Experimental Report

Proposal: **8-02-666** **Council:** 10/2012

Title: The effect of pH on biomimetic membranes containing novel lysylphosphatidylglycerol analogues.

This proposal is continuation of: 8-02-485

Researh Area: Biology

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Experimental Team: HARVEY Richard
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Local Contact: BARKER Robert

Samples: hDPPG
h3adLPG
Magainin peptide (GIGKWLHSAKKFGKAFVGEIMNS)
h3aLPG

Instrument	Req. Days	All. Days	From	To
D17	10	3	18/03/2013	21/03/2013

Abstract:

The bacterial lipid lysylphosphatidylglycerol (LPG) is a major component of the plasma membranes of a number of important human pathogens including Staphylococcus aureus, Mycobacterium tuberculosis and Bacillus anthracis. LPG is thought to alter the charge and order properties of the bacterial plasma membrane in order to render it resistance to a range of important cationic antimicrobials,
including the defensive peptides of our own innate immune system. We are currently investigating the regulation of expression of this lipid in Staphylococcus aureus, and its influence on membrane physical properties, in particular the molecular mechanisms by which it facilitates resistance to drug partitioning. The labile nature of LPG has meant that to date, biophysical studies of its role in drug resistance have been flawed and inconclusive. At KCL we have synthesized a number of novel stable LPG analogues specifically for
the purpose of modeling its role in defensive peptide resistance, using neutron reflectometry and allied techniques. In this investigation we are interested in studying the effect of environmental pH on the structure of LPG-containing bilayers and peptide resitance.

Introduction

The bacterial lipid lysyl-phosphatidylglycerol (LPG) (fig. 1) is a major component of the plasma membranes of important pathogens such as *Staphylococcus aureus*, *Bacillus anthracis* and *Mycobacterium tuberculosis*, the expression of which has been associated with resistance to antimicrobial peptides and antibiotics such as gentamycin and vancomycin [1]. The mechanism by which LPG confers resistance to all these cationic antimicrobials is primarily by dampening the anionic charge of the membrane and thus attenuating drug adsorption and membrane partitioning [1]. What this proposed mechanism ignores, however, is the fact that the ionisable groups of the LPG headgroup have different pK_as; the pK_a of the phosphate is ~3.0, the α amine pK_a is ~7.0 and the ε amine pK_a is ~10.0 [2]. This means that at pH ~6.5 (the pH of the outer leaflet of the plasma membrane in *S. aureus*) a proportion of the LPG will be zwitterionic, ensuring that the charge of the anionic lipids of the membrane (phosphatidylglycerol and cardiolipin) [3] predominates.

Environmental pHs below that of 6.5 induce increased expression of LPG is *S. aureus*, which can then mop-up excess environmental protons as its α amines become increasingly ionised. This in turn induces ion-pairing between the fully-ionised cationic LPG molecules and their anionic neighbours. This ion-pairing may affect the membrane structure through the formation of more rigid domains, providing another mechanism whereby drug partitioning is reduced, in conjunction with charge dampening. The biophysical experiments required to test such a hypothesis have yet to be conducted on LPG, due to its highly labile nature [4]. To this end we have synthesised a range of stable LPG analogues (Fig. 1), with similar physic-chemical properties specifically for the purpose of studying the behaviour of LPG in membranes under conditions which mimic those encountered by bacteria, in their natural habitats and when under attack by the body's defences.

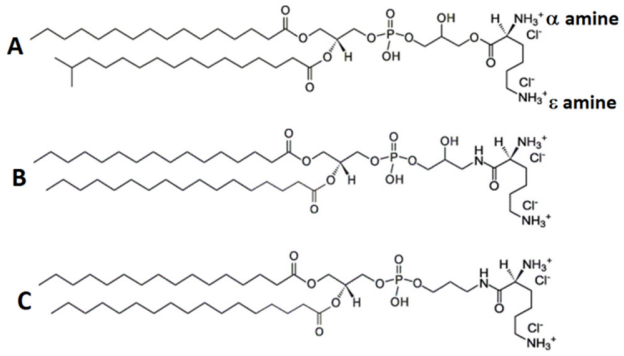

Fig. 1 Structures of (**A**) native bacterial lysyl-phosphatidylglycerol, (**B**) synthetic 3-aza-lysyl-phosphatidylglycerol (3aLPG) and (**C**) synthetic 3-aza-dehydroxy-lysyl-phosphatidylglycerol (3adLPG).

In this experiment we have studied the effect of pH on membranes containing LPG analogues to provide insight into the differential effects of environmental pH upon membrane structure.

Methods

A floating biomimetic bilayer composed of d_{62}DPPG/d_{62}3adLPG (70:30 mol%) was deposited onto a polished silicon block which had previously been silanized with 3-(trimethoxysilyl)propyl acrylate (TMPA) and derivatised with 1-palmitoyl-2-[16-(acryloyloxy)hexadecanoyl]-*sn*-glycero-3-phosphorylcholine (al-PC) [5], using Langmuir-Blodgett followed by Langmuir-Schaefer

deposition. The deposited bilayer was characterised on the D17 reflectometer at 55°C (to ensure that the lipids were in the fluid phase) using three solvent contrasts (H_2O, silicon-matched water and D_2O) over a range different pHs from 7.4 to 5.5 (specifically pHs: 7.4, 7.2, 6.8, 6.5, 6.2, 6.0, 5.8 & 5.5). All the solvent contrasts were buffered with Tris(hydroxymethyl)aminomethane (TRIS)–glacial acetic acid (AcOH) 1:1 mol/mol adjusted to the required pH with concentrated HCl or DCl.

Results and Discussion

The reflectivity data obtained from the bilayer at each different pH were simultaneously fitted for the three solvent contrasts using a least squares simplex algorithm with the RasCal software (v1.0.0), in order to obtain specific bilayer parameters and to construct SLD profiles (Fig. 2 and Table 1). What is clear from the results is that the ordering of the Staphylococcal mimetic bilayers is greatly influenced by the ionization state of the d_{62}3adLPG. In its predominantly zwitterionic state at pH 7.4, the bilayer structure is more disordered, exhibiting greater roughness and solvent penetration.

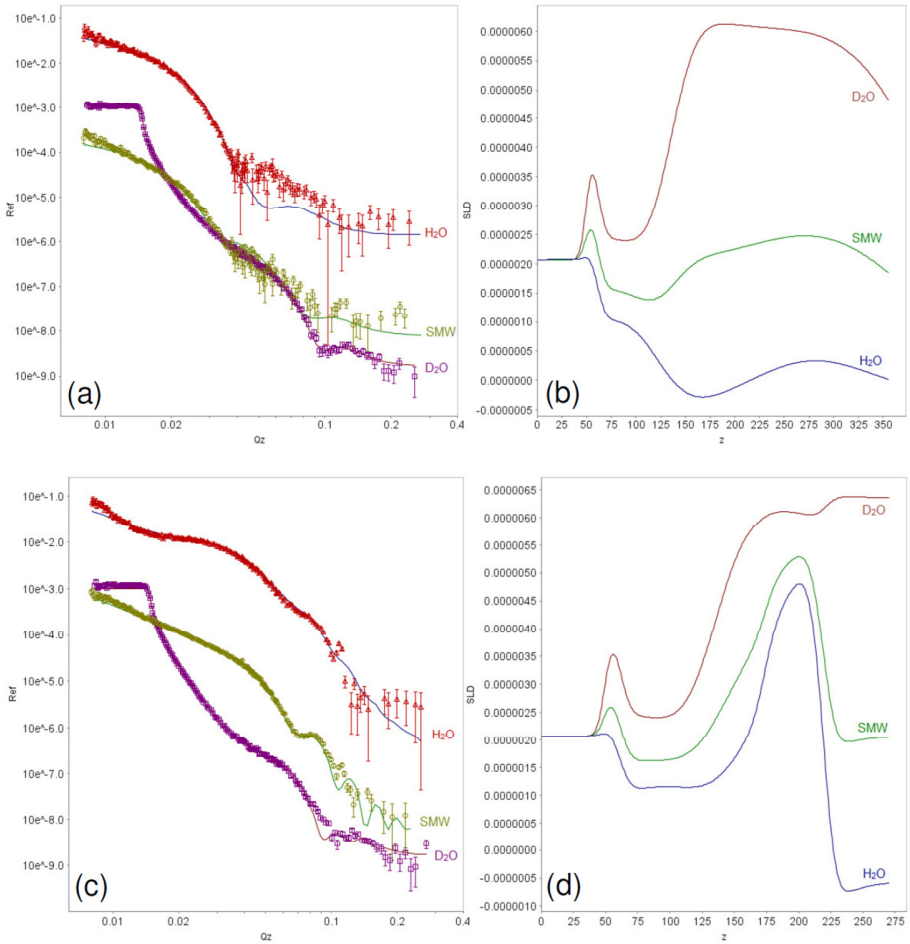

Fig. 2 Fitted neutron reflectivity curves of d_{62}PG/d_{62}3adLPG 7:3 at 55 °C, with (**a**) a pH 7.4 buffering system and (**b**) the derived fitted SLD profiles of each contrast used and with (**c**) a pH 5.5 buffering system and (**d**) the derived fitted SLD profiles of each contrast used.

At pH 5.5, where the majority of the headgroups of d_{62}3adLPG would be expected to be cationic, the bilayers present a much more ordered structure than was observed under neutral conditions. The bilayer exhibits reduced undulation and roughness and has less solvent penetration. This pH dependent ordering is probably due to the formation of ion-pairs between the two lipid components, reducing lateral packing stress caused by the excess of anionic lipid.

Table 1 Bilayer structural parameters for d62PG/d623adLPG 7:3 at 55 °C, in pH 5.5 and 7.4 buffers, obtained from the fitted reflectivity curves in Fig. 2.

pH	Layer	Thickness (Å)	SLD ($\times 10^{-6}$ Å^{-2})	Hydration (%)	Roughness (Å)
7.4	Water Layer	118.77 ± 19.6		100	
7.4	Head groups	8.00 ± 1.3	1.89	53.86 ± 7.4	23.14 ± 14.8
7.4	Chains	32.35 ± 3.4	6.79	26.44 ± 7.5	23.14 ± 14.8
5.5	Water Layer	28.99 ± 5.6		100	
5.5	Head groups	8.09 ± 1.3	4.08	16.23 ± 3.6	7.22 ± 2.70
5.5	Chains	31.40 ± 2.6	7.06	0.74 ± 0.4	7.22 ± 2.70

The threshold pH at which the bilayers began to adopt their more ordered conformation was pH 6.5, close to the pK_a of the α amine of the 3adLPG. This suggests that the bilayer requires a certain degree of acidity to reduce the intrinsic disorder present from containing a high proportion of anionic lipids. Below pH 6.5, there appears to be a gradual increase in the ordering effect which might be expected to be proportional to the increase in 3adLPG α amine ionization. Above pH 6.5 there was little observed difference in the reflectivity profiles or the bilayer parameters obtained from them, in comparison with the bilayer status at pH 7.4.

References

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[5] Hughes, A. *et al.* (2008) *Langmuir* **24**: 1989-1999