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Historical perspective

The enzymatic sphingomyelin to ceramide conversion increases the shear membrane viscosity at the air-water interface

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ABSTRACT

Whereas most of lipids have viscous properties and they do not have significant elastic features, ceramides behave as very rigid solid assemblies, displaying viscoelastic behaviour at physiological temperatures. The present review addresses the surface rheology of lipid binary mixtures made of sphingomyelin and ceramide. However, ceramide is formed by the enzymatic cleavage of sphingomyelin in cell plasma membranes. The consequences of the enzymatically-driven ceramide formation involve mechanical alterations of the embedding membrane. Here, an increase on surface shear viscosity was evidenced upon enzymatic incubation of sphingomyelin monolayers. The overall rheological data are discussed in terms of the current knowledge of the thermotropic behaviour of ceramide-containing model membranes.

1. Introduction

From the seminal paper of Singer and Nicholson [1], cell membranes were postulated to be two-dimensional fluid structures. Through hydrophobic and hydrophilic interactions, the lipid bilayer structure could thus accommodate other biomolecules such as integral membrane proteins. Lipid fluidity was therefore invoked to be the dynamic property responsible for protein translational diffusion within the membrane. Moreover, functional membranes were conceived in terms of the lipid viscosity and changes in membrane fluidity, such as it would be triggered by changes in temperature or by different compositions of membrane phospholipids, might lead to membrane dysfunction. For long time, cell membranes were hence considered to be a short –ranged oriented solution of interacting integral proteins embedded in a continuous viscous phospholipid bilayer solvent.

Very early, thermotropic transitions from a gel (Lβ, ordered-chain) state to a liquid disordered (Lα, disordered-chain) state of lipid bilayers was detected for saturated phospholipids such as the canonical dipalmitoylphosphatidylcholine (DPPC) [2]. It was already established that the temperature for the gel to liquid transition (T_m or melting temperature) depends largely on the chain-length and unsaturation of the acyl chains and on the chemistry of the polar head-group [3]. Thus, DPPC undergoes a phase transition at approximately 42 °C. Saturated phospholipids with longer acyl chains have indeed higher T_m. Remarkably, biological membranes are preserved fluid at physiological temperatures thanks to the presence of membrane proteins and Chol [4].

Simons and Ikonen revisited in 1990s the classical picture of the fluid plasma membrane. The idea that the plasma membrane could be marbled with smaller and more ordered domains able to laterally move within the lipid bilayer originated the so-called “raft hypothesis” [5]. Based on the particular interactions that govern the sphingolipid and cholesterol (Chol) interactions, lipid rafts were hypothesized to be enriched in sphingomyelin (SM) and chol, originating the denominated liquid ordered (l_o) phase [6]. In terms of viscous flowing, the l_o phase behaves as an intermediate state between Lα and Lβ phases. Although lipid rafts were attributed to play an important role in many cellular processes their existence of l_o phases in biological membranes remains still controversial [7].

However, the role played by solid phases in biological membranes has been recently revisited as the existence of ceramide (Cer)-enriched domains has been reported in different metabolic and cellular events as cell proliferation, apoptosis or disease [8,9,10]. The biological function of Cer-enriched domains could depend on the alterations of membrane biophysical properties [11] that occur upon Cer enzymatic formation [12,13,14,15]. Besides the *de novo* synthesis pathway of ceramides in the endoplasmic reticulum [16], ceramides can be generated in the plasma membrane through the action of the enzyme sphingomyelinase

Abbreviations: bCer, brain ceramide; bSM, brain sphingomyelin; Cer, Ceramide; SM, Sphingomyelin; eggCer, egg ceramide; eggSM, egg sphingomyelin; pSM, palmitoyl sphingomyeline; pCer, palmitoyl ceramide; SMase, sphingomyelinase

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(SMase) that hydrolyses the SM to phosphocholine and Cer [17]. The Cer levels in the plasma membrane are extremely low but can rapidly increase by the action of SMase in response to different stimuli [18], eliciting a number of different biological responses [8,9,10]. Accordingly, the binary mixture SM + Cer is the structural basis of Cer-enriched domains in cell membranes. Thus, the alterations of the membrane might be in part related to the unique viscoelastic properties of ceramides. Whereas most of lipids have viscous properties and they do not have significant elastic properties, ceramides behave as very rigid solid assemblies, displaying viscoelastic features at physiological temperatures [19]. Several rheological studies have been addressed to understand the physico-chemical interactions between SM and Cer and their implications in the Cer-induced alteration of the viscoelastic membrane properties.

In this review we first summarize the relevant information obtained by compression and shear surface rheology of premixed SM/Cer monolayers (Sections 2 and 3). We then present new data describing the flowing properties of SM monolayers upon SMase-driven conversion to Cer (Section 4), emphasizing the correlation between the topographical phase information of SM/Cer monolayers (Section 5) with the mechanical perturbations associated to enzymatic SM to Cer conversion (Section 6). Finally, we discuss the role played of the viscoelastic alterations by the SM to Cer conversion in modulating the mechanical and physical properties of model bilayers composed of more complex mixtures.

2. Π-A compression isotherms of SM/Cer mixtures

The monolayer isotherms of mixtures of SM/Cer at different proportions have been studied earlier for different alkyl species [20,21,22]. A liquid-expanded (LE, chain-disordered) to liquid-condensed (LC, chain-ordered) phase transition is found for intermediate chain SMs (from C14:0 to C26:0, and C24:1) at low lateral pressures. The lateral pressure of the two-dimensional transition clearly depends on the acyl composition of SMs and the temperature. The lower the temperature and the longer the acyl chain, then the lower the surface pressure (and larger the molecular area) that are observed at the onset of the two-dimensional phase transition [23]. A different surface behaviour has been found for unsaturated (C18:1) and very long chain polyunsaturated SMs. For this case, the compression isotherms were compatible with a LE state and showed large molecular areas [24]. In contrast, a LE behaviour, is observed for short 12:0 SM at all temperatures in the 10–30 °C range without any indication of two-dimensional phase transitions [23].

Air/water monolayers of natural ceramides present a continuous isotherm in the whole range of surface pressures at room temperatures. For larger molecular areas, isotherms remain at a near-zero pressure that is compatible with a diluted state. Upon compression, the isotherms suddenly rise, eventually entering a collapse regime, characterized by a constant pressure. Generally, the isotherms display a condensed-like shape characterized by a high slope, typical of solid phases [20,21]. Synthetic ceramides such as pCer can exhibit expanded, condensed and solid states. This polymorphic behaviour depends on the temperature and reflected an accurate phase diagram [25]. A similar multiphasic behaviour has been found for natural ceramides [26] and mixed (short and longer) ceramides [27].

The addition of increasing amounts of Cer generally produces a condensing effect on SM monolayers. As an example, Fig. 1 shows the isotherms obtained for mixtures of pSM/pCer in different proportion. In this case, the LE-LC phase transition of SM occurred at 25 mN/m and the condensing effect of pSM by the presence of pCer was more pronounced at lower lateral pressures [20]. A similar condensing effect has been reported for eggSM/eggCer monolayers [21].

The compression response is mainly driven by membrane compactness, *i.e.* a high compression modulus describes the ability of lipids to densely pack in such a way that molecular hard cores resist against

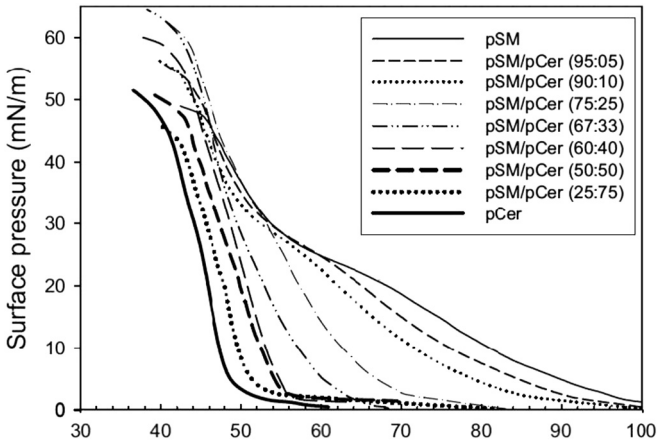

Fig. 1. Compression isotherms of pSM-pCer mixed monolayers: pure pSM (thin solid line), pure pCer (thick solid line), and pSM-pCer mixtures at 5 mol% (thin short-dashed line), 10 mol% (thin dotted line), 25 mol% (thin dot-dashed line), 33 mol% (thin double-dot-dashed line), 40 mol% (thin long-dashed line), 50 mol% (thick short-dashed line), and 75 mol% (thick dotted line) of pCer.
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further compression. The equilibrium compression modulus C^{-1} can be easily calculated from the numerical derivative of the experimental π-A isotherms as

$$C^{-1} = -A\frac{\partial \pi}{\partial A}$$

As expected from the isotherm curves, ceramides are characterized by a much higher modulus ($C_{Cer}{}^{-1} \approx 300$ mN/m for natural bCer, eggCer or pCer) than SMs ($C_{SM}{}^{-1} \approx 100$ mN/m, for natural bCer, eggCer or pCer) at lateral pressures ranging from 25 to 35 mN/m, corresponding to the biologically relevant surface packing [28]. Even higher values of the compression modulus for other synthetic ceramides have also been reported (400 mN/m and 600 mN/m for C24:1Cer and C16Cer respectively) [29]. Fig. 2 shows the compression modulus of eggSM/eggCer mixtures [21]. Note that the addition of increasing amounts of ceramides raises the compression modulus on SM monolayers, a fact related to the condensation effect induced by ceramides.

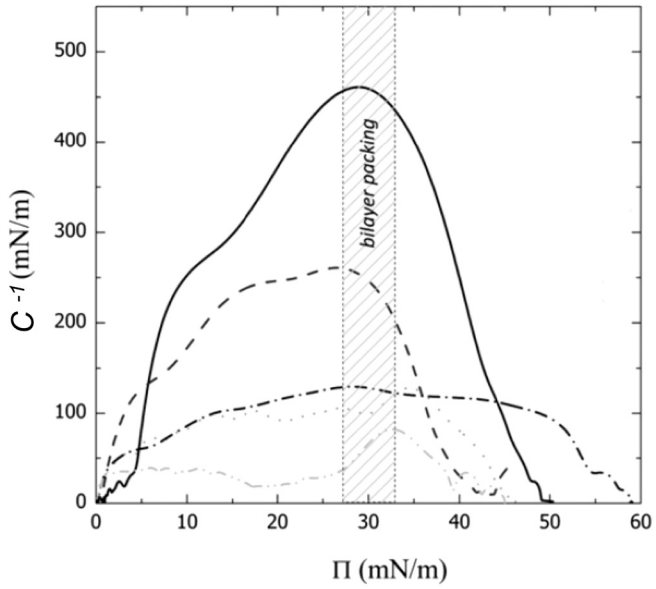

Fig. 2. π-Dependence of the compression modulus, C^{-1}, for lipid monolayers made of eggSM/eggCer mixtures: (solid line) 1:0 mol; (dashed line) 2:1 mol; (dash-dotted line) 1:1 mol; (dotted line) 1:2 mol; and (dashdot- dot-dashed line) 0:1 mol).
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