Biophysical Chemistry 252 (2019) 106210

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: www.elsevier.com/locate/biophyschem

Cholesterol modulates the pressure response of DMPC membranes

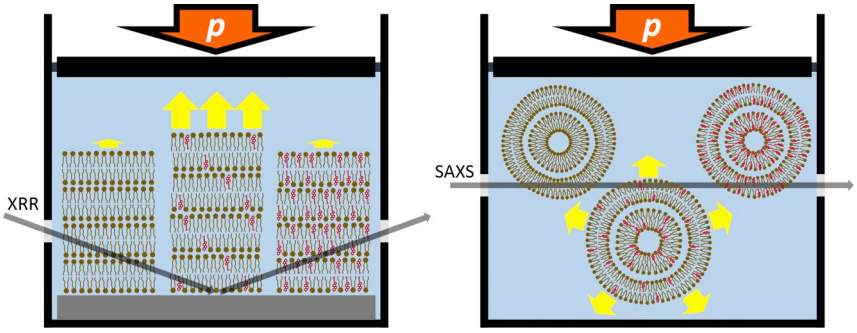
Göran Surmeier, Michael Paulus, Paul Salmen, Susanne Dogan, Christian Sternemann, Julia Nase*

Fakultät Physik/DELTA, Technische Universität Dortmund, 44221 Dortmund, Germany

HIGHLIGHTS

- Low cholesterol content causes strong pressure-induced expansion of DMPC multi-layers
- At high cholesterol concentrations, the membrane structure remains largely stable
- The structure of single solid-supported bilayers is largely unaffected by pressure
- High cholesterol content stabilizes solid-supported multilayers in an aqueous phase

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
DMPC/cholesterol membranes
High hydrostatic pressure
Solid-supported multilayers
Multi-lamellar vesicles
X-ray reflectometry
Small angle X-ray scattering

ABSTRACT

In this work, the effect of cholesterol on the pressure response of solid-supported phospholipid multilayers is analyzed. It is shown that DMPC multilayers become highly pressure-responsive by the incorporation of low amounts of cholesterol, resulting in a strong pressure-induced expansion of the bilayer spacing. This is accompanied by a high tendency of the multilayer system to detach from the substrate. Increasing the cholesterol concentration reduces the pressure-induced expansion and the membrane structure remains largely unchanged upon pressurization, consequently the stability of the multilayers improves. For a determination of the influence of the substrate, the pressure-dependent behavior of multilayers is compared to that of solid-supported bilayers and multi-lamellar vesicles in bulk solution. While single-supported bilayers remain largely unaffected by external pressure independent of their cholesterol content, multi-lamellar vesicles and multilayers behave similarly.

1. Introduction

Lipid bilayers constitute the basic structure of cell membranes and control the information and mass transfer between the intracellular and extracellular region [1,2]. Their structural properties are decisive for the ability of the cell to fulfil its biological functions. The structural properties of lipid bilayers vary depending on external conditions and they undergo pressure- and temperature-induced phase transitions. Typically, the high flexibility that is provided by the fluid L_α phase is essential for their biological functionalities [3,4]. However, below a certain temperature or above a certain pressure, the main phase transition from fluid into a gel-like state occurs. Nevertheless, some organisms survive at surprisingly harsh conditions like in the Mariana trench at low temperatures and pressures of more than 1 kbar [5,6]. In order to understand how living cells can withstand these conditions, it is necessary to study their composition and the interaction between different components of cell membranes. A constituent of particular interest in this context is cholesterol, which occurs in cell membranes

* Corresponding author.
E-mail address: julia.nase@tu-dortmund.de (J. Nase).

https://doi.org/10.1016/j.bpc.2019.106210
Received 6 May 2019; Received in revised form 12 June 2019
Available online 20 June 2019

typically in concentrations of 4 to 50 mol%. Due to its rigid sterol rings, it affects the mobility and order of the surrounding lipids. While cholesterol reduces the mobility of lipids in the fluid phase, it hinders the crystallization of the tails groups in gel-like phases [7]. Previous studies have shown that the incorporation of cholesterol into lipid membranes leads to a broadening of the phase transition regime. Above a certain cholesterol concentration, the fluid and gel-like phase cannot be distinguished anymore [4,8–10]. This state is referred to as liquid-ordered L_o phase and shares properties of fluid and gel-like phases [11]. It is characterized by low positional order and fast diffusion, but high conformational and orientational order.

The phase behavior of bulk phospholipid systems has already been investigated in detail, e.g., DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) [4,12] or DMPC (1,2-dimyristoylsn-glycero-3-phosphocholine) [13–15] vesicles. Besides the fluid L_α phase, DMPC forms several gel-like phases, like the rippled $P_{\beta'}$ phase [16] or the lamellar $L_{\beta'}$ phase. Furthermore, an interdigitated phase $L_\beta i$ [17] and the so-called GEL-III phase [18], in which the hydrocarbon tails are completely stretched and not tilted or interdigitated, have been observed at high pressure. DMPC bilayers containing cholesterol have been studied in experiments [19,20] and simulations [21]. Beneath the liquid ordered L_o phase, mentioned above, the crystalline non-tilted L_c phase has been found at low temperatures. For high cholesterol contents, a separation of the molecules in pure cholesterol and DMPC clusters occurred. However, most of the studies involving cholesterol examine bilayers at ambient pressure. There are only a few high hydrostatic pressure studies available, typically focusing on the effect of cholesterol on the pressure-dependent main phase transition [4,22].

In the past, much effort has been made to find and improve model membranes that can be prepared on solid surfaces. Solid-supported lipid bilayers can be used to study basic membrane processes, since multiple surface-sensitive methods are available, and they also have biotechnical applications [23–28]. A major challenge is to minimize the influence of the substrate on the structure of model membranes, while still maintaining stability in an aqueous environment. A simple way to reduce the influence of the substrate is the preparation of lipid multilayers. In previous studies, DMPC multilayers were investigated under high humidity gas phase, e.g., to study the temperature-dependent phase behavior or effects of adding cholesterol [29,30]. For high hydrostatic pressure studies up to several kbar, multilayers have to be fully immersed in water. Investigations on the behavior of DMPC multilayers at the solid-liquid interface show that they are highly unstable at ambient pressure, especially when they are heated above the main phase transition temperature [24,31]. Nevertheless, using DMPC multilayers, we observed that in the L_α phase regime, the abrasive effect of water can be reduced by increasing the pressure to the main phase transition boundary [32].

In this work, the pressure-dependent structural properties of solid-supported DMPC multilayers containing cholesterol was studied in a pressure range from 50 bar to 5 kbar by X-ray reflectometry (XRR) at a constant temperature of 293 K. At this temperature, DMPC adopts the $P_{\beta'}$ phase at low pressures. Phase transitions have been observed at 0.4 kbar from $P_{\beta'}$ to $L_{\beta'}$ and at 3 kbar from $L_{\beta'}$ to GEL-III both in optical transmittance measurements [18]. In a small angle X-ray scattering (SAXS) study performed at a slightly higher temperature (21 °C), the $P_{\beta'}$ to $L_{\beta'}$ transition occurred at 2.4 kbar and no indications of a further transition were found up to 3.8 kbar. We applied cholesterol concentrations of 0 to 23 wt%. At ambient pressure, a transition from the $P_{\beta'}$ into the L_o phase is expected at approximately 12 wt% cholesterol [20,21]. We deposited 10 to 15 bilayers on hydrophilic silicon wafers, which were then transferred into aqueous solution. For a determination of the influence of the substrate in this model system, the pressure-dependent behavior of multilayers was compared to solid-supported bilayers and multi-lamellar vesicles in bulk solution. The structure of the latter was examined with SAXS.

We found that low cholesterol concentrations cause a strong vertical expansion of solid-supported DMPC multilayers under pressurization, triggering a detachment of the layers from the substrate surface. At high concentrations, cholesterol suppresses structural changes induced by external pressure to a large extent and promotes stability. The structural changes that occurred in solid-supported multilayers at pressurization were very similar to those in multi-lamellar vesicles. In contrast, single-supported bilayers remained largely unaffected in the entire considered pressure range.

2. Samples and methods

2.1. Sample preparation

For the preparation of solid-supported multilayers, we followed the procedure described by Nowak et al. [32]. DMPC, cholesterol and 2-propanol were purchased from Sigma-Aldrich (Munich, Germany) and were used without further purification. DMPC was dissolved in 2-propanol at a concentration of 10 mg/mL and cholesterol was added in concentration of 0–23.1 wt% in relation to the total lipid mass (yielding 0–34 mol%). The lipids were deposited on the hydrophilic surface of a silicon wafer by spin-coating. Therefore, 60 μL lipid solution were pipetted onto a wafer (7.6 × 7.6 mm^2), which was then rotated for 30 s at 4000 rpm. During this process, the solvent evaporates and 10–15 oriented bilayers remain on the surface [24]. For an examination of single solid-supported bilayers, the upper layers of the multilayer stack were removed by immersion in water. The wafers were mounted in a sample cell filled with aqueous buffer solution. We used pressure-stable Bis-Tris buffer solution with a concentration of 25 mmol/L to achieve a constant pH value of 7 also at high pressures [33].

Multi-lamellar vesicles were prepared by dissolving DMPC and cholesterol in different ratios in chloroform. Then, the solvent was evaporated under a gentle stream of nitrogen before it was completely removed in a desiccator. Afterwards, Bis-Tris buffer solution at pH 7 was added. To obtain a homogeneous mixture, five freeze-thaw cycles were conducted. The water content of all samples was 80 wt%.

2.2. X-ray reflectometry

The vertical structure of the solid-supported multilayers was studied with XRR. This method provides insight into the laterally averaged electron density profiles $\rho_e(z)$ perpendicular to the sample interface [34]. Therefore, the reflected intensity of a monochromatic X-ray beam with a wavelength λ is recorded as a function of the angle of incidence α_i. In this scattering geometry, the wave vector transfer $\boldsymbol{q}$ has only a vertical component $q_z = 4\pi \sin(\alpha_i)/\lambda$.

XRR experiments were performed at beamline ID31 [35] of ESRF (Grenoble, France) with a photon energy of 70 keV (λ = 0.177 Å) and at beamline BL9 [36] of DELTA (Dortmund, Germany) with a photon energy of 27 keV (λ = 0.459 Å). These energies ensure adequate transmission trough the liquid bulk phase and the diamond windows of the applied high hydrostatic pressure cell. The cell enables XRR measurements at the solid-liquid interface up to a maximum pressure of 5 kbar in a controlled sample environment that is separated from the pressure transmitting liquid by Kapton windows. A detailed description is given by Wirkert et al. [37]. The sample temperature was controlled by a circulating water flow and was set to 293 K for all experiments. At this temperature, pure DMPC bilayers are in the gel-like $P_{\beta'}$ regime [18]. The addition of cholesterol leads to a transition into the liquid ordered L_o phase [21]. The reflectivities were recorded with area detectors (MAXIPIX, PILATUS 100 k) to capture the specularly reflected and diffusely scattered radiation simultaneously. The diffusely scattered intensity was used to determine the background. To avoid radiation damage, the beam was blocked during motor movements and the sample was laterally shifted after every scan.

The periodicity of the multilayers causes the occurrence of Bragg reflections [38]. A reflection at a position q_{Bragg} of the order n

corresponds to a multilayer spacing of $d = \frac{2\pi\, n}{q_{\mathrm{Bragg}}}$. In order to determine q_{Bragg}, we normalized the reflectivities to the Fresnel reflectivity of an ideally flat silicon − water interface and fitted a Gaussian distribution to the first order Bragg reflections. The layer thickness of solid-supported bilayers $d = \frac{2\pi}{q_{\mathrm{period}}}$ was calculated from the period of oscillation q_{period} of the corresponding reflectivity curves. Entire reflectivity curves were fitted using the Parratt algorithm in combination with the effective density model [39,40]. This procedure yields thickness and electron density of all sublayers and the roughness of the interfaces between them. We modelled the structure of a bilayer by six sublayers representing the two head and two tail groups, a water layer between the head groups and a gap between the tail groups. This pattern was periodically repeated to obtain a model of the whole multilayer. Prerequisite for this approach is a high lateral homogeneity of the sample. The electron density of the surrounding water was calculated from pressure-dependent density values available at the NIST data base [41].

2.3. Small angle X-ray scattering

The structure of multi-lamellar vesicles was determined via SAXS. The diffraction patterns were recorded by an area detector (MAR345) covering a q-range of 0.03 to 0.6 Å^{-1}. The setup was calibrated with silver behenate [42]. The measurements were conducted with a custom-made high hydrostatic pressure cell [43], which is essentially identical in construction to the high hydrostatic pressure XRR cell. Pressures between 50 bar and 3.5 kbar were applied at a temperature of 293 K. The SAXS experiments were performed at beamline BL9 [44] of DELTA (Dortmund, Germany) with a photon energy of 10 keV (λ = 1.240 Å). The spacing d was calculated from the Bragg reflection position q_{Bragg}. To determine q_{Bragg} the two dimensional diffraction patterns were integrated azimuthally. In the region of the first Bragg reflection, a linear background was subtracted and a Pearson type VII distribution [45] was fitted to the data.

3. Results and discussion

3.1. SAXS on multi-lamellar vesicles

The first order Bragg reflections of multi-lamellar vesicles with selected cholesterol concentrations are shown in Fig. 1. Based on the depicted fits, we obtained the pressure dependence of the vesicle spacing d as displayed in Fig. 2. The phases were identified by comparison with literature values [14,18,21].

Pure DMPC vesicles exhibited two coexisting phases, indicated by two Bragg reflections. The more intense reflection is related to a spacing of 66 Å at 50 bar and corresponds to the rippled gel phase $P_{\beta'}$ [14,18]. The less intense reflection is related to a lattice constant of 58 Å, which probably refers to the lamellar gel phase $L_{\beta'}$ [14]. At pressurization, the width of the reflections increases, indicating that the coherence length of the multi-lamellar structure decreases. Up to 1.5 kbar, the spacing of the $L_{\beta'}$ phase slightly increases, while the $P_{\beta'}$ spacing remains constant. Beyond that pressure, the reflections strongly overlap and both phases are barely distinguishable. A transition into the GEL-III phase which was observed at around 3 kbar by Prasad et al. [18], can neither be confirmed nor excluded on the basis of the data. The addition of 4.8 wt % cholesterol suppresses the $L_{\beta'}$ phase. However, we still observe two coexisting phases. A very weak reflection occurs at a slightly higher q value that overlaps with the main reflection. This observation can be explained by a slight separation of a cholesterol-poor phase, which was also observed for DPPC vesicles containing 10 mol% cholesterol [46]. The corresponding spacing of 66–68 Å (not shown in Fig. 2 for clarity), which is close to the value of pure DMPC, supports this interpretation. The dominant phase strongly expands upon pressurization up to a lattice constant of more than 72 Å at 1.75 kbar. Further pressure increase induced a strong compression which can be assigned to a phase transition. A similar behavior was observed for 9.1 wt% cholesterol concentrations.

At 14 wt% cholesterol, a phase transition into the liquid-ordered phase L_o [10,21] was observed. The transition occurred at 0.75 kbar, indicated by an abrupt reversal of slope of $d(p)$. The width and intensity of the reflections barely change beyond that pressure. At higher cholesterol concentrations, the system was already in the L_o phase at 50 bar. At 23.1 wt%, the SAXS patterns did not change over the entire pressure range.

3.2. XRR on solid-supported bi- and multilayers

The first order Bragg reflections of solid-supported DMPC multilayers with selected cholesterol concentrations, as they appeared in the reflectivity curves, are presented in Fig. 3. In contrast to bulk vesicles, coexistence of phases was not found in the solid-supported multilayer system.

Fig. 4 shows the spacing d of solid-supported multilayers at the lowest measured pressure of 50 bar in direct comparison to multi-lamellar vesicles. In solid-supported multilayers and multi-lamellar vesicles alike, low amounts of cholesterol increased the lattice constant by approximately 4 Å. The addition of more cholesterol led to a reduction of spacing slightly below the value of pure DMPC. Volume and surface measurements showed good agreement over the entire concentration range and the comparison provides a strong indication that the deposition of 10–15 bilayers is sufficient to largely suppress the influence of the substrate.

Fig. 5 shows the pressure dependence of the spacing of solid-supported multilayers (*filled markers*) and the thickness of solid-supported bilayers (*open markers*). At low cholesterol concentrations (*blue symbols*), the multilayers became highly pressure-responsive compared to pure DMPC (*black circles*) and we observed a strong expansion of the layer system with increasing pressure. This behavior reduced the

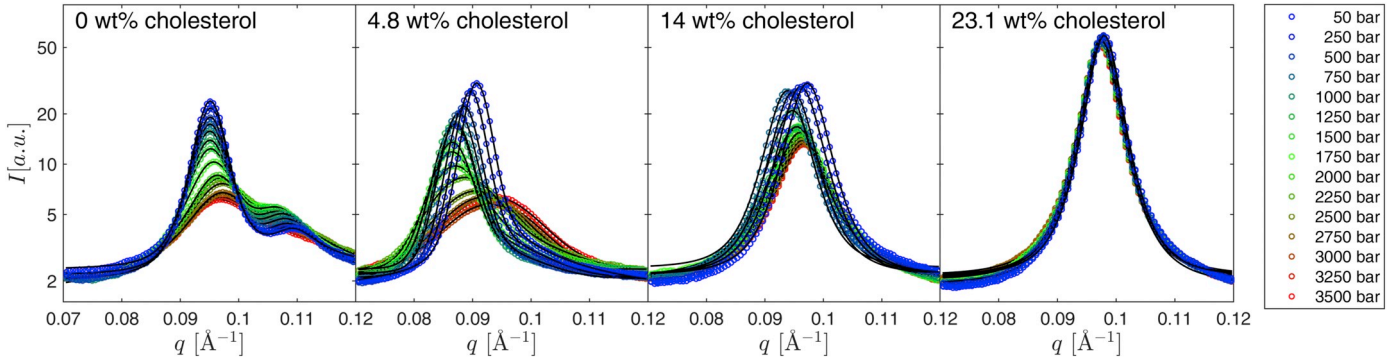


Fig. 1. SAXS data (*colored points*) and fits (*black lines*) of first order Bragg reflections of multi-lamellar DMPC vesicles containing different amounts of cholesterol upon pressurization.

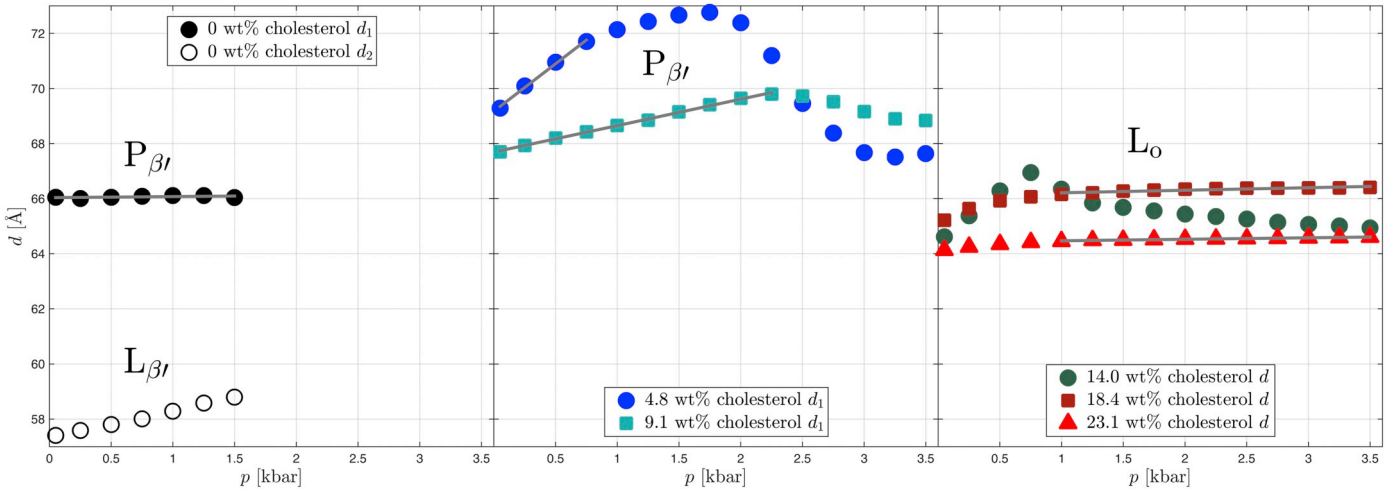


Fig. 2. Pressure dependence of the spacing d for different cholesterol concentrations. If two reflections occurred, d_1 denotes the more intense and d_2 the less intense reflection. The grey lines mark the linear regions that were considered for determining the compressibilities.

stability of the multilayer stacks, since the multilayers had a high tendency to detach in this concentration regime. Partial detachment of the multilayers led to a decrease of the intensity of the Bragg reflections in Fig. 3. Missing data points at high pressures in Fig. 5 indicate that no Bragg reflections were observed. In this case, typically only the bottom layer remained on the surface.

With further increase of the concentration (*red symbols*), the multilayers became less pressure-responsive and the pressure-induced vertical expansion diminished. At the same time, their stability improved. Therefore, we were able to obtain more in-depth information on the structure of DMPC multilayers at high cholesterol concentrations by modelling their electron density profiles. Fig. 6 shows the reflectivity curves of DMPC multilayers with 18.4 wt% cholesterol at pressures up to 4 kbar. The high quality of the fits proves that the assumption that the structure of all bilayers is identical is reasonable. The corresponding electron density profiles of a single repetition unit of the multilayers are illustrated in Fig. 7. We observed an increase of the electron density in all sublayers at pressurization, which was more pronounced in the tail groups than in the head groups. The lipid bilayers were very slightly compressed in the vertical direction. This can be seen from a small shift of the maxima of the electron density in the head groups towards the centre of the bilayers with increasing pressure. At the same time, the minima in the water sublayers shift outwards, indicated by black arrows. This enlargement of the water layer between the head groups caused the spacing to increase in total, in agreement with Fig. 5 (full brown triangles).

Comparing the pressure dependence of the multilayer spacing to that of the thickness of single substrate-bound bilayers (Fig. 5, *open markers*) reveals that direct contact to the substrate exerts strong influence on the pressure response of lipid bilayers upon pressurization. The differences in the absolute values of bi- and multilayers of 15–20 Å

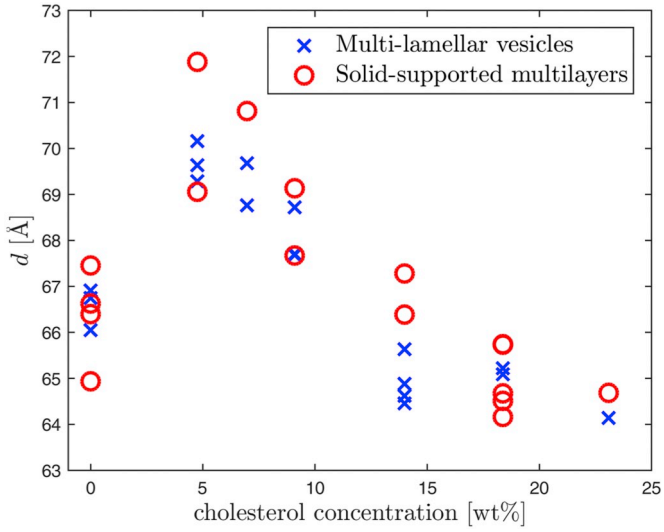


Fig. 4. Spacing of multi-lamellar vesicles and solid-supported multilayers at 50 bar. Different data points at the same concentration represent different samples. At concentrations where two Bragg reflections were observed in the SAXS data, only the spacing corresponding to the dominant phase is shown.

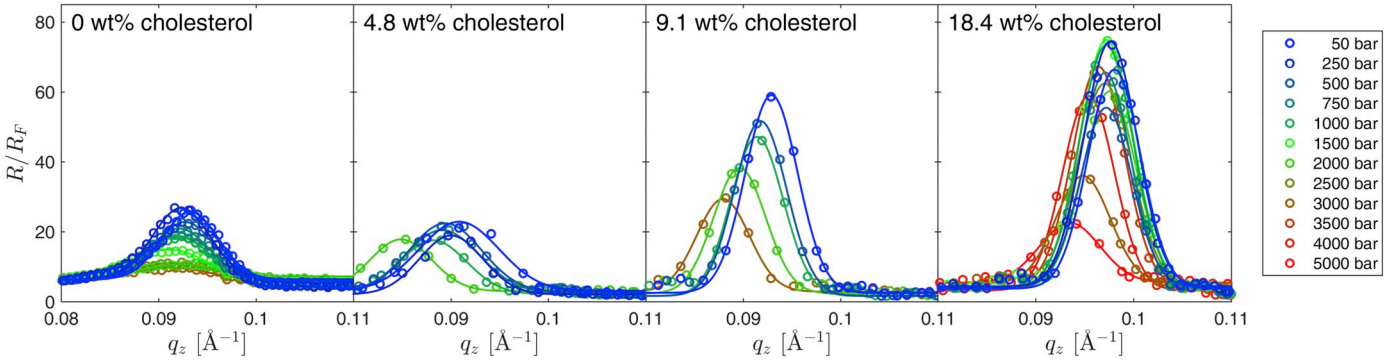


Fig. 3. First order Bragg reflections of reflectivity curves (*colored points*) and fits (*colored lines*) of solid-supported DMPC multilayers containing different amounts of cholesterol upon pressurization.

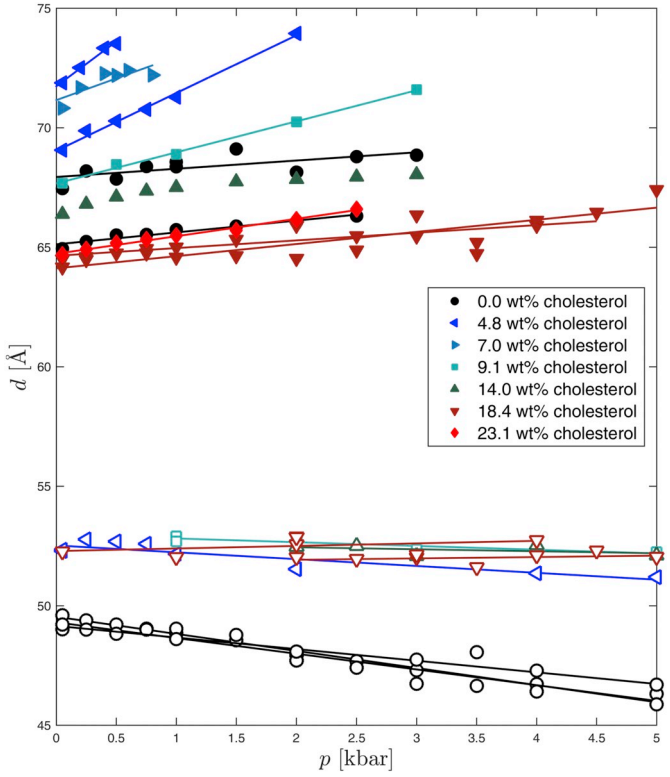


Fig. 5. Spacing of solid-supported multilayers (*filled markers*) and thickness of single bilayers (*open markers*) as function of pressure with linear fits used for determination of compressibilities.

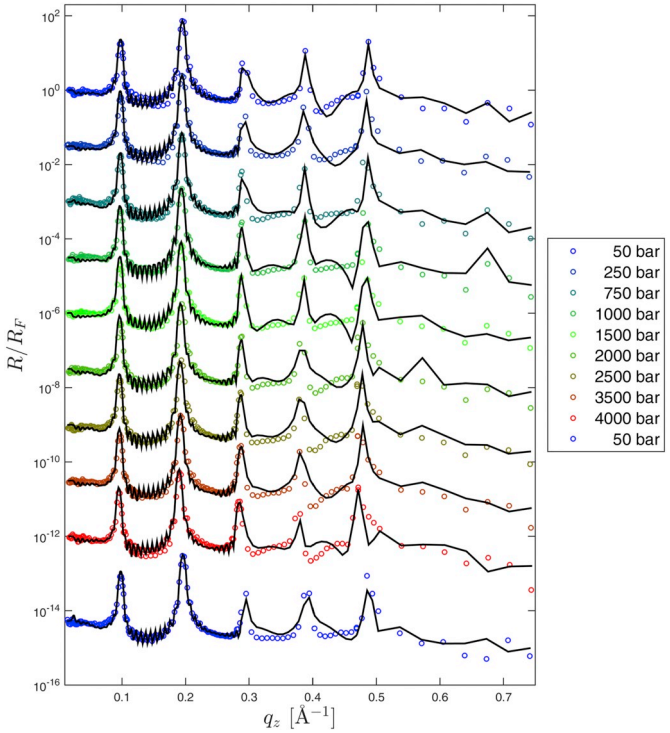


Fig. 6. Reflectivity curves (*colored*) of DMPC multilayers containing 18.4 wt% cholesterol and fits (*black*). The reflectivity curves are shifted vertically for better visualization.

can be attributed mainly to the water layers between adjacent head groups that add to the multilayer spacing. Furthermore, the thickness of substrate-bound phospholipid bilayers is reduced by a few Å compared

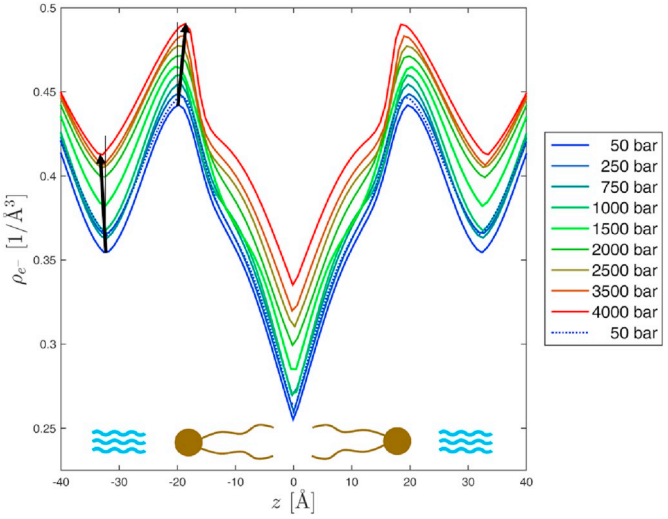


Fig. 7. Electron density profiles of one repetition unit, corresponding to the fits shown in Fig. 6. The black arrows highlight the slight shifts of the minima and maxima at increasing pressures.

to bilayers without direct contact to the substrate [47]. The data in Fig. 5 show clearly the different pressure response of substrate-bound bilayers. The layer thickness of single solid-supported pure DMPC bilayers (*black symbols*) decreased slightly at pressurization. Once cholesterol was added, d remained essentially constant throughout the entire pressure range. This behavior indicates that structural changes due to external pressure are mostly suppressed in close proximity to the substrate.

For a quantitative comparison of the pressure-dependent behavior of the three examined sample systems we determined their linear compressibilities $\kappa = -\frac{1}{d0}\frac{\Delta d}{\Delta p}$. A similar analysis of the compressibility in other lipid systems has been performed in literature [48,49]. Therefore, we fitted the spacing d as a function of pressure with a linear model. The linear regions that were considered for the determination of κ are indicated in Figs. 2 and 5. While the spacing of solid-supported membranes changed linearly in the entire pressure range (as long as they were stable), only a selected area was taken into account for multilamellar vesicles: we fitted only the linear increase in the low-pressure regime at low cholesterol concentrations. This pressure regime overlaps with the pressure regime where no detachment of the multilayers occurred. Due to the phase transition at 14 wt% cholesterol, no compressibility was determined for this concentration. Fig. 8 shows that the compressibilities of multi-lamellar vesicles and solid-supported multilayers exhibit a very similar concentration dependence. In both cases, the compressibility shows large negative values at low concentrations and approaches zero, when more cholesterol is added. In contrast, the compressibilities of single bilayers are close to zero in the entire concentration range as the interaction with the substrate hinders structural changes.

4. Conclusion

In conclusion, the behavior of DMPC membranes exposed to external pressures depending on their cholesterol content was analyzed by studying multi-lamellar vesicles and solid-supported membranes. In multi-lamellar vesicles, a transition from the $P_{\beta'}$ to the L_o phase at 14 wt % cholesterol and 0.75 kbar was observed. A strong expansion of the spacing in the low-pressure regime and an increase of the width of the Bragg reflections in the entire pressure range showed that pressurization induces pronounced structural changes below 14 wt% cholesterol. In contrast, in the L_o phase, the lipid layers were highly pressure resistant. Shape and positions of the reflections remained constant within

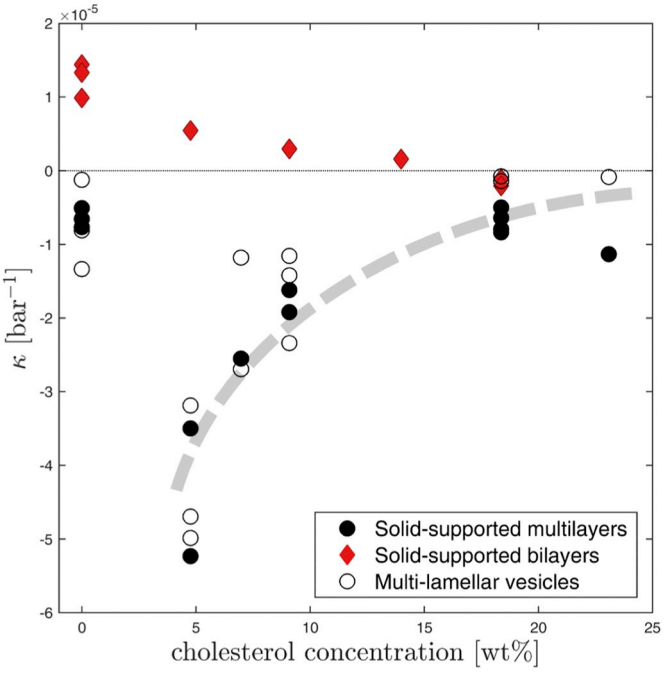


Fig. 8. Comparison of compressibilities of different types of model membranes. The dashed line illustrates the course of the data points.

the experimental resolution. The reduced pressure response of cholesterol-rich membranes translates into improved stability of solid-supported multilayers. The influence of the substrate seems to be largely suppressed within the examined parameter range at a deposition of 10–15 bilayers. In this concentration regime of cholesterol, multilayers and multi-lamellar vesicles showed similar compressibilities. In contrast, the investigation of solid-supported single bilayers has shown how strongly the solid support affects the membranes. The pressure response in this case was small and almost cholesterol-independent. This behavior can be explained by the strong substrate – lipid interaction [27,50] that confines the vertical flexibility of the film.

The results confirm that cholesterol-containing lipid multilayers under full hydration are well suited for the structural investigation by XRR. We demonstrated that cholesterol in concentrations that are typical for biological systems reduces their tendency to detach, even under pressurization stress. This opens up the way to more detailed studies applying reliably stable model membranes with optimized properties in the future, e.g., on the interactions of membranes with proteins at high pressure conditions, and might thus finally contribute to an enhanced understanding of life in the deep sea.

Notes

The authors declare no competing financial interest.

Acknowledgment

The authors thank the DFG (FOR 1979) for financial support. We acknowledge the European Synchrotron Radiation Facility ESRF and DELTA for providing synchrotron radiation and Dr. Veijo Honkimäki and the ID31 staff for the assistance during our experiment at the ESRF. We thank Prof. Dr. Roland Winter and Samy Al-Ayoubi (TU Dortmund, Germany) for the support with the vesicle preparation. Also, we gratefully acknowledge Yury Forov, Christian Albers, Lukas Tepper, Julian Schulze, Steffen Bieder, Jennifer Bolle, Mike Moron and Marc Moron (all of TU Dortmund, Germany), who were involved in the execution of the synchrotron experiments.

References

[1] S. Christoforidis, H.M. McBride, R.D. Burgoyne, The Rab5 effector EEA1 is a core component of endosome docking, Nature 397 (1999) 621.
[2] S.D. Conner, S.L. Schmid, Regulated portals of entry into the cell, Nature 422 (2003) 37.
[3] S.J. Singer, G.L. Nicolson, The fluid mosaic model of the structure of cell membranes, Science 175 (1972) 720–731.
[4] R. Winter, C. Jeworrek, Effect of pressure on membranes, Soft Matter 5 (2009) 3157–3173.
[5] P.M. Oger, M. Jebbar, The many ways of coping with pressure, Res. Microbiol. 161 (2010) 799–809.
[6] Paul F. McMillan, David C. Clary, R. Winter, W. Dzwolak, Exploring the temperature-pressure configurational landscape of biomolecules: from lipid membranes to proteins, Philos. Trans. R. Soc. A Math. Phys. Eng. Sci. 363 (2005) 537–563.
[7] S.A. Kotenkov, et al., Effect of cholesterol and curcumin on ordering of DMPC bilayers, Appl. Magn. Reson. 50 (2019) 511–520.
[8] M.R. Vist, J.H. Davis, Phase equilibria of cholesterol/dipalmitoylphosphatidylcholine mixtures: deuterium nuclear magnetic resonance and differential scanning calorimetry, Biochemistry 29 (1990) 451–464.
[9] M. Rappolt, et al., Structural, dynamic and mechanical properties of POPC at low cholesterol concentration studied in pressure/temperature space, Eur. Biophys. J. 31 (2003) 575–585.
[10] J. Peters, et al., Thermodynamics of lipid multi-lamellar vesicles in presence of sterols at high hydrostatic pressure, Sci. Rep. 7 (2017) 15339.
[11] T.T. Mills, J. Huang, G.W. Feigenson, J.F. Nagle, Effects of cholesterol and unsaturated DOPC lipid on chain packing of saturated gel-phase DPPC bilayers, Gen. Physiol. Biophys. 28 (2009) 126.
[12] S. Karmakar, B.R. Sarangi, V.A. Raghunathan, Phase behaviour of lipid–cholesterol membranes, Solid State Commun. 139 (2006) 630–634.
[13] D. Needham, E. Evans, Structureand mechanical properties of giant lipid (DMPC) vesicle bilayers from 20 °C below to 10 °C above the liquid crystal-crystalline phase transitionat 24 °C, Biochemistry 27 (1988) 8261–8269.
[14] J. Eisenblätter, R. Winter, Pressure effects on the structure and phase behavior of DMPC-gramicidin lipid bilayers: a synchrotron SAXS and 2H-NMR spectroscopy study, Biophys. J. 90 (2006) 956–966.
[15] M. Trapp, et al., High hydrostatic pressure effects investigated by neutron scattering on lipid multilamellar vesicles, Phys. Chem. Chem. Phys. 15 (2013) 20951–20956.
[16] K. Akabori, J.F. Nagle, Structure of the DMPC lipid bilayer ripple phase, Soft Matter 11 (2015) 918–926.
[17] H. Ichimori, T. Hata, H. Matsuki, S. Kaneshina, Barotropic phase transitions and pressure-induced interdigitation on bilayer membranes of phospholipids with varying acyl chain lengths, Biochim. Biophys. Acta Biomembr. 1414 (1998) 165–174.
[18] S. Krishna Prasad, R. Shashidhar, B.P. Gaber, S.C. Chandrasekhar, Pressure studies on two hydrated phospholipids — 1,2-dimyristoyl-phosphatidylcholine and 1,2-dipalmitoyl-phosphatidylcholine, Chem. Phys. Lipids 43 (1987) 227–235.
[19] K. Mortensen, W. Pfeiffer, E. Sackmann, W. Knoll, Structural properties of a phosphatidylcholine-cholesterol system as studied by small-angle neutron scattering: ripple structure and phase diagram, Biochim. Biophys. Acta BBA-Biomembr. 945 (1988) 221–245.
[20] D.J. Recktenwald, H.M. McConnell, Phase Equilibriain binary mixtures of phosphatidylcholine and cholesterol, Biochemistry 20 (1981) 4505–4510.
[21] F.J.-M. de Meyer, A. Benjamini, J.M. Rodgers, Y. Misteli, B. Smit, Molecular simulation of the DMPC-cholesterol phase diagram, J. Phys. Chem. B 114 (2010) 10451–10461.
[22] C. Bernsdorff, A. Wolf, R. Winter, E. Gratton, Effect of hydrostatic pressure on water penetration and rotational dynamics in phospholipid-cholesterol bilayers, Biophys. J. 72 (1997) 1264–1277.
[23] P. Losada-Pérez, M. Khorshid, D. Yongabi, P. Wagner, Effect of cholesterol on the phase behavior of solid-supported lipid vesicle layers, J. Phys. Chem. B 119 (2015) 4985–4992.
[24] U. Mennicke, T. Salditt, Preparation of solid-supported lipid bilayers by spin-coating, Langmuir 18 (2002) 8172–8177.
[25] E. Sackmann, Supported membranes: scientific and practical applications, Science 271 (1996) 43–48.
[26] R.P. Richter, R. Bérat, A.R. Brisson, Formation of solid-supported lipid bilayers: an integrated view, Langmuir 22 (2006) 3497–3505.
[27] U. Mennicke, D. Constantin, T. Salditt, Structure and interaction potentials in solid-supported lipid membranes studied by X-ray reflectivity at varied osmotic pressure, Eur. Phys. J. E 20 (2006) 221–230.
[28] L. Perino-Gallice, G. Fragneto, U. Mennicke, T. Salditt, F. Rieutord, Dewetting of solid-supported multilamellar lipid layers, Eur. Phys. J. E 8 (2002) 275–282.
[29] W.-C. Hung, M.-T. Lee, F.-Y. Chen, H.W. Huang, The condensing effect of cholesterol in lipid bilayers, Biophys. J. 92 (2007) 3960–3967.
[30] T.A. Harroun, M. Koslowsky, M.-P. Nieh, V.A. Raghunathan, J. Katsaras, Finite-size effects do not reduce the repeat spacing of phospholipid multibilayer stacks on a rigid substrate, Eur. Phys. J. E 13 (2004) 359–362.
[31] T. Salditt, Thermal fluctuations and stability of solid-supported lipid membranes, J. Phys. Condens. Matter 17 (2005) R287.
[32] B. Nowak, et al., Solid-supported lipid multilayers under high hydrostatic pressure, Langmuir 32 (2016) 2638–2643.
[33] R.C. Neuman Jr., W. Kauzmann, A. Zipp, Pressure dependence of weak acid ionization in aqueous buffers, J. Phys. Chem. 77 (1973) 2687–2691.
[34] U. Pietsch, V. Holy, T. Baumbach, High-Resolution X-Ray Scattering: From Thin

Films to Lateral Nanostructures, Springer Science & Business Media, 2013.
[35] V. Honkimäki, Engineering materials research at ESRF, Synchrotron Radiat. News 30 (2017) 48–54.
[36] M. Paulus, et al., An access to buried interfaces: the X-ray reflectivity set-up of BL9 at DELTA, J. Synchrotron Radiat. 15 (2008) 600–605.
[37] F.J. Wirkert, et al., X-ray reflectivity measurements of liquid/solid interfaces under high hydrostatic pressure conditions, J. Synchrotron Radiat. 21 (2014) 76–81.
[38] Bragg William Henry, Bragg William Lawrence, The reflection of X-rays by crystals, Proc. R. Soc. Lond. Ser. Contain. Pap. Math. Phys. Character, vol. 88, 1913, pp. 428–438.
[39] L.G. Parratt, Surface studies of solids by total reflection of X-rays, Am. Phys. Soc. 95 (1954) 359–369.
[40] M. Tolan, X-Ray Scattering from Soft-Matter Thin Films: Materials Science and Basic Research, Springer, 1998.
[41] P.J. Linstrom, W.G. Mallard, The NIST chemistry WebBook: a chemical data resource on the internet, J. Chem. Eng. Data 46 (2001) 1059–1063.
[42] T.C. Huang, H. Toraya, T.N. Blanton, Y. Wu, X-ray powder diffraction analysis of silver behenate, a possible low-angle diffraction standard, J. Appl. Crystallogr. 26 (1993) 180–184.
[43] C. Krywka, et al., Effect of Osmolytes on pressure-induced unfolding of proteins: a high-pressure SAXS study, ChemPhysChem 9 (2008) 2809–2815.
[44] C. Krywka, et al., The small-angle and wide-angle X-ray scattering set-up at beamline BL9 of DELTA, J. Synchrotron Radiat. 14 (2007) 244–251.
[45] P.S. Prevey, The use of person VII distribution functions in X-ray diffraction residual stress measurement, Adv. X-ray Anal. 29 (1985) 103–111.
[46] R. Winter, Synchrotron X-ray and neutron small-angle scattering of lyotropic lipid mesophases, model biomembranes and proteins in solution at high pressure, Biochim. Biophys. Acta Protein Struct. Mol. Enzymol. 1595 (2002) 160–184.
[47] T. Charitat, E. Bellet-Amalric, G. Fragneto, F. Graner, Adsorbed and free lipid bilayers at the solid-liquid interface, Eur. Phys. J. B - Condens. Matter Complex Syst. 8 (1999) 583–593.
[48] M. Pisani, et al., Compressing inverse lyotropic systems: structural behavior and energetics of dioleoyl phosphatidyl ethanolamine, Phys. Rev. E 68 (2003) 021924.
[49] L. Paccamiccio, et al., Pressure effects on lipidic direct phases: the dodecyl trimethyl ammonium chloride− water system, J. Phys. Chem. B 110 (2006) 12410–12418.
[50] C. Naumann, T. Brumm, T.M. Bayerl, Phase transition behavior of single phosphatidylcholine bilayers on a solid spherical support studied by DSC, NMR and FT-IR, Biophys. J. 63 (1992) 1314–1319.