Experimental report

08/02/2016

Proposal: 9-13-611 **Council:** 4/2015

Title: Neutron reflection to study the interaction of designed -helix peptides with model lipid monolayers at the air/water interface

Research area: Physics

This proposal is a new proposal

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Samples: DOPC
Sm
Cholesterol
DPPC
DPPE
Cl
DPPG
DOPG
LPS
LTA
RaLPS
Buffer: PBS
Peptide: G4
Surfactants: hydrogenated and deuterated Tween 80
For buffers: L-histidine and sodium phosphate

Instrument	Requested days	Allocated days	From	To
FIGARO Langmuir trough	5	4	10/09/2015	14/09/2015

Abstract:

Increase of antibacterial resistance to antibiotics has led to huge international demand for developing new antibacterial agents with different mechanisms of action. Many natural antimicrobial peptides (AMPs) can disrupt bacterial membranes and kill them without causing resistance. Over the past 10 years or so, we have developed a series of rationally designed peptides with the general sequence G(IIKK)nI-NH2, with n-the number of coils of α-helix (n=2-4, denoted as G2, G3 and G4). These peptides are benign to mammalian cell hosts, exhibiting high mildness or biocompatibility. To help understand their membrane lytic actions and selective responses, we have developed lipid monolayer models using Langmuir film technique and characterized how our peptides interact with model G+, G- and red blood cell monolayer models consisting of 1, 2 and 3 membrane components. In the first part of our neutron reflection work, we aim to quantity how peptide G4 penetrated and associated with lipid monolayers focusing on the examination of charge and unsaturation. The known antimicrobial peptides will be used as controls, thus enabling us to make better comparison.

Proposal: 9-13-611

Dates: 10-14.09.2014

Title: Neutron reflection to study the interaction of designed α-helix peptides with model lipid monolayers at the air/water interface

Introduction

Increase of antibacterial resistance to antibiotics has led to huge demand for developing new antibacterial agents with different mechanisms of action. Many natural antimicrobial peptides can disrupt bacterial membranes and kill them without causing resistance. Over the past 10 years or so, we have developed a series of rationally designed peptides with the general sequence $G(IIKK)_nI$-NH2, with n-the number of coils of α-helix (n=2-4, denoted as G_2, G_3 and G_4). These peptides are benign to mammalian cell hosts, exhibiting high mildness or biocompatibility.[1] To help understand their membrane lytic actions and selective responses, we have developed lipid monolayer models using Langmuir film technique and characterized how our peptides interact with model G+, G- and red blood cell monolayer models consisting of 1, 2 and 3 membrane components. By means of NR experiments we intended to understand how the lipids charge difference change the peptide-lipid binding affinity by examining the nano-scale resolution of interfacial peptide-lipids structures. For comparison we used as model monolayers DPPC (red blood cells model) and DPPG (bacteria cell membranes model).

Materials and Methods

In this experiment we examined the interaction of the G_4 peptide with model lipid monolayers at the air-water interface. We used a Langmuir trough with a subphase volume of 80 ml. The peptide solution was made using a PBS buffer (10mM, pH = 7.4). The experiments were carried out using either null reflecting water (NRW) with zero scattering length density as the subphase or D_2O. The lipid monolayer was created on the surface of water and after the solvent evaporation it was compressed and held at the required surface pressure. Then, the concentrated peptide solution was injected in the subphase underneath the lipid layer via a syringe with a curved needle from the outer side of the trough barrier with the final peptide concentration in the subphase of 3μM. Data was obtained using d_{62}-lipids and a mixture of deuterated and hydrogenated lipids (so called "contrast matched", cm-lipid, with about 5% d-lipid mixed with 95% h-lipids) to give a SLD of the tails matched to air or to NRW. The data was acquired at two incident angles of 0.62° and 3.8°. All the measurements were carried out at room temperature at 23±2°C. 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.

In our study we used a full Q-range analysis for the data to evaluate the structure characteristics of the layer, and 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.[2,3] In this work the approach is applied to a time-resolved adsorption process for the first time. For the low Q analysis we used the data from cm-DPPC, d-DPPC, cm-DPPG and d-DPPG, all measured in NRW, before and after injecting the peptide. For the structural analysis we used four contrasts: cm-lipid/NRW, cm-lipid/D_2O, d-lipid/NRW, and d-lipid/D_2O. 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 a 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 pressure before injection was 15 mN/m. At equilibrium, for DPPC monolayers the surface pressure increase was 10 ± 2 mN/m, and for DPPG monolayers the increase was 21 ± 3 mN/m.

The compositional analysis for the lipid-peptide mixtures, which enabled us to follow the individual surface excess values for lipids and the peptide over time was done using the low Q analysis method.[2,3] Figure 1 shows the surface lipid concentrations 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. It was found that the initial amount of DPPC and DPPG in the respective monolayer was similar. The dynamic peptide adsorption or binding to DPPC over a time period of 80 min was barely noticeable with a total peptide adsorbed amount of 0.13 μmol/m^2. On the other hand, in the case of the peptide-DPPG system, there was a clear time-dependent increase of the total peptide surface excess, with a final surface excess for the peptide reaching 0.73 μmol/m^2, with a simultaneous decrease of the amount of the lipid component.

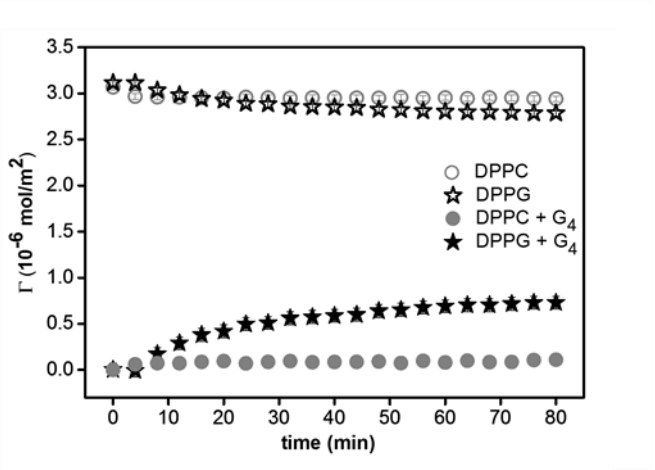

Figure 1 Surface concentration values as a function of time representing simultaneous peptide binding to the interface of DPPC and DPPG monolayers, together with the lipid loss for the DPPC and DPPG monolayers. Time zero is chosen for the pure lipids with the next point showing the first surface amount of peptide binding after the injection.

The structural analysis of the equilibrium adsorbed peptide DPPG interfacial layer was done on the full Q-range data. Figure 2a and 2b show the reflectivity profiles with the model-to-data fits for a two-layer model and the respective SLD profiles for a tilted condensed phase DPPG monolayers at surface pressure of 15 mN/m. Before the peptide addition the acyl chain thickness was ~15.8 Å and the head group has a thickness of ~8 Å, with the solvent content of ~33%. These values are in good agreement with results reported form previous studies.[4] For the equilibrium adsorbed peptide to DPPG monolayer, the fitted values from the three-layer model were: 16 Å for the chains region, 12.3 Å for the head group and 18.5 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 was calculated to be 0.67 μmol/m^2 (in agreement with the values calculated form the low Q analysis) with 13% adsorbed to the lipid acyl chain, 18% in the head group region, and 68% in the peptide only region.

Conclusion

In summary, the results presented here, using the monolayer model system, together with data obtained from other techniques such as Brewster angle microscopy, enabled us to understand the lipid differentiation and selectivity to the G_4 peptide, as well as the early stages of the peptide-membrane interactions. From our results, the peptide could clearly discriminate between the models for mammalian and bacterial cell membranes by lipid types. Nevertheless, at this stage of our research, it's too early to propose a specific action mechanism of membrane

disruption of these peptides. More studies and techniques are to be employed in order to fully characterise the $G(IIKK)_4I\text{-}NH_2$ peptide interaction with model cell membranes for different cell types which will help us to improve the performance of these peptides for producing future therapeutic agents in the ongoing antibiotic resistance battle. A manuscript is in preparation, which presents more detailed results obtained using the beam time allocated in September 2015.

The data obtained from two other initial surface pressures for the peptide/DPPG system are currently being analysed and will constitute an important part of the second paper related to this ongoing study.

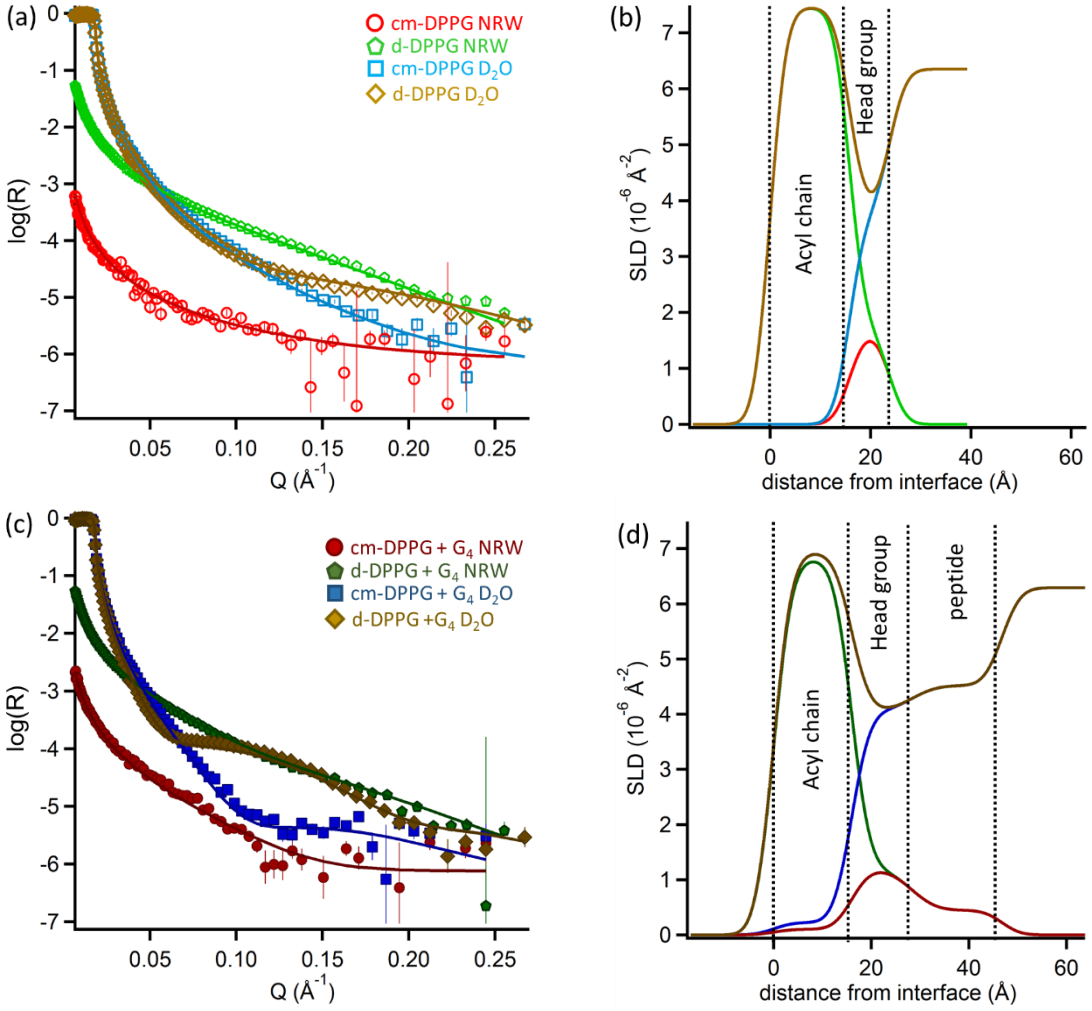

Figure 2. NR profiles for (a) a best two-layer fit for a DPPG monolayer at 15 mN/m and (b) the related SLD profile. (c) and (d) represent the NR profile for the best three-layer model fit to data of an equilibrium G_4 adsorbed DPPG monolayers at 15 mN/m and the associated SLD profile.

References

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