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On the miscibility of cardiolipin with 1,2-diacyl phosphoglycerides: Binary mixtures of dimyristoylphosphatidylethanolamine and tetramyristoylcardiolipin ☆

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ABSTRACT

The thermotropic phase behavior and organization of model membranes composed of binary mixtures of the quadruple-chained, anionic phospholipid tetramyristoylcardiolipin (TMCL) with the double-chained zwitterionic phospholipid dimyristoylphosphatidylethanolamine (DMPE) were examined by a combination of differential scanning calorimetry (DSC) and Fourier-transform infrared (FTIR) spectroscopy. After equilibration at low temperature, DSC thermograms exhibited by binary mixtures of TMCL and DMPE containing <80 mol DMPE exhibit a fairly energetic lower temperature endotherm and a highly energetic higher temperature endotherm. As the relative amount of TMCL in the mixture decreases, the temperature, enthalpy and cooperativity of the lower temperature endotherm also decreases and is not calorimetrically detectable when the TMCL content falls below 20 mol%. In contrast, the temperature of the higher temperature endotherm increases as the proportion of TMCL decreases, but the enthalpy and cooperativity both decrease and the transition endotherms become multimodal. The FTIR spectroscopic results indicate that the lower temperature endotherm corresponds to a lamellar crystalline (L_c) to lamellar gel (L_β) phase transition and that the higher temperature transition involves the conversion of the L_β phase to the lamellar liquid-crystalline (L_α) phase. Moreover, the FTIR spectroscopic signatures observed at temperatures below the onset of the L_c/L_β phase transitions are consistent with the coexistence of structures akin to a TMCL-like L_c phase and the L_β phase, and with the relative amount of the TMCL-like L_c phase increasing progressively as the TMCL content of the mixture increases. These latter observations suggest that the TMCL and DMPE components of these mixtures are poorly miscible at temperatures below the L_β/L_α phase transition temperature. Poor miscibility of these two components is also suggested by the complexity of the DSC thermograms observed at the L_β/L_α phase transitions of these mixtures and with the complex relationship between their L_β/L_α phase transition temperatures and the composition of the mixture. Overall, our data suggests that TMCL and DMPE may be intrinsically poorly miscible across a broad composition range, notwithstanding the homogeneity of the fatty acid chains of the two components and the modest (~10 °C) difference between their L_β/L_α phase transition temperatures.

1. Introduction

The structure and organization of lipid bilayer model membranes derived from mixtures of two or more lipids are of special biological importance because the lipid components of all cell membranes are heterogeneous mixtures of different classes and molecular species of lipids. This fact has provided the impetus for numerous studies of the thermotropic phase behavior and mixing properties of a wide range of lipid mixtures [see 1]. The results of such studies are usually expressed as temperature-composition pseudo phase diagrams, from which important information about the miscibility of specific lipid components can be obtained. The importance of such studies has now come into sharper focus because of suggestions that cell membranes may contain laterally segregated, compositionally distinct domains that may be essential for the normal functioning of membrane proteins and transmembrane signaling systems [see 2–6].

Abbreviations: DMPE, dimyristoylphosphatidylethanolamine; CL, cardiolipin; PE, phosphatidylethanolamine; DMPG, dimyristoylphosphatidylglycerol; DMPC, dimyristoylphosphatidylcholine; TMCL, 1,2,-1′,2′-tetramyristoylcardiolipin; DSC, differential scanning calorimetry; IR, infrared; FTIR, Fourier-transform infrared; C=O, carbonyl; CH_2, methylene; L_α, lamellar liquid-crystalline; L_β, lamellar gel; L_c, lamellar crystalline; T_m, gel/liquid-crystalline phase transition temperature

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Of the limited number of studies of mixtures of different classes of phospholipids that have been performed, relatively few have been on cardiolipin (CL[1])-containing systems, presumably because this anionic, quadruple-chained lipid is not a major component of most mammalian cell membranes. However, CL is an important component of the plasma membranes of many types of Gram-negative and Gram-positive bacteria, and of the mitochondrial and chloroplast inner membranes of eukaryotes [7–11]. CL is usually a relatively small component (≤10 mol%) of such membranes, where its primary role appears to be that of supporting the function of key membrane proteins [7]. The consensus of currently available data is that CL is important for the structural stabilization and activation of many mitochondrial enzymes, especially those involved in ATP synthesis and energy transduction [12–18], and for maintaining the structure and function of the Type II photoreaction center of photosynthetic bacteria and plants [19]. In higher eukaryotes, CL is an effector of the cytochrome P-450-dependent cholesterol side-chain cleavage enzyme and activates cytochrome c oxidase and the mitochondrial phosphate carrier protein [20–22]. In fact, it has been suggested that the capacity for structurally specific interactions with membrane CL may actually be highly conserved in such proteins [23]. CL may also be found in substantially higher quantities (~55–60 mol%) in the membranes of some microbial organisms, wherein elevated levels of CL and its derivatives appear to enhance the tolerance of these organisms to halophilic and resource-depletion stress [11,24–26]. The study of CL-containing membranes is thus an important aspect of the understanding of the membrane properties required to support the function of many of cellular mechanisms, including those involved in energy procurement and transduction [26,27].

Relatively few studies of CL membranes, and of membranes composed of mixtures of CL with other lipids, have been performed. In part, this is because the chemical synthesis of stereochemically pure CL has not been as straight forward as with most other naturally occurring phospholipids, so that only a few species of stereochemically pure, synthetic CL are currently commercially available. Thus, most of the studies of CL-containing membranes have been performed with mixtures composed of highly unsaturated CLs isolated from natural sources (predominantly beef heart) and various other types of naturally occurring and synthetic phospholipids, and the general consensus of such studies have been that CL is highly immiscible with most other lipids [see 28 and references cited therein]. However, an unambiguous interpretation of many of these results is often difficult, because there are considerable differences between the length and degree of unsaturation of the hydrocarbon chains of the CL and non-CL components of the mixture. It is therefore difficult to disentangle the potential effects of variations in the polar headgroup composition of the mixture from the known effect of variations in fatty acyl group length and degree of unsaturation on phospholipid miscibility [see 28 and references cited therein]. To circumvent such problems, we are studying the interactions of stereochemically pure, synthetic TMCL with various dimyristoyl glycerophospholipids. We present here the results of a detailed study of binary mixtures of di-anionic TMCL with zwitterionic DMPE using a combination of high-sensitivity DSC and FTIR spectroscopy. This study encompasses all aspects of the polymorphic phase behavior exhibited by these mixtures and addresses a number of fundamental issues related to the overall miscibility of these two lipids and the general principles underlying lipid/lipid interactions in all of the polymorphic phases formed. PE is a major component of many eukaryotic membranes, and PE and CL are both found in many prokaryotic surface membranes and in the mitochondrial inner membranes of most eukaryotic cells. The study of the structure and organization of PE/CL model membranes and of the miscibility of these lipids is thus biologically relevant.

[1] See Abbreviations.

2. Materials and methods

The phospholipids TMCL and DMPE were obtained from Avanti Polar Lipids Inc. (Alabaster, AL) and used without further purification. Binary mixtures were prepared by mixing chloroform:methanol (9:1) solutions of the lipids in the amounts required to obtain the desired composition. The solvent was then slowly removed in a stream of nitrogen such that the lipid mixture was cast as a thin film on the sides of a clean glass tube whilst maintaining a temperature near 60 °C. The lipid film was then dried *in vacuo* overnight to ensure removal of the last traces of solvent. Hydration of the lipid film was achieved by placing some wet cotton wool into the tube (without contacting the lipid film) and allowing the sample to absorb water from the water vapor-saturated air by warming the sample to temperatures near 60 °C. Subsequently, the cotton wool was removed and the hydrated sample was quickly dispersed in pre-warmed buffer composed of 100 mM phosphate, 5 mM NaN_3, 150 mM NaCl, pH 7.4 and mixed by vigorous vortex mixing at temperatures near 60 °C. In one set of measurements, samples containing 3–4 μmol of lipid were dispersed in 600 μl of buffer and a 500 μl aliquot was introduced into the hastelloy capsule of a Calorimetry Sciences Corporation (Spanish Fork, UT) high-sensitivity, heat-conduction Multi-Cell DSC instrument. Prior to initial data acquisition, the sample was preconditioned by heating to 60 °C followed by cooling to −7 °C at scanning rates near 60 °C/h. Data acquisition scans were then performed at scanning rates near 10 °C/h. Typically, the process first involves three cycles of heating and cooling between −7 °C and 60 °C. Subsequently, the samples were cooled to −30 °C and incubated at that temperature 12 h, then heated to 5 °C and incubated at that temperature to ensure that all ice had completely melted. This low temperature incubation protocol was repeated twice and the sample was then cooled to −7 °C and a further set of data acquisition runs, consisting of two cycles of heating and cooling between −7 °C and 60 °C, were performed. In a companion set of DSC measurements, samples containing ~1 mg of lipid were dispersed in 1 ml of buffer by methods similar to those described above. Subsequently, 323 μl aliquots were loaded into a high-sensitivity power-compensation nano-DSC instrument (Calorimetry Sciences Corporation) and data were acquired between 0 and 60 °C at heating and cooling rates near 10 °C/h. The measurements performed in the Multi-Cell DSC enabled an accurate mapping of the gel phase polymorphic behavior of these samples, whereas those in the Nano-DSC measurements enabled more accurate mapping of the boundaries of the gel/liquid-crystalline phase transitions of the various lipid mixtures examined. The data acquired were analyzed and plotted with the Origin software package (OriginLab Corporation, Northampton, MA).

FTIR spectroscopy was performed on samples containing 2–4 mg of lipid. Samples were dispersed as described above in 50–75 μl of a D_2O-based buffer containing 100 mM phosphate, 150 mM NaCl, pD 7.4. The dispersion was squeezed between the CaF_2 windows of a heatable, demountable liquid cell (NSG Precision Cells, Farmingdale, NY) equipped with a 25 μM Teflon spacer. Once mounted in the sample holder of the spectrometer, the sample could be heated between −20 °C and 90 °C by an external, computer-controlled water bath. Prior to initial data acquisition, samples were subject to the same low temperature incubation protocol described above for the DSC samples. Infrared spectra were then acquired as a function of temperature with a Digilab FTS-40 Fourier-transform Spectrometer (Biorad, Digilab Division, Cambridge, MA) using data acquisition parameters similar to those described by Mantsch et al. [29]. The experiment involved a sequential series of 2 °C temperature ramps with a 20-minute inter-ramp delay for thermal equilibration, and was equivalent to an effective scanning rate of 4 °C/h. The data obtained were analyzed using computer programs obtained from the instrument manufacturer and from the National Research Council of Canada. In cases where absorption bands appeared to be a summation