Inorganic Cadmium affects the fluidity and size of phospholipid based liposomes

Inorganic Cadmium (Cd) is a non-essential metal and a toxic environmental pollutant that induces multiple biochemical dysfunctions. Due to industrial activities since the 1800s, exposure to ever increasing levels of Cd has resulted in a significant impact on human health which requires a better understanding of metal induced changes to cell structure and function. While many studies have analyzed metal-protein interactions, fewer have investigated interactions of metals with lipids. The lipid bilayer is a selectively permeable membrane that acts as a barrier separating the inside and outside of all cells and lipids comprise about 50% of the dry weight of many cell membranes. The cell barrier represents a significant metal target but can contain more than 1000 lipids, thus it is necessary to investigate the interactions of Cd with simpler systems of initially only 1-2 lipids.

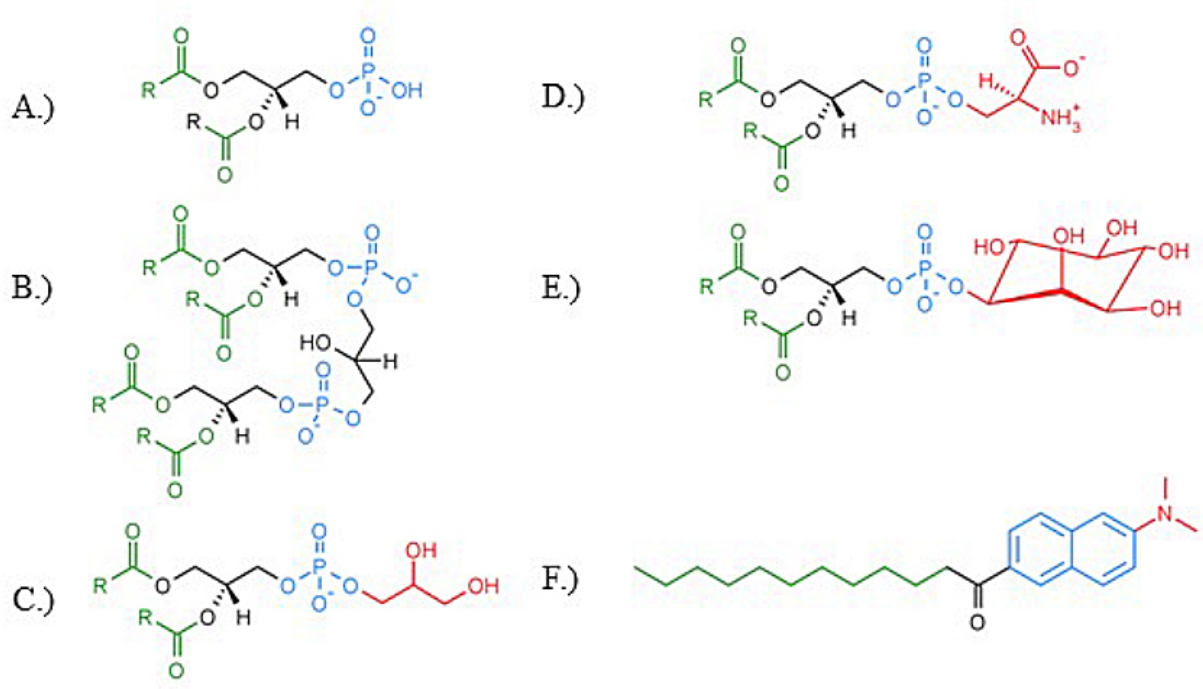

Fig. 1. Structures of phosphatic acid (A), cardiolipin (B), phosphatidylglycerol (C), phosphatidylserine (D), phosphatidylinositol (E) and Laurdan (F).

Positively charged Cd ions in 100 mM NaCl at pH 7.40 were predicted to electrostatically target negatively charged lipids. Our paper in BBA Biomembranes (2016) focused on Cd interactions with various negatively charged Biomimetic model membranes containing 1-2 lipids for which different locations of the negative charge affecting its accessibility to Cd were varied under strict control of pH, salt content and lipid concentration. Multi-layered lipid structures (Multilamellar Vesicles) were

passed through polycarbonate filters with defined pore sizes to produce a solution of single lipid bilayer structures called Large Unilamellar Vesicles (LUVs) in the 100-nm range.

To study metal-lipid interactions, we utilized the fluorescent molecule Laurdan which readily incorporates into lipid bilayers and acts as a polarity sensor. Laurdan fluoresces more at 440 nm in tightly packed bilayers in the gel phase, where it is less accessible to water, while due to increased water exposure, Laurdan fluoresces more at 490 nm in the less tightly packed liquid-crystalline phase. These Fluorescence intensity changes can be related to membrane fluidity and quantified by using the generalized polarization (GP) equation.

$$GP = \frac{I_{440\,nm} - I_{490\,nm}}{I_{440\,nm} + I_{490\,nm}} \quad (1)$$

GP was used to monitor fluidity changes versus temperature and to calculate membrane phase transitions temperatures (T_m) between the gel and liquid crystalline phases. We quantified Cd effects on both fluidity and T_m.

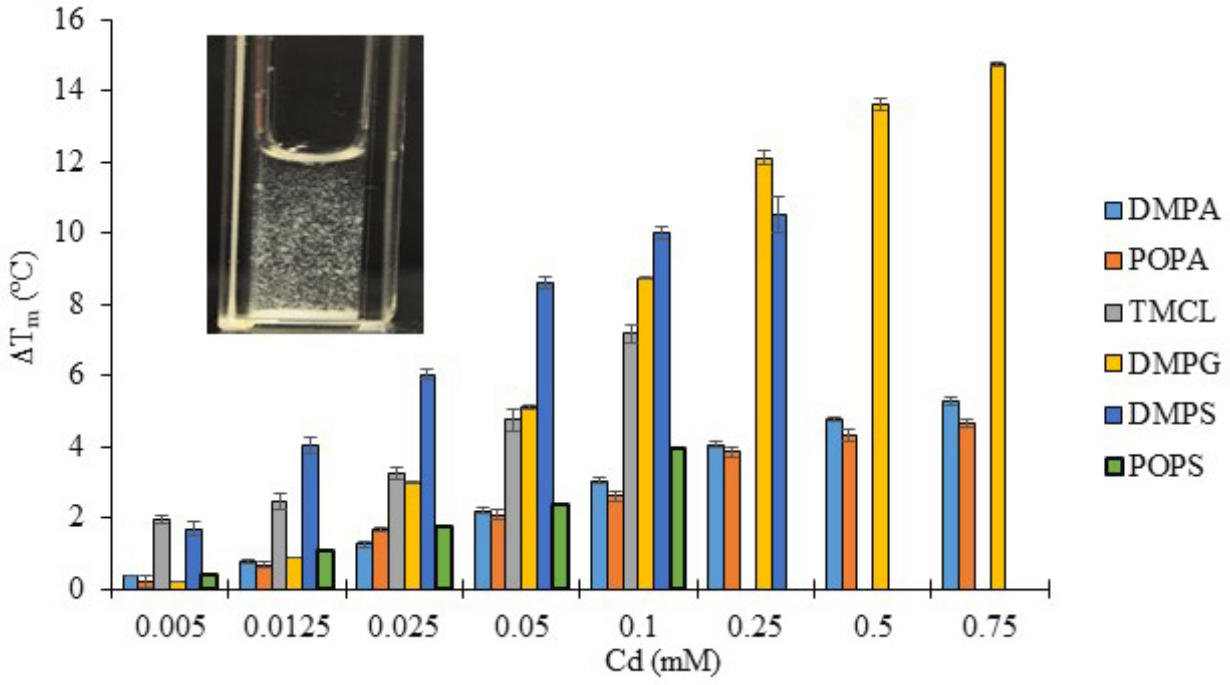

Fig. 2. Cd-induced changes in the phase transitions of DMPA, DMPG, DMPS and TMCL. The dotted line shows the lipid concentration. Results are the average of 3 replicates + standard deviation. (DM=di-14:0 fatty acids (2 saturated chains); TM= tetra-14:0 fatty acids (4 saturated chains). Insert: aggregation of DMPA by Cd.

We found that Cd interacted with biologically relevant negatively charged phosphatidic acid (PA), cardiolipin (CL), phosphatidylglycerol (PG), phosphatidylserine (PS) and phosphatidylinositol (PI) lipids (Fig. 1). These Cd-lipid interactions increased the GP indicating increased membrane rigidity. Moreover, Cd consistently induced less rigidity in the gel phase and much more at the T_m and in the liquid crystalline phase. This lead to concentration dependent Cd-induced T_m increases (Fig. 2). Overall, the highest T_m increases were observed for Cd and fully saturated PS (DMPS) and PG (DMPG) lipids below and above a metal/lipid ratio of 1, respectively (Fig. 2). Furthermore, GP data showed that Cd targeted the saturated negatively charged lipids more strongly than their partially unsaturated forms and even induced LUV aggregation at higher metal concentrations in most saturated systems (except PG).

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