Experimental report

08/03/2017

Proposal: TEST-2552 **Council:** 4/2015

Title: NR study of short antimicrobial peptides

Research area:

This proposal is a new proposal

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Experimental team: Daniela CIUMAC

Local contacts: Richard CAMPBELL

Samples: d-DPPG & h-DPPG Lipids
h-peptide: G(IIKK)4-I-NH2
Phosphate buffer solution

Instrument	Requested days	Allocated days	From	To
FIGARO	1	5	16/11/2015	17/11/2015
			05/12/2015	07/12/2015
			27/08/2016	29/08/2016

Abstract:

Experimental report: N° **TEST-2552**

Dates of experiment **16-17.11.2015; 5-7.12.2015; 27-28.08.2016**

Title: **NR study of short antimicrobial peptides**

Introduction

The field of antibacterial resistance is gaining more and more attention after facing more than 30 years of lack of discovery of new antibiotics [1] and an increase in resistant superbugs [2]. Antimicrobial peptides (AMPs) are promising antibacterial agents, due to their membrane disruptive mechanism of action. Extensive studies have been undertaken to search for novel AMPs from various origins and sources. Rational design of new peptides aims to achieve high bactericidal activity and low toxicity. In this respect, we have designed, synthesised, and examined a series of cationic AMPs with the sequence of G(IIKK)n-I-NH_2 where n denotes the number of α-helical repeats (n=2-4, denoted as G2, G3 and G4) [3][4]. G3 and G4 peptides possess strong antibacterial activity against both gram-positive and gram-negative bacteria, and we have shown that bacteria can't easily acquire resistance against them [5]. However, we still do not have the molecular level of understanding about the exact mechanistic processes that lead to the selective killing of bacteria cells. By means of neutron reflection (NR) experiments we intended to study how the lipids charge, packing density and acyl chain saturation influence the G4 peptide binding affinity. This was done using model lipid monolayers created using a Langmuir trough.

Materials and methods

In all experiments the lipid monolayers were created at the air/water interface using a special Langmuir trough purposely designed to facilitate the neutron reflection measurement with due consideration of the sufficient beam footprint and liquid volume (80ml). The experiments were carried out using either null reflecting water (scattering length density = 0), or D2O as subphase. The lipids, dissolved in a suitable solvent, were spread on the surface of the buffer, and after solvent evaporation, they were compressed to the surface pressure of interest. Then the peptide solution (dissolved in PBS buffer) was injected in the subphase under the monolayer on the other side of the barrier (the final peptide concentration was 3 μM). This was done using a syringe with a long curved needle. The interactions were studied for different initial surface pressures: 8, 15, and 28 mN/m. During the beam time allocated for experiment #test2552 we examined the selective binding of G4 to anionic POPG and DPPG, and mixtures of DPPG/DPPE (30/70) and DPPG/TMCL (60/40), with the mixtures serving as bacterial membrane models. A first study performed in September 2015 (#9-13-611) involving DPPC and DPPG monolayers interaction with G4 injected at initial pressure of 15 mN/m has now been published in Colloids and Surfaces B: Biointerfaces [6].

In our studies we used hydrogenous and deuterated lipids (where available), on NRW and D2O. All the measurements were carried out at room temperature at 22±2°C. The data was acquired at two incident angles of 0.62° and 3.8°. The NR profiles were recorded firstly for the equilibrated lipid monolayers, then the peptide was injected in the subphase and the reflectivity was updated every 4 minutes for 80 minutes. We have used two approaches in our studies. The first one involved a low Q analysis to follow the compositional changes over time. The approach comprises resolution of the scattering excesses of two isotopic contrasts of lipid with peptide in NRW to give the surface excess of each component [7]. In the full Q analysis for the pure lipids a two layer model was used to fit the

data (the chain layer in air and the head group layer inside water), whereas for the peptide/lipid systems either a two or three layer model was needed in order to resolve the interfacial layer structure.

Results

The NR data for the peptide binding experiments was recorded at the same time as the surface pressure. The initial surface pressures before injection were 8 and 28 mN/m. Fig.1 shows the surface pressure changes against time over the first 90 min after peptide injection into the subphase under DPPG and POPG monolayers. After peptide injection, the surface pressure increased with different kinetics depending on the initial pressure. For both lipids there was a slow increase for low initial injection pressure, with the equilibrium in the pressure rise being reached after 40min, whereas in the case of high initial injection pressure, the penetration kinetics was much faster and went through a phase of overpressure. This can be explained by an orientation change of the peptide from "flat" to an "edgewise" position in the monolayer.

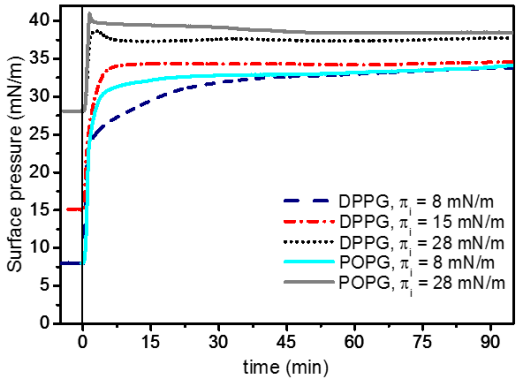

Figure 1 Surface pressure vs time changes after injection of G4 under DPPG and POPG at initial surface pressures of 8, 15 and 28 mN/m.

The low Q and full Q analysis on this system revealed that whilst less peptide is bound to POPG, the amount of lipid removed is similar to the amount of DPPG removed. An example of NR results is presented in Fig. 2, which shows the NR profiles for 4 contrasts for DPPG before and after peptide injection at the initial surface pressure of 28 mN/m. For the lipid only (Fig 2a) a two layer model fit revealed an acyl chain thickness of ~17.5 Å, and head group thickness of ~10.5 Å. For the equilibrium adsorbed peptide to the monolayer, the fitted values from the three-layer model were: 19.3 Å for the chains region, 13.2 Å for the head group and 13.9 Å for the peptide only layer. The peptide was found to have penetrated the lipid tails and head group regions. The total peptide adsorbed amount to DPPG monolayers was 0.98 μmol/m^2, whereas for POPG the peptide amount was 0.35 μmol/m^2 and a two layer model was found satisfactory to fit the full Q structural features of equilibrium adsorbed peptide to POPG monolayers. The results of this study involving monolayers composed of anionic DPPG and POPG for the study of acyl chain saturation influence and lipid packing density on the peptide binding affinity form the second manuscript soon to be send for publication.

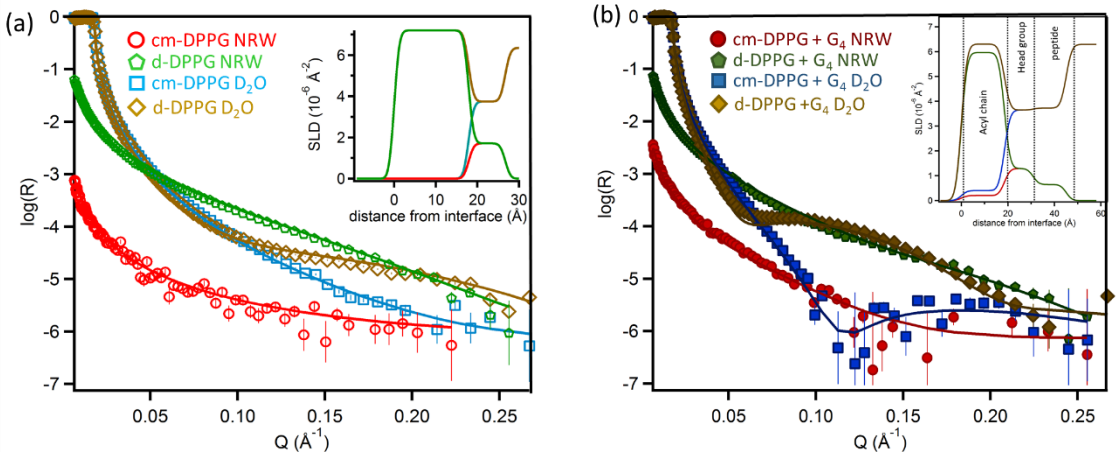

Figure 2. NR profiles with best fits and the associated SLD profiles for (a) DPPG and (b) equilibrium G4 adsorbed to DPPG monolayers at the initial surface pressure of 28 mN/m.

The real cellular membranes are complex systems, composed of many lipid types, accounting for about half the mass of the membrane. In order to gain a closer insight into how the G4 peptide selectively interacts with the red blood cells and bacterial membranes, more complex model membranes must be used. We have developed monolayers comprised of two and three components, mimicking the gram bacteria membrane. Fig. 1 shows the surface concentration values as a function of time for one component (DPPG and DPPC) and two component (DPPG/DPPE and DPPG/TMCL) monolayers plotted against time, with the amount of peptide binding shown simultaneously. The results clearly indicate the lipid removal from the interface whilst the peptide became associated with them. Moreover, we can see that the exact choice of the model system is very important in the characterization of the peptide interaction with the cell membranes.

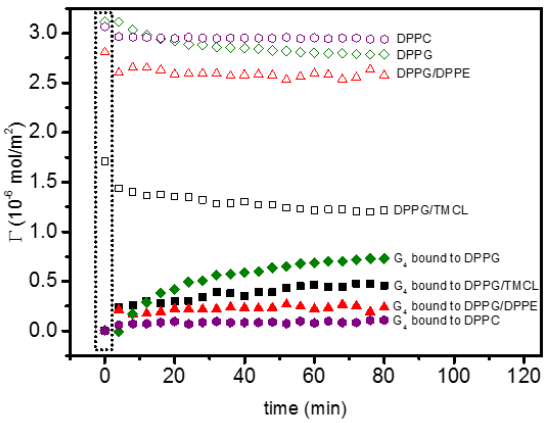

Figure 3. Surface concentration values as a function of time for one and two component model lipid monolayers representing simultaneous peptide binding together with the lipid loss over time scale of 80 min.

Conclusion

In summary, the results presented here, together with those obtained from other methods like FTIR (Fourier Transform Infrared Spectroscopy), and Brewster angle microscopy enabled us to understand the selective binding of G4 peptide to different lipid monolayer mimics. The first study involving charge characteristics implications on the selective interactions between G4 with DPPC and DPPG monolayers has been recently published. The second study regarding the effects of lipid packing density and tail saturation upon peptide binding to POPG and DPPG monolayers comprise the second manuscript ready to be send for publication.

References

[1] J. Davies, Microbiol. Mol. Biol. Rev., 417-433, 2006.
[2] F.C. Tenover, The American Journal of Medicine, 3-10, 2006.
[3] J. Hu, C.X. Chen, S. Zhang, X. Zhao, H. Xu, X.B. Zhao, J.R. Lu, Biomacromolecules, 3839−3843, 2011.
[4] C. Chen, J. Hu, P. Zeng, F. Pan, M. Yaseen, H. Xu, J. R. Lu, Biomaterials, 1552-1561, 2014
[5] C. Chen, J. Hu, P. Zeng, Y. Chen, H. Xu, J.R. Lu, ACS Appl. Mater. Interfaces, 16529−16536, 2014.
[6] D. Ciumac, R.A. Campbell, H. Xu, L. Clifton, A. Hughes, J.R.P. Webster, J.R. Lu, Colloids Surf. B, 308-316, 2017.
[7] R.A. Campbell, A. Tummino, B.A. Noskov, I. Varga, Soft Matter, 5304–5312, 2016.