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X-ray structure, thermodynamics, elastic properties and MD simulations of cardiolipin/dimyristoylphosphatidylcholine mixed membranes

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ABSTRACT

Cardiolipins (CLs) are important biologically for their unique role in biomembranes that couple phosphorylation and electron transport like bacterial plasma membranes, chromatophores, chloroplasts and mitochondria. CLs are often tightly coupled to proteins involved in oxidative phosphorylation. The first step in understanding the interaction of CL with proteins is to obtain the pure CL structure, and the structure of mixtures of CL with other lipids. In this work we use a variety of techniques to characterize the fluid phase structure, material properties and thermodynamics of mixtures of dimyristoylphosphatidylcholine (DMPC) with tetramyristoylcardiolipin (TMCL), both with 14-carbon chains, at several mole percentages. X-ray diffuse scattering was used to determine structure, including bilayer thickness and area/lipid, the bending modulus, K_C, and S_{Xray}, a measure of chain orientational order. Our results reveal that TMCL thickens DMPC bilayers at all mole percentages, with a total increase of ~6 Å in pure TMCL, and increases A_L from 64 Å^2 (DMPC at 35 °C) to 109 Å^2 (TMCL at 50 °C). K_C increases by ~50%, indicating that TMCL stiffens DMPC membranes. TMCL also orders DMPC chains by a factor of ~2 for pure TMCL. Coarse grain molecular dynamics simulations confirm the experimental thickening of 2 Å for 20 mol% TMCL and locate the TMCL headgroups near the glycerol-carbonyl region of DMPC; i.e., they are sequestered below the DMPC phosphocholine headgroup. Our results suggest that TMCL plays a role similar to cholesterol in that it thickens and stiffens DMPC membranes, orders chains, and is positioned under the umbrella of the PC headgroup. CL may be necessary for hydrophobic matching to inner mitochondrial membrane proteins. Differential scanning calorimetry, S_{Xray} and CGMD simulations all suggest that TMCL does not form domains within the DMPC bilayers. We also determined the gel phase structure of TMCL, which surprisingly displays diffuse X-ray scattering, like a fluid phase lipid. A_L = 40.8 Å^2 for the ½TMCL gel phase, smaller than the DMPC gel phase with A_L = 47.2 Å^2, but similar to A_L of DLPE = 41 Å^2, consistent with untilted chains in gel phase TMCL.

Abbreviations: CL, cardiolipin; TMCL, tetramyristoylcardiolipin; TMCL, ½TMCL; DMPC, dimyristoylphosphocholine; PC, phosphocholine; DPPC, dipalmitoylphosphocholine; DPPE, dipalmitoylphosphoethanolamine; DMPE, dimyristoylphosphocholine; DLPE, dilauroylphosphocholine; LAXS, low-angle X-ray scattering; WAXS, wide-angle X-ray scattering; CGMD, coarse grain molecular dynamics; TFE, trifluoroethanol; MLVs, multilamellar vesicles; CHESS, Cornell High Energy Synchrotron Source; DSC, differential scanning calorimetry; NVT, canonical ensemble (*N*, number of moles, *V*, volume, *T*, temperature, all conserved); NPT, isobaric–isothermal ensemble; IMMs, inner mitochondrial membranes.

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1. Introduction

Cardiolipin (CL) refers to a group of unusual glycerophospholipids which contain four instead of two acyl chains. The acyl chain composition varies with species and cell state. CL's headgroup is negatively charged at pH 7.0 due to two phosphate groups with pK_a's ~3 and >8 (Kates et al., 1993) connected by a glycerol moiety. Many investigations have been carried out to characterize CL's overall structure (Lewis et al., 2007), headgroup structure (Haines, 2009; Tarahovsky et al., 2000), thermodynamic properties (Lewis and McElhaney, 2009; Nichols-Smith et al., 2004), water permeability (Shibata et al., 1994), membrane fluidity (Yamauchi et al., 1981), ability to form the hexagonal II (H_{II}) phase (Powell and Hui, 1996; Powell and Marsh, 1985; Rand and Sengupta, 1972; Seddon et al., 1983) and domains when mixed with other host phospholipids (Domenech et al., 2007; Frias et al., 2011; Lupi et al., 2008; Pinheiro et al., 1994; Sennato et al., 2005). Molecular dynamics simulations have been carried out to determine the structure of CL in bilayers (Dahlberg, 2007; Dahlberg and Maliniak, 2008) and the hydrogen-bonding capability of its headgroup (Dahlberg et al., 2010). However, comparisons between different studies and techniques are difficult due to different chain compositions. In this work we present results from experiments and simulations; in both cases, CL contains the same chains as the host lipid, DMPC (dimyristoylphosphatidylcholine).

The keen interest in CL emanates from its important biological roles. CLs are unique to biomembranes that couple phosphorylation and electron transport: bacterial plasma membranes, chromatophores, chloroplasts and mitochondria (Hoch, 1992). CL is necessary for proper ADP/ATP carrier function and/or formation of protein supercomplexes (Claypool et al., 2008; Hoffmann et al., 1994; Pfeiffer et al., 2003; Zhang et al., 2002). CL has been shown to co-isolate with each of the proteins that participate in oxidative phosphorylation: cytochrome oxidase (Robinson, 1993), ATP/ADP exchange protein (Horvath et al., 1990), F_0F_1 ATP synthase (Eble et al., 1990), the orthophosphate transporter (Kaplan et al., 1986) and the cytochrome bc_1 complex (Yu et al., 1978). It has been suggested that CL's role is to serve as a proton trap for oxidative phosphorylation due to the acid-anion nature of its unique headgroup (Haines and Dencher, 2002). Alternative roles for CL include the formation of cubic phases which can efficiently store lipid for the dynamic cristae (Deng and Mieczkowski, 1998) and apoptosis signaling (Kagan et al., 2009). Especially, CL seems to play a crucial role in the assembly of a passive docking platform for the binding of Bid, and the initiation of the apoptotic process (Jalmar et al., 2013). Finally, a defect in CL synthesis is the cause of Barth's syndrome, which is a sex-linked recessive disorder, clinically characterized by the classical symptoms of cardiomyopathy, neutropenia and delayed growth with a lethal effect on young boys (Barth et al., 1983). It is thought that a defective CL cannot interact with the proteins involved in energy production in the mitochondria. In rat liver mitochondria, CL contains chains with primarily C18:2 fatty acids (Daum, 1985), symmetrically (Schlame et al., 2005). In Barth's syndrome, chain symmetry is lost, which could account for CL's inability to interact with proteins (Schlame et al., 2005).

CL is synthesized exclusively on the inner mitochondrial membrane (IMM) (Hostetler and Van den Bosch, 1972; Jelsema and Morre, 1978) and except for a small amount of phosphatidylinositol, CL is the only negatively charged lipid in mitochondria (Hovius et al., 1990). There is ~20% (by weight) CL in eukaryotic mitochondria, where ~3/4 resides in the IMM (Daum, 1985). The major portion (75–90%) of CL in the IMM is located on the matrix side (Daum, 1985). CL is 9.2 mol% of total IMM lipids (Gomez and Robinson, 1999). Since most CL is located on the matrix side, the local concentration of CL could be as high as ~20 mol% CL, but because CL can be found associated with proteins and is also transferred across the membrane, its concentration will vary based on location and state of the cell. In this work we therefore explored many mole ratios of tetramyristoylCL (TMCL) including pure TMCL for comparison, but focused on 20 mol% for the coarse-grained MD simulations as a matrix side mimic of IMM. We mixed TMCL with dimyristoylphosphatidylcholine (DMPC), where TMCL contains the same chains as DMPC, C14:0. Although the chains of CL in eukaryotic mitochondria are primarily unsaturated, investigation of mixtures with identical chains rules out chain immiscibility as a cause of phase separation. We use low-angle X-ray diffuse scattering (LAXS) to obtain the structure (bilayer thickness and area) and bending modulus (K_C), and wide-angle X-ray scattering (WAXS) to obtain the S_{Xray} order parameter of TMCL/DMPC mixtures in the fluid phase. Densimetry determines the molecular volumes which are needed for electron density profiles, while differential scanning calorimetry determines the T_m's (main transition melting temperatures). LAXS is also used to determine the structure of pure TMCL in the gel phase. The experimental work was complemented with coarse-grain Martini molecular dynamics simulations to obtain a comprehensive view on the underlying processes.

2. Materials and methods

2.1. Samples

Purified lipids were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. DMPC Lots #140PC-226 and #140PC-256, and TMCL Lots #140CA-40 and #140CA-51 as the NH_4^+ salt were used. Thin layer chromatography (TLC) revealed that Lot #140CA-40 TMCL contained <1% lysolecithin and that Lot #140CA-51 TMCL contained <0.1% lysolecithin before the experiments. Lipid stocks solutions were prepared by precisely weighing lyophilized lipid into glass vials and HPLC chloroform was added; these stock solutions were aliquoted into glass test tubes to generate the mole percentages: 0, 0.7, 2.7, 4.4, 10, 20 and 100 TMCL/(DMPC + TMCL). When these concentrations are converted into chain concentrations, 2TMCL/(DMPC + 2TMCL), where TMCL = ½TMCL, they become 0, 1.39, 5.26, 8.43, 33.33 and 100. For X-ray scattering, 4 mg lipid mixture in 200 μl HPLC chloroform:trifluoroethanol (TFE) (1:1, v:v) was plated onto silicon wafers (15 mm × 30 mm × 1 mm) via the rock and roll method (Tristram-Nagle, 2007) to produce ~1800 well-aligned bilayers. Solvents were thoroughly removed, first by evaporation for one day in the fume hood, then by evaporation under vacuum for at least two hours. Samples were hydrated through the vapor in a thick-walled X-ray hydration chamber (Kučerka et al., 2005a) for 0.5–1 h. For X-ray capillary experiments, multilamellar vesicles (MLVs) were prepared by mixing dried lipid mixtures with MilliQ water or 50 mM Hepes buffer, pH 7.0, to a final concentration of 25 wt% and cycling three times between −20 °C and 50 °C for ten minutes at each temperature with vortexing; this suspension was loaded into X-ray capillaries (Charles Supper, Cambridge, MA). For densimetry, MLVs at 5 wt% in water were hydrated as above. For calorimetry, MLVs at a concentration of 0.1% in water were hydrated as for densimetry.

2.2. Volume determination

Volumes of fully hydrated MLVs of DMPC/TMCL lipid mixtures and pure lipids were determined as a function of temperature from 20 ± 0.01 °C to 55 ± 0.01 °C using an Anton-Paar USA DMA5000M (Ashland, VA) vibrating tube densimeter (Raghunathan et al., 2012). After densimetry, TLC revealed ~2% lysolecithin.