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Aspirin inhibits formation of cholesterol rafts in fluid lipid membranes

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ABSTRACT

Aspirin and other non-steroidal anti-inflammatory drugs have a high affinity for phospholipid membranes, altering their structure and biophysical properties. Aspirin has been shown to partition into the lipid head groups, thereby increasing membrane fluidity. Cholesterol is another well known mediator of membrane fluidity, in turn increasing membrane stiffness. As well, cholesterol is believed to distribute unevenly within lipid membranes leading to the formation of lipid rafts or plaques. In many studies, aspirin has increased positive outcomes for patients with high cholesterol. We are interested if these effects may be, at least partially, the result of a non-specific interaction between aspirin and cholesterol in lipid membranes.

We have studied the effect of aspirin on the organization of 1,2-dipalmitoyl-*sn*-*glycero*-3-phosphatidylcholine (DPPC) membranes containing cholesterol. Through Langmuir–Blodgett experiments we show that aspirin increases the area per lipid and decreases compressibility at 32.5 mol% cholesterol, leading to a significant increase of fluidity of the membranes. Differential scanning calorimetry provides evidence for the formation of metastable structures in the presence of aspirin. The molecular organization of lipids, cholesterol and aspirin was studied using neutron diffraction. While the formation of rafts has been reported in binary DPPC/cholesterol membranes, aspirin was found to locally disrupt membrane organization and lead to the frustration of raft formation. Our results suggest that aspirin is able to directly oppose the formation of cholesterol structures through non-specific interactions with lipid membranes.

1. Introduction

Lateral heterogeneity in lipid composition in membranes permits the existence of so-called lipid rafts: regions of the membrane believed to contain elevated cholesterol composition and increased molecular order [1–4]. Domains serve a functional purpose as they are thought to take part in membrane-associated events such as lipid/protein sorting and signal transduction, among other roles [5–10]. Experimental observation of rafts has been challenging as they are believed to be both small and short lived [11–14].

Cholesterol is suggested to drive domain formation through lipid interactions with cholesterol's stiff ring structure leading to the so-called liquid ordered (l_o) phase [15–18]. Small, transient cholesterol domains in binary lipid bilayers at physiological levels of cholesterol were recently reported from computer simulations and experiments [18–23]. At high concentrations of cholesterol, above ~40 mol%, immiscible cholesterol bilayers were reported to form spontaneously in model lipid bilayers [24–28].

There is growing evidence for an influence of various pharmaceuticals on lipid membrane organization and stability [29]. In particular, non-steroidal anti-inflammatory drugs (NSAID's) have been shown to disturb bilayer structures in real and model membranes [30,31]. Aspirin (acetylsalicylic acid, ASA) is the most common NSAID and has been shown to have strong interactions with bilayer structures [30,32]. Aspirin strongly perturbs model membrane structures in a concentration dependent manner and influences human erythrocyte shape [33]. As well, aspirin decreases the hydrophobic surface barrier in mucosal membranes, leading to a diffusion of acid and gastrointestinal injury [34] and has an effect on protein sorting [35]. Recently, a direct interaction between aspirin and cholesterol was reported as aspirin was observed to reduce the volume of cholesterol plaques in model membranes with elevated cholesterol concentrations of 40 mol% [36].

Abbreviations: DPPC, dipalmitolyphosphatidylcholine; ASA, acetylsalicylic acid; DSC, differential scanning calorimetry; L-B, Langmuir–Blodgett; PG, pyrolytic graphite; TFE, trifluoroethanol; RH, relative humidity; COX, cyclooxygenase

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However, an influence of aspirin on membranes with physiological levels of cholesterol has not been explored.

We examined the effect of aspirin on membranes composed of DPPC and physiological levels of cholesterol. The molecules are depicted in Fig. 1. By combining Langmuir–Blodgett isotherms, calorimetric measurements and neutron diffraction, we present evidence that the presence of 10 mol% ASA leads to a significant re-fluidification of DPPC/32.5 mol% cholesterol bilayers. The neutron diffraction patterns are indicative of ASA super-structures in the fluid bilayers, which seem to frustrate the formation of cholesterol rafts.

2. Results

2.1. Langmuir–Blodgett monolayer compression isotherms

Pressure versus area isotherms were recorded for DPPC monolayers with and without cholesterol in order to determine molecular areas and compressibility. ASA was dissolved in the aqueous sub-phase at concentrations of 0 mM and 3 mM. All experiments were performed with a sub-phase temperature of 50 °C. Fig. 2a) shows the compression isotherms for all experiments. The addition of 32.5 mol% cholesterol to the monolayer shifts the isotherm towards lower pressures and lower area per lipid compared to a monolayer composed of pure DPPC. However, with the addition of 3 mM ASA into the water sub-phase, the compression isotherm shifted to higher pressures and higher area per lipid, for both pure DPPC monolayers and monolayers containing 32.5 mol% cholesterol. To best capture trends observed in the compression isotherms, a pressure of 27 mN/m was chosen to compare the changes in the monolayer area per molecule as well as in the compressibility modulus due to the incorporation of cholesterol and ASA.

The mean molecular area for all isotherms at a pressure of 27 mN/m is plotted in Fig. 2b). At this pressure, a DPPC monolayer has a mean area of $A_L = 71.9 \pm 0.5$ Å^2. The addition of 32.5 mol% cholesterol to the DPPC monolayers decreases A_L significantly to 38.6 ± 0.2 Å^2. Inclusion of 3 mM ASA in the sub-phase leads to an increase of the area per lipid to 74.4 ± 0.7 Å^2. The same effect is observed in the presence of cholesterol: the addition of 3 mM ASA in the sub phase leads to a significant increase of the area per lipid in DPPC/32.5 mol% cholesterol from 38.6 ± 0.2 Å^2 to 44.3 ± 0.3 Å^2.

The compressibility of the monolayers is determined by the slope of the isotherms (as detailed in the Materials and methods section, Section 4). The addition of ASA to the water sub-phase decreases the elastic compressibility modulus, C_S^{-1}, from 84 ± 1 mN/M for pure DPPC to 63 ± 1 mN/M for DPPC/3 mM ASA as depicted in Fig. 2c), at a pressure of 27 mN/m. This is in strong contrast to cholesterol, which increases the compressibility modulus when added to the monolayer from 84 ± 1 mN/M to 89.8 ± 0.3 mN/M, as reported previously [37,38]. Addition of ASA to the DPPC/32.5 mol% cholesterol monolayers reduces the compressibility from 89.8 ± 0.3 mN/M to 80.6 ± 0.5 mN/M.

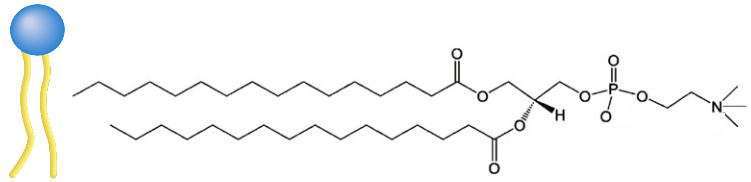
A decreased compressibility modulus is indicative of membranes, which are softer and more compressible. The increase in molecular area and decrease in compressibility for a pure DPPC bilayer in the presence of aspirin points to a general increase in fluidity of the membranes. The increase in molecular area and decrease in compressibility for DPPC/32.5 mol% cholesterol bilayers is strong evidence for a re-fluidification of the bilayers at a physiological cholesterol concentration with ASA.

2.2. Differential scanning calorimetry

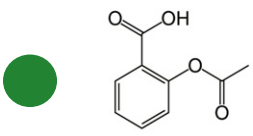
The effect of ASA on the phase behavior of membranes was examined using differential scanning calorimetry. Multi-lamellar vesicles (MLVs) of different composition were prepared for these experiments. Heating thermograms are plotted in Fig. 3a). A thermogram taken from a pure DPPC sample shows two endothermic transitions. There is a pre-transition from the gel to ripple phase ($L_\beta \rightarrow P_{\beta'}$) at T ~ 308 K. The main transition to the fluid phase (L_α) occurs at 314 K, in agreement with literature values [39]. Addition of ASA resulted in a decrease in the main transition temperature and broadened the main transition peak (and eliminated the pre-transition). Similar results have been reported when ASA is added to DMPC liposomes [33]. A reduction or suppression in transition temperatures and transition enthalpy is evidence that ASA reduces the cooperativity of the main and pre-transitions, indicative of a more fluid structure.

As expected, thermograms of samples composed of DPPC with 32.5 mol% cholesterol in Fig. 3b) show no transitions, as was reported previously for the l_o phase [20,40]. The absence of a transition proves that a cholesterol concentration of 32.5 mol% is high enough to induce the l_o phase in DPPC bilayers. No change was observed after addition of 1 mM ASA (data not shown). However, as shown in Fig. 3b), addition of 6 mM ASA led to the appearance of an exothermic transition at $T = 313.6$ K.

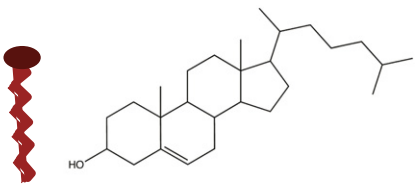
The bilayers were then cooled and the heating thermogram was repeated. After this second cycling the transition peak shifted to $T = 308$ K and significantly broadened. The temperature cycling was repeated, and a third thermogram shows no transition peak. We note that no peak was observed upon cooling, regardless of cycle number. The dependence of the thermogram upon temperature cycling indicates the presence of inhomogeneities and kinetically trapped states existing on



1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC)



Acetylsalicyclic acid (ASA)



Cholesterol

Fig. 1. Schematic representations of the 1,2-dipalmitoyl-*sn-glycero*-3-phosphatidylcholine (DPPC), cholesterol, and acetylsalicylic acid (ASA, aspirin) molecules used in this study.