Experimental Report

Proposal: **8-02-653** **Council:** 4/2012

Title: Interaction of a novel quinolone analogue with model bacterial membranes

This proposal is a new proposal

Researh Area: Biology

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Samples: HT61 quinolone analogue
d62-DPPG
Trilysine peptide
d62-DPPC

Instrument	Req. Days	All. Days	From	To
D17	4	3	08/11/2012	11/11/2012

Abstract:

Persistent infections caused by non-reproducing bacteria are very difficult to treat with conventional metabolism-targetting antibiotics, because the cells are metabolically inert. This allows opportunistic pathogens such as Staphylococcus aureus to cause chronic colonisation and infections, which are a major problem for long-term hospital patients. HT61 is a novel quinolone analogue which has been shown to act preferentially against non-reproducing bacteria, and therefore shows promise as a therapeutic weapon against recalcitrant infections caused by S. aureus. Although the mode of action of HT61 is as yet unknown, evidence suggests that it may act through a membrane-disrupting mechanism, since it has been shown to depolarise S. aureus cell membranes, and to partition into bacterial-mimetic anionic monolayers at the air/water interface. We propose a neutron reflectometry experiment to determine the degree to which the membrane partitioning of HT61 may alter lipid bilayer parameters to be consistent with the observed cell depolarisation. This will provide important data for the elucidation of this novel antibiotic's bactericidal mechanism.

Introduction

Chronic infections caused by non-dividing "persister" bacteria of the species *Staphylococcus aureus*, are difficult to treat with conventional antibiotics. Quite apart from the increasing incidence of resistance to antibiotics amongst common strains of pathogenic staphylococci, the metabolism of non-dividing cells operates at such a reduced level that it ceases to function as a viable target for therapeutics [1]. One approach to tackling this problem of eradicating persistent infections has been to target the plasma membrane of non-dividing organisms, as functional integrity of the cell envelope remains a requirement for cell survival, even in cells which are otherwise metabolically inert [2]. Membrane active antimicrobials are not new and include widely studied substances such as chlorhexidine and an ever-increasing number of antimicrobial peptides. The use of such antimicrobials against persister staphylococci has innate limitations, not the least of them being the narrow selective toxicity of chlorhexidine [3] and the efficacy and formulation issues which are currently impeding the development of peptide-based antibiotics, even for use against topical infections [4]. One novel approach to tackle the problem of persister infections has been to develop membrane-active analogues of conventional antibiotics, which have selective toxicity against bacteria and lack the formulation and stability problems inherent with antimicrobial peptides. One such modified antibiotic is the quinolone analogue HT61, which has shown excellent activity against non-dividing staphylococci [1, 2]. Although the mechanism of action of HT61 is currently unknown, there is evidence to suggest that its bactericidal effect involves membrane activity, since it causes depolarisation of the plasma membrane of *S. aureus* cells when they are challenged by the drug [2].

Whether the membrane-activity phenomena exhibited by HT61 contribute directly to cell death or not, requires a more detailed examination of the interaction between the drug and *S. aureus*-mimetic membranes. If membrane partitioning is linked to the putative disruption of the plasma membrane required to cause depolarisation through the dissipation of ion gradients, then the minimum concentration of the drug required for bactericidal activity should elicit a gross alteration of membrane morphology [3]. Such morphological changes can include the induction of domain formation, an increase in bilayer disorder through lipid packing discontinuities, membrane thickening or thinning or the formation of pores.

Methods

Floating biomimetic bilayers composed of three mixtures of d_{62}DPPC/d_{62}DPPG (75:25, 50:50 and 25:75 mol%) were 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 bilayers were 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). After characterisation, each of the PC/PG bilayers was exposed to the quinolone analogue HT61 for one hour prior to flushing away excess drug and a repeat of the reflectivity measurements using the three solvent contrasts mentioned above.

Results and Discussion

The main aim of this study was to use neutron reflectometry to examine the interaction between HT61 and bacterial-mimetic bilayers, by determining the extent to which its association with

these membranes caused measurable effects upon parameters such as layer thickness, average area per molecule, SLD and tail hydration. With respect to the biological relevance of the different model membranes used in this study, the one composed of d62DPPC/d62DPPG (75:25 mol %) (Fig. 1) is closer in terms of the amount of net charge present in the membrane, to the situation which would be expected in bacterial cells.

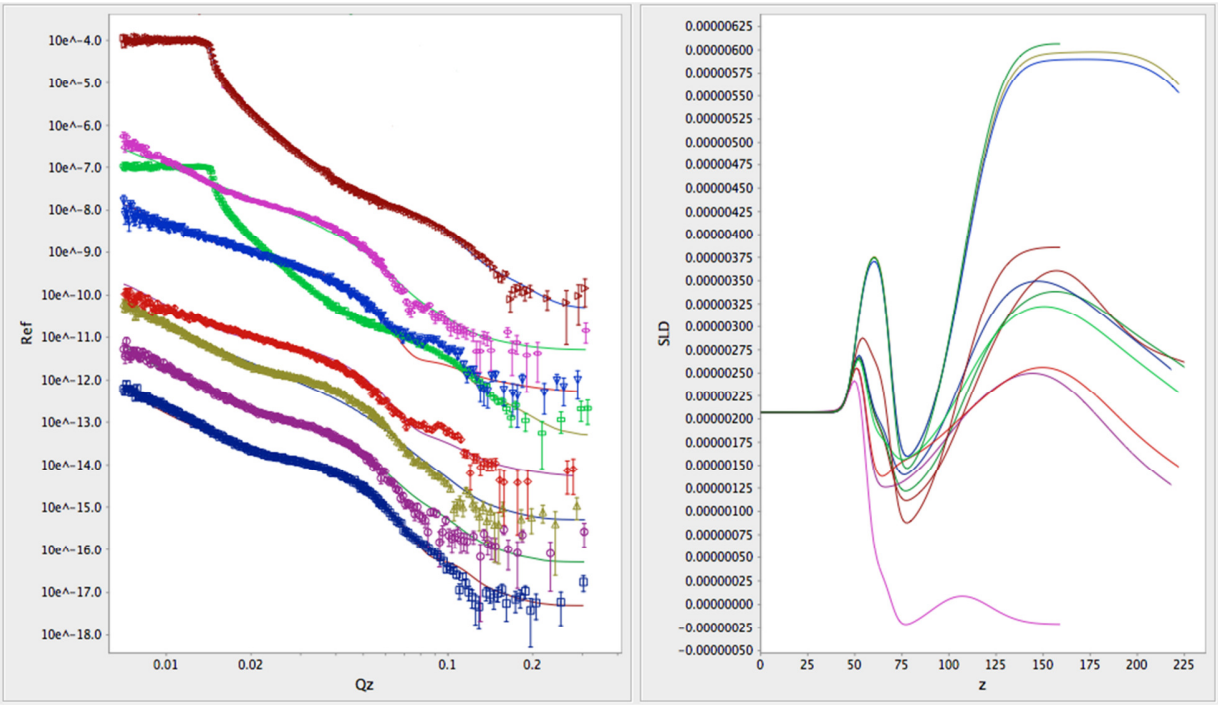

Fig. 1 Neutron reflectivity profiles obtained from floating bilayers formed of d_{62}DPPC/d_{62}DPPG (75:25 mol %) in different solvent contrasts (at 55°C) together with their derived scattering length density profiles.

The bilayer parameters obtained for the three floating bilayers from the fitting and analysis of the reflectivity profiles (Table 1) show some variability for the characterised samples prior to challenge with the drug, which may be attributed to the effects on lipid packing of altering their composition with increasing amounts of charged lipids. Increasing the amount of PG in the system would have an effect on bilayer stability due to the increased lateral repulsion introduced, which could have resulted in the formation of interdigitated bilayers, especially in the case of the sample containing 75% DPPG. It is reassuring to note that the solvent penetrating into the hydrophobic core of the bilayers does not increase with the addition of more PG, suggesting that any disruption caused in the packing does not destabilise the bilayer to a large degree and that any packing inhomogeneity would be compensated for by the putative interdigitation.

Table 1 Bilayer parameters derived from the fitted reflectivity curves and their associated SLD profiles for three d_{62}DPPC/d_{62}DPPG membrane mimetic floating bilayers pre- and post-exposure to the quinolone analogue HT61.

Sample	Bilayer thickness (Å)	Area per molecule (Å^2)	Tail hydration (%)
PC/PG (25:75)	44.2	102.9	2.0
PC/PG (25:75) + HT61	38.4	112.3	33.6
PC/PG (50:50)	35.6	90.6	0.0
PC/PG (50:50) + HT61	45.6	106.9	58.8
PC/PG (75:25)	27.9	97.4	0.0
PC/PG (75:25) + HT61	50.4	119.9	69.3

In all three cases, exposure to HT61 caused an increase in the average area per molecule and a large increase in the hydration of the tail region of the bilayers. Changes were also noted in the bilayer thickness, which may have been due to the action of the drug in disrupting the membrane and causing the formation of blebs from the surface. What is clear from these results is the relationship between the amount of PG present in the sample and the extent of the damage. One reasonable assumption which can be made is that the percentage tail hydration is directly related to the amount of membrane disruption caused, since this implies that the coverage of the block by the lipid bilayer is decreasing and being replaced by bulk solvent. This implies that major damage has been incurred as a result of exposure to HT61.

The obvious conclusions which can be drawn from these results are that the increase in DPPG results in a greater extent in membrane damage because of the affinity of HT61 for this anionic lipid. We can also be quite certain that HT61 causes catastrophic damage to the membrane through either the removal of lipids or the formation of pores.

References

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3. Gilbert, P. & L.E. Moore (2005) *J App Microbiol* **99**: 703-715.
4. Fjell, C.D., *et al.* (2012) *Nat Rev Drug Discov* **11**: 37-51.
5. Hughes, A.V., *et al.* (2008) *Langmuir* **24**: 1989-1999.