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Experimental Report

PROPOSAL NUMBER	2019-04-11-16-46-17
PROPOSAL TITLE	The effect of metal cations on the structure of model phospholipid membranes

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ABSTRACT

We have studied changes in the structural parameters of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) unilamellar vesicles due to the addition of divalent metal cations Ca^{2+}, Mg^{2+} and Co^{2+} at a concentration series 0–30 mM by means of small-angle neutron scattering (SANS). The membrane structural parameters (thickness and area per lipid) were obtained at different concentrations of cations in the gel and fluid phases of membrane. Both Ca^{2+} and Mg^{2+} ions at the concentrations of 0–1 mM increase the membrane thickness by 1.9 Å and 2.9 Å in fluid and gel phase, respectively. Then a weak tendency towards a decrease of membrane thickness in the fluid phase is observed. In the case of Co^{2+} ions, all changes are somewhat weak. We advocate a model of electrostatic interactions for these systems that assumes a formation of ion bridges between lipid head-groups. The developed model is of interest to future studies of membrane interactions with various charged peptides.

EXPERIMENT

To prepare samples of DMPC vesicles, the dehydrated lipid was mixed in the chloroform / methanol solvent. Then it was evaporated under a flow of argon to create a lipid film. Complete removal of solvent traces was carried out in a vacuum chamber. The salts of the studied ions $CaCl_2$, $MgCl_2$, and $CoCl_2$ were used to prepare solutions in D_2O to take a full advantage of SANS experimental approach for maximizing the contrast effect. We proposed to examine 2 phases, i.e. DMPC in gel, and DMPC in fluid phase (the main phase transition temperature is 24 C), 3 types of ions on the DMPC membrane of each phase added at 10 different concentration points. The salt solutions with DMPC lipid dissolved were thoroughly mixed in a shaker and passed through the phase transition temperature by freeze-thawing cycles. The resulting multilayer vesicles were extruded to obtain unilamellar (ULV) vesicles.

RESULTS AND DISCUSSIONS

We have observed changes in the bilayer thickness and lateral area per lipid discussed previously for DPPC-ion systems[1-2]. The revealed effect on the bilayer structural properties confirms the direct interactions between ions and lipids. Namely, fig. 1 shows graphs of changes in the thickness of DMPC bilayers in the fluid and gel phases as a function of added Ca^{2+}, Mg^{2+}, Co^{2+} concentration.

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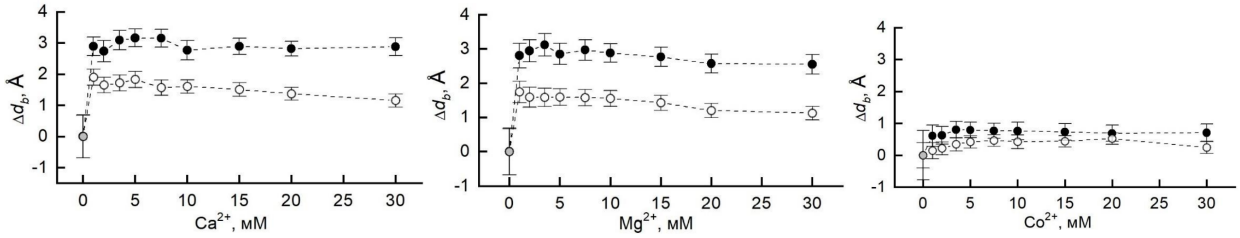

(A) (B) (C)

Figure 1: Changes in the membrane thickness obtained for DMPC bilayers in fluid (○) and gel (●) phases as a function of added (A) Ca^{2+}, (B) Mg^{2+}, (C) Co^{2+} concentration.

Based on the experimental data, we propose a model of lipid-ion interactions. Due to the ion binding to the membrane, the electric field reorganizes the orientation of PN dipole from it being parallel with membrane plane, which is accompanied with the formation of ion bridges. It leads to an increase in the thickness[4]. Further increase in the concentration of ions leads to the saturation of changes in the membrane structure. This can be explained by the influence of spatial restrictions occurring here, which does not allow further changes in the lateral structure[4].

On the other hand, Co^{2+} ions have an insignificant effect on the structural parameters of DMPC bilayers. This can be explained by different hydration, strength and binding sites of Co^{2+} ions to the head groups of phospholipids[2]. Moreover, in aqueous solutions Co^{2+} forms high-spin complexes with an ordered octahedral arrangement of water molecules[5] because of cobalt is a transition metal. This probably reduces the extent of Co^{2+} binding to the headgroups.

The proposed interaction model of ion bridges appears closely correlated to ion charge density at the DMPC bilayer surface and it is in a good agreement with characteristic average distances between phospholipids in the membrane that are indeed comparable to calculated Debye screening lengths for ions[4].

CONCLUSION

It was shown that Ca^{2+} and Mg^{2+} cations lead to the structural changes in DMPC bilayers due to conformational changes in the head groups and the formation of ion bridges throughout the range of the studied concentration of reference points. In the concentration range of 1–30 mM, phospholipid molecules are reorganized with a tilt of hydrophobic tails. However, in the case of Co^{2+} cations, all these changes are extremely weak, which may be explained by the binding sites of Co^{2+}, which are different from Ca^{2+} and Mg^{2+}. It was also shown that the model of electrostatic behavior, previously developed for DPPC+Ca^{2+} systems, can be extended to other PC systems.

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Important: The experimental report must be no more two pages.