

Analysis of Interfacial Water Structure close to Mixed Langmuir Monolayers by Sum-Frequency Spectroscopy

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ABSTRACT

Sum frequency spectroscopy (SFS) in the OH stretching region was employed to obtain structural information about the interfacial water structure underneath Langmuir monolayers on H₂O subphase. The films were composed by the model lipid dioctadecyldimethylammonium bromide (DOMA or DODAB), the surfactants methyl stearate (SME) and stearic acid (STA), and their mixtures. The results demonstrated that the uncompensated head group charge of DODAB at the interface is the reason for structuring of interfacial water close to the mixed DODAB-methyl stearate monomolecular films. To get insight into this charge-induced effect SFS studies of the structure of the stearic acid monolayer – water interface as a function of pH were performed. At low pH values the stearic acid monolayer is neutral and the water structure close to it is mostly disordered, while at high pH values it is negatively charged and interfacial water structure is strongly promoted by the surface electric field.

Keywords: sum-frequency spectroscopy, interfacial water structure, Langmuir monolayers, surfactants-lipid films.

1 INTRODUCTION

Studies of the liquid/gas interface are of great interest to biology (biomembranes, bilayers, vesicles) and colloid science (reverse micelles, foams, self-assembled films, froth floatation). Most frequently, a bidimensional confinement of amphiphilic molecules with specific structural organization (Langmuir film) is used as a simple but accurate model system for an ordered liquid/gas interface. At high enough surface pressure the Langmuir film – water – air interface consists of three components: solid-like hydrophobic part (the alkyl tails), solid-like hydrophilic part (the head groups) and the interfacial water layer structured to an extent depending on the interactions of water molecules with the head groups. In situ nondestructive characterization of the Langmuir film–water interface can be obtained by monitoring surface pressure and area isotherms, by Brewster angle and fluorescence microscopies. The molecular structure of the film can be deduced by IR external reflection spectroscopy, and by

X-ray and neutron diffractions. All those techniques, however, are not sensitive to the state and structure of interfacial water. Sum-frequency generation spectroscopy (SFG) is an inherently interface specific method [1, 2] and is capable to provide structural information not only about the monolayer, but also about the water layer immediately beneath it.

It is well known that water structure at interfaces is very different compared to bulk water, because of the asymmetry of the interface and often due to its charged nature. Strong electrical fields can exist at an aqueous interface with an ordered Langmuir or self-assembled monolayer and lead to ordering effects in the interfacial water molecules. The ordered water layers have in turn a strong SFG signal in the OH stretching region which can be used to construe the hydrogen bonding patterns and orientation between the water molecules.

Adsorption of proteins at interfaces (lipid membranes) and other biomaterials is influenced by the interface properties: surface potential, lipid density and heterogeneity. These properties often depend on the interfacial water structure [3, 4]. Here we report SFG studies of water structure adjacent to simplified cell membrane models consisting of single surfactant or mixed lipid–surfactant Langmuir monolayers.

2 EXPERIMENTAL DETAILS

2.1 Sum-Frequency Spectroscopy

SFG principles have been described in detail elsewhere [1, 2]. In brief, SFG is a surface specific method based on second order nonlinear optical mixing in which two pulsed laser beams, one visible and the second infrared (IR), at frequencies ω_{vis} and ω_{IR} , overlap temporarily and spatially on an interface to generate an output at the sum frequency $\omega_{SF} = \omega_{IR} + \omega_{vis}$. The underlying principle for the surface specificity of SFG is that second order nonlinear optical processes are forbidden in bulk media with inversion symmetry, but at a surface or interface the inversion symmetry is broken and sum-frequency generation becomes possible. If the IR frequency is tunable, the technique becomes sensitive to the molecular structure of

the interface by detecting vibrational resonances of interfacial molecules.

In our experiments, we have used an EKSPLA, Ltd. picosecond sum-frequency (SF) generation spectrometer [5]. The visible beam is at 532 nm, and the tunable IR beam covers the range 2.3 – 10 μm (4350 – 1000 cm^{-1} in vibrational frequencies). The tunable IR laser is a combination of an optical parametric generator - difference frequency generator. The visible and IR beams were overlapped spatially at the liquid/air interface over an area of ca. 500 μm in diameter. Both pulses also overlapped temporally by using an optical delay line in the path of the visible beam. The incident angles of the visible and IR beams at the interface were 60° and 55° degrees, respectively. The SF signal was collected in reflection at an angle of ca. 60°. A monochromator, photomultiplier and a gated electronics system were used for detection. The sum-frequency spectra were collected in *ssp* polarization combination of the three beams (*s* - sum-frequency, *s* - visible, *p* - infrared). Data were collected at 6 cm^{-1} increments and each point is the average of 100 laser shots. The spectral resolution was determined by the spectral width of the IR beam which is $\leq 6 \text{ cm}^{-1}$. All spectra were recorded at constant temperature of 24 $^{\circ}\text{C}$.

2.2 Materials and Monolayer Preparation

Milli-Q water from a Millipore filtration system with a resistivity of 18 $\text{M}\Omega\cdot\text{cm}$ (pH=5.6) was used as a subphase in the preparation of the Langmuir films. Dioctadecyldimethylammonium bromide, DOMA or DODAB, $(\text{CH}_3(\text{CH}_2)_{17})_2\text{N}(\text{CH}_3)_2\text{Br}$, of 99% purity was obtained from Sigma, methyl stearate (SME, $\text{CH}_3(\text{CH}_2)_{17}\text{COOCH}_3$) was a GC standard from Merck, and stearic acid (STA, $\text{CH}_3(\text{CH}_2)_{17}\text{COOH}$), 99% pure was from Aldrich. Chloroform (Sigma, HPLC grade) solutions of these lipids with concentration of 1 mM were prepared and stored at 4 $^{\circ}\text{C}$ until use. SME-DOMA and STA-DOMA mixtures with different mole ratios were prepared volumetrically from the stock solutions.

A Langmuir trough (MicroTrough S, Kibron, Inc.) was used for the preparation of the monolayers. Due to its compact size it was easily integrated in the SF spectrometer detection scheme without compromising the alignment of the beams. Surface pressure isotherms on water subphases (20 $^{\circ}\text{C}$) were obtained by spreading the studied films into their “gaseous” (uncompressed) phases, waiting 10 min then compressing at 5 $\text{\AA}^2 \text{ molecule}^{-1} \text{ min}^{-1}$ until collapse. Surface pressures, at which the SF spectra were recorded were between 5 and 35 mN/m . The surface pressures were kept constant during spectra collection with the help of the integrated feedback of the trough.

3 RESULTS AND DISCUSSION

3.1 Interfacial Water OH Stretching Spectra at Mixed Lipid Monolayers

The OH stretching spectra of interfacial water beneath the pure DOMA monolayers, SME:DOMA monolayers at mixing ratios 2:1, 6:1 and 10:1, and pure SME monolayers are shown in Figure 1, a-e. Significant O–H stretching band signals (3100 – 3700 cm^{-1}) from the DOMA–water interface, Figure 1a, suggest that a highly ordered layer of water molecules forms beneath the DOMA monolayer. Usually SFS spectra of interfacial water structure have two characteristic regions, one centered at ca. 3240 cm^{-1} , and a second at ca. 3440 cm^{-1} [6]. The first one is assigned to the OH vibrations of tetrahedrally coordinated water molecules, and is often referred to as “ice-like” structure component to account for the fact that the vibrational spectrum of ice is characterized with strong intensity in this region [7]. The second is attributed to water molecules that are involved in not so well ordered structures, connected to other water molecules with distorted, weaker H-bonds. Either of these two assignments is, however, characteristic of water molecules with more disordered hydrogen bonding than in the solid (ice) and the term “water structure” should be accepted as describing a time averaged picture of the hydrogen bonding between water molecules in the liquid state. In addition, we should have in mind that due to its inversion symmetry bulk water does not generate SF signal.

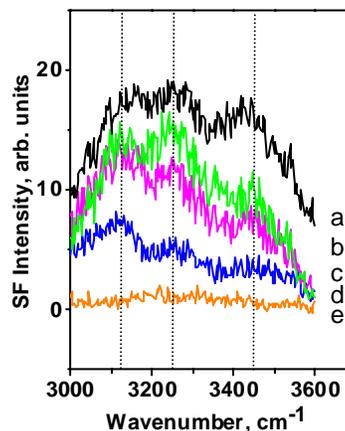


Figure 1: Sum-frequency spectra of interfacial water close to model mixed lipid monolayers at pH=5.6 a) Pure DOMA; b) SME:DOMA=2:1; c) SME:DOMA=6:1; d) SME:DOMA=10:1; e) Pure SME. Dashed lines indicate wavenumbers of the vibrational modes discussed in the text

It is believed that the orientation and ordering of water molecules at an interface is most often a result of the alignment of their molecular dipoles with the electric field present in the interfacial region. In the case of the DOMA–water interface this field is due to the presence of $\text{N}^+(\text{CH}_3)_2$ ions in the head groups of the DOMA molecules arranged

in the two-dimensional ordered lattice of the Langmuir film. Thus, we will refer to the interfacial water structure promoted by the charged monolayer interface as “electric field induced”.

When SME–DOMA mixed monolayers are studied one more component in the OH band of interfacial water appears, located at ca. 3130 cm^{-1} , Figure 1a - 1d, in addition to the two OH band components normally observed at water/air interfaces at ca. 3240 and 3440 cm^{-1} . The lower wavenumber of this component characterizes it as corresponding to OH oscillators taking part in hydrogen bonding with increased strength. We assign this component to clusters of water molecules, which are directly bonded to the head groups of the DOMA molecules. Although this component can be noticed in the OH band of the pure DOMA–water interface, Figure 1a, it is not well resolved. With the increase of the SME fraction in the mixed films, the structured interfacial water layer becomes thinner as seen by the decrease in intensity of the total OH band signal, Figure 1b - 1d. Interestingly, the intensity in the low frequency part of the OH band (3130 cm^{-1}) increases its contribution relative to the components at ca. 3240 and 3440 cm^{-1} with SME increase, Figure 1c, 1d. Finally, there is no OH signal from the pure SME–water interface, Figure 1e. The decrease in the total intensity of the OH band can be explained by the reduced DOMA head group dipole concentration which results in decreased thickness of the electric field induced ordered water layers. Most probably at ratios SME:DOMA 10:1 most ordered water molecules at the interface are in clusters bonded to the head groups. The absence of OH band signal in the spectrum of the pure SME–water interface can be explained by the nonpolar nature of the SME molecules. The SME head groups, in addition to being neutral in water, are somewhat hydrophobic due to the presence of a methyl group. Apparently, this also contributes for a more disordered water structure close to the mixed films and for completely disordered (bulk-like) water structure beneath the pure SME film.

3.2 Interfacial Water OH Stretching Spectra at Stearic Acid Monolayers

To support the hypothesis that the structuring of interfacial water close to the studied monomolecular Langmuir films is mostly due to the uncompensated head group charge of DOMA we have examined the interfacial water SF spectra close to a mixed stearic acid (STA)–DOMA monolayer and compared it to the spectra of the pure STA monolayer–water interface. There was no signal in the OH stretching band region of the mixed monolayer suggesting that the water molecules at the interface are completely disordered and thus, having inversion symmetry, are not capable of producing an SF signal. In contrast, the pure STA–water interface showed a weak but noticeable OH band signal characteristic of water structuring. The structuring of water at the STA–water

interface is believed to be due to the partial deprotonation of the STA head groups at the natural pH of 5.6, which results in partial negative charge of the film. The negatively charged STA film orients the water dipoles with their hydrogen atoms pointing at the interface. To further investigate this effect we have studied the dependence of the interfacial water structure SF spectra on the pH of the subphase in the conditions of fully compressed (well ordered) STA monolayers, Figure 2.

At $\text{pH}\approx 3$ we have observed no SF signal in the OH stretching region and therefore assume that the water structure close to it is completely disordered. This is a proof that the STA monolayer has no charge and is electrically neutral at these conditions. At pH values between 3 and 9, Figure 2, the stearic acid head groups are progressively deprotonated and some of them become negatively charged. This leads to the formation of structured water layer beneath the STA monolayer. Its thickness increases with the increase in pH as demonstrated by the increased intensity of the SF signals. At very high pH values ($\text{pH}>10$) the monolayer is completely ionized: ice-like water signal dominates the SF spectra and the thickness of the ordered interfacial water layer is maximized: the SFS signal is maximal, Figure 2, (a, b).

The interfacial water spectra close to STA monolayers at different pH values of the subphase are also characterized by the two basic regions, here at ca. 3210 and 3440 cm^{-1} . Interestingly, at pH values of 8 and 4.3, Figure 2, b and e, the maximum of the not completely tetrabonded water component shifts to ca. $3500 - 3550\text{ cm}^{-1}$. At $\text{pH}=4.3$ this is the only type of structured interfacial water just starting to form. At $\text{pH}=8$, Figure 2,b the biggest fraction of interfacial water molecules assume tetrabonded arrangement characterized by the strong peak at 3210 cm^{-1} , but the appearance of the weaker and clearly defined peak at 3500 cm^{-1} needs further analysis.

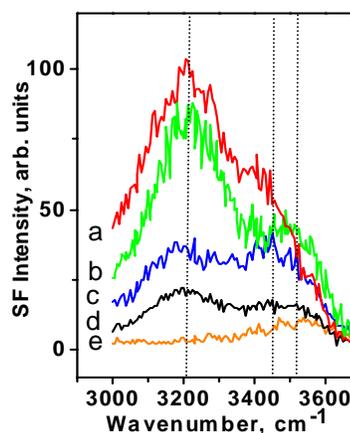


Figure 2: Sum-frequency spectra of interfacial water close to stearic acid monolayers at different pH: a) $\text{pH}=11.5$; b) $\text{pH}=9.8$; c) $\text{pH}=8$; d) $\text{pH}=5.6$; e) $\text{pH}=4.3$. Dashed lines indicate wavenumbers of the vibrational modes discussed in the text

4 CONCLUSIONS

Sum frequency spectroscopy proves to be a very successful and the only available method to study effectively the structure of interfacial water close to model biomimetic structures (Langmuir films). The results from SFS analysis of interfacial water help obtain a better understanding of the conditions necessary to design mixed monolayers with enhanced properties for effective protein adsorption. The high sensitivity of SFS to interfaces of lipid monomolecular films with water and the capabilities to perform in situ nondestructive measurements offers significant, often unique, advantages, over other surface sensitive techniques used in similar studies before. More SFS studies at the lipid (protein) monolayer-water interface are needed to explore the full potential of the method for bio-nanotechnology and medical applications.

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