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Mixing behaviour of asymmetrical glycerol diether bolalipids with saturated and unsaturated phosphatidylcholines

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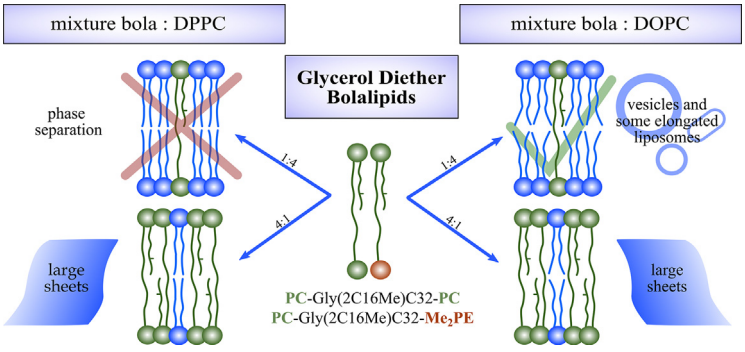
HIGHLIGHTS

- Asymmetrical glycerol diether bolalipids show different miscibility with DPPC/DOPC.
- An excess of bolalipid in the mixtures leads to the formation of large sheets.
- An excess of DPPC in the mixtures leads to phase separation.
- The bolalipid:DOPC 1:4 mixtures show the formation of stable liposomes.

GRAPHICAL ABSTRACT



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ABSTRACT

Liposomes have received attention as a promising class of drug delivery vehicles. To date, many approaches have been tried developing liposomes for oral use. However, no liposomal formulation is on the market so far that is approved for oral application. In this study, we investigate the miscibility of two glycerol diether bolalipids with classical saturated and unsaturated phosphatidylcholines by means of differential scanning calorimetry (DSC), transmission electron microscopy (TEM), and dynamic light scattering (DLS). Our bolalipids contain a long C32 alkyl chain bound to glycerol in the *sn*-3 position and a short C16 in the *sn*-2 position, which further carries a racemic methyl branch. The *sn*-1 position of the glycerol as well as the end of the long C32 alkyl chain contain polar headgroups: either two phosphocholine headgroups (**PC-Gly(2C16Me)C32-PC**) or a phosphocholine and a phosphodimethylethanolamine headgroup (**PC-Gly(2C16Me)C32-Me$_2$PE**). We demonstrate that glycerol diether bolalipids show better miscibility with unsaturated phosphatidylcholines than with saturated ones. Both bolalipids in mixture with the unsaturated 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) form liposomes, which are stable in size upon storage. These mixed bolalipid/phospholipid vesicles could be used as an oral liposomal formulation in the future.

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1. Introduction

Liposomes are considered to be one of the most successful drug delivery systems, not least because of the outstanding encapsulation efficiency of hydrophilic as well as lipophilic compounds [1,2]. However, liposomal formulations, which are currently available, are mostly developed for parenteral, pulmonal, nasal, and/or topic applications [2–4]. Because phospholipids are highly susceptible to conditions found in the gastrointestinal tract (GIT), i.e. the presence of bile salts, gastric acid, or lipases [5–7], the oral application of liposomes consisting of classical phospholipids is not promising at all. As a consequence, various approaches were developed to improve the stability of orally administered liposomes: Besides the coating with polymers such as polyethylene glycols [8–10] and other materials including chitosan [11,12], polysaccharides [13], and proteins [14], the stabilization of liposomes using cholesterol [15] is known to date. Finally, also long-chain bipolar lipids (bolalipids) that are able to span and, hence, stabilize a phospholipid bilayer membrane can be used to enhance to integrity of liposomes. This approach was already tested for a wide range of natural and artificial bolalipids [16–23].

Bolalipids are molecules consisting of a long alkyl chain with two hydrophilic headgroups attached to each end [24,25]. This special class of lipids can be found in the membranes of certain species of archaea, e.g. *thermoacidophiles*, where they are responsible for the outstanding stability of archaea against harsh living conditions, such as low pH values, high temperatures, or high salt concentrations [26–28]. Therefore, it stands to reason to use these bolalipids to stabilize liposomal formulations composed of classical phospholipids against the conditions found in the GIT.

Two approaches exist to obtain sufficient amounts of archaeal lipid material. First, bolalipids can be extracted from the archaeal membranes by organic solvents. It has already been shown in previous studies that the obtained bolalipid mixtures can be used for the formulation of liposomes [17–20,23]. However, this extraction procedure is expensive and time consuming. Second, due to the complex chemical structure of these lipid substances, the total synthesis of the naturally occurring bolalipids, published by Kakinuma and co-workers [29–31], is also time-consuming and, hence, not suitable for production of bolalipids on a larger scale. Therefore, we, and also other groups, have tried to simplify the chemical structure of bolalipids while maintaining the stabilizing properties of natural bolalipids [22,32,33].

We have shown previously that artificial bolalipids composed of one long saturated alkyl chain of 32 carbon atoms and two phosphocholine (PC) headgroups—the PC-C32-PC representing a very simple archaeal model lipid—self-assemble in water into nanofibers and micelles [34,35]. Vesicular structures were not observed. This single-chain bolalipid has shown no tendency to be incorporated into bilayers of different saturated and unsaturated phosphatidylcholines [36,37]. The reason for this observation is the larger space requirement of the PC headgroup of the bolalipid compared to the small cross-sectional area of its single alkyl chain. As a consequence, void volume is produced when the bolalipid is inserted in a stretched manner into a phospholipid bilayer, which can be filled by neither phospholipid nor bolalipid. This packing frustration finally leads to the separation of the mixed bolalipid/phospholipid system into PC-C32-PC nanofibers and phospholipid vesicles [36].

To overcome these packing problems, the expansion of the cross-sectional area of the bolalipid' alkyl chain in order to fill the void volume is one possibility. This "enlargement" can be done by inserting heteroatoms [38,39], acetylene [35] or diacetylene [40] groups, methyl branches [35], and phenyl- [41–45] or biphenyl [41] moieties into the long alkyl chain. The insertion of these perturbations led to a certain miscibility of the single-chain alkyl-modified bolalipids with saturated phosphatidylcholines, e.g. 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) [45], 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) [37,43–45], or 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) [43–45]. In some cases, an increased stability of the ordered gel phase of the lipid mixture (higher T_m value) was observed [44]. However, the vesicular aggregate structure of classical phosphatidylcholines was destroyed in nearly every single-chain bolalipid/phospholipid mixture and EM images revealed the presence of small bilayer fragment and disks-like aggregates. Thus, complete miscibility of artificial single-chain bolalipids and classical phospholipids, which will lead to stabilized liposomes, was not observed up to now.

Besides the single-chain bolalipids, we recently introduced a novel class of bolalipid, termed glycerol diether bolalipids [46]. These lipids are composed of an unmodified alkyl chain with 32 carbon atoms (C32) in the *sn*-3 position, a methyl-branched C16 alkyl chain in the *sn*-2 position, and a zwitterionic PC headgroup in the *sn*-1 position of a glycerol moiety. The long C32 alkyl chain is terminated either by a second PC headgroup, leading to **PC-Gly(2C16Me)C32-PC**, or by a phosphodimethylethanolamine (Me_2PE) headgroup, **PC-Gly(2C16Me)C32-Me$_2$PE** (see Fig. 1) [46].

Investigations regarding the aggregation behaviour of these novel glycerol diether bolalipids in aqueous suspension was part of previous work [46]. We could show that **PC-Gly(2C16Me)C32-PC** forms lamellar structures with a high bending stiffness. The analogue **PC-Gly(2C16Me)C32-Me$_2$PE** self-assembles at pH 5, where the Me_2PE headgroup is fully protonated, into large lamellar aggregates as well. These sheet-like structures are stable up to a pH value of 9. Above a pH value of 9, the aggregates became smaller and they appeared more rounded and disk-like. Beginning with a pH value of 11, we observed a lamella/nanofiber transformation; and at pH 12, nanofibers became the dominant aggregate form of **PC-Gly(2C16Me)C32-Me$_2$PE** [46].

In this study, we investigated the miscibility of the two glycerol diether bolalipids (see Fig. 1) with bilayer forming phosphatidylcholines: either with the saturated DPPC or with the unsaturated 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC). Investigations were carried out by means of differential scanning calorimetry (DSC), transmission electron microscopy (TEM) of negatively stained samples as well as vitrified specimens (cryo-TEM), and dynamic light scattering (DLS). Since the protonation state of the Me_2PE headgroup is pH-dependent, miscibility studies using the **PC-Gly(2C16Me)C32-Me$_2$PE** were performed at different pH values, namely pH 5.0, 7.6, and 10.0.

2. Materials and methods

2.1. Chemicals

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) were purchased from Avanti Polar Lipids (Alabaster, USA) and used without further purification.

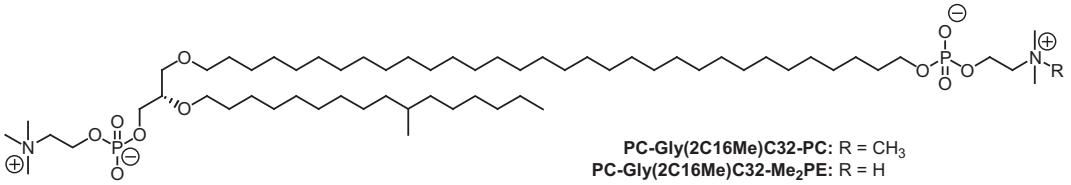

Fig. 1. Chemical structure of **PC-Gly(2C16Me)C32-PC** and **PC-Gly(2C16Me)C32-Me$_2$PE** investigated in this work.