^{17}O RESIDUAL QUADRUPOLE COUPLINGS AS A PROBE OF MEMBRANE HYDRATION

E.Y. Chekmenev (NHMFL, CIMAR/NMR); T.A. Cross (NHMFL/FSU, Chemistry and Biochemistry)

Introduction

Structural and functional studies of membranes and membrane associated proteins significantly rely on an accurate knowledge of the membrane hydration level. Specifically, the lipid phase and dynamic properties of the lipid molecules are different at various hydration conditions. In protein studies, the situation is additionally complicated by protein structural and dynamic changes mediated by water. Thus, accurate control and measurement methods are of high importance in such studies.

Results and Discussion

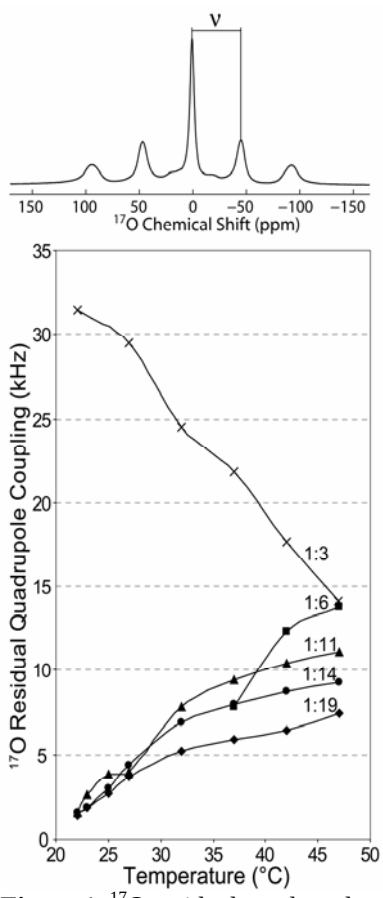

Figure 1. ^{17}O residual quadrupole couplings (RQC) of water in DMPC bilayers at various temperatures and hydration levels observed at 19.6 T.

We are particularly interested in the structural and functional characterization of membrane proteins performing biological functions such as proton conductance (M2 protein of Influenza A virus) and ion conductance (gramicidin A) across cell membranes. These biological targets are very difficult to crystallize and are thus unapproachable by widely used X-ray diffraction methods, while solid-state NMR has been proven as one of the most powerful tools for structural and functional studies of membrane associated proteins in the native lipid environment. High resolution solid-state NMR efforts frequently require preparation of macroscopically aligned protein samples, where the hydrated and oriented lipid bilayers are sandwiched between glass slides to aid protein alignment. One of the most challenging steps in such sample preparation is the control and monitoring of hydration levels in the sample.

It was shown that the residual quadrupole coupling (RQC) of water can be detected in aligned systems with residual order. The interface of lipid bilayers is solvated by water molecules with tiny residual order. Here, we show that the ^{17}O RQC of water can be observed in such oriented lipid bilayers at moderate hydration levels typical of our experiments. Such spectra (Figure 1, top) exhibit the characteristic quintet, because ^{17}O nuclei is spin 5/2, illustrating that water molecules are associated with the lipid interface and retain a small degree of order. While the RQC measured as the frequency separation between the peaks of the quintet is temperature dependent near the phase transition temperature of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) between gel and liquid crystalline phases, ~24ºC, there is little dependence upon hydration level. However, above the phase transition temperature the ^{17}O RQC increases both with a decrease in the number of water molecules per DMPC and increases modestly with increasing temperature. This trend is observed down to the low hydration limit of 3 water molecules per lipid, where the bilayers no longer exist and the material becomes an amorphous solid losing the cooperative properties of the bilayer.

Conclusions

^{17}O RQC is a highly sensitive probe of hydration level in the lipid bilayers in liquid crystalline phase. Moreover, our experiments with DMPC demonstrate that they can be quantitatively used to determine water content in experimentally relevant conditions. We successfully demonstrated that ^{17}O RQC of interfacial water in the lipid bilayers can be used to determine the hydration level of DMPC bilayers.