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Imaging of the domain organization in sphingomyelin and phosphatidylcholine monolayers

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Abstract

The lateral organization of biomembranes has gained significant interest when the fluid mosaic model was challenged by the model of "lipid rafts". Several lipid classes like cholesterol and sphingolipids are considered to be essential for their formation. Here we investigate the lateral domain formation in binary mixtures of sphingomyelin and phosphatidylcholine. Both are major lipid components of lipoproteins and mammalian cell membranes at various molar ratios. Surface pressure–area isotherms and surface potential–area isotherms of monolayers composed of these lipids clearly indicated non-ideal mixing. In addition, Brewster angle microscopy provided a well-suited approach to image the formation of lateral domains. These images demonstrated that pure sphingomyelin forms very stable finger-like domains that exhibit a distinct internal organization suggesting an anisotropic orientation of the acyl side chains. Similar behavior was found for mixtures containing more than 60 mol% sphingomyelin. With increasing content of phosphatidylcholine the domain size decreased and the surface pressure, where domain formation occurred, increased. At lower sphingomyelin content (30–60 mol%) rather round-shaped, smaller domains were observed. Thus, the potential of sphingomyelin domains as potentially important building blocks for actual domains that could be building blocks for raft formation is suggested, even without the presence of cholesterol. In addition, these observations may suggest a role for the distinct molar ratio of these key lipids frequently found in physiologically relevant particles such as low and high density lipoproteins or the outer leaflet of the human erythrocyte membrane.

Keywords: Brewster angle microscopy; Lipid packing; Lipid domain formation; Monolayer; Membrane mimetic system

1. Introduction

Glycerol-based phospholipids and sphingomyelins are among the most abundant lipids in mammalian plasma membranes. Within the last decade, sphingolipids have emerged as active participants in cell regulation since these lipids and their metabolites are involved in differentiation, cellular senescence or apoptosis (Ohanian and Ohanian, 2001). Furthermore,

Abbreviations: SM, sphingomyelin; PC, phosphatidylcholine; CHOL, cholesterol; POPC, 1-palmitoyl-2-oleoyl-phosphatidylcholine; BAM, Brewster angle microscopy; LDL, low density lipoprotein; HDL, high density lipoprotein

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the fusion of some viruses is enhanced or dependent on sphingolipids being present in the target membrane (Van-Mau et al., 2000; Saez-Cirion et al., 2002).

Recently the classical fluid bilayer model was challenged by the concept of lipid islands termed "rafts" supported by findings of detergent insoluble membrane fragments enriched in certain lipids like sphingomyelin (SM) and cholesterol (CHOL) (Brown and London, 1998). The model of functional lipid rafts as outlined previously (Simons and Ikonen, 1997) suggested various biological functions for these moieties and thus emphasized the importance of lipid organization in various biological systems (see also e.g. Anderson and Jacobson, 2002; Harder, 2003; Simons and Vaz, 2004). A lipid domain structure for bilayers was earlier suggested from lateral diffusion studies of lipids in sarcoplasmic reticulum membranes that was shown to be area-limited (Laggner, 1981).

Biophysical investigations of model systems have made significant contributions to the understanding of lipid rafts (for recent reviews see Simons and Toomre, 2000; London, 2002). For example, Silvius and coworkers showed differential partitioning of SM into SM/CHOL domains (Wang and Silvius, 2000) and partitioning of lipidated peptides into ordered lipid domains (Wang et al., 2001). Many contributors to the field discussed a vital role for cholesterol in the formation and stability of such membrane rafts (Radhakrishnan et al., 2000; Xu et al., 2001; Veatch and Keller, 2003) and initial imaging of raft structures by atomic force microscopy was reported (Rinia et al., 2001; Giocondi et al., 2004). But interestingly, an attenuating effect of cholesterol on domain formation was also reported for renal brush border membranes (Milhiet et al., 2001). Moreover, phosphatidylcholine and sphingomyelin, characterized by the same headgroup but different backbone organization, are major components of the outer membrane layer of platelets and erythrocytes (Cesar et al., 1993) or the monolayer of lipoproteins (Barenholz and Gatt, 1982). They are abundant in different characteristic molar ratios like 70% PC and 30% SM for low density lipoprotein (LDL) (Sommer et al., 1992), about 55% PC to 45% SM for the human erythrocyte membrane (Cesar et al., 1993) or about 15% PC and 85% SM in certain high density lipoprotein subclasses (HDL) (Barenholz and Gatt, 1982).

Therefore, our working hypotheseis has been that these obvious significant differences in the PC/SM ratio are not random, but will impact bilayer properties, and hence membrane functionality, as well as the monolayer properties in lipoproteins. In particular, we are interested in the possible existence of any SM domains, even without the interaction with cholesterol that is commonly seen to induce lateral demixing (Radhakrishnan et al., 2000). In this work we report on the mixing behaviour of two major mammalian lipids, PC and SM as deduced from the surface pressure and potential versus area isotherms. In addition, the formation of distinct SM domains as a function of PC admixture was imaged by Brewster angle microscopy. This technique, developed independently by two groups (Henon and Meunier, 1991; Hönig and Möbius, 1991), is an important imaging tool that allows the direct visualization of monolayers at the air/water interface without the addition of a potentially perturbing fluorescent probe.

2. Materials and methods

2.1. Phospholipids

1-Palmitoyl-2-oleoyl-phosphatidylcholine (POPC) and egg-sphingomyelin (SM), containing primarily N-linked palmitate, were purchased from Avanti Polar Lipids (Alabama, USA). Purity of both lipids (>99%) was checked by thin layer chromatography using $CHCl_3$:MeOH:H_2O (75:25:6, v/v) as solvent, whereby only one single spot was detected. The exact amount of phospholipid stock solutions was determined according to the method of Bartlett (1959).

2.2. Monolayer experiments

Surface pressure–area (π–A) isotherms were recorded using a customer-designed rectangular Teflon trough (Nanotechnology, MDT Corp., Moscow, Russia) thermostated at 25 °C. A moveable Teflon barrier compressed the monolayer with compression velocity between 0.07 and 0.12 nm^2/(min molecule). A Wilhelmy balance (20 mm wide filter paper) was used to measure the surface pressure. All glass was cleaned with chromosulfuric acid and rinsed extensively with highly purified water to avoid any traces of impurities. The subphase was highly purified water, prepared with the Milli-RO4 + Milli-Q water purification system. The monolayers were prepared by spreading a defined volume of a μM lipid solution in chloroform (Baker analyzed HPLC grade) onto the aqueous subphase using a Hamilton syringe. The system was enclosed in a box in order to minimize water evaporation, to ensure high humidity, and to avoid trace pollution of the system. All values displayed are highly reproducible and represent the average of at least three experiments and the standard deviation is less than 1% for surface pressure and less and 5% for the surface potential values.