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Intermolecular interactions of lysobisphosphatidic acid with phosphatidylcholine in mixed bilayers

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Abstract

Lysobisphosphatidic acid (LBPA) can be regarded to represent a unique derivative of phosphatidylglycerol. This lipid is highly enriched in late endosomes where it can comprise up to 10–15 mol% of all lipids and in these membranes, LBPA appears to be segregated into microdomains. We studied the thermotropic behavior of pure dioleoyl-LBPA mono- and bilayers using Langmuir-lipid monolayers, electron microscopy, differential scanning calorimetry (DSC), and fluorescence spectroscopy. LBPA formed metastable, liquid-expanded monolayers at an air/buffer interface, and its compression isotherms lacked any indication for structural phase transitions. Neat LBPA formed multilamellar vesicles with no structural transitions or phase transitions between 10 and 80 °C at a pH range of 3.0–7.4. We then proceeded to study mixed LBPA/dipalmitoylphosphatidylcholine (DPPC) bilayers by DSC and fluorescence spectroscopy. Incorporating increasing amounts of LBPA (up to X_{LBPA} (molar fraction) = 0.10) decreased the co-operativity of the main transition for DPPC, and a decrease in the main phase transition as well as pretransition temperature of DPPC was observed yet with no effect on the enthalpy of this transition. In keeping with the DSC data for DPPC, 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC)/LBPA mixed bilayers were more fluid, and no evidence for lateral phase segregation was observed. These results were confirmed using fluorescence microscopy of Langmuir-lipid films composed of POPC and LBPA up to $X_{\mathrm{LBPA}} = 0.50$ with no evidence for lateral phase separation. As late endosomes are eminently acidic, we

Abbreviations: C_s^{-1}, elastic modulus of area compressibility; DPH, 1,6-diphenyl-1,3,5-hexatriene; DPPC, 1,2-dipalmitoylphosphatidylcholine; DSC, differential scanning calorimetry; LBPA, lysobisphosphatidic acid; $\mathrm{LBPA}_{18:1}$, *sn*-(3-oleoyl-2-hydroxy)-glycerol-1-phospho-*sn*-3′-(1′-oleoyl-2′-hydroxy)-glycerol; LUV, large unilamellar vesicle; MLV, multilamellar vesicle; NBD-PC, 1-palmitoyl-2-{12-[(7-nitro-2-1,3-benzoxadizole-4-yl)amino]dodecanoyl)-*sn*-glycero-3-phosphocholine; POPC, 1-palmitoyl-2-oleoyl-phosphatidylcholine; PPDPC, 1-palmitoyl-2[10-(pyren-1-yl)]decanoyl-*sn*-glycero-3-phosphocholine; T_{m}, main phase transition temperature; T_{p}, pretransition temperature; π, surface pressure; ΔH, enthalpy change

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examined the effect of lowering pH on lateral organization of mixed PC/LBPA bilayers by DSC and fluorescence spectroscopy. Even at pH 3.0, we find no evidence of LBPA-induced microdomain formation at LBPA contents found in cellular organelles.
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Keywords: Differential scanning calorimetry; Fluorescence spectroscopy; Langmuir-lipid monolayers; Lipid–lipid interactions

1. Introduction

Lipids are not just simple structural components of cellular membranes. These molecules show a plethora of distinct functions by acting as cellular signaling molecules by binding to their cellular receptors and also by activating proteins through changes in the biophysical state of the cellular membranes embedding proteins. Biological membranes can be described as liquid-crystalline, adaptive, and co-operative supramolecular assemblies that are highly dynamic and apt to regulation by various membrane binding ligands, membrane lipid composition, and a number of physical parameters. Biologically, the most common lipid phase is the lamellar and fluid (liquid-disordered) phase. However, the latter phase is just one of a number of lamellar phases found. Accordingly, lamellar lipid bilayers undergo a number of different lyotropic and thermotropic phase transitions that also involve major inplane structural reorganizations (Kinnunen and Laggner, 1991). The lateral organization in a multicomponent system is a complex process as several features, such as dehydration, pH, hydrophobic mismatch of the constituents, and charge contribute (Holopainen et al., 1999; Lehtonen and Kinnunen, 1995; Lehtonen et al., 1996; Leventis et al., 1986; Mustonen et al., 1987; Tilcock and Cullis, 1981; Tuominen et al., 1999).

Bis(monoacylglycerol) phosphate or lysobisphosphatidic acid (LBPA) was discovered by Body and Gray in pig lung homogenate in 1967 and since then, it has been found in most tissues and cell types. It usually represents less than 1% of the total phospholipid mass (Mason et al., 1972; Rouser et al., 1969), but increased LBPA contents have been found in several lipidosis and in response to some pharmaceutical agents (Adachi et al., 1972; Brotherus et al., 1974; Cochran et al., 1985; Yamamoto et al., 1970). In several models, LBPA was shown to have a *sn*-1:*sn*-1 stereoconfiguration (Brotherus et al., 1974) distinguishing this phospholipid from other cellular phospholipids. The stereoconfiguration, however, remains a matter of controversy and Kobayashi et al. (2002) proposed recently a *sn*-2:*sn*-2 stereoconfiguration in BHK cells. In these cells, immunocytochemical and labeling studies suggested that LBPA is located in the endocytic/endosomal membranes, particularly in late endosomes (Kobayashi et al., 1998a), and is involved in cholesterol transport (Kobayashi et al., 1999) and protein/receptor trafficking (Kobayashi et al., 1998b) possibly via formation of lateral microdomains. Due to its peculiar molecular structure, we can expect that the effective shape of LBPA to be a cone or an inverted cone. Accordingly, in the latter case, increasing contents of LBPA would result in altered lateral pressure profile (Cantor, 1997) within the membrane leading to higher H_{II} propensity (Kinnunen, 1996). This effect is of importance since this may promote the binding of peripheral membrane proteins to the membrane surface (Kinnunen, 1996) and can also provide a molecular level explanation (Holopainen et al., 1999) for the observed fusogenic properties of LBPA (Kobayashi et al., 2002).

This study was undertaken to elucidate the biophysical characteristics of pure $LBPA_{18:1}$ in aqueous solutions and to study the effects of $LBPA_{18:1}$ in saturated and unsaturated phosphatidylcholine bilayers, at contents found in late endosomes. We show using Langmuir-lipid films, fluorescence microscopy of lipid monolayers, electron microscopy, differential scanning calorimetry, fluorescence spectroscopy, and static light scattering that pure $LBPA_{18:1}$ forms metastable monolayers at the air–water interface and multilamellar vesicles in aqueous solutions. Neat dioleoyl-LBPA does not show any phase transitions either as monomolecular films or as bilayers. Furthermore, using differential scanning calorimetry, we show that $LBPA_{18:1}$-containing DPPC membranes are more fluid and that in fluid POPC bilayers, $LBPA_{18:1}$ is not enriched into microdomains at physiological pH or at acidic pHs found in late endosomes.

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