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Properties of Water at the Lipid-DNA Interfaces Revealed by Heterodyne-Detected Vibrational Sum Frequency Generation (HD-VSFG) Spectroscopy

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[Introduction]

Cationic lipids are widely used as in vitro DNA transfection vectors and show promise as non-viral carriers for in vivo gene therapy. In solution, DNA and cationic lipids form complexes that can enter the cell through endocytosis. Understanding and ultimately controlling the electrostatic interactions between these lipids and DNA is therefore essential to optimize these genetic carriers. Although numerous in vivo and in vitro studies have been performed to explore the structural properties of the lipid-DNA interaction, yet the molecular level understanding of this interaction is very limited.¹ Vibrational spectroscopy is a useful tool to get the information about the molecular level interactions in lipoplexes. Several groups have conducted infrared absorption measurements of various DNA/lipid systems. However, due to the lack of inherent surface sensitivity/specificity of infrared absorption, we still do not get clear understanding of the complexation of DNA with lipids. In this study, we overcome the surface specificity/sensitivity challenge by applying heterodyne-detected vibrational sum frequency generation (HD-VSFG)² spectroscopy to gain a molecular level picture of the interaction between DNA, lipids, and interfacial water.

[Experimental]

A narrow band visible (ω_1) and broadband IR (ω_2) beams are used for the HD-VSFG. The SF, visible and IR beams were s-, s-, and p-polarized, respectively, (ssp polarization). All the spectra were normalized to a quartz spectrum. 1,2-dipalmitoyl-3-trimethylammonium propane (DPTAP) was purchased as lyophilized powders from Avanti Polar lipids. λ -phage DNA (each molecule specified as 48502 base pairs in length by the manufacturer) was purchased from Sigma Aldrich. The surface pressure was monitored continuously during the HDVSFG measurement. All the measurements were done in liquid condensed (LC) phase of DPTAP with surface pressure of 31 ± 3 mN/m.

[Results and Discussion]

Figure 1 shows complex $\chi^{(2)}$ spectra in the OH stretching region of the DPTAP monolayer/ aqueous solution interfaces containing DNA at various concentrations ranging from 0 to 220 pM. Two broad negative band around 3200 cm^{-1} and 3450 cm^{-1} in the $\text{Im}\chi^{(2)}$ spectra at 0 pM DNA have been assigned to the symmetric OH stretching of hydrogen bonded water, split

by Fermi resonance with the bending overtone. The sign of the $\text{Im}\chi^{(2)}$ spectra in the OH stretching region is negative, indicating that the water molecules at these interfaces are oriented with their hydrogen pointing downward to bulk water. When no DNA is added, the OH band intensity is about five times larger than that at the neat water surface because the static electric field created by the DPTAP monolayer induces orientation of more water molecules along the field. Addition of DNA to the DPTAP subphase results in a reduction of water signals even at concentrations as low as 10 pM. Around 100 pM, the signal reaches to the level of the neat air/ water interface. This implies that already at these low concentrations, the static electric field is fully screened by the polyanionic DNA. This result indicates that DNA reaches the interface and has specific interaction with the lipid. As the DNA concentration increases further ($>200\text{ pM}$), the sign of $\text{Im}\chi^{(2)}$ in the OH stretch region becomes completely positive which shows that the orientation of the water molecules change from hydrogen down to hydrogen up at the DPTAP interface. This flipping of water orientation at the interface depicts that DNA makes a contact adsorption to the lipid head group and net surface charge of the interface turn to be negative. This study clearly exhibits the applicability and novelty of the HD-VSFG technique to get the inherent molecular level understanding of biological interfaces.

[References]

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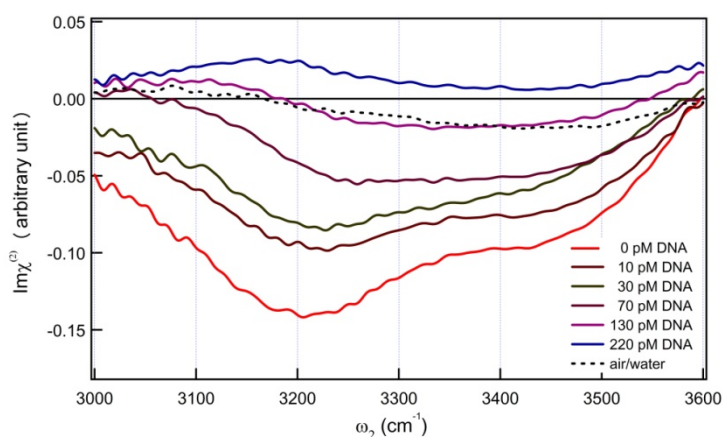


Figure 1. $\text{Im}\chi^{(2)}$ spectra of air / DPTAP monolayer / H_2O interfaces in the OH stretching frequency region containing DNA of the concentrations at 0, 10, 30, 70, 130, 220 pM. Black dotted line shows the $\text{Im}\chi^{(2)}$ spectrum of air/water interface.