Experimental report

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Proposal: 9-13-730 **Council:** 4/2017

Title: NR study into the selective binding of designed short amphiphilic antimicrobial peptides into model lipid membranes

Research area: Physics

This proposal is a continuation of 9-13-611

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Samples: Rc-LPS
G(IIKK)3-4-I-NH2
lipids and sterols: POPE, POPG, CL, DOPC, DPPC, chol
GIGAVLKVLTTGLPALTSWIKRKRQQ (melittin)
G(ILKK)3L-NH2
KKK(KKII)2II-NH2
KI(KKII)2KI-NH2
C8-G(IIKK)2I-NH2
LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES (LL37)
GIGKFLHSAKKFGKAFVGEIMNS (magainin 2)

Instrument	Requested days	Allocated days	From	To
FIGARO Langmuir trough	4	3	20/03/2018	23/03/2018

Abstract:

Bacterial infections are becoming increasingly complicated to treat due to the ability of bacteria to gain resistance against many of currently used antibiotics. Antimicrobial peptides have been widely studied as potential antibacterial drugs to combat antimicrobial resistance. In our previous studies we have examined a series of designed amphiphilic AMPs with the sequence of G(IIKK)n-I-NH2 where n denotes the number of α-helical repeats (n=2-4, denoted as G2, G3 and G4). The G3 and G4 peptides have been shown to possess strong but also different antibacterial activity against both gram-positive and gram-negative bacteria. These designed peptides were structurally optimised, so they show better efficacy and better biocompatibility than most AMPs reported, natural or designed. However, we still do not have the molecular level of understanding about the exact mechanistic processes that lead to the effective killing of bacteria cells and mildness to mammalian host cells. Neutron reflection (NR) work as described here aims to provide more direct insight regarding the membrane disruption mechanism of G4 using model lipid monolayers mimicking the membrane of gram-negative bacteria.

NR Study into the Selective Binding of Designed Short Amphiphilic Antimicrobial Peptides into Model Lipid Membranes

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Introduction

Antimicrobial peptides (AMPs) disrupt bacterial membranes to kill pathogenic microbes. Because such structural damage is physical and difficult for microbes to evolve process to counter-act it, it is therefore hard for them to develop antimicrobial resistance. Natural AMPs are widely found in living species to fight against microbial invasions but they are restricted in their low efficiency, long sequence, and cost. In our AMP research over the past 10-15 years, we have developed a series of *de Novo* surfactant-like AMPs with the general formula $G(IIKK)_nI=NH_2$, where n indicates the number of α-helix repeats. Extensive studies have proved that when n=3, 4 (denoted as G_3, and G_4), peptides have potent antimicrobial activity and good biocompatibility [1]. Lipid monolayers can be constructed onto the air/water interface to provide a simple and straightforward model to help examine how different AMPs interact with model lipid membrane leaflets focused on lipid charge and saturation by neutron reflection (NR) on FIGARO [2, 3]. In this experiment we have studied the interactions of G_3 and G_4 with membrane compositions mimicking Gram-negative bacteria (lipopolysaccharide from the outer leaflet and the ternary membrane components from the inner leaflet). NR experiments helped unravel different structural features underlying membrane disruptions leading to the killing of the bacteria.

Materials and Methods

We constructed the inner and outer leaflets of the Gram-negative bacteria by using the POPE/DPPG/CL (7:2:1), rough C type lipopolysaccharide (RcLPS), respectively. Additionally, the ternary mixture of DOPC/DPPC/chol (1:1:1) has been used as the mimetic of the generally non-charged red blood cell membrane. Langmuir trough (Nima Technology Ltd, Coventry, UK) was used to create the air/water interface upon a subphase of 80 ml PBS buffer (pH=7.4). 4 isotropic contrasts were carried out by producing either deuterated or protonated lipid monolayers onto the null reflecting water (NRW) or D_2O, respectively. The lipid monolayers were spread onto the air/water interface and compressed to the surface pressure at 28 mN/m, followed by measuring the structures of the lipid monolayers by NR. Concentrated peptide solutions were then injected underneath the lipid monolayers to produce the required peptide concentrations. The interactions of the G_4 peptide with the ternary lipid mixtures were examined to continue the previous work to understand its selective affinity to the lipid membrane models. Binding of G_3 and G_4 peptides with the RcLPS monolayers was also studied to understand how the *E. coli* outer membrane responded to different AMPs. The NR experiments were carried out at two incident angles at 0.62° and 3.8°, respectively.

In NR data analysis, the dynamic lipid loss and peptide adsorption upon peptide binding was derived by the low Q-range data treatment of both deuterated and hydrogenated lipids with and without peptides in NRW. The structural characteristics of the lipid monolayers with and without peptides were resolved by the full Q-range data analysis involving all 4 isotropic contrasts. In the absence of peptide, lipid monolayers were treated by a 2-layer model consisting of the top layer in air and the bottom layer immersed in water. In contrast, the peptide-lipid systems were either fitted by a 2-layer or 3-layer model depending on the extent of peptide distributed underneath the head group region.

Results

To mimic the physiological condition of real membranes, the lipid monolayers were compressed to the high pressure of 28 mN/m under which the monolayer structures were determined by NR with and without peptide binding. The main structural parameters obtained are shown in Table 1. The area per molecule (A) of the pure RcLPS, POPE/DPPG/CL and DOPC/DPPC/chol monolayers at 28 mN/m was

determined to be 199 ± 2 Å^2, 65 ± 2 Å^2, and 53 ± 2 Å^2, respectively. Figure 1(A) shows the reflectivity profile of the RcLPS monolayers at the initial surface pressure at 28 mN/m under 4 isotropic contrasts. A 2-layer model fit and the SLD profiles were shown in Figure 1(C). The head group of the RcLPS layer was characterized to be 23 ± 2 Å, larger than the natural phospholipids with the size of around 10 Å.

The 2-layer fit was also appropriate to the binding of the peptide to the LPS monolayer as illustrated in Figure 1(B) and 1(D). After injections of G_3 at 3 μM, 20% of the LPS molecule was found to be removed. Due to the strong amphiphilicity of G_3, peptide molecules can quickly penetrate the lipid monolayer to the acyl chain reion. As a result, G_3 was found in both top layer and bottom layer with volume fractions of 20% and 15%, respectively. In contrast, G_4 at 1 μM was found to be more potent, with 45% of LPS molecules being removed. Additionally, more G_4 was found to bind to the LPS monolayer than G_3, with the surface concentrations of 0.23×10^{-6} and 0.34×10^{-6} mol/m^2 to the tail and head groups, respectively. Because LPS molecules are large and can strongly attract G_4 peptides via electrostatic and hydrophobic interactions, the LPS monolayer as the outer leaflet of the Gram-negative bacteria can hinder the antimicrobial efficacy of AMPs. The results clearly indicate that the amount of lipid loss and the extent of membrane structural damage cause by AMP binding are crucial parameters to account for their antimicrobial activities. Hence, the cationicity and hydrophobicity of the AMPs cannot provide improved antimicrobial efficiency due to their strong binding affinity to the bacterial defense molecules. Although G_3 is less effective in breaking the LPS integrity, its prominent mobility among the LPS molecules confers it with more competitive antimicrobial activity than G_4 peptides.

The structure analysis of the ternary lipid mixtures was carried out, and their interactions with G_4 peptides were compared to the previous studied unitary model [2, 3]. As demonstrated in Table 1, the peptide was distributed to both head and tail regions of POPE/DPPG/CL monolayer and peptide binding led to the removal about 10% of lipids in the process. By comparison with the previous published DPPG and POPG monolayers, the real *E. coli* inner membrane model (POPE/DPPG/CL) was less bound by G_4 at 3 μM. The red blood cell membrane model as composted of DOPC/DPPC/chol was rigid, and difficult to be disrupted by G_4 at 3 μM. As a result, little peptide binding was detected.

Sample	Layer	τ (Å)	Φ_{lipid}	$\Phi_{peptide}$	$\Phi_{solvent}$	A_{lipid} (Å^2)	Γ_{lipid} (10^{-6} mol/m^2)	$\Gamma_{peptide}$ (10^{-6} mol/m^2)
RcLPS (28mN/m)	Tail	13 ± 1	0.9 ± 0.11	N/A	N/A	199 ± 2	0.83 ± 0.08	N/A
	Head	23 ± 2	0.51 ± 0.05	N/A	N/A	198 ± 2	0.83 ± 0.08	N/A
G_3 3μM +RcLPS	Tail	13 ± 1	0.6 ± 0.06	0.2 ± 0.02	N/A	N/A	0.51 ± 0.05	0.19 ± 0.02
	Head	23 ± 2	0.55 ± 0.05	0.15 ± 0.02	0.4 ± 0.04	N/A	0.67 ± 0.06	0.28 ± 0.03
G_4 1μM +RcLPS	Tail	13 ± 1	0.5 ± 0.05	0.3 ± 0.03	N/A	N/A	0.43 ± 0.04	0.23 ± 0.02
	Head	23 ± 2	0.28 ± 0.03	0.25 ± 0.03	0.47 ± 0.05	N/A	0.46 ± 0.05	0.34 ± 0.03
POPE/DP PG/CL	Tail	16 ± 2	0.86 ± 0.01	N/A	N/A	63 ± 3	2.58 ± 0.2	N/A
	Head	9.5 ± 1	0.5 ± 0.05	N/A	0.5 ± 0.05	61 ± 3	2.75 ± 0.2	N/A
+ G_4 3μM	Tail	17 ± 2	0.76 ± 0.07	0.12 ± 0.01	N/A	69 ± 3	2.40 ± 0.2	0.11 ± 0.01
	Head	13 ± 2	0.33 ± 0.02	0.13 ± 0.01	0.54 ± 0.05	66 ± 3	2.52 ± 0.2	0.10 ± 0.01
DOPC/DP PC/chol	Tail	16 ± 2	0.92 ± 0.09	N/A	N/A	53.3 ± 2	3.12 ± 0.3	N/A
	Head	10 ± 1	0.57 ± 0.06	N/A	0.43 ± 0.04	53.2 ± 2	3.12 ± 0.3	N/A
+ G_4 3μM	Tail	15 ± 2	0.92 ± 0.09	N/A	N/A	56.7 ± 2	2.93 ± 0.3	N/A
	Head	12 ± 1	0.44 ± 0.03	0.05 ± 0.005	0.51 ± 0.05	59.9 ± 2	2.92 ± 0.3	0.03 ± 0.003

Table 1: Parameters of the two-layer model fits to the RcLPS, POPE/DPPG/CL, and DOPC/DPPC/chol monolayers at the surface pressure of 28 mN/m. Peptide adsorption and lipid removal were noticed after peptide injections.

Conclusion

Lipid monolayers as the mimicry of the cell membrane systems enabled us to characterize the structures upon AMP binding. NR results demonstrated that cationic G_4 peptides can selectively target at the anionic lipid membranes with lower binding affinity to the electrically neutral lipid systems. The electrostatic interactions of the positively charged AMPs and the negatively charged lipids endow the further peptide penetration and hydrophobic interaction. The charge differences of the bacterial membranes and the mammalian cell membranes were considered to be the origin of the peptide selective activity and biocompatibility. The ternary lipid model with 30% anionicity was less destructed

than the fully charged unary model by the G_4 peptides. LPS lipid was extracted from *E. coli* and the monolayer was formed by spreading the lipid on surface of water. G_4 can strongly disrupt the LPS layer even at 1 μM, but lose mobility once bound to the LPS molecules. In contrast, G_3 was found to be less disruptive to the LPS monolayer, and less binding to the lipid than G_4 but more potent than G_4. A manuscript is in preparation to describe the antimicrobial mechanisms of these AMPs with the outer and inner membranes of *E. coli*.

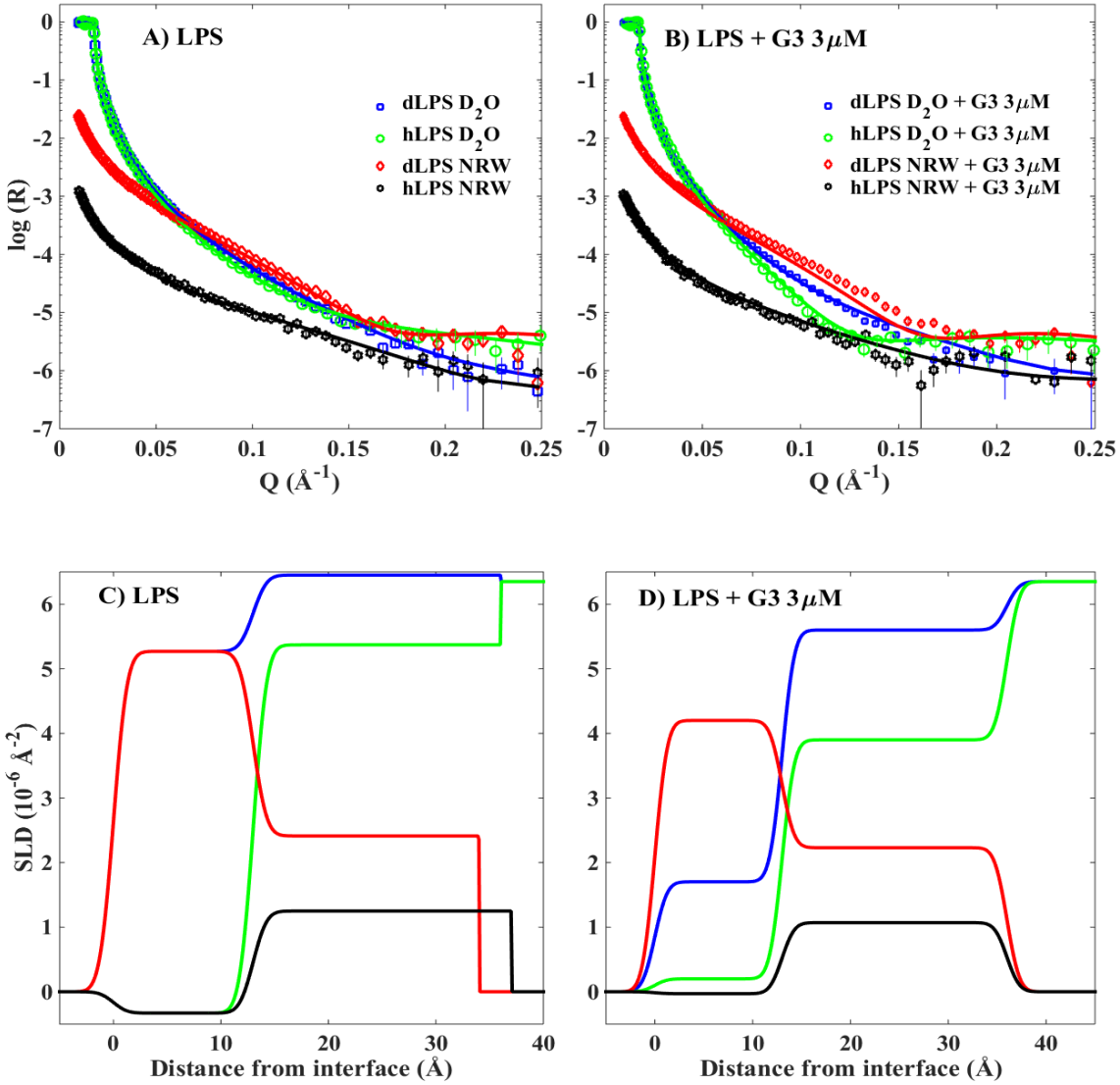

Figure 1: NR profiles and fits for (A) LPS monolayers at 28 mN/m and (B) with the addition of G_3 at 3 μM. A t2-layer model was used to fit the LPS monolayers with and without peptides, and the related SLD profiles were presented in (C) and (D).

References:

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