

## DISSOLVED MAGNESIUM AS AN ENVIRONMENTAL SELECTION PRESSURE IN MIXED LIPID VESICLES: EVOLVING PROTOCELL MEMBRANES TO MODERN MEMBRANES

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**Introduction:** Proto-cell membranes on early Earth may have been composed of single chain amphiphiles (SCAs) due to their prebiotic availability [1] and ability to self-assemble into bilayer structures. However, SCA membranes are easily disrupted by divalent cations in aqueous solutions. Mixed SCA vesicles are known to be more resistant to the fatal effects of dissolved salts [2, 3]. Here we examined the potential role of  $Mg^{2+}$  as an environmental selection pressure in the transition of fatty acid membranes to mixed SCA-phospholipid membranes and, finally, modern phospholipid membranes. Additionally, the Mg-tolerance of binary mixtures of fatty acid and phospholipid vesicles was determined by various analytical methods.

**Methods:** We examined oleic acid (OA, carboxylic acid head group) and palmitoyl-2-oleoylphosphatidylcholine (POPC, zwitterionic head group) vesicles. The stability of pure OA, pure POPC and mixed OA-POPC vesicles at different stoichiometric ratios was investigated in the presence of different concentrations of dissolved  $MgCl_2$  at pH 8.5. The fatal magnesium concentration,  $[Mg^{2+}]_{fatal}$ , was defined as the concentration of  $Mg^{2+}$  required to disrupt ~ 100% of the vesicles. Membrane disruption was determined by measuring the decrease in fluorescence intensity of a membrane-soluble dye, naphthopyrene (NP), that had been previously entrapped in the vesicle membranes. In the NP fluorescence assay, vesicle membranes are characterized by the formation of NP dimers in the excited state (“excimers”) with emission at 465 and 495 nm. However, in micelle or monomer solutions have a strong NP “monomer” emission at 390 nm. The  $I_{EXC}:I_{MON}$  ratio decreases relative to its initial value is identified as the  $[Mg^{2+}]_{fatal}$ .  $[Mg^{2+}]_{fatal}$  was also confirmed by dynamic light scattering and optical microscopy. Quantitation of OA and POPC concentration in the vesicles was achieved by filtration (0.22  $\mu m$  pore) to remove  $Mg^{2+}$ -lipid aggregates, followed by HPLC analysis of the filtrate.

**Results:** The zeta potential ( $\zeta$ ) of various lipid vesicles at various  $Mg^{2+}$  concentrations (0, half-fatal and fatal) and pH 8.5 was determined by dynamic light scattering. As anticipated, OA vesicles showed the most negative  $\zeta$  and it becomes less negative with the incorporation of POPC into the membrane.

The relative amount of OA/POPC in vesicles in the presence of  $Mg^{2+}$  compared to the stoichiometric ratio

in the original vesicles in the absence of  $Mg^{2+}$  was determined by HPLC. A significant decrease in the ratio of OA-POPC was noted after the addition of  $MgCl_2$  to various mixed lipid vesicles. Furthermore, the  $[Mg^{2+}]_{fatal}$  increased drastically with increasing relative POPC content from 5 mM for pure OA, to ~ 30 mM for  $[OA]/[POPC] = 5:1$ , and > 80 mM for pure POPC at 2 mM total lipid concentration. The effect was the most dramatic when the initial OA/POPC values were high (10:1), which is the condition with the greatest relevant to the early stages of primitive cell membrane evolution.

**Discussion:**  $Mg^{2+}$  - binding to the negatively-charged head groups of OA disrupted vesicles. The  $[Mg^{2+}]_{fatal}$  drastically increased as the relative POPC content increased. We propose two distinct mechanisms for these observations. First, as confirmed by  $\zeta$ -potential measurements, POPC (zwitterionic head group) stabilized the mixed-lipid vesicles by decreasing the relative negative charge density of the vesicles, so more  $Mg^{2+}$  was needed to disrupt the vesicles. Second,  $Mg^{2+}$  was found to preferentially bind to and abstract OA from OA-POPC mixed lipid membranes, resulting in lower  $[OA]/[POPC]$  ratio in the vesicles as compared to the initial ratio.

This is the first time that a divalent cation has been shown to directly affect the composition of a mixed lipid membrane by selectively removing one lipid. The significantly greater Mg-tolerance of SCA-phospholipid vesicles may hold implications for the evolutionary selection of phospholipid membranes and for accommodating  $Mg^{2+}$ -promoted processes such as RNA polymerization.

### References:

- [1] Yuen G.U. and Kvenvolden K.A. (1973). *Nature* **246**, 301-303. [2] Monnard P.-A., Apel C.L., Kanavarioti A. and Deamer D.W. (2002). *Astrobiology* **2**, 139-152 [3] Namani T. and Walde P. (2005). *Langmuir* **21**, 6210-6219.