propane (DPTAP), and dipalmitoyl-phosphatidylcholine (DPPC) were used for measurements. DPPG has a negative charge, DPTAP has a positive charge, and DPPC has both positive and negative charges in the head group. The net charge of the DPPC head group is zero. The structures of the hydrophobic group of these lipids are same. The lipids were dissolved in chloroform/methanol mixture or chloroform and the lipid solution was spread on an ultrapure water surface to form a lipid monolayer.

Figure 1 shows the Im$\chi^{(2)}$ spectra of the DPPG, DPTAP, and DPPC interfaces. The CH stretching bands are observed at 2875, 2937, and 2962 cm$^{-1}$. The shapes of the CH bands are identical among three interfaces, indicating the similar structure of the hydrophobic group of the lipids at the lipid/water interfaces. In the region between 3000 and 3500 cm$^{-1}$, the bands of the symmetric OH stretching of hydrogen-bonded water below the lipid head group are observed. The sign of the Im$\chi^{(2)}$ indicates the
orientation of molecules at the interface. The positive sign of the OH band for the DPPG interface represent a H-up orientation of water (Figure 2). The sign of the OH band for the DPTAP interface is opposite to DPPG, indicating that the orientation of water below the head group is determined by the net charge of the lipid, as reported previously. The positive sign of the OH band for the DPPC interface agree with the previous work, which indicates the net H-up orientation of water at the DPPC interface.

In the region between 3500 and 3800 cm\(^{-1}\), new features are observed; a negative band for the DPPG interface, a positive band for the DPTAP interface, and no band for the DPPC interface. For the DPPG and DPTAP interfaces, the sign of the bands are opposite to those of the OH bands between 3000 and 3500 cm\(^{-1}\). This result means that the bands above 3500 cm\(^{-1}\) are due to water oriented oppositely to that below the head group. Since oppositely oriented water is not expected below the head group, the bands observed for the DPPG and DPTAP interfaces are attributed to water above the head group (Figure 2). Water above the head group is likely to be bound to carbonyl and/or ester moieties of lipids. These results indicate that the net orientation of water above the head group is also determined by the net charge of the lipid. The observed zero Im\(\chi(2)\) for the DPPC interface suggests that water above the head group has a random orientation because of the electric neutrality of the DPPC head group.

**REFERENCES**