Chemistry and Physics of Lipids 167–168 (2013) 33–42

Contents lists available at SciVerse ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

Visualizing a multidrug resistance protein, EmrE, with major bacterial lipids using Brewster angle microscopy

Safia Nathoo, Jennifer K. Litzenberger, Denice C. Bay, Raymond J. Turner, Elmar J. Prenner*

Department of Biological Sciences, Faculty of Science, University of Calgary, 2500 University Drive NW, Calgary, Alberta, Canada T2 N 1N4

ARTICLE INFO

Article history:
Received 27 September 2012
Received in revised form
22 December 2012
Accepted 18 January 2013
Available online 4 February 2013

Keywords:
Lipid domains
EmrE protein
Lateral membrane architecture
Brewster angle microscopy
Monolayer
Lipid–protein interactions

ABSTRACT

Understanding lipid–protein interactions to enhance our knowledge of membrane architecture is a critical step in the development of novel therapeutic measures to respond to the drastic rise of drug resistant microorganisms. *Escherichia coli* contains a small archetypal inner membrane multidrug resistance protein, EmrE, that must multimerize to be functional but this multimerization is difficult to demonstrate *in vivo*. We studied three major *E. coli* lipids (phosphatidylethanolamine, phosphatidylglycerol and cardiolipin) that varied in head group structure, acyl chain length and saturation. These were investigated both in the presence and absence of EmrE to determine which lipid(s) EmrE influenced most strongly. Langmuir monolayers and Brewster angle microscopy demonstrated that varying each head group, acyl chain length and saturation contributed to differences in membrane packing and affected lipid–protein associations. Long unsaturated anionic lipids were influenced most strongly by EmrE. Shorter acyl chains initiated string-like formations of EmrE clusters, whereas longer chains contributed to enhance protein clustering. Longer partially unsaturated acyl chains in phosphatidylglycerol showed a significant surface pressure decrease in the presence of the protein, indicating that the monolayer was destabilized. Interestingly, longer unsaturated chains of cardiolipin formed the most stable monolayer in the presence of EmrE. These studies indicate cardiolipin acyl chains that hydrophobically match protein helical lengths stabilize EmrE structural forms.

1. Introduction

Biological membranes are important dynamic structures that not only provide structural support, but are also implicated in a variety of cellular processes including cell signalling and protein and lipid trafficking (Schug et al., 2012; Dietrich et al., 2001; Simons and Ikonen, 1997). To better understand the various roles of a biomembrane, it is important to study its major components: lipids and proteins. Extensive work has been done in the area of lipid domain architecture in eukaryotes (Simons and Ikonen, 1997). Most eukaryotic domains are comprised of specific lipids, including sphingolipids and sterols such as cholesterol. These specialized sites may be important for cell functions like signalling and transport (Lingwood and Simons, 2010). A similar membrane domain signalling hypothesis has been suggested for prokaryotes (Epand and Epand, 2009; Mileykovskaya and Dowhan, 2000) but even for well-studied organisms such as *E. coli*, much less is known.

E. coli are gram-negative bacteria that contain two lipid membranes separated by a thin layer of peptidoglycan. The inner membrane of the organism is made up of zwitterionic phosphatidylethanolamine (~75–80%), anionic phosphatidylglycerol (~20–25%) and anionic cardiolipin (~5–15%) (Cronan, 2003; Dowhan, 1997). Each of these phospholipids has the potential to provide different physical properties to a membrane, and some are involved in many cellular processes. Phosphatidylethanolamine (PE) usually serves as a bilayer-forming lipid, but can also assist in creating membrane curvature by adopting the H_{II} phase depending on the length and saturation of the acyl chains, as well as surrounding ions (Haney et al., 2010). Additionally, PE has been associated with membrane protein assembly and certain enzymatic functions in *E. coli* (Dowhan, 1997). Many proteins rely on lipids such as PE that are able to form hydrogen bonding for proper activity (Bazzi et al., 1992). Other proteins, such as the lactose permease Y protein (LacY), require PE for proper folding and activity (Dowhan and Bogdanov, 2009).

Phosphatidylglycerol (PG) is an abundant bacterial lipid with a negatively charged head group that may be an essential component of the *E. coli* membrane. Previous studies have shown that PG deficient mutants cannot replicate DNA properly, leading to

Abbreviations: BAM, Brewster angle microscopy; CL, Cardiolipin; DM, Dimyristoyl; DP, Dipalmitoyl; *E. coli*, *Escherichia coli*; EmrE, *Escherichia coli* multidrug resistance protein E; H_{II}, Inverted hexagonal phase; LacY, Lactose permease Y protein; L_C, Liquid condensed; L_E, Liquid expanded; PO, Palmitoyl oleoyl; PE, Phosphatidylethanolamine; PG, Phosphatidylglycerol.

* Corresponding author. Tel.: +1 403 220 7632.
E-mail address: eprenner@ucalgary.ca (E.J. Prenner).

0009-3084/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.chemphyslip.2013.01.007

cell apoptosis. The charged lipids PG and CL are also important for protein translocation and membrane stability, and have been implicated in interactions with proteins and peptides (Kusters et al., 1991; Xia and Dowhan, 1995). The lateral organization of PE and PG mixtures seems to be mainly driven by head group interaction and much less by lateral acyl chains (Pozo Navas et al., 2005). Recently, the preferential formation of 1:1 complexes of PE:PG has been reported (Wydro and Witkowska, 2009).

Cardiolipin (CL) can carry two negative charges depending on environmental conditions. The lipid has a larger cross-sectional area ratio of acyl chains (4 total) to head group (2 phosphates), allowing for non-lamellar phase formations (Schug et al., 2012; Dowhan, 1998; Mukhopadhyay et al., 2008). The relatively small head group allows for stronger intermolecular interactions (Lewis and McElhaney, 2009), and in combination with the four acyl chains, may make the phosphate groups more accessible to ligands such as drugs or metals as well as peptides or proteins (Lewis and McElhaney, 2009). CL can interact with protein amino acid residues through different means such as hydrogen bonding, electrostatic and van der Waals forces (Arias-Cartin et al., 2011; Romantsov et al., 2009, 2010), and several protein complexes require CL for proper folding and function (Lee, 2003; Hoch, 1992; Nichols-Smith et al., 2004). Some bacteria may control their lamellar/non-lamellar phase transition by their CL content (Lewis et al., 2007). CL domain formation was reported in mixtures with saturated PC and PE (Sennato et al., 2005). CLs can undergo rapid membrane modifications to form lateral domains and play significant roles in many cellular processes, including energy-dependent reactions (Gold et al., 2010; Mileykovskaya and Dowhan, 2000; Rietveld et al., 1993). It has been hypothesized that lateral lipid sorting may be driven in part by specific interactions with integral and peripheral proteins (Lehtonen and Kinnunen, 1997; Marsh, 1995). The strong impact of mitochondrial creatine kinase on model mitochondrial membranes has been demonstrated by the formation of protein-CL clusters (Maniti et al., 2011).

Although integral membrane proteins are difficult to purify and characterize because of their hydrophobic properties (Seddon et al., 2004), many have now been extensively studied with a variety of biophysical and structural approaches. Our model system, the *E. coli* EmrE protein, is a 12 kDa inner membrane multidrug resistance transporter that removes quaternary ammonium compounds from the cell (reviewed by Bay et al., 2008; Bay and Turner, 2009). The protein is part of the small multidrug transporter family and is made up of 110 amino acid residues. EmrE monomers oligomerize to form a functional unit, but details of this complex structure have remained elusive *in vivo*. It has been shown *in vitro* that 2–10 monomers form functional units, depending on experimental conditions (Bay et al., 2008; Bay and Turner, 2009). EmrE oligomerization is generally studied from a protein-protein interaction perspective; however, recent studies suggest that certain lipids influence protein multimerization and activity (Charalambous et al., 2008; Miller et al., 2009; Gröger et al., 2012). Charalambous et al. (2008) have proposed that anionic PG may alter EmrE transport activity in liposomes and Miller et al. (2009) suggested that PE lipids influence protein refolding because these lipids are known to increase lateral pressure in liposome experiments.

In a monolayer, short-range attractions between lipid molecules can lead to the formation of coexisting thermodynamic phases at the air–water interface (McConnell and Koker, 1996; Heinig et al., 2004). Monolayer formation depends on the degree of molecular interaction, from limited interactions in gas phase that increase consistently from liquid-expanded (L_E) to liquid-condensed (L_C), and finally to a solid phase. These phases often separate into distinct lateral domains, the size and shape of which is determined by the energetic compromise between repulsive and attractive forces (Heinig et al., 2004). Energetically unfavourable dipole–dipole forces and chemical potential gradients destabilize the domains, while attractive short-range interactions like van der Waals forces promote phase separation (Heinig et al., 2004). Increased line tension favours circular domains, while electrostatic repulsion between molecular dipoles favours non-circular architecture (Hu et al., 2006).

Lipid monolayer systems allow the study of the biological membrane processes and interactions in a highly controlled environment (Brockman, 1994). They have also been used for a variety of investigations using model mammalian and bacterial systems (Le et al., 2011). The stringent compositional control in mixed systems allows for highly reproducible data. The use of monolayers for the investigation of membrane proteins requires the selection of suitable protein targets. Several groups have published seminal work on the investigation of membrane proteins such as c-Fos (Borioli et al., 2001), rhodopsin (Lavoie et al., 2002) and bacteriorhodopsin (Lavoie et al., 1999). Salesse and co-workers have provided a comprehensive report on the use of membrane proteins in monolayers, indicating that protein stability is important when using this technique and care should be taken when selecting the protein of interest, as some are more prone to denaturation (Boucher et al., 2007). EmrE is an excellent candidate due to its highly hydrophobic composition that increases its stability in denaturing conditions, at 10 M urea and 5% SDS (Miller et al., 2009) or when the protein is dissolved in chloroform (Winstone et al., 2002).

Brewster angle microscopy allows label-free imaging of such lateral domains at the air–water interface (Nandi and Vollhardt, 2003; Hoenig and Moebius, 1991). The reflection of laser light at the Brewster angle is restored upon the spreading of a monofilm and the captured light is used for the real-time imaging of lateral architecture and changes upon compression or addition of membrane proteins. Whereas domain formation of mammalian lipids (Prenner et al., 2007; Milhiet et al., 2001) or proteins (Cruz et al., 2004; Maggio et al., 2005) has been extensively investigated, much less is known about the lateral organization of bacterial systems although domains have been proposed as well (Epand and Epand, 2009; Mileykovskaya and Dowhan, 2000). We have previously shown the formation of distinct protein clusters in bacterial models systems. These structures clearly protruded from the monolayer and exhibited a narrow size distribution, largely independent of protein concentration (Gröger et al., 2012). Overall, work in this area has demonstrated that the chemical nature of the different lipid head groups as well as the acyl chain length and saturation determine both lipid–lipid and lipid–protein interactions, and thus the formation of lipid domains and/or protein clusters. Such interactions also affect the shape and size of these structures.

To examine the influence of EmrE on the lateral lipid sorting hypothesis in more detail, *E. coli* lipids with different head groups, acyl chain lengths and degree of saturation were investigated *in vitro* using model membranes in the absence and presence of EmrE. Here we investigated lipid film stability and lateral domain formation of the major bacterial lipids (PE, PG and CL) with varying side chain architecture in the absence and presence of the integral membrane protein EmrE.

2. Materials and methods

2.1. Lipid solutions

All lipids (1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine [DMPE], 1,2-dimyristoyl-*sn*-glycero-3-phospho-(1'-rac-glycerol) [DMPG], 1,1′,2,2′-tetratetradecanoyl cardiolipin [TMCL], 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine [DPPE], 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) [DPPG],