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Temperature and pressure dependent growth and morphology of DMPC/DSPC domains studied by Brewster angle microscopy

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Abstract

In this work, the temperature and pressure dependent growth of domains in DMPC/DSPC monolayers at various molar ratios was studied by Brewster angle microscopy. Upon compression, roughly discoidal domains with some branching are formed. Further compression leads to an increase in both the number and the average size of the domains, which range between ca. 5 and 20 μm. The isobaric heating of the monolayers results in a gradual decrease of the domain size until their disappearance. The size and morphology of the domains depend not only on equilibrium parameters such as temperature, pressure and composition, but appear to be also strongly dependent on non-equilibrium parameters such as the rate of perturbation. The comparison between our results and those previously published for bilayers allows us to infer that the growth behaviour in monolayers can be qualitatively but not quantitatively extrapolated to bilayers.

Keywords: Brewster angle microscopy; Lipid monolayers; Domains; DMPC; DSPC; Nucleation theory

1. Introduction

The lateral heterogeneity of biological membranes is now a well-established property (Vereb et al., 2003). It is believed to play a key role in important biological processes such as membrane protein binding, insertion and function (Brown and London, 2000; Mouritsen and Jørgensen, 1997; Simons and Ikonen, 1997), membrane permeability (Mouritsen and Jørgensen, 1995), and in plane molecular reactions (Edidin, 1997). In order to understand the physical processes which rule the existence, size and shapes of these domains, a series of model lipid mixtures have been studied by several techniques such as for example, atomic force microscopy (AFM) (Kaasgaard et al., 2001; Ratto and Longo, 2002; Sanchez and Badia, 2003), two-photon fluorescence spectroscopy (Bagatolli and Gratton, 2000b; Baumgart et al., 2003) and Monte Carlo simulations

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(Jørgensen and Mouritsen, 1995; Sugár et al., 1999). Among these model membranes, the binary mixture of dimyristoylphosphatidylcholine (DMPC) and distearoylphosphatidylcholine (DSPC) stands out as one of the most studied (Leidy et al., 2001; Morrow et al., 1991; Sankaram et al., 1992; Sugár et al., 1999; Vaz et al., 1989). The temperature-composition phase diagram of this system displays a broad biphasic region in which gel and liquid crystalline domains coexist (Foster and Yguerabide, 1979; Knoll et al., 1981; Shimshick and McConnell, 1973). The lateral structure of this system in supported or non-supported bilayers has recently been observed by AFM (Giocondi and Le Grimellec, 2004; Giocondi et al., 2001; Kaasgaard et al., 2003; Leidy et al., 2002) and two-photon fluorescence microscopy (Bagatolli and Gratton, 2000a).

Monolayers at the air–water interface are extremely valuable model membranes (McConnell, 1991; Möhwald, 1990; Möhwald et al., 1995; Vollhardt, 2002). Experiments in which molecular area, surface pressure, temperature and chemical nature of the subphase are varied can easily be performed and by this means, a broad set of thermodynamic parameters, which characterize the monolayer can be accurately determined (Adamson, 1982; Gaines, 1966). Although transmembrane processes cannot be studied in monolayers, this system is very well suited to study processes at the membrane surface (Brezesinski and Möhwald, 2002). In addition to their role as model membranes, the monolayer is the biological state of pulmonary surfactants, which coat the alveolar air spaces in lungs (Discher et al., 1999; Veldhuizen and Haagsman, 2000).

Since its initial development, Brewster angle microscopy (BAM) (Hénon and Meunier, 1991; Hönig and Möbius, 1991) has established itself as a simple and non-perturbing technique to characterize the lateral structure of monolayers on the μm length scale. Its non-perturbing character makes it an ideal technique to study phenomena in which nucleation processes are of major importance, such as domain formation. In the present study, we have used BAM to monitor domain formation in DMPC/DSPC monolayers at the air–water interface. Experiments in which the monolayers were compressed or heated were performed at different DMPC/DSPC molar ratios. We have also determined the importance of the history of the sample in terms of the conditions of deposition and rate of perturbation and provided a qualitative and semi-quantitative explanation of these effects in the context of classical nucleation theory. In order to determine to what point and in which conditions the growth behaviour in monolayers can be extrapolated to bilayers, a strong emphasis is put on determining the similarities and differences between these two model membranes. It should be noted that BAM images of the same system have recently been published (Kubo et al., 2001). In that work however, a single temperature was probed at the extreme low end of the phase coexistence region. In the present study, we provide results at variable surface pressures at a temperature, which is well within the biphasic region as well as results of variable temperature experiments, both of which are necessary to discuss the relationship with bilayers.

2. Experimental

The lipids dimyristoylphosphatidylcholine and distearoylphosphatidylcholine were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. Solutions with different DMPC/DSPC molar ratios in chloroform were prepared at a fixed concentration of $1\,\mathrm{mg\,ml^{-1}}$. For all the experiments, the solutions were spread on distilled water purified with a Millipore Milli-Q filtering system with a resistivity of $\geq 18.1\,\mathrm{M\Omega\,cm}$. In all cases, the pH of the subphase was 5.5 and the chloroform was left to evaporate for 30 min before the beginning of the experiments. The experimental setup consisted of a commercial computerized Langmuir trough (NIMA technology, Coventry, England) upon which was mounted a BAM 2 plus Brewster angle microscope manufactured by Nanofilm Technologie (Göttingen, Germany). The isothermal experiments were carried out by setting the subphase at the desired temperature prior to the deposition of the lipid solution and subsequent compression. For the isobaric experiments, the solutions were spread at 20 °C, the monolayer compressed to the target surface pressure, and the Langmuir trough controller set to the isobaric mode. Surface pressures were measured using a filter paper Wilhelmy plate. The Langmuir trough and the Brewster angle microscope being in a closed environment, no significant evaporation of the subphase was observed during the experiments.