Experimental report

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Proposal: 9-13-748 **Council:** 4/2017

Title: Cation-induced changes in the structure of lipid membranes

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: DOPC
DPPC
DOPC+Mg2+
DPPC + Mg2+

Instrument	Requested days	Allocated days	From	To
D16	8	7	18/04/2018	25/04/2018

Abstract:

There has been a renewed interest in the studies of membrane structure and interaction properties in the presence of salt solutions. The divalent metal cations attract a special attention due to their peculiar properties. In spite of their importance however, their adsorption to membranes and their influence on lipid bilayers is far from being understood. We have detected previously a compacting effect of Ca2+ on the bacterial membrane structure. Its peculiarity seems to result from specific interactions with membrane. Intriguingly, a much lower specificity has been shown by Zn2+ ions that are also much smaller. These recent results suggest two parameters playing a key role in the mechanism of ion-membrane interaction. First, it is the size and hydration properties of ions themselves. Second, the specific interactions may depend on the density of lipid-ion interactions per lipid, thus correlating with the lipid lateral area. We propose to expand the previously examined divalent cations by Mg2+, which has a different size. The structural changes due to ions will be scrutinized for DPPC and DOPC oriented multilayers for addressing the effect of area per lipid.

Experimental Report

Experiment title Cation-induced changes in the structure of lipid membranes

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Abstract
We detected previously a compacting effect of Ca^{2+} on the bacterial membrane structure. Its peculiarity seems to stem from specific interactions with membrane. Intriguingly, a much lower specificity has been shown by Zn^{2+} ions that are also much smaller. These recent results suggested two parameters playing a key role in the mechanism of ion-membrane interaction. First, it is the size and hydration properties of ions themselves. Second, the specific interactions may depend on the density of lipid-ion interactions per lipid, thus correlating with the lipid lateral area. We have expanded the previously examined divalent cations by Mg^{2+}, which is characteristic of a different size. In addition, the structural changes due to ions have been scrutinized in the cases of two lipids (i.e., DPPC and DOPC) forming oriented bilayers of the similar transversal characteristics, yet very different in the lateral direction.

Cell membranes are selectively permeable barriers and can thus control the movement of substances in and out of cells. Membrane properties such as, membrane fluidity, bending and rigidity moduli, electrostatics, and aggregation and fusion are tightly associated with ions that are prevalent in both the cytosol and the exterior of the membrane. Not surprisingly then, differences in biological activity of various ions have been correlated to the effects that these ions have on the structure of bacterial mimic bilayers.[1] Importantly, the examined cations are thought to play a number of significant physicochemical roles in the molecular organization of the bacterial outer membrane. Agreeably with this notion, Ca^{2+} was found to alter the structure of smooth lipopolysaccharide (LPS) bilayers in a manner that water did not penetrate the outer/inner core to the same extent as for example, Na^{+}- and Mg^{2+}-LPS bilayers. This difference in water penetration was concluded as a result of calcium "compacting" the LPS molecules in the membrane-water interface region.[1]

Intriguingly, the same conclusions have been obtained from experiments looking at the effects of Ca^{2+} and Zn^{2+} on the model membranes made of synthetic dipalmitoylphosphatidylcholine (DPPC) bilayers.[2-4] The differences between the hydration properties of bilayers with various ions may be rationalized by the physical differences between the cations themselves:[5] 1) Ca^{2+} has a larger ionic radius than Mg^{2+} or Zn^{2+}; 2) the preferred coordination number for hydrated calcium ions is at least 6 (8 under crystalline form), and 6 for magnesium and zinc ions; 3) as a consequence of their larger ionic radius, calcium ions exhibit a lower hydration energy requiring smaller amounts of energy for the removal of their hydration shells. As a result of these characteristics, Ca^{2+} appears to bind to phosphate, carboxylate, or sulfonate groups with higher specificity, affecting the lipid bilayer thickness differentially when compared to Zn^{2+}.[4] Whether such specificity is unique to calcium or it pertains to other cations remains to be explored. We continue expanding our studies by looking at another biologically interesting cations, namely Mg^{2+}.

The cation binding depends not only on the properties of the cations themselves but also on the lipids. For instance, the cation binding constant has been correlated to a relatively loose packing of lipids with unsaturated tails that increases the area per lipid headgroup.[6] We have therefore expanded our previous measurements based on the DPPC bilayers by measurements utilizing also DOPC bilayers. The main difference between the structures formed by the two lipids is in the lateral area per lipid, which modifies consequently the density of lipid-cation interactions.

The experimental technique employed in our investigations continues to be the neutron diffraction due to its ability to provide important characterization of model membranes.[7] The configuration of D16 instrument[8] with 2D detector was utilized in the mode of constant scattering vector size in which the sample was rotated through the series of Bragg angles. Fig. 1 shows an example of rocking curves measured for DPPC sample while hydrated by 70, 40, and 8% D_2O water.

The number of Bragg peak orders reaching 7 in our measurements suggests the high quality samples with respect to the parallel orientation of bilayers. This is further corroborated by very narrow widths of the peaks. Occurrence of two characteristic minima along the rocking curve recognizable in particular for the 1st order peak is the consequence of neutron absorption by the sample when the incident or diffracted beam is oriented along the substrate surface.[7] The best model for describing the shape of obtained peaks was found to consist of the sum of Gauss and Lorentz function (Fig. 2). The width of central Lorentz peak for all the samples was about 0.06 degree confirming the high level of membrane alignement.[9]

The samples were held during the measurements vertically at 25°C in an air-tight hydration chamber provided by ILL. The chamber's bottom was filled with a saturated K_2SO_4 (97% RH)[10] solution of different D_2O/H_2O mixtures. The well-controlled hydration conditions slightly below the full hydration assure the sample stability throughout the course of the experiment. Under these conditions, the amount of inter-bilayer hydration water is sufficient to avoid structural changes

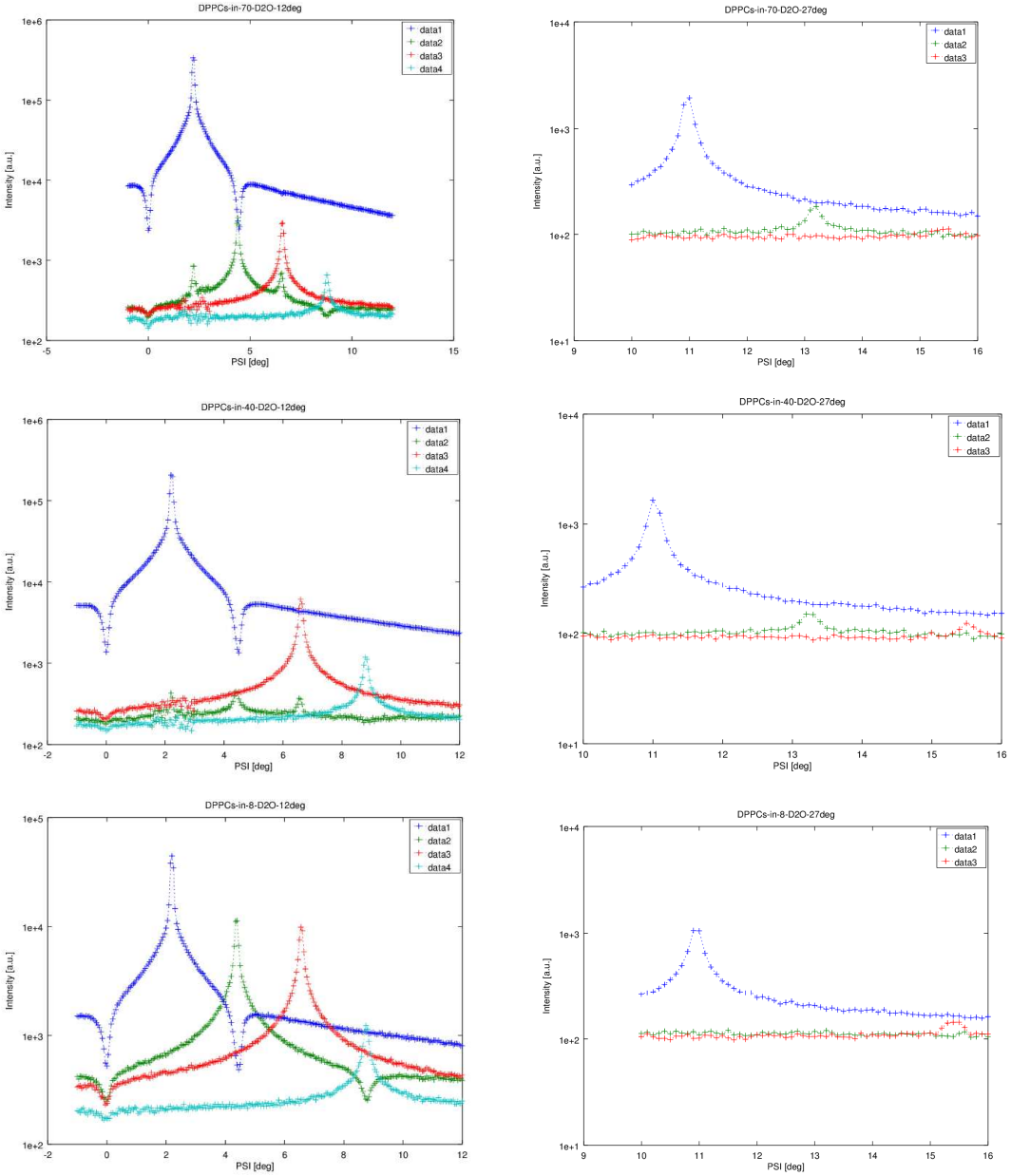

Figure 1: Uncorrected rocking curves measured for DPPC samples hydrated with 70% (top panels), 40% (middle panels), and 8% (bottom panels) D_2O water mixture. The diffraction intensity is collected as a function of the sample angle PSI, while the detector active area is fixed at the position of the various diffraction orders.[8] Typically, 4 peaks were measured at the detector angle of 12 degrees (left-hand panels), and another 3 peaks at the angle of 27 degrees (right-hand panels).

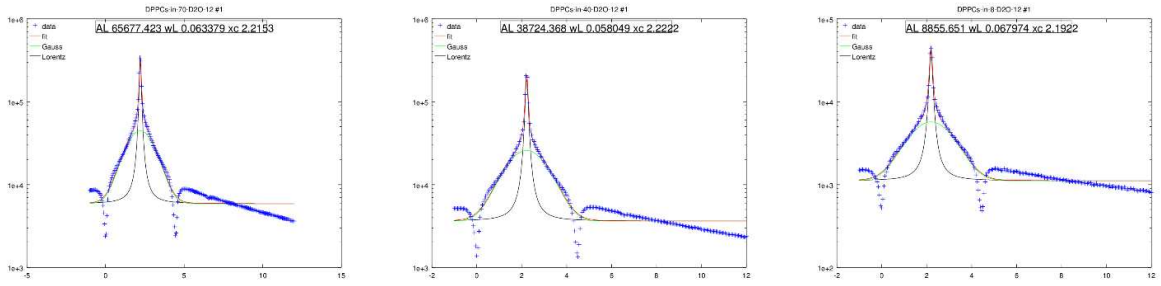

Figure 2: 1st order peak rocking curves measured for DPPC samples hydrated with 70% (left-hand panel), 40% (middle panel), and 8% (right-hand panel) D_2O water mixture. The presence of very narrow central peak fitted by Lorentz function confirms the high quality of the bilayers alignment.

due to steric constraints.[7] Consequently, the interlamellar repeat d-spacing represents a good measure of bilayer steric thickness. We have evaluated this parameter first.

The repeat d-spacing relates directly to an inverse peak position ($2\pi/q$). We improve the reliability of its calculation by utilizing the entire set of measured diffraction orders (thus $d=2\pi n/q_n$). The evaluated d-spacings for various samples are visualized in Fig. 3 and compared to the results published previously.[4]

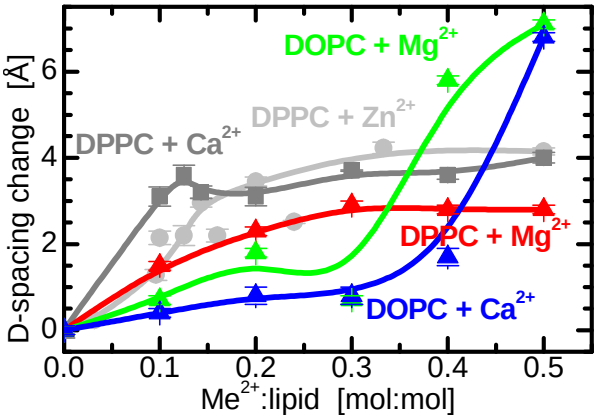

Figure 3: Changes to the transversal lamellar D-spacing in the various multilayered model membranes studied. The changes appear to group by lipids (i.e., DOPC vs. DPPC) rather than by the cations added (i.e., Ca^{2+}, Zn^{2+}, and Mg^{2+}).

It is obvious from our analysis of lamellar D-spacing that all of the systems studied are sensitive to the increasing concentration of cations. The extent of the changes shows however much stronger dependence on the lipids than on the cations themselves. While DPPC bilayers utilized both in previous (DPPC+Ca^{2+}, and DPPC+Zn^{2+})[4] and present (DPPC+Mg^{2+}) measurements tend to group together with maximal changes up to 4 Å, systems based on DOPC bilayers swell by more than 6 Å. The behaviour of the changes are also very different for the two lipids examined.

Our preliminary results thus confirm the strong dependence of lipid-ion interactions on the physico-chemical properties of lipids. It is nevertheless important to look closely at the details of bilayer structure which is obviously a place where the interactions of interest happen. Since D-spacing evaluated in the above analysis consists of two components corresponding to the thickness of bilayer and that of water layer, it will be important to deconvolute this information. It is of our further interest to reveal structural details based on the neutron scattering length density profiles that can be calculated from our current data.

Sample preparation

Dipalmitoyl-phosphatidylcholine (DPPC) and dioleoyl-phosphatidylcholine (DOPC) were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. $CaCl_2$ and $MgCl_2$ salts, and organic solvents were obtained from Sigma-Aldrich. Approximately 12 mg of lipid (thin film comprising of 2,000-3,000 bilayers having a total thickness ~15 μm when spread onto a 25 x 50 mm^2 silicon wafer) was solubilized with appropriate amounts of salt in deionized water and mixed thoroughly when following several freeze-thaw cycles and vortexing vigorously. The dispersions were deposited on leveled silicon wafers that were heated to 50°C, and excess water left to evaporate. The care was taken to form lipid multilayers in the fluid phase, and to anneal the samples for several hours upon rehydration. High quality oriented stacks were confirmed by observing up to 7 orders of diffraction peaks, and the narrow width of the central peak in the rocking curves shown in e.g., Fig. 1.

Small-Angle Neutron Diffraction

Neutron diffraction data were collected at the Institut Laue-Langevin (ILL) in Grenoble, France on D16 small momentum transfer diffractometer with variable vertical focusing.[8] Neutrons of 4.518Å wavelength were selected by the (002) reflection of a pyrolytic graphite (PG) monochromator. Incoming beam was formed by the set of slits (S_1=150x6 mm^2 and S_2=25x6 mm^2) and sample-to-detector distance was 0.95 m. All samples were measured at two detector positions with ^{3}He position sensitive detector. Γ_1=12° was utilized for the detection of up to 4th order diffraction peak, and Γ_2=27° for the detection of higher order peaks. The intense first order peak, which appeared for most of the samples, was measured with the 5 mm attenuator reducing the intensity by factor of 11.5. The data of area detector were visualized and reduced by an in-house written routine and the Lamp software provided by ILL.[11]

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