Experimental report

07/08/2017

Proposal: 9-13-659 **Council:** 4/2016

Title: SANS study of the interaction between antimicrobial peptides and model phospholipid liposomes

Research area: Physics

This proposal is a new proposal

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Samples: DOPC
d-DOPC
DOPG

Instrument	Requested days	Allocated days	From	To
D33	2	0		
D11	2	2	08/09/2016	10/09/2016

Abstract:

We have previously demonstrated that designed cationic AMPs with the general sequence of G(IIKK)nI-NH2 (n=3,4) show great selective antibacterial ability in co-culturing experiments. The high selectivity is thought to be related to the difference in charges between different cell membranes. We have developed lipid vesicle models mimicking mammalian and bacterial (G-, G+) outer cell membranes by controlling charges, saturation and membrane composition. In our previous lab work including zeta potential measurements, liposomes’ size variation, Cryo-TEM and encapsulation of (6)-carboxyfluorescein (CF) leakage, we found that the liposome systems undergo charge reversal, leakage, damage or fusion upon interacting with our peptide G4. Small angle neutron scattering (SANS) is a powerful and unique technique to determine the variations of vesicular lipid bilayer structure and composition associated with these phenomena upon G4 peptide binding. Furthermore, it is possible to determine the peptide location by a combination of several isotopic contrasts involving deuterated lipids and different H2O:D2O ratios.

Proposal: 9-13-659
Dates: 08/09/2016-10/09/2016
Title: Interaction between antimicrobial peptides and phospholipid liposomes by SANS

In the previous studies undertaken in the Manchester Biological Physics Group, we have demonstrated that a newly designed cationic peptides G(IIKK)$_4$I-NH$_2$ (G4) is effective at killing Gram-positive and Gram-negative bacteria and inhibiting cancer cell growth. [1,2] Under co-culturing conditions, they can selectively recognise and attack bacteria while showing no affinity to mammalian cell hosts. Further work using mouse and Xenograft models also confirmed these observations, further demonstrating the high selectivity and high potency of the peptides while displaying little toxicity. [3,4]

The aim of the SANS work was to illustrate the structural implications of the peptides toward lipid bilayers. In the first part of this work, we focused on studying the electrostatic initiation of the lipid-peptide interaction because one of the common differences between human red blood cells (hRBCs) and bacteria/cancerous cells is the negative surface charges distributed on the outer membranes. DOPC liposomes were made with diameters around 100nm by lipid extrusion and used as a control with no net surface charge. DPPG/DPPC mixture (20% DPPG) liposomes with the same size were used to mimic the bacterial membranes with negative surface charges.

The SANS data obtained from DOPC liposomes (LOWQ, ISIS) show little change with 0, 5 and 50 μM G4, confirming little association of the peptide to the membrane. However, the SANS profiles for the 20% DPPG/DPPC liposomes (ILL) are significantly different with 0, 1，3 and 5 μM G4. A simple lamellar model was used to fit the data from the negatively charged liposomes. Over the G4 concentration range studied, the thickness of the bilayer increased from 37 to 39 Å and the corresponding SLD increased from 0.88 to 1.02 × 10^{-6} Å^{-2}, showing a clear trend of increased peptide binding onto the bilayer as peptide concentration rose.

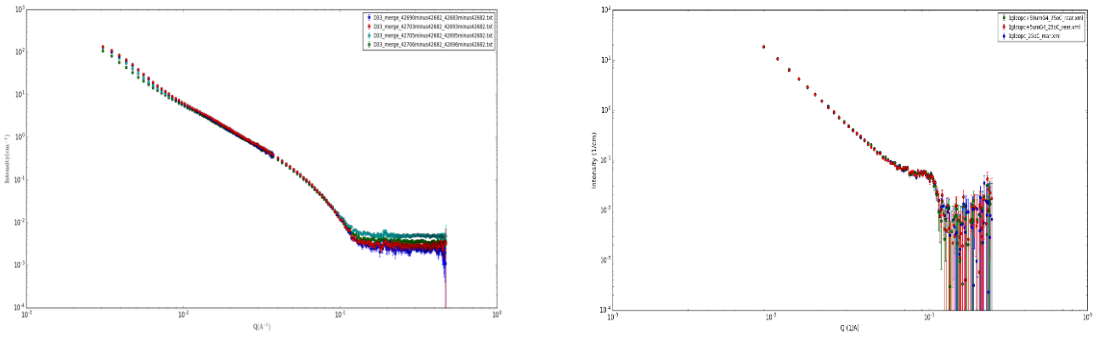

Figure 1. The SANS profiles on the left were from the liposomes of 20% DPPG/DPPC with 0, 1, 3 and 5 μM G4 peptide and those on the right were from the liposome data of DOPC with 0, 5 and 50 μM G4 peptide.

Another distinct difference between mammalian cells and bacteria is the presence of cholesterol in the mammalian cells. Cholesterol can play an important role in maintaining cell membrane structural integrity and fluidity. The outer membrane surface of cancerous cells may well contain charges as well as cholesterol. In this case, we studied the effect of the addition of cholesterol on the antibacterial interaction. Four liposomes with different component proportions were used in this study: 100%DOPC, 80%DOPC+20% Cholesterol, 60%DOPC+40%DOPG and 40%DOPC+40%DOPG+20% Cholesterol. From the SANS profiles obtained (not shown here), we could clearly see that liposomes with different components have different sizes and shapes, even though the method used to prepare the liposomes was the same. [5,6] These size and shape changes complicated the fitting process, but luckily these changes do not conceal the impact of peptide binding and association to the membrane bilayers.

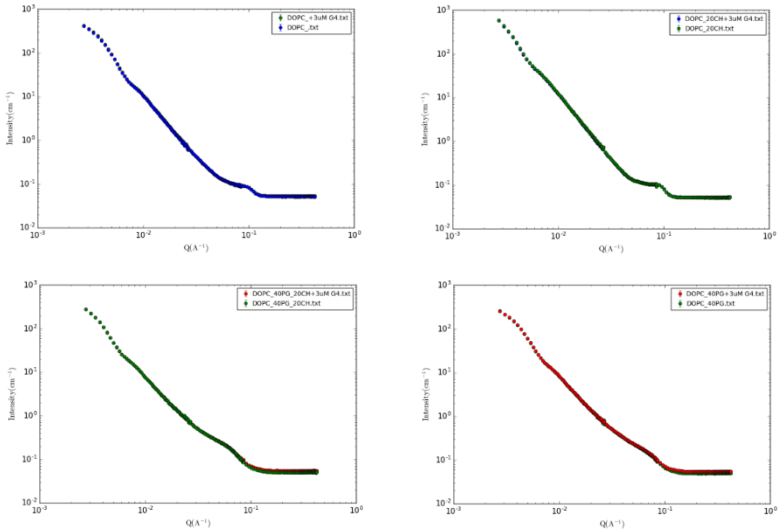

Figure 2. SANS data profiles showed the four different types of liposomes with and without 3 μM peptide G4.

As shown in Figure 2, there's little effect from the peptide G4 on the liposome containing only DOPC or DOPC and cholesterol. For the liposomes with 40% negatively charged lipids, the interaction can be observed from the profiles over the large Q range, indicating the peptide at this concentration didn't change the size and shape of the liposomes but already vary the structure of the liposomes' lipid bilayers. The negatively charged liposomes without cholesterol have a more obvious change with the peptide G4 binding than the negatively charged ones with cholesterol, meaning that cholesterol can influence cell membrane structural integrity by mediating peptide binding.

From the features of the SANS profiles, we can see that the liposomes are not simply unilaminar; other features will also need to be considered in fitting, especially for the liposomes without charge. A combined fitting model by considering the bilayer structure and the size and shape of the liposomes needs to be generated in the future to fit the data better.

References

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