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Bilayer undulation dynamics in unilamellar phospholipid vesicles: Effect of temperature, cholesterol and trehalose

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ABSTRACT

We report a combined dynamic light scattering (DLS) and neutron spin-echo (NSE) study on the local bilayer undulation dynamics of phospholipid vesicles composed of 1,2-dimyristoyl-glycero-3-phosphatidylcholine (DMPC) under the influence of temperature and the additives cholesterol and trehalose. The additives affect vesicle size and self-diffusion. Mechanical properties of the membrane and corresponding bilayer undulations are tuned by changing lipid headgroup or acyl chain properties through temperature or composition. On the local length scale, changes at the lipid headgroup influence the bilayer bending rigidity κ less than changes at the lipid acyl chain: We observe a bilayer softening around the main phase transition temperature T_m of the single lipid system, and stiffening when more cholesterol is added, in concordance with literature. Surprisingly, no effect on the mechanical properties of the vesicles is observed upon the addition of trehalose.

1. Introduction

In mammal organisms, lipid vesicles very often serve as natural carriers, for instance in the case of red blood cells or synaptic vesicles. Their functional properties strongly depend on the composition of their membranes [1–4]. In order to achieve valuable insight into that functionality, materials' properties such as the bending rigidity κ of the lipid membranes can be tuned by changes of temperature or composition. For the purpose, in this study we look at unilamellar vesicles (ULVs) of 1,2-di-myristoyl-glycero-3-phosphatidylcholine (DMPC) with the membrane additive cholesterol (Chol) or trehalose (Treh) added, (see Fig. 1). In the composite systems,the two additives can be considered complementary: due to its hydrophobic nature, cholesterol preferably arranges along the lipid acyl chains [4], whereas trehalose locates near the lipid headgroups [5]. An overview on how changes in temperature or amount of cholesterol or trehalose affect the membrane structure along the bilayer normal is given by Doxastakis et al. [6]. Temperature in fact plays an important role, in particular around the main phase transition. In single lipid systems, minor changes in temperature in close vicinity to the main phase transition temperature T_m produce a critical swelling of the lamellae associated with a lipid bilayer softening [7–10].

The co-surfactant cholesterol has been extensively studied with regard to its effects on bilayer self-assembly, phase state and structure [11–15], as well as on its mobility within the membrane [16,17]. Cholesterol inserts into the lipid bilayer membrane in concentrations of up to 50 mol% and is known to regulate membrane fluidity [18], permeability [19], rigidity [20–22] and the lateral mobility of proteins [23,24]. Its effect on membrane dynamics has been scrutinized over a broad range of time and length scales using quasi-elastic and inelastic scattering experiments [4,20–22,25,26]. The insertion of cholesterol into the lipid bilayer reduces free volume in the membrane plane, and thus influences collective in-plane dynamics [4,26].

The disaccharide trehalose has attracted comparable interest due to its bioprotective properties. Organisms that are able to survive potentially damaging conditions produce trehalose in high quantities during stress periods [27]. MD simulations predict that at low concentration trehalose stabilizes membrane structures by partially replacing water molecules in the hydration shell of the lipid headgroups via hydrogen bonding [6,27–29]. At high trehalose concentrations, the disaccharide can serve as a replacement for water under anhydrous conditions, which explains its effectiveness as lyophilization agent for vesicles and cells [28]. In the latter cases, the sugar molecules form a glassy state by interlinking different lipid headgroups, which prevents membrane fusion processes and reduces dehydration-induced stresses [30–34]. The MD simulations at low to moderate trehalose concentration (up to 30%) are in-line with experimental findings on the partitioning of trehalose into the headgroup region [5,35,36]. However, also partial

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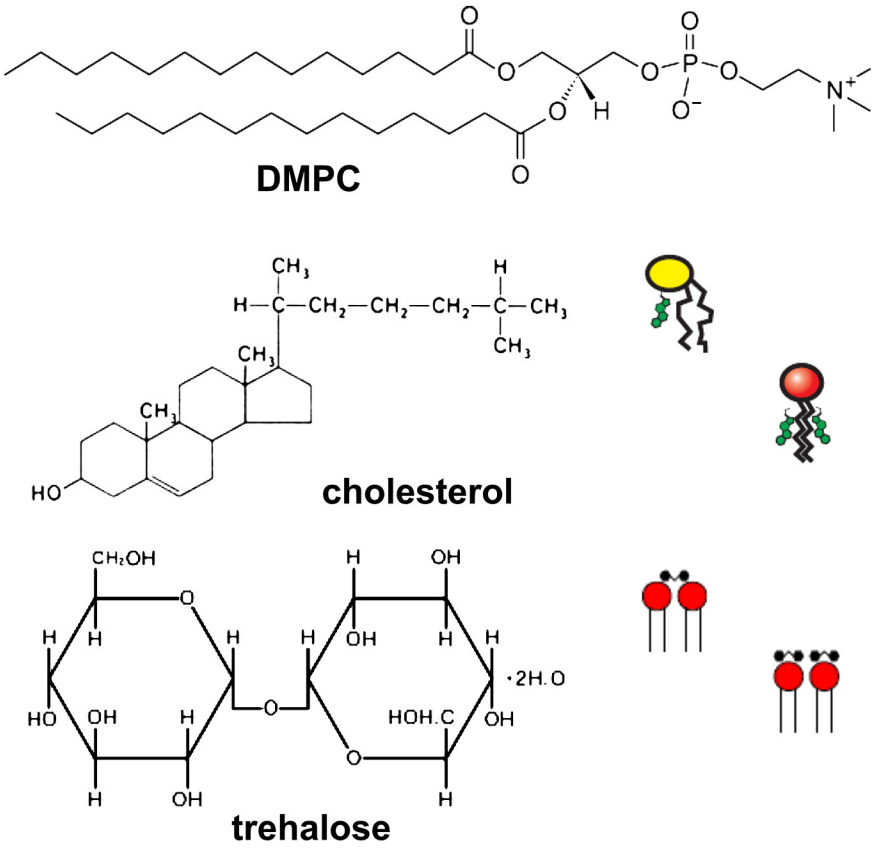

Fig. 1. Phospholipid molecule and membrane additives used in this study (*from top*): DMPC, cholesterol and trehalose. Due to its hydrophobic nature, cholesterol preferably locates near the DMPC acyl chain region, whereas trehalose attaches to the phospholipid headgroups (see schemes on the right of the respective structural formulae).

depletion of trehalose from the hydration layer was observed experimentally [37,38]. The concentration dependent change-over of membrane–sugar interactions, partitioning vs. depletion, was recently investigated by Andersen et al. [34]. The authors studied the interaction of trehalose with unilamellar vesicles of DMPC in D_2O by small-angle neutron scattering (SANS) and thermodynamic measurements. They observed strong binding of trehalose tothe interface, which made the membrane thinner and laterally more expanded at low trehalose concentration. Their experimental findings were confirmed by MD simulations by Kapla and co-workers who interpreted the experimental results as an intercalation effect of the trehalose molecules into the polar part of the lipids [39]. In the simulation, a decrease of lateral lipid mobility in the membrane plane was predicted in the presence of trehalose, as well as an increase in the bilayer bulk modulus.

The choice of appropriate means for the characterization of membrane mechanical properties such as bulk modulus and bending rigidity is strongly dependent on the system under investigation. In the case of giant unilamellar vesicles (GUVs) on the length scale of 10–50 μm, the membrane fluctuation dynamics is widely studied by optical and microscopy techniques [40–47]. Also dynamic light scattering (DLS) has occasionally been used [48].

X-ray and neutron diffraction techniques come into play whenever lipid systems get smaller and organized as multilamellar vesicles (MLVs). The elastic constants are obtained from the line shape analysis of the lipid bilayer Bragg peaks [49–51]. Neutron spin-echo [12,14] and MD simulations [13] further allow direct monitoring of membrane thickness fluctuations in that regime.

When it comes to unilamellar vesicles (ULVs) of the order of 50 nm in radius, which in a biological environment would correspond to sizes close to the ones of synaptic vesicles [52], long wavelength neutron spin-echo spectroscopy (NSE) is a well-suited experimental approach for a direct investigation of local lipid bilayer undulation dynamics and the membranes' corresponding mechanical properties. The ULVs we use are smaller than GUVs by three orders of magnitude and thus are subject to significantly different curvatures and interface line tensions.

In this work we use neutron spin-echo spectroscopy (NSE) to study the local lipid bilayer undulations and bending rigidities as a function of temperature or composition. We unfold the bilayer undulations and the vesicle center-of-mass diffusion by complementary dynamic light scattering (DLS) measurements and a combined analysis of NSE and DLS data [20,53,54]. Since cholesterol perturbs lipid bilayers non-universally, i.e. depending on lipid acyl chain saturation [40,55–57], we extend previous long wavelength NSE work by investigating how the sterol molecule affects the local undulations of lipid bilayers containing a fully saturated instead of a mono-unsaturated phospholipid. Moreover, a very interesting aspect to the topic is the influence of trehalose on bilayer undulations and bending rigidity directly.

2. Material and methods

2.1. Sample preparation

The lipids (DMPC, cholesterol) were purchased from Avanti (Alabaster, AL, USA) and dissolved in chloroform/trifluoroethanol (1:1) in the desired molar proportions. The solvent was subsequently evaporated slightly above room temperature in a vacuumoven and the dry lipids were hydrated with heavy water (D_2O) at a concentration of 10 mg/ml, heated from room temperature up to 30°C, ultrasonicated in a bath and cooled down to room temperature. This procedure was repeated three times. Trehalose was obtained from Merck (Darmstadt, Germany), dissolved in D_2O, and subsequently added to the dried phospholipid. In order to obtain unilamellar vesicles (ULVs), the suspension consisting of multilamellar vesicles (MLVs) was passed ten times through a polycarbonate filter with 50 nm pore diameter using