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# Structure of Water at Nonionic Lipid/Water Interfaces Revealed by Heterodyne-Detected Vibrational Sum Frequency Generation Spectroscopy

(Molecular Spectroscopy Laboratory, RIKEN) ADHIKARI, Aniruddha; NIHONYANAGI, Satoshi; YAMAGUCHI, Shoichi; TAHARA, Tahei

**[Introduction]**

Despite its ubiquitous presence in nature and importance in many processes of biological relevance, our knowledge about lipid/water interfaces remains rather limited. This is chiefly due to difficulties in experimentally detecting and characterizing such interfaces with sufficient molecular level sensitivity. Heterodyne-detected vibrational sum frequency generation (HD-VSFG) is a novel nonlinear spectroscopic tool which allows us to overcome these difficulties and gain useful insights into lipid/water interfaces. The HD-VSFG experiment is capable of determining the net orientation of water molecules at a given interface. The sign of the OH band as revealed in the imaginary $\chi^{(2)}$ spectrum of interfacial water provides a direct evidence about whether the water molecule is oriented with its H atoms pointing towards or away from the interface. Using HD-VSFG, we have previously studied interfaces between water and anionic, cationic and zwitterionic lipids and clarified the orientation and hydrogen-bond structure of water at these ionic lipid/water interfaces.[1,2] Our studies revealed that for cationic lipid interfaces comprising of 1,2-dipalmitoyl-3-(trimethylammonium) propane (DPTAP), the water exists in the H-down orientation whereas the net orientation of interfacial water molecules is opposite (H-up) to that of the cationic lipids for anionic lipids such as 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (DPPG). These observations can be readily rationalized by the direction of static electric field created by the charge of the headgroup at the interface.[1] For a zwitterionic lipid such as 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), the situation is more complex. However, the peculiar double peaked imaginary $\chi^{(2)}$ spectrum indicates that there are 3 different types of water molecules that are associated with the negatively charged phosphate, positively charged choline and the weakly interacting hydrophobic region of the lipid.[2]

In this work, we extend our HD-VSFG study of lipid/water interfaces and study the structure of water at a nonionic lipid/water interface.

**[Experiment]**

We chose 1,2-dipalmitoyl-*sn*-glycerol (DPG) for our study beacause it is nonionic and bears no charge. This is in contrast to a zwitterionic lipid where the headgroup bears moieties with opposite charges thus making the lipid net neutral. Figure 1 shows the chemical structure of DPG. It belongs to the

biologically important Diacyl Glycerol (DAG) class of lipids. We used isotopically diluted water in our studies to minimize the effect of intra/intermolecular couplings in the imaginary $\chi^{(2)}$ spectra and thus allow a straightforward interpretation of the spectrum.

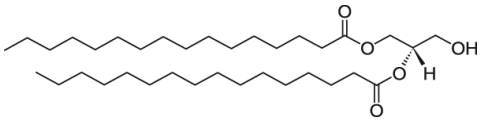


**Figure 1**. Chemical structure of 1,2-dipalmitoyl-*sn*-glycerol (DPG).

**[Results and Discussion]**

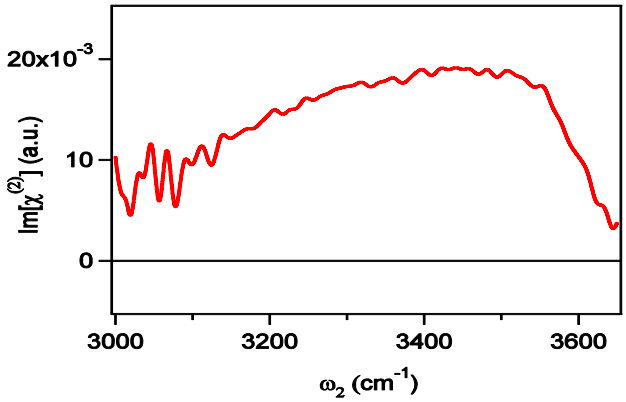


**Figure** 2. Im $\chi^{(2)}$ spectra of 1,2-dipalmitoyl-sn-glycerol (DPG) in 20% HOD/$D_2O$ at the air/water interface. The sum frequency, $\omega_1$, $\omega_2$ beams were s-, s-, p-polarized, respectively.

Figure 2 shows the imaginary $\chi^{(2)}$ spectrum of the nonionic DPG/water (HOD) interface in the OH stretch region. The positive sign of the OH band indicates that the water at the interface of this nonionic lipid interface takes net H-up orientation despite the absence of a charge on the lipid headgroup. To verify the generality of this trend we also studied monolayers of two other nonionic OH headgroup bearing amphiphilic molecules, octadecanol and cholesterol at the air/water (HOD) interface. Similar positive sign for the OH band was obtained in both cases. These results suggest that the hydroxyl oxygen in the headgroup behaves as an acceptor of hydrogen bond from the interfacial water molecules and makes their H atoms directed towards the headgroup's oxygen (thus favoring a net H-up orientation).

It is concluded that the orientation and structure of interfacial water molecules in the vicinity of nonionic lipids is very different from that of zwitterionic lipids.

**[References]**


1. Mondal, J. A.; Nihonyanagi, S.; Yamaguchi, S.; Tahara, T. *J. Am. Chem. Soc*. **2010**, *132*, 10656.
2. Mondal, J. A.; Nihonyanagi, S.; Yamaguchi, S.; Tahara, T. *J. Am. Chem. Soc*. **2012**, *134*, 7842.