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Inorganic cadmium affects the fluidity and size of phospholipid based liposomes

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ABSTRACT

Following intake and absorption of Cd into the bloodstream, one possible target is the lipid membrane surrounding erythrocytes as well as kidney and liver cells where Cd accumulates. We investigated the interactions of Cd with model membranes from a biophysical perspective by using fluorescence spectroscopy and dynamic light scattering to monitor changes in liposome size, membrane fluidity and lipid phase transition. The fluorescent probe Laurdan was incorporated into liposomes and used to quantitate cadmium induced fluidity changes in model systems hydrated in 20 mM HEPES, 100 mM NaCl pH 7.4. The metal effects on membranes composed of the zwitterionic phosphatidylcholine were compared to the negatively charged lipids phosphatidic acid (PA), cardiolipin (CL), phosphatidylglycerol (PG), phosphatidylserine (PS) and phosphatidylinositol (PI). The data showed that 5–2000 μM Cd electrostatically targeted negatively charged lipids and increased the rigidity of these membranes whereby the gel to liquid crystalline phase of fully saturated anionic lipids was increased following the order: PG > PS > CL ~ PA. In addition, dynamic light scattering showed that Cd induced liposome aggregation in all negatively charged systems except for the PGs. Moreover, both effects were much stronger for saturated acyl chains versus unsaturated species. Finally, charge localization was important as lipids carrying the charge more distant from the hydrophobic core of the bilayer showed stronger interactions with Cd.

1. Introduction

Inorganic cadmium (Cd) is a non-essential metal and a highly toxic environmental pollutant known to induce multiple biochemical dysfunctions [1]. Due to the anthropogenic activities since the 1800s, organisms are exposed to ever increasing levels of toxic heavy metals [2]. Cd was classified as a human carcinogen in 1993 and has since been shown to induce cancers in the liver, kidney and stomach [3]. Furthermore, Cd has an ionic radius similar to that of calcium and an electronegativity similar to zinc leading to a disruption of the normal functions of these metals which are essential for human health. Absorption of Cd into the bloodstream can occur through inhalation or ingestion. The former requires Cd to pass through the air-blood barrier in the lungs while the latter requires passage through the gastrointestinal tract. Inhalation depends on factors such as particle size and can vary between 20 and 50% of the amount that is inhaled [4]. An average absorption of 5% Cd through ingestion can increase up to 20% in conditions of a calcium or iron deficiency [5,6]. Ingestion has been mostly attributed to food with an estimated daily uptake ranging from 10 to 40 μg/day in nonpolluted areas to several hundred micrograms/day in Cd-polluted areas [7].

Elucidating the mechanisms leading to cadmium toxicity is difficult as this metal has many different molecular targets leading to multiple biochemical dysfunctions [8]. As such, both the acute and chronic exposure and the significant impact on human health require a better understanding of the molecular basis of the metal induced changes to cell structure and function. Whereas more work has been reported on metal protein interactions considerably less is known about toxic metal lipid interactions which is the focus of this study. There is a precedent for the consideration of lipids as a molecular target for Cd as one study found that Cd concentrations as low as 50 μM induced membrane rigidity in human erythrocyte and rap hepatocyte membranes although these membranes contain proteins which cannot be ruled out as targets

Abbreviations: Cd, Inorganic cadmium; CL, Cardiolipin; DM, Dimyristoyl; DMPA, 1,2-Dimyristoyl-*sn*-glycero-3-phosphate; DMPC, 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine; DMPG, 1,2-Dimyristoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol); DMPS, 1,2-Dimyristoyl-*sn*-glycero-3-phospho-L-serine; DO, Dioleoyl; DOCP, 2-((2,3-Bis(oleoyloxy)propyl) dimethyl ammonio)ethyl hydrogen phosphate; DPPA, 1,2-Dipalmitoyl-*sn*-glycero-3-phosphate; DSC, Differential scanning calorimetry; GP, General polarization; GUVs, Giant unilamellar vesicles; Laurdan, 6-Dodecanoyl-2-dimethylaminonapthalene; LUVs, Large unilamellar vesicles; PO, Palmitoyl-oleoyl; POPA, 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphate; POPC, 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPE, 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine; POPG, 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol); PI, 1,2-Dioleoyl-*sn*-glycero-3-phospho-(1′-myo-inositol); PI(3)P, 1,2-Dioleoyl-*sn*-glycero-3-phospho-(1′-myo-inositol-3′-phosphate); PI(3,5)2P, 1,2-Dioleoyl-*sn*-glycero-3-phospho-(1′-myo-inositol-3′,5′-bisphosphate); POPS, 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine; PS, 1′, phosphatidylserine; TMCL, 3′-Bis [1,2-dimyristoyl-*sn*-glycero-3-phospho]-*sn*-glycerol; TOCL, 1′,3′-Bis [1,2-dioleoyl-*sn*-glycero-3-phospho]-*sn*-glycerol.

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as well [9]. Similar results have been observed with Cd and membranes containing the negatively charged phosphatidylserine lipid at 200 μM metal which represents a protein free system.

In reality, metal-lipid interactions are more complex since heavy metals such as Cd are found as different species as a function of pH and the presence of chlorides [10]. In the absence of phosphate and under otherwise physiological conditions (pH 7.4, 100 mM NaCl), Cd is found as 66% $CdCl^+$, 27% $CdCl_2$ and 7% Cd^{2+} [10]. Under these experimental conditions, Cd forms predominantly positively charged species which are predicted to favor electrostatic interactions between Cd and negatively charged lipids.

The complexity of metal lipid interactions and their strong dependence on the experimental conditions and the metal/lipid mole ratio have recently been reviewed [11] and thus the current work investigates the interactions of cadmium with a selection of structurally diverse negatively charged lipids under stringent control of pH, ionic strength and lipid concentration. Biomimetic model membranes varied in terms of charge density and localization as well as acyl chain saturation and the results clearly demonstrated that the metal effects significantly depend on the detailed lipid structure as well as the metal/lipid mole ratio in solution.

2. Materials and methods

2.1. Reagents

1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 2-((2,3-bis(oleoyloxy)propyl)dimethylammonio)ethyl hydrogen phosphate (DOCP), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphate (POPA), 1,2-dimyristoyl-*sn*-glycero-3-phosphate (DMPA), 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine (DMPS), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG), 1,2-dimyristoyl-*sn*-phosphoglycerol (DMPG), 1,3-bis[1,2-dimyristoyl-*sn*-glycero-3-phospho]-*sn*-glycerol (TMCL), 1,3-bis[1,2-dioleoyl-*sn*-glycero-3-phospho]-*sn*-glycerol (TOCL), 1,2-dioleoyl-*sn*-glycero-3-phospho-(1′-myo-inositol) (PI), 1,2-dioleoyl-*sn*-glycero-3-phospho-(1′-myo-inositol-3′-phosphate) (PI(3)P) and 1,2-dioleoyl-*sn*-glycero-3-phospho-(1′-myo-inositol-3′,5′-bisphosphate) (PI(3,5)P2) were obtained from Avanti Polar Lipids (Alabaster, AL). $CdCl_2$ and 6-dodecanoyl-2-dimethyl-aminonaphthalene (Laurdan) were purchased from Sigma-Aldrich (Oakville, ON). Spectrosil Far UV Quartz cuvettes with a path length of 5 mm were purchased from Starna Cells, Inc. (Atascadero, CA). All reagents and lipid solutions were made using doubly distilled water (ddH_2O) filtered by a Millipore Synergy 185 water purification system (Billerica, MA).

All chemicals were used without further purification.

2.2. Sample preparation

Dry lipid powders used in the preparation of large unilamellar vesicles (LUVs) were analytically weighed using a Sartorius Microbalance MC 5 (Göttingen, Germany). Liposomes composed of DMPC, POPC, DOCP, POPE, POPA, DMPA, TOCL, TMCL, POPG, DMPG, POPS, DMPS, PI, PI(3)P, and PI(3,5)2P were prepared by co-dissolving the required amounts of lyophilized lipid powder(s) and fluorophore in chloroform/methanol (1:1 *v*/*v*). This solvent was removed using argon gas followed by applying-vacuum overnight to ensure complete evaporation of organic solvent traces. Lipid films were then hydrated in 20 mM HEPES, 100 mM NaCl at pH 7.4 followed by heating and sonication resulting in a suspension of multilamellar vesicles (MLVs). Samples were then further sonicated and exposed to 5 freeze thaw cycles to ensure that the films were completely removed from the glass surface. LUVs were prepared by using an extruder (Avanti Mini Extruder, Alabaster, AL) and by passing the MLVs through 100 nm Nuclepore polycarbonate membranes at least 20 times. For each preparation, the solution was kept 5–10 °C above the T_m of the lipid during extrusion. The final concentration of lipid used for fluorescent measurements was 0.3 mM with vesicles containing a molar ratio of 1:550 Laurdan/lipid.

2.3. Laurdan background

Laurdan (see Fig. 2) has two main parts, a long chain that facilitates rapid uptake into the liposomes and a fluorescent naphthalene group that is anchored in the polar region of the bilayer and can thus respond to changes in hydration. In a lipid bilayer Laurdan has a high quantum yield and hence a molar ratio of 1 Laurdan:550 mol lipid provides a sufficient signal while not significantly impacting the properties of the membrane [12].

The fluorescent moiety of Laurdan contains a dipole moment due to partial charge separation between the carbonyl group and the 2-dimethylamino head group. The energy required for solvent reorientation lowers the energy of the excited state of Laurdan resulting in a red shift of the probes emission spectrum in more polar solvents. With respect to lipid bilayers, Laurdan's emission spectrum has been shown to be sensitive to the phase of the membrane. Bilayers in the more ordered gel phase exhibit a maximum emission at 440 nm, whereas the peak emission of membranes in the more fluid liquid crystalline phase is found at 490 nm [13]. Intensity readings at these two wavelengths are used to calculate the generalized polarization by using Eq. (1):

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

Based on this formula, GP values vary between +1 to −1 and can be used to monitor fluidity changes as a function of temperature. In order to avoid misinterpreting changes in the GP during lipid trials as metal-induced changes in the fluorescence of Laurdan, control experiments were conducted with the water soluble analogue Prodan. In order to determine if Cd affects the fluorescence intensity of this class of dye molecules at 440 and 490 nm, experiments were conducted in 9:1 (*v*/*v*) isopropyl alcohol: 100 mM NaCl pH 7.40 (see Fig. S1). Results showed that 2 mM Cd did not affect the intensity of 500 nM Prodan at 440 or 490 nm in either solvent that was tested. These results show that changes in the GP of Laurdan are not an artifact of the interactions of these metals with the dye.

2.4. Fluorescence measurements in the presence of cadmium

Zwitterionic PC membranes were used as negative controls to assess the Cd induced changes of membrane fluidity in negatively charged liposomes. LUVs containing Laurdan were studied using a Varian Cary Eclipse Spectrofluorometer (Agilent Technologies, Santa Clara, CA). The temperature of the sample was controlled within ±0.1 °C using a circulating water bath (Agilent Technologies, Santa Clara, CA). Each sample was allowed to equilibrate to a temperature at least 10 °C below the T_m of the system for 10 min before measurements were initiated. Each sample was incubated with Cd at room temperature then immediately adjusted to the first temperature in the melting curve. Fluorescence measurements were recorded using an excitation wavelength of 340 nm and emission intensities at 440 nm and 490 nm using 5 nm bandwidth for both the excitation and emission slits. Scattering contributions in the data were corrected by subtracting readings for dye-free LUVs. Experiments lasted between 30 and 45 min.

Data was then used in Eq. (1) to calculate the GP value at a particular temperature. These GP values were then plotted versus temperature and the sigmoidal Boltzmann function (Eq. (2)) was fit to the data using Origin Pro 8.0 software [14] to determine the T_m of the sample