Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochimica et Biophysica Acta 1758 (2006) 248–258
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
http://www.elsevier.com/locate/bba

Temperature and pressure effects on structural and conformational properties of POPC/SM/cholesterol model raft mixtures—a FT-IR, SAXS, DSC, PPC and Laurdan fluorescence spectroscopy study

Chiara Nicolini [a], Julia Kraineva [a], Monika Khurana [a], Nagarajan Periasamy [a], Sérgio S. Funari [b], Roland Winter [a,*]

[a] University of Dortmund, Department of Chemistry, Physical Chemistry I-Biophysical Chemistry, Otto-Hahn-Straße 6, D-44227 Dortmund, Germany
[b] Hasylab, c/o DESY, Notkestrasse 85, D-22607 Hamburg, Germany

Received 21 December 2005; received in revised form 24 January 2006; accepted 26 January 2006
Available online 20 February 2006

Abstract

We report on the effects of temperature and pressure on the structure, conformation and phase behavior of aqueous dispersions of the model lipid "raft" mixture palmitoyloleoylphosphatidylcholine (POPC)/bovine brain sphingomyelin (SM)/cholesterol (Chol) (1:1:1). We investigated interchain interactions, hydrogen bonding, conformational and structural properties as well as phase transformations of this system using Fourier transform-infrared (FT-IR) spectroscopy, small-angle X-ray scattering (SAXS), differential scanning calorimetry (DSC) coupled with pressure perturbation calorimetry (PPC), and Laurdan fluorescence spectroscopy. The IR spectral parameters in combination with the scattering patterns from the SAXS measurements were used to detect structural and conformational transformations upon changes of pressure up to 7–9 kbar and temperature in the range from 1 to about 80 °C. The generalized polarization function (*GP*) values, obtained from the Laurdan fluorescence spectroscopy studies also reveal temperature and pressure dependent phase changes. DSC and PPC were used to detect thermodynamic properties accompanying the temperature-dependent phase changes. In combination with literature fluorescence spectroscopy and microscopy data, a tentative *p*,*T* stability diagram of the mixture has been established. The data reveal a broad liquid-order/solid-ordered (l_o+s_o) two-phase coexistence region below 8±2 °C at ambient pressure. With increasing temperature, a $l_o+l_d+s_o$ three-phase region is formed, which extends up to ~27 °C, where a liquid-ordered/liquid-disordered (l_o+l_d) immiscibility region is formed. Finally, above 48±2 °C, the POPC/SM/Chol (1:1:1) mixture becomes completely fluid-like (liquid-disordered, l_d). With increasing pressure, all phase transition lines shift to higher temperatures. Notably, the l_o+l_d ($+s_o$) phase coexistence region, mimicking raft-like lateral phase separation in natural membranes, extends over a rather wide temperature range of about 40 °C, and a pressure range, which extends up to about 2 kbar for $T=37$ °C. Interestingly, in this pressure range, ceasing of membrane protein function in natural membrane environments has been observed for a variety of systems.

Keywords: Lipid bilayer; Model raft mixture; Phase transition; Pressure; SAXS; DSC; PPC; FT-IR

1. Introduction

Lipid bilayer systems are valuable model systems for biological membrane. They provide a variety of polymorphic phases, depending on their molecular structure and environmental conditions, such as the water content, pH, ionic strength, and temperature [1–6]. The importance to understand not only the physical behavior of simple one-component systems, but also of heterogeneous membranes is due to the importance to understand many aspects of the biological membranes, such as membrane mechanical and thermotropic properties, lateral diffusion of membrane components and the existence of segregated membrane domains with distinct lipid compositions [7–9]. Formation of lipid domains is a mere consequence of the many-particle nature of biological membranes.

Simple, one-component saturated phospholipids often exhibit two thermotropic lamellar phase transitions, a gel to gel

* Corresponding author.
E-mail address: roland.winter@uni-dortmund.de (R. Winter).

0005-2736/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2006.01.019

($L_{\beta'}/P_{\beta'}$) pretransition and a gel to liquid-crystalline (L_α or l_d) main transition at a higher temperature T_m. In the fluid-like L_α-phase, the acyl chains of the lipid bilayers are conformationally disordered, whereas in the gel phases, the chains are more extended and ordered [1,2,10]. In recent years, studies carried out on binary mixtures combining cholesterol with different saturated and unsaturated phosphatidylcholines showed the coexistence of a cholesterol-enriched "intermediate" phase, often called liquid-ordered (l_o) phase, which coexists with a cholesterol-depleted liquid-crystalline or gel phase over a wide range of temperatures and sterol contents [11,12]. Other studies have also examined the phase behavior of cholesterol-containing ternary mixtures, generally containing an unsaturated lipid like phosphatidylcholine and a saturated lipid like sphingomyelin [13–17]. Such lipid systems are supposed to mimic distinct liquid-ordered lipid regions, called "rafts", which seem to be also present in cell membranes. They are rich in sphingomyelin and cholesterol and they are thought to be important for cellular functions such as signal transduction and the sorting and transport of lipids and proteins [18–21]. Such "artificial rafts" could be expected to mimic some features of mammalian plasma membranes.

In this study, we explored the temperature and pressure dependent structure, phase behavior and fluidity of such a canonical lipid raft model system, the POPC/SM/Chol (1:1:1) mixture, using Fourier transform-infrared (FT-IR) spectroscopy, small-angle X-ray scattering (SAXS), differential scanning calorimetry (DSC), pressure perturbation calorimetry (PPC) and fluorescence spectroscopy using Laurdan as dye. FT-IR spectroscopy is a non perturbing technique that monitors molecular vibrations and thus operates on a very short time scale. It has been extensively shown in the literature that many IR spectral parameters, particularly the frequencies, widths, intensities, shapes and splitting of IR bands, are very sensitive to the structural and dynamical properties of membrane lipid molecules [22–32]. Additionally, in our study, we used the SAXS technique that allows to follow changes of membrane structural properties [33]. Besides these two techniques, we also applied thermodynamic methods to detect temperature-dependent phase changes, DSC and PPC. The latter is a rather new tool that measures the heat consumed or released by the sample after sudden small pressure jumps of a few bar, yielding precise values of the apparent coefficient of thermal expansion of the lipid bilayer [34,35]. It was already shown by Heerklotz [34] that DSC and PPC together are able to detect thermotropic changes in membrane order in ternary lipid systems. The emission spectrum of the environmentally sensitive fluorescence probe Laurdan (6-dodecanoyl-2-dimethyl-aminonaphtalene) was used to detect changes of phase behavior of the lipid bilayer system as a function of temperature and pressure. The spectral changes of the emission spectrum of Laurdan are generally quantified by the so-called generalized polarization function (*GP*). The measured *GP* values of our system reflect the overall phase behavior and fluidity of the membrane as a function of temperature and pressure.

The rationale for using pressure as an additional experimental variable in studies of biomolecular systems next to temperature has already been discussed by a number of authors (see, e.g., [36–39]). Hydrostatic pressure has essentially been used as a physical parameter for studying the stability and energetics of membranes and proteins, but also because high pressure is an important feature of certain natural membrane environments and because the high pressure phase behavior of biomolecules is of biotechnological and pharmacological interest. Temperature has been one of the most often used parameters to study the thermodynamic, structural and dynamic properties of membranes. A change in temperature of a system leads to changes of the thermal energy and density at the same time, whereas pressure dependent studies at constant temperature introduce only changes in density and change intermolecular separations of the system, hence providing additional information about the energetics and phase behavior of the system without disturbing thermally activated processes. Compared to other biomolecules such as proteins or DNA, lipid bilayers have been shown to respond most sensitively to hydrostatic pressure [36–39]. Considerable knowledge exists about pressure effects on simple, one-component lipid bilayer systems, very little is known about complex lipid mixtures, however [38–52]. Hence, by using SAXS at a synchrotron facility, DSC, PPC, FT-IR and fluorescence spectroscopy we have determined the structure and temperature–pressure phase diagram of POPC/SM/Chol (1:1:1) in a temperature range from ~1 to 70 °C and in a pressure range up to 9 kbar. Up to these pressures, the solvent, water, is still in the liquid state at these temperatures.

2. Materials and methods

2.1. Sample preparation

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC) and bovine brain sphingomyelin (SM) were purchased from Avanti Polar Lipids (Birmingham, AL), cholesterol (Chol) from Sigma-Aldrich (Steinheim, Germany). The lipids were used without further purification. The POPC/SM/Chol lipid mixtures were prepared by first dissolving the required amounts of lipids in chloroform/methanol (4:1). The solvent was then completely removed under vacuum using a Speed Vac Sc 110 (Savant, Farmingdale, NY, USA) before adding water (D_2O or H_2O). Homogeneous lipid dispersions were obtained after five freeze–thaw vortex cycles. Depending on the method, either large unilamellar or multilamellar vesicles have been prepared.

2.2. FT-IR spectroscopy

The lipids were dissolved at a concentration of 20% (w/w) in D_2O. The FT-IR spectra were recorded with a Nicolet MAGNA 550 spectrometer equipped with a liquid nitrogen cooled MCT (HgCdTe) detector. For the pressure-dependent measurements, the infrared light was focussed by a spectral-bench onto the pin-hole of a diamond anvil cell (DAC) with type IIa diamonds [32,53,54]. Each spectrum was obtained by co-adding 512 scans at a spectral resolution of 2 cm^{-1} and was apodized with a Happ–Genzel function. The sample chamber was purged with dry carbon dioxide free air. Powered α-quartz was placed in the hole of the steel gasket of the DAC and changes in pressure were quantified by the shift of the phonon band of quartz appearing at 695 cm^{-1} [54]. The temperature dependent measurements were carried out using a cell with CaF_2 windows separated by 50 μm teflon spacers. An external circulating