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VIBRATIONAL SPECTROSCOPIC STUDIES TO ELUCIDATE THE STRUCTURE OF WATER AT BIOLOGICAL INTERFACES

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Abstract- In biological systems, water takes up to 80% of the volume inside a cell. This water solubilizes the biological macromolecules such as the DNA, proteins and lipids. Recent advancements have shown that the water at the interface of a lipid membrane is structured, as five layers of structured water have been found at this solvent cage. Steady state Raman spectroscopy of water in lipids was performed in an attempt to elucidate the structure of water at the biological interface. Deuterium oxide (heavy water) was employed to hydrate lipid biomolecules. The heavier deuterium atom shifts the molecular vibrations and renders them distinct from conventional OH vibrations. Raman spectroscopy was used to probe the difficulties of observing the vibrational signature of the water molecule at low hydration limits. It was demonstrated that Raman can identify signatures of potential structured forms of water at the interface with lipid membranes.

Key Words: Structured water, lipid interface, Raman spectroscopy, deuterium oxide, heavy water.

I. INTRODUCTION

The Structure and Properties of Water

Water is an unusual molecule that takes up more than 70% of the earth's surface. This unusual substance is essential to life on earth, and is a main component of almost all forms of life. Each water molecule consists of two hydrogen atoms covalently bonded to a single oxygen atom. Due to the nature of the atomic structure of water, it has very unique electrochemical properties. In a water molecule, oxygen has a higher electronegativity than the hydrogen, thus electrons are more attracted to the oxygen atom. Because of this, the oxygen atom has a partial negative charge while the hydrogen has a partial positive charge. As a result, water is considered to be a polar molecule with a large permanent electric dipole moment, and this property leads to a very large dielectric constant in the bulk form^[1].

In biology, water takes up to 80% of the volume in a cell. This water acts as an environment for the DNA, proteins and lipids, and in fact solubilises these biological macromolecules. Despite the prevalence of water in a cell, very little is known about its structure at the liquid/molecular interface^[2]. The lack of understanding of water at biological interfaces hinders our understanding of the various biomolecular associations within a cell^[3].

Liquid water is normally considered to be amorphous, meaning there is no long range atomic order. Conversely, solid water (ice) is crystalline, indicating molecular organization. For this reason, water that is amorphous is known as unstructured water, and water that is crystalline is structured water. During a phase change, water molecules orientate themselves in distinctly different patterns. Frozen water molecules arrange themselves in a

highly ordered geometric pattern, whereas liquid water molecules are arranged in small clusters of joined particles. Notably, water has been demonstrated to exhibit at least fifteen different crystalline forms^[4], the basis for example of the many different patterns of snowflakes. This variability in structural forms, coupled with the large molecular electric dipole moment lead to the suggestion that a layer of water around specific biomolecules may have a characteristic structure and potentially play a functional role in the biochemistry of the molecule.

The Structure and Properties of Lipid

Lipids are organic molecules that consist of carbon, hydrogen and oxygen. The main classes of lipids include fats, waxes, steroids, and phospholipids. Phospholipids, also referred to as glycerophospholipids, are a class of lipids that are key components of biological membranes. Both extracellular cellular plasma membranes and intracellular organelle membranes are predominantly made up of phospholipids.

Phospholipids are amphipathic bio-molecules constituted of hydrophilic and hydrophobic components. The hydrophilic head group contains one or more phosphate groups, while the hydrophobic tail is made up of two fatty acyl chains. It is through the presence of water that the molecules go through a natural process and spontaneously self-assemble into a bi-layer. The hydrophilic heads are polar and face towards the water, while the hydrophobic tails are non-polar and are directed inwards to the core of the bi-layer.

An important feature of the lipid bi-layer is its highly impermeable structure, meaning that it allows only certain molecules to freely pass through, while inhibiting others.

Only water and gases are easily able to diffuse through the bi-layer. Because of this, large molecules cannot cross the bi-layer without the assistance of other structures. For example, lipids play a major role in the barrier function of skin^[5]. Other classes of lipids are important for biological functions such as energy storage, cell signalling and providing nutrients. Hydrophobic hydrations are critical in biological functions such as ligand binding, membrane formation, and protein folding^[6].

II. THE EXPERIMENT

The purpose of this study was to examine the potential role of structured water in biological systems, using the example of the interface with lipid membranes. Previous studies using Atomic Force Microscopy have indicated that up to five well defined layers of water are present at the interface. Raman spectroscopy was chosen as the analytical technique as it is increasingly being explored for potential diagnostic and biochemical applications.

Raman Spectroscopy

Raman spectroscopy is a spectroscopic technique, which relies on the Raman effect that is based on an inelastic scattering of photons of light by material vibrations. The Raman effect was proposed and demonstrated by Sir C.V. Raman in 1928^[2] and independently by G. Landsberg and L. Mandelstam^[3]. Inspired by the accepted inelastic scattering of X-rays, Raman proposed a "New type of secondary radiation" or "modified" scattering which resulted from the effect of the fluctuations from the normal state of atoms and molecules associated with vibrations. He demonstrated that, in addition to elastic (Rayleigh or Mie) scattering, in which radiation scattered by a material has the same energy (frequency/wavelength), light could be inelastically scattered through a gain or loss of photon energy to the molecular vibrations of the material. The spectrum of the inelastically scattered radiation represented a fingerprint of the molecular vibrations within a material. The observation of the Raman effect gave rise to the field of Raman spectroscopy, a versatile alternative to infrared absorption spectroscopy and now a common analytic laboratory tool. C.V. Raman was awarded the Nobel prize in physics in 1930 for his work and in 1998 the Raman Effect was designated an ACS National Historic Chemical Landmark in recognition of its importance in materials and process analysis.

Raman spectroscopy is a great way of obtaining detailed chemical information because it is a non-destructive and non-invasive technique. It is used to find crystallographic arrangements of molecules within a sample. The vibrations of a molecule can be simplified as a harmonic oscillator, governed by Hooke's law, in which the bond strength is represented by the spring constant, k , and the atoms by an effective mass μ . The frequency of a bond can therefore be represented by $\nu = (k/\mu)^{1/2}$ and is therefore characteristic of the molecular vibration. However, for a molecule to be Raman active, there must be change in the polarizability. Symmetric stretches normally imply that the molecule is Raman active.

Raman spectroscopy was employed during this study in order to obtain spectra of deionized and deuterium oxide both in their liquid and solid forms. The heavier hydrogen in deuterated water render the vibrations distinct from any environmental or other water. In addition, spectra of lipid and water (isolated in different ways) were obtained.

Data Presentation and Interpretation of Results

Spectra of Water

Spectra of deionized water and deuterium oxide at room temperature (RT) and at 77K were obtained using Raman spectroscopy and are plotted in Figures 1 and 2. At room temperature, the deuterated and deionized water have broad spectra. However, when they are brought to a very low temperature (77 K), both spectra dramatically sharpen. The spectra of both types of water at 77K are considered to be representative of mixed crystalline forms of water.

In biological systems, it is anticipated that the majority of water will be unbound. Structured water at a biological interface however should be similar to crystalline water. Frozen water should contain a range of crystalline forms. From previous studies of water in skin, it was concluded that bound water has a characteristic vibration at 3212 cm^{-1} , partially bound water and 3280 cm^{-1} , partially unbound water at 3343 cm^{-1} , and unbound water is 3465 cm^{-1} ^[7]. The lowering of the frequencies of the O-H vibrations in the bound case is the result of an increase of the effective mass of the bond due to coupling with the neighboring environment. In figure 1, the largest Raman peak is at $\sim 3450\text{cm}^{-1}$, consistent with the dominance of unbound water in skin. In the frozen water, there is a Raman peak at $\sim 3210\text{cm}^{-1}$, consistent with bound water in skin, although the dominant peak is shifted to even lower frequencies $\sim 3020\text{cm}^{-1}$, indicating even tighter binding in crystalline water.

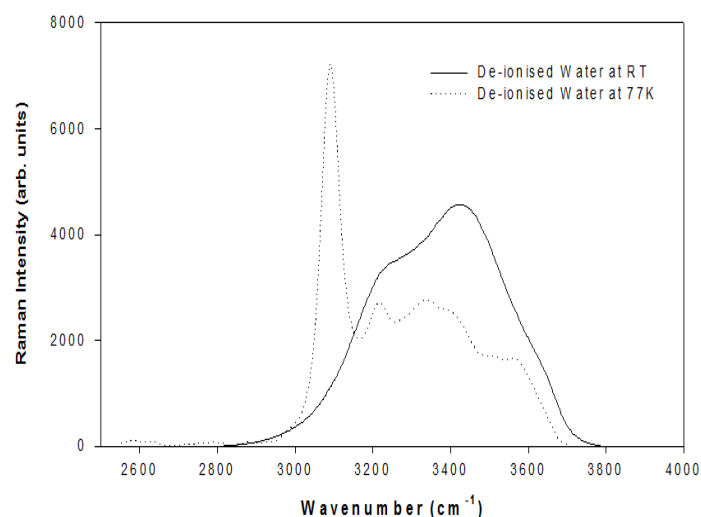


Figure 1: Raman Spectrum of Deionized Water, is RT defined as room temp in the text

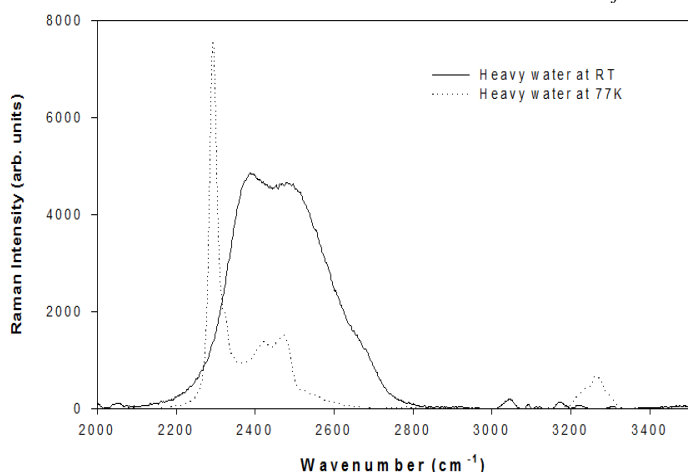


Figure 2: Raman spectrum of deuterium oxide

Figure 2 shows the Raman spectra of deuterium oxide at room and low temperature. As expected, due to the heavier hydrogen atom, the Raman features are shifted to lower frequencies. A similar sharpening of the features and shift to lower frequencies is observed at low temperatures. Importantly, the distinctive features are significantly shifted from the frequencies of normal unbound water, and therefore deuterium oxide could be a potential probe of water structure at the biological interface.

Lipid

Figure 3 shows the characteristic spectrum of a lipid layer deposited on a quartz disk by spin coating. The spectrum in this region is dominated by CH vibrations of the aliphatic chain, which are down shifted from the OH vibrations of water shown in figure 1. It should be noted also that CH vibrations are considerably more Raman active than OH vibrations, as the bond is much less polar and therefore more polarizable. Furthermore, the vibrational frequencies are well shifted from those of deuterium oxide, shown in Figure 2. Also plotted in figure 3 is the spectrum of the lipid layer, which was rehydrated using a drop of heavy water and allowed to evaporate. Notably, the spectrum is significantly different from that of the subsequent spin coated, indicating a significant difference in the morphology of the lipid layers.

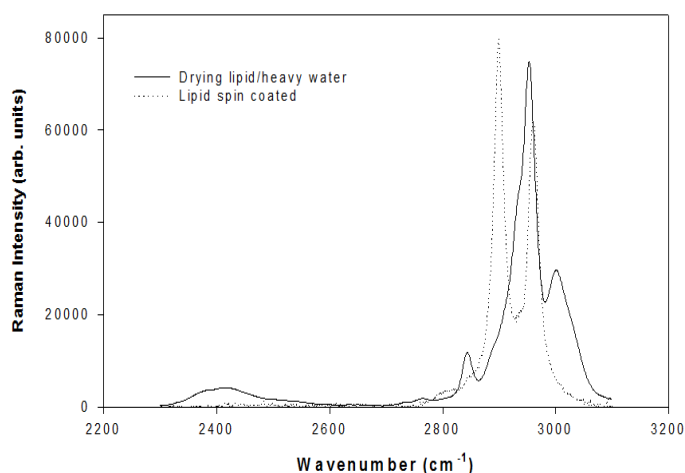


Figure 3: Raman spectra of spin coated lipid on a quartz disc dried (dotted line) and rehydrated (solid line).

In the spectrum of the rehydrated and dried lipid, trace amounts of D_2O are indicated by the features at $\sim 2400\text{cm}^{-1}$. Closer examination of these features, as shown in Figure 4, reveals however that they cannot be singularly ascribed to vibrations of heavy water at room temperature. Furthermore, they cannot be ascribed to vibrations of the lipid molecules themselves. The spectral features are down shifted compared to the average vibration features of the heavy water at room temperature, a phenomenon similar to that observed upon freezing. Although the spectral shifting is not as extreme as that observed at low temperatures, it is noted that the features ascribed to structured water in skin are also not as dramatically shifted. It is therefore proposed that the observed behaviour can be ascribed to the presence of some degree of structured (bound or partially bound) water at the water lipid interface.

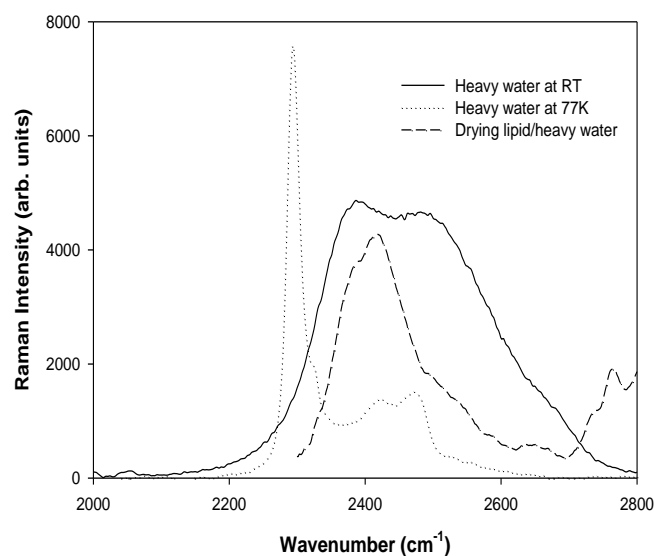


Figure 4: Raman spectra of drying lipid with deuterium oxide water on slide.

CONCLUSION

From the drying deuterated water and lipid spectrum, it can be concluded that there is an indication of change in the spectrum, likely due to the water, although this has to be further investigated under more controlled conditions in order to prevent atmospheric water hydrating the lipid specimen. Nevertheless, the proof of concept study demonstrates the potential for Raman spectroscopy to probe the structure of water at the biological surface. As an optical method, Raman spectroscopic microscopy can be performed across the visible spectrum, where most biomolecules are transparent, and thus could be used to probe the structure of water within the subcellular environment, potentially opening up an additional dimension of understanding biochemical function.

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