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Long-timescale motions in glycerol-monopalmitate lipid bilayers investigated using molecular dynamics simulation

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ABSTRACT

The occurrence of long-timescale motions in glycerol-1-monopalmitate (GMP) lipid bilayers is investigated based on previously reported 600 ns molecular dynamics simulations of a $2 \times 8 \times 8$ GMP bilayer patch in the temperature range 302–338 K, performed at three different hydration levels, or in the presence of the cosolutes methanol or trehalose at three different concentrations. The types of long-timescale motions considered are: (i) the possible phase transitions; (ii) the precession of the relative collective tilt-angle of the two leaflets in the gel phase; (iii) the *trans–gauche* isomerization of the dihedral angles within the lipid aliphatic tails; and (iv) the flipping of single lipids across the two leaflets. The results provide a picture of GMP bilayers involving a rich spectrum of events occurring on a wide range of timescales, from the 100-ps range isomerization of single dihedral angles, *via* the 100-ns range of tilt precession motions, to the multi-μs range of phase transitions and lipid-flipping events.

1. Introduction

The cell membrane defines the boundary of a cell and works as the separating barrier and first interaction site of the cell with its surroundings [1]. The basic component of this membrane is a lipid bilayer [2–5]. Several phases are known for lipid bilayers in aqueous environments [6,7], their prevalence being determined by the types of the lipid molecules, their concentrations, the possible presence of cosolutes (CSLs), as well as pressure and temperature. The corresponding phase-transition temperatures and mechanisms are of fundamental importance for both biology and technology [6,8].

The biologically most relevant phases of lipid bilayers are the liquid crystal (LC) and the gel (GL) phases [9,10]. In the presence of short-chain aliphatic alcohols, a third type of phase can appear, the interdigitated (ID) phase [11–13]. These three phases are sometimes referred to as L_α, L_β (or $L_{\beta'}$), and $L_{\beta I}$, for LC, GL and ID, respectively [9], and can be distinguished by the arrangement of the lipids within the bilayer and by differences in the area per lipid, in the bilayer thickness, in the distribution of the headgroup and tail atoms along the bilayer normal, and in the methylene carbon–hydrogen order parameters [6,14,15].

In the LC phase, the tails are conformationally disordered, involving a mixture of *trans* and *gauche* conformations. The methylene groups present low order parameters [9] and no preferential collective orientation of the chains relative to the bilayer normal (collective tilting) is observed.

In the GL phase, the aliphatic lipid tails are arranged in nearly all-*trans* conformations and the methylene groups present high order parameters [9]. In the $L_{\beta'}$ form of the GL phase, the tails are tilted with respect to the bilayer normal [9,10]. In the L_β form, the tails are oriented perpendicularly to the bilayer midplane [16]. The prevalence of either $L_{\beta'}$ or L_β is determined by the ratio of the effective headgroup to tail cross-sections [16], a high ratio favoring tilting [9,16–18], *i.e.* $L_{\beta'}$ over L_β. Compared to the LC phase, the GL phase is also laterally more compact and transversely more expanded, the difference being most pronounced when the GL phase involves a low tilt angle [18,19].

In the ID phase, the aliphatic tails of the two leaflets interpenetrate in such a way that the terminal methyl groups of one leaflet are located close to the headgroups of the other [19–25]. The ID bilayer presents a lateral expansion and transversal compaction comparable to the LC phase, along with a tight chain packing and nearly all-*trans* conformations of the tails similar to the GL phase. To our knowledge, the extent of tilting of a bilayer in the ID phase has not been determined experimentally. However, considering that the

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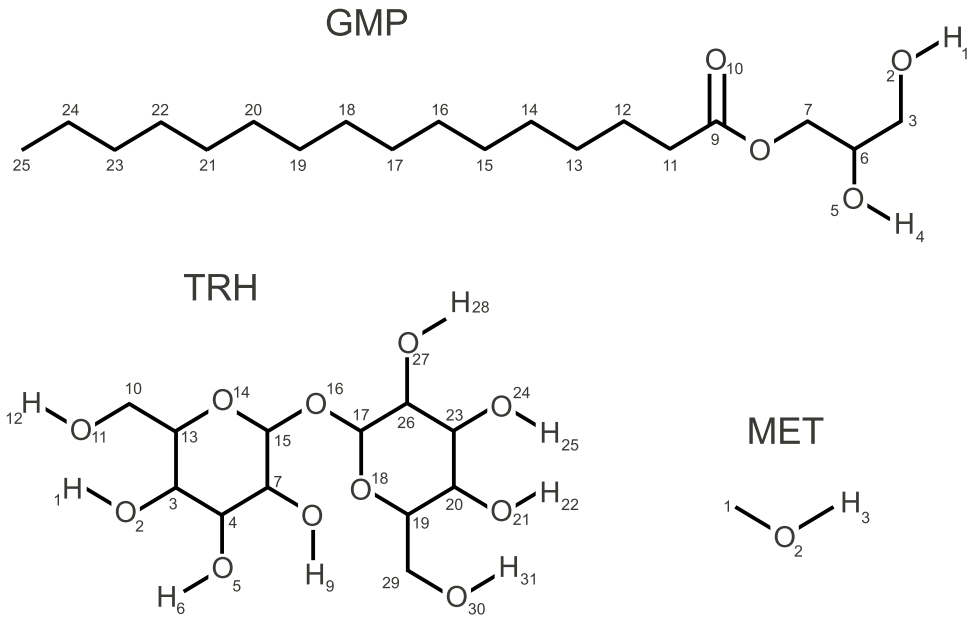

Fig. 1. Chemical structures of the monoglyceride and cosolutes considered in the present study. The compounds are the lipid glycerol-1-monopalmitate (GMP) and the cosolutes (CSLs) trehalose (TRH) and methanol (MET). The numbering refers to the GROMOS molecular topology used in the simulations. See Ref. [19] and Suppl. Mat. therein for detailed force-field information.

headgroup spacing is largely increased compared to that in the GL phase, it seems reasonable to assume that the ID phase generally presents little or no tilting, a suggestion supported by simulations of monoglyceride bilayers [19]. The methylene order parameters are typically even higher in the ID than in the GL phase [26–28], which results from both a reduced tilting and a tighter chain packing [19,29].

Atomistic molecular dynamics (MD) simulations have greatly contributed to the characterization and understanding of the structure, thermodynamics and dynamics of lipid bilayers under various conditions [19,30–49]. These simulations provide information at a spatial (atomic level) and temporal (femtosecond) resolution inaccessible to experiment, concerning system sizes (∼10 nm) and timescales (∼1 μs) already relevant for the evaluation of thermodynamic properties *via* statistical mechanics and the comparison with experimental data. Most of these studies have been carried out in the context of biologically relevant lipids, typically diglycerides with functionalized headgroups such as dipalmitoylphosphatidylcholine (DPPC) [31,33–35,37,38,41,42,45,47]. However, these lipids remain relatively challenging to simulate, owing to difficulties in the force-field design [50,51] and treatment of electrostatic interactions [50,52–57], and to the slow convergence of system properties with respect to both system size [18,49–51,58–60] and simulation timescale [18,58,61–63].

For this reasons, it is also of interest to consider less complex bilayer systems such as monoglyceride lipid systems [19,43,47–49,64,65]. In addition to being relevant in the context of prebiotic research [66,67] and technological applications [68,69], these lipids present a number of key advantages compared to *e.g.* DPPC for a computational investigation of lipid phase transitions: (i) the presence of only one aliphatic tail per headgroup, leading to a faster relaxation; (ii) the limited role of electrostatic interactions (uncharged, non-zwitterionic and moderately polar headgroup); (iii) the absence of a ripple phase [70] as an intermediate state between the GL and LC phases; and (iv) the availability of experimental structural and thermodynamic data [14,68,69,71–77].

In a series of previous studies by our group, glycerol-1-monopalmitate (GMP; Fig. 1) was chosen as a test system to investigate the phase characteristics and phase-transition properties of a simple model lipid [19,43,47–49,64,65]. In particular, the effects of the hydration level and of the possible presence of the CSLs methanol and trehalose (MET and TRH, respectively; Fig. 1) on the bilayer properties and on the main transition temperature T_m for the GL ↔ LC transition were investigated in detail [19]. The three phases LC, GL (tilted) and ID (only in the presence of MET) of GMP bilayers, as well as their interconversion, were observed in these simulations, as illustrated in Fig. 2. This previous study was based on 83 MD simulations, each of 600 ns duration, of a GMP bilayer patch of 2 × 8 ×8 lipids carried out at different temperatures in the range of 302–338 K, either in the absence of CSL at three different hydration levels (simulations previously reported in Ref. [49]) or in the presence of MET or TRH at three different concentrations.

In the present article, these simulations are further analyzed to investigate the occurrence of long-timescale motions in the bilayer and the influence of hydration and CSLs on these motions. The slow motions considered are: (i) the possible phase transitions; (ii) the precession of the relative collective tilt-angle of the two leaflets in the GL phase; (iii) the *trans-gauche* isomerization of the dihedral angles within the lipid tails; and (iv) the flipping of single lipids across the two leaflets.

2. Methods

2.1. Molecular dynamics simulations

All the MD simulations considered were reported previously in Ref. [19]. The corresponding force-field information and simulation details can be found therein, and only the essential points will be repeated here.

The MD simulations were performed using the GROMOS MD++ program [78–80], with the 53A6$_{OXY}$ force field [81] for GMP, TRH and MET, along with the simple point charges (SPC) water model [82]. They were carried out under periodic boundary conditions based on rectangular boxes containing a hydrated GMP bilayer patch of 2 × 8 ×8 lipid molecules in the *xy*-plane, leading to a total number of 128 lipid molecules in the systems. Both leaflets consisted of a racemic mixture of the *R* and *S* enantiomers of the GMP molecule. A variable number of CSL molecules was added: