Lipid membranes loaded with Ca^{2+} and Zn^{2+} cations

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Abstract. We have studied the interactions of calcium and zinc with the biomimetic membrane made of dipalmitoyl-phosphatidylcholine (DPPC). The small angle neutron experiment on oriented multilamellar samples showed clearly differences in the effects of the two cations. For both, a bilayer thickness increases due to divalent metal ion (Me^{2+}) binding, reaching the maximum at stoichiometry Me^{2+}:DPPC~1:7 mol/mol. However, while the further increase in Ca^{2+} results in a bilayer thinning down to the level of pure DPPC, the Zn^{2+} binding indicates the behaviour of a typical isotherm, reaching a level of saturation.

1. Introduction

It is well known that divalent metal cations play important role in cell's physiology and biochemistry. In addition to calcium and magnesium, metals such as iron, manganese, copper, zinc, nickel and cobalt are essential at the appropriate concentration, yet toxic beyond normal levels. Among the first-row transition metals, zinc is second only to iron in terms of abundance and importance in biological systems. Zn^{2+} plays a fundamental role in several critical cellular functions such as protein metabolism, gene expression, structural and functional integrity of biomembranes, and in metabolic processes [1]. Compared to other micronutrients, zinc exists in biological systems in high concentrations, particularly in biomembranes. Concentration of zinc in animal organels ranges from $<10^{-9}$ M in cytoplasm to $>10^{-3}$ M in some membrane vesicles [2].

Cell 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. Interestingly, the divalent metal cations were found to play a dominant role in affecting bilayer structure. For example, it is well known that Zn^{2+} plays a fundamental role in several critical cellular functions such as protein metabolism, gene expression, structural and functional integrity of biomembranes, and in metabolic processes, while Ca^{2+} was shown to alter a bacterial membrane in a manner limiting its water penetration. The cation binding depends strongly on the property of the cation and the membrane lipid head-group.

Our observations agree well with the notion that the effect of Ca^{2+} and Zn^{2+} cations on the structure of lipid bilayer can most likely be rationalized in terms of electrostatic interactions, rather than that of geometrical constraints due to bilayer curvature, and thus reinforcing the special importance of these cations. The electronic structure of calcium results in its high affinity to

electronegative groups PO_4 and CO_2 and therefore, also other electronegative moieties such as ester oxygens and/or carbonyl groups of the lipid headgroup can be directly involved in complex formation. The molecular dynamics simulations have been performed to gain more detailed information.

2. Results and Discussion

We have performed the small angle neutron experiment on oriented multilamellar samples to decouple effects due to electrostatic interactions from those of geometrical constraints found in curved vesicular bilayers. The experiment involved the contrast variation measurements, in which the series of D_2O/H_2O solutions were utilized for the sample hydration. This approach allows to solve the scattering phase problem by requiring the scattering form factors to change linearly as a function of D_2O content [3]. We also employ the procedure suggested by Leonard et al. [4] for unambiguous phase determination as described in details elsewhere [5].

The neutron scattering length density (NSLD) profiles of bilayers were obtained by a successful Fourier transformation of corrected contrast varied diffraction data [5]. While the profile corresponding to the 100% D_2O contrast condition provides the best estimate of bilayer steric thickness, lower contrast profiles reveal more details in the region of lipid head group. This is best seen in the case of 8% D_2O measurements, where the net NSLD of water equals to zero, providing essentially the lipid only NSLD profile of bilayer (Fig. 1a). In addition, the contrast varied diffraction data can be subtracted from each other either, providing thus effectively the probability distributions of water and lipid molecules. Figure 1b shows graphically the example of water probability distributions resulting from the subtraction. This approach does not require any assumptions on the functional form of NSLD profiles nor on the probability distributions resulting from their direct subtractions. The water distribution is obtained from averaging all of the separately subtracted pairs of contrast varied NSLD profiles, providing also an estimate on standard deviation error.

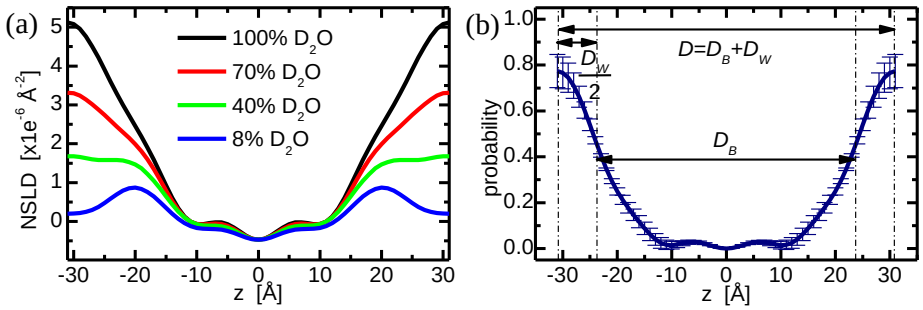

Figure 1. (a) Bilayers prepared of DPPC mixed with Zn^{2+} at 10.4:1 ratio shown through the NSLD profiles calculated from measurements at various contrast conditions. (b) Water probability distribution obtained by the NSLD subtraction procedure.

The obtained results confirm clearly the binding of both cations to the lipid bilayers. It is reflected in the increased lamellar repeat distance D that most likely results from the charge induced repulsion of bilayers. Interestingly, the further scrutiny of parameters reveals these changes in D, that is a sum of D_B and D_W (see Fig. 1b), are not distributed between D_B and D_W in a same manner for different ion concentrations. In addition, there is an apparent difference in the influence of the two ions examined. Let us first explore closer the effect of Zn^{2+} ions. The Fig. 2a shows a monotonic increase of D and D_B over the concentration range up to 0.14 mol/mol (i.e., DPPC:Zn^{2+}=7:1), after which the parameters seem to reach plateau.

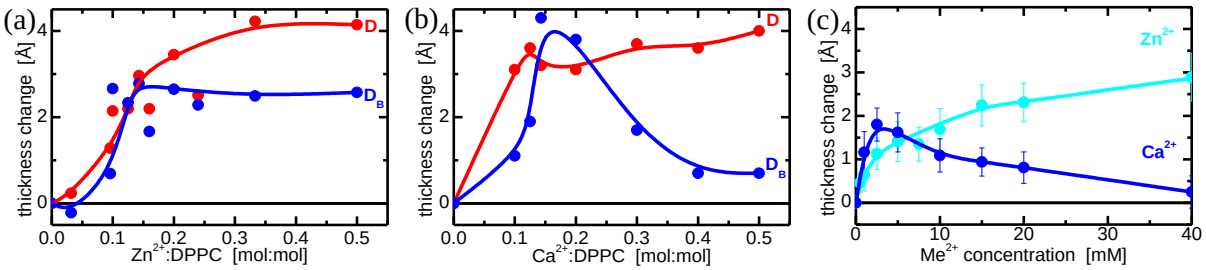

Figure 2. (a) Zn^{2+} and (b) Ca^{2+} cation-induced changes to bilayer structure expressed through lamellar repeat distance D (red) and bilayer thickness D_B (blue). (c) The results of cation-induced changes to the bilayer structure of unilamellar vesicles dispersed in water solution as published previously [6].

Ca^{2+} loaded bilayers clearly response to the increasing amount of cations differently when compared to Zn^{2+}. While the changes to the bilayer structure follow the same trend for both ions in the range up to 0.14 mol/mol, they differ at higher concentrations. The most pronounced difference is observed in the bilayer thickness D_B that decreases with further ionic increase, displaying a maximum at the 7:1 ratio of DPPC:Ca^{2+}. The D_B decreases almost to the level of pure DPPC bilayer at ratios above 2.5:1. This is supported also by the previously published results reproduced in Fig. 2c, in which unilamellar vesicles dispersed in the water solution loaded with either Zn^{2+} or Ca^{2+} ions displayed the same trends as in our results [6]. In addition, the same effect in the case of planar and curved bilayers rules out the involvement of the curvature impact similarly to conclusions for charged lipid bilayers [7].

We utilized molecular dynamics simulations for obtaining more structural details on the submolecular level in the most intriguing concentration point of 7:1 ratio of DPPC:Me^{2+}. Figure 3 focuses on the two major head group components represented by phosphate and carbonyl that are anticipated to be the primary targets for cation bindings due to their electronegativity.

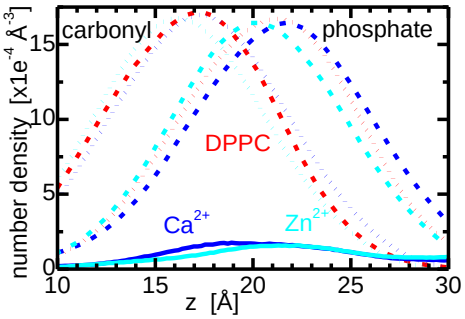

Figure 3. Number density of carbonyl (dotted lines) and phosphate groups (dashed lines) simulated for pure DPPC bilayer (red) and bilayers loaded with Ca^{2+} (blue) or Zn^{2+} (cyan) ions.

The addition of Ca^{2+} or Zn^{2+} ions affects clearly the DPPC head group as is evident by the positional change of its two components. While Ca^{2+} causes a small shifting of both carbonyl and phosphate components outwards, about twice as big shift towards the bilayer center is observed in the case of Zn^{2+}. On the other hand, the cations themselves seems to be localized in opposite directions, where Ca^{2+} penetrates deeper into the lipid bilayer.

3. Conclusions

The effects of Ca^{2+} and Zn^{2+} on the DPPC bilayer are different, as shown in changes of the lipid bilayer thickness. The radial distribution functions of cation-lipid pairs calculated for several lipid hedgroup atoms reveal a strong binding of Ca^{2+} to phosphate group (forming a contact pair Ca^{2+}-P and non contact pair Ca^{2+}-O) at low concentrations, while likely contributing to a screening effect at higher

concentrations. The location of Zn^{2+} shows more-less uniform correlation with various lipid headgroup atoms. The weak correlation of zinc with lipid headgroup then results into the saturation of structural changes with its increasing concentration.

Acknowledgments
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