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Archaeal lipids in oral delivery of therapeutic peptides

Ann-Christin Jacobsen [a], Sara M Jensen [a,b], Gert Fricker [c], Martin Brandl [a,*], Alexander H. Treusch [b]

[a] Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Odense DK-5230, Denmark
[b] Department of Biology and Nordic Center for Earth Evolution, University of Southern Denmark, Odense DK-5230, Denmark
[c] Department of Pharmaceutical Technology, Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg, Heidelberg D-69120, Germany

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ABSTRACT

Archaea contain membrane lipids that differ from those found in the other domains of life (Eukarya and Bacteria). These lipids consist of isoprenoid chains attached via ether bonds to the glycerol carbons at the *sn*-2,3 positions. Two types of ether lipids are known, polar diether lipids and bipolar tetraether lipids. The inherent chemical stability and unique membrane-spanning characteristics of tetraether lipids render them interesting for oral drug delivery purposes. Archaeal lipids form liposomes spontaneously (archaeosomes) and may be incorporated in conventional liposomes (mixed vesicles). Both types of liposomes are promising to protect their drug cargo, such as therapeutic peptides, against the acidic environment of the stomach and proteolytic degradation in the intestine. They appear to withstand lipolytic enzymes and bile salts and may thus deliver orally administered therapeutic peptides to distant sections of the intestine or to the colon, where they may be absorbed, eventually by the help of absorption enhancers. Archaeal lipids and their semisynthetic derivatives may thus serve as biological source for the next generation oral drug delivery systems.

The aim of this review is to present a systematic overview over existing literature on archaea carrying diether and tetraether lipids, lipid diversity, means of lipid extraction and purification, preparation and in vitro stability studies of archaeal lipid-based liposomal drug carriers and in vivo proof-of concepts studies.

1. Introduction & background

Despite tremendous research efforts undertaken in recent years, oral delivery of labile drug compounds such as therapeutic proteins and peptides still is challenging primarily for two reasons: 1) due to the harsh environment of the lumen of the gastrointestinal (GI) tract, including acidic pH and proteolytic enzymes, which the drug needs to withstand and 2) the intestinal barrier, which the drug needs to come across on its way to the site of action. The GI tract's physiological role is the digestion of nutrients, including proteins and peptides, and subsequent absorption of digested fragments, including amino acids and short peptides. To this end, the GI tract plays an important role in preventing "foreign" compounds, including proteins and larger peptides, from entering the body as these may be pathogenic or allergenic. These essential features of the GI tract account for the challenges encountered with the oral administration of peptide drugs.

On the other hand, therapeutic peptides represent a rapidly growing class within the pharmaceutical market with currently 60 US Food and Drug Administration (FDA) approved peptide drugs and 140 and over 500 peptide drugs in clinical and preclinical trials, respectively (Fosgerau and Hoffmann, 2015). The oral route in general is the preferred way of administration as it combines ease and convenience of self-application with low production cost. However, routine oral application of therapeutic peptides has been accomplished in only a few cases (e.g. Desmopressin, Cyclosporin A) with a few more in various phases of clinical trials. Despite major research efforts in recent years, oral administration of peptide and protein drugs has not been accomplished for the vast majority of compounds to date (Renukuntla et al., 2013). Clearly, novel and more efficient oral formulation strategies suitable for this class of compounds would yield a tremendous step forward in treatment options for a number of diseases where peptide based drugs are essential.

Different formulation strategies, for example the use of absorption enhancers, enzyme inhibitors, bioadhesive systems, site specific delivery systems and particulate carrier systems, such as liposomes or solid lipid nanoparticles, have been investigated to enable the oral drug

Abbreviations: A. fulgidus, Archaeoglobus fulgidus; CF, 5(6)-carboxyfluorescein; Chol, Cholesterol; CpCl, cetylpyridinium chloride; DEL, diether lipid; DGD, dialkyl-glycerol-diether; EPC, Egg-phosphatidylcholine; FDA, Food and Drug Administration; FITC, fluorescein isothiocyanate; GCTE, glycerylcaldityl tetraether; GDD, Glycerol-dialkanol diether; GDGT, Glycerol-dialkyl-glycerol-tetraethers; GDNT, Glycerol-dialkyl-nonitol tetraethers; GI tract, Gastro-intestinal tract; hGH, Human growth hormone; *M. espanolae*, *Methanobacterium espanolae*; *M. jannaschi*, *Methanococcus jannaschii*; MLV, multilamellar vesicles; *M. mazei*, *Methanosarcina mazei*; PC, phosphocholine; PEG, polyethylene glycol PLFE; PLFE, polar lipid fraction E; rRNA, ribosomal ribonucleic acid; SHB, simulated human bile; *T. acidophilum*, *Thermoplasma acidophilum*; TEL, tetraether lipid; TPL, total polar lipid fraction; ULV, unilamellar vesicle.

* Corresponding author.
E-mail address: mmb@sdu.dk (M. Brandl).

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delivery of peptide based drugs, for recent reviews see (Choonara et al., 2014; Renukuntla et al., 2013) Varamini and Toth (2016); Gupta et al. (2013).

Nanoparticulate carriers are regarded as promising for several reasons: 1) they may protect their drug load against the harsh environment of the upper intestine 2) they may release their drug load in a sustained or site specific manner (e.g. where the pH value is close to neutral and/or the activity of proteolytic enzymes reduced) 3) they may slow down the drug's passage along the GI tract and/or convey intimate contact with the mucosa (e.g. through muco-adhesive surface characteristics). We shall focus here on a specific type of nanoparticulate carrier, liposomes.

Liposomes are (sub-)microscopic vesicular structures composed of one or several phospholipid (PL) bilayer(s) enclosing an aqueous core. Both, the aqueous core and the bilayer(s) can accommodate drug compounds. Hydrophilic compounds like most peptides may be encapsulated and retained within the aqueous core for extended periods of time if they are sufficiently large and charged, such that leakage across the bilayer(s) is slow. Hydrophobic compounds may be incorporated within the bilayer(s) and their apparent solubility thus improved (Brandl, 2001). The general structure of liposomes is shown in Fig. 1.

Encapsulation of therapeutic peptides within liposomes may offer some protection against the harsh environment in the GI tract. Thereby, the premature degradation of the therapeutic compound can be prevented. Recent studies support this by indicating that oral bioavailability of poorly soluble and low bioavailable drugs could be enhanced by liposome formulations (Fricker et al., 2010). Even though these results are promising, conventional liposomes only exhibit a limited stability at low pH in the presence of bile salts and lipases (Lasic, 1998), thus demonstrating the clear need for an improvement of liposomal formulations for oral use. Different strategies to stabilize liposomes in the GI tract environment have been investigated. For example, the linking of polymers such as polyethylene glycol (PEG), the sugar chain portion of mucin or polyvinyl alcohol to the liposome surface (surface coating), has a stabilizing effect on liposomal formulations (Silva et al., 2012). Another promising technique to increase liposomal stability, which will be the focus of this review, is the use of archaeal membrane lipids in the formulation of liposomes (Jacquemet et al., 2009).

Archaea are a diverse group of prokaryotic microorganisms. Even though several archaeal species were already identified at the beginning of the 20th century, (for a recent review see (Petitjean et al., 2015), archaea were not classified as a separate group of prokaryotes and were initially called *archaebacteria*. With the introduction of a novel technique to reconstruct evolutionary relationships that is based on the comparison of 16S ribosomal RNA (rRNA) sequences (Woese and Fox, 1977), archaea were found to be different from bacteria. Subsequently, Woese and coworkers proposed the three domain model based on the 16S rRNA phylogeny, which divides the tree of life into three main domains, Bacteria, Eukaryota and Archaea (Woese et al., 1990). With the separation of Archaea into a distinct domain, the epithet "*bacteria*" became redundant and hence literature uses the term *Archaea* instead of *archaebacteria* today.

Originally, two major kingdoms (same as phyla in Bacteria) were recognized for the domain Archaea, Euryarchaeota and Crenarchaeota. The kingdom Euryarchaeota consists of cultivated species that are methanogens, halophiles or thermophiles and is a phenotypically heterogeneous group. The kingdom Crenarchaeota is a more homogenous group, mainly consisting of thermoacidophiles and thermophiles (Woese et al., 1990). With the use of environmental molecular techniques 'non-extreme' archaea (mainly mesophiles) were found in many environments, including the ocean, freshwater and soils. Research concerning archaea is rapidly progressing and, besides mesophile Eury-

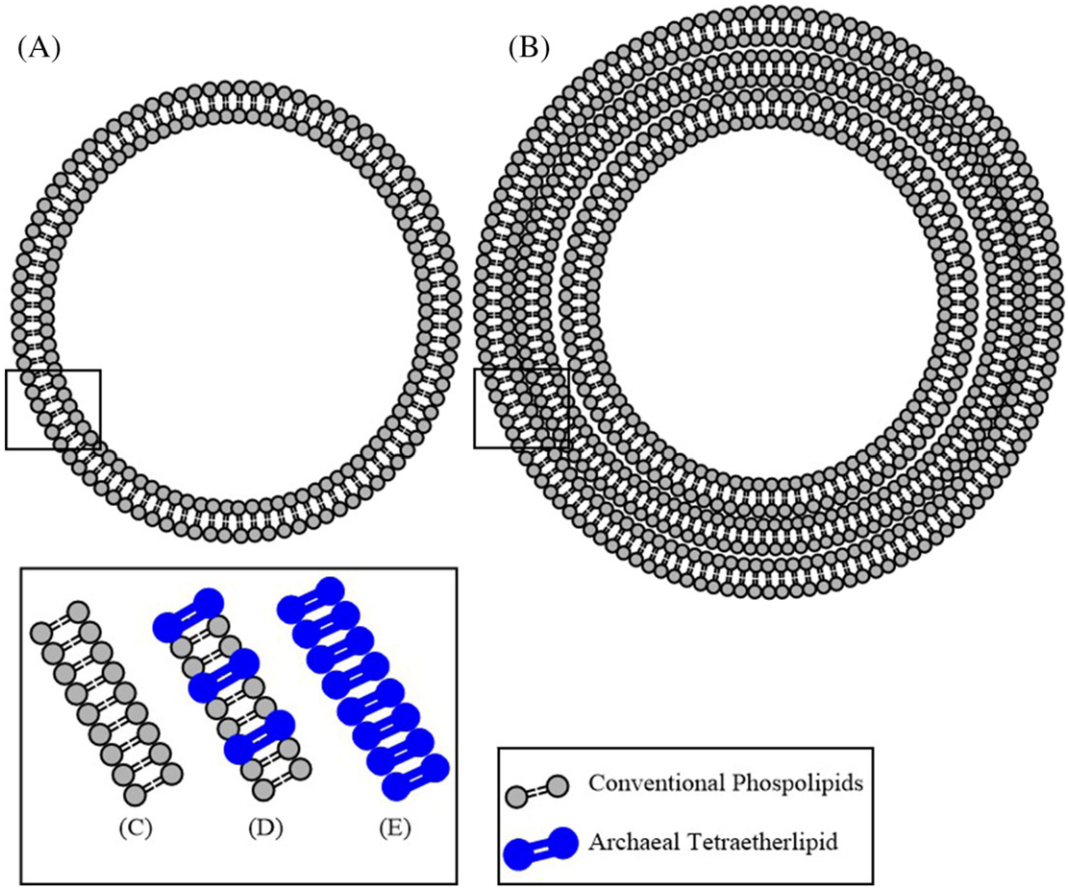

Fig. 1. General structure of liposomes. (A) ULV liposome, (B) MLV liposome, (C) Conventional PL liposome, (D) Mixed vesicle and (E) Tetraether lipid archaeosome.