Experimental report

03/04/2016

Proposal: 8-02-693 **Council:** 4/2014

Title: Interaction between TAT peptide and neutral phospholipid membrane

Research area: Biology

This proposal is a continuation of 8-02-555

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Samples: synthetic peptide/ synthetic phospholipid

Instrument	Requested days	Allocated days	From	To
D16	14	9	27/04/2015	05/05/2015

Abstract:

We are currently investigating the transmembrane mechanism of the cell-penetrating peptide TAT from human immunodeficiency virus. We have used D16 to determine the concentration-dependent distribution of peptide across negatively-charged bilayers and now wish to determine the corresponding distribution for zwitterionic bilayers. The data will complement, and aid in the interpretation of data from other biophysical methods, including differential scanning calorimetry and circular dichroism.

Interaction of TAT peptide with neutral phospholipid bilayer as revealed by neutron diffraction

Peptide distribution in DOPC lipid bilayer

A series of experiments have been conducted to compare the peptide distribution at two different concentrations (0.1 mol% and 1 mol%) in the DOPC lipid bilayers. As shown in Figure 1 and Table 1, the best fit to the observed peptide profile clearly shows that the interaction between TAT peptide and neutral DOPC lipid bilayer takes place at two discrete positions at low peptide concentration (0.1 mol%). A fraction of the peptides were deeply inserted into the hydrophobic core of lipid bilayer at around 5.8 Å from the center of the bilayer. The balance of the peptide remained in contact with the glycerol backbone region of lipid bilayer at 17.8 Å from the center of the bilayer. When the peptide concentration further increased (1 mol%), the peptide distribution at the hydrophobic core region completely disappeared and only one broad distribution was detected at 16 Å from the center of the bilayer. Compared to the distribution at 0.1 mol% peptide concentration, the center mass of the peptide shifts 1.8 Å toward the center of the bilayer and the width also expands from 4.2 Å to 6.4 Å, indicating that more TAT peptides are occupying the glycerol backbone region of the lipid bilayer. More importantly, no peptide was found in the peripheral aqueous phase between adjacent bilayers in any of the peptide concentrations investigated, which is a marked contrast to the previous neutron diffraction study on anionic DOPC/DOPS lipid bilayer. This supports the previous assumption that the electrostatic attraction between the negatively-charged headgroups of phospholipid and the positively charged TAT peptide is an obstacle rather than a facilitator for the intrinsic deep location of the TAT peptide in the glycerol backbone region of lipid bilayer. In addition, TAT peptide preferentially inserts into the glycerol backbone region and the insertion is independent of the negative-charged headgroups of DOPS phospholipid.

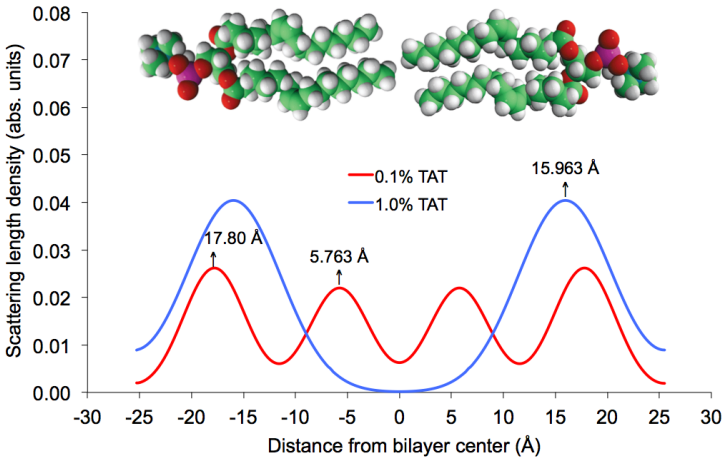

Figure 1. Summary of the Gaussian fits to observed peptide (difference SLD) profiles at a series of peptide concentrations, representing TAT peptide distributions in neutral DOPC bilayers. The interbilayer water compartment is at the two edges of the graph. A pair of phospholipid molecules is shown above the graph to illustrate the orientation of the lipid bilayer. The comparison is set to the same *D*-repeat.

TAT peptide distribution		L/P=100:0.1	L/P=100:1
Gaussian 1	Position (Å)	17.8	16.0
	Width (Å)	4.2	6.4
	Occupancy (%)	55.0	100
Gaussian 2	Position (Å)	5.8	
	Width (Å)	4.1	
	Occupancy (%)	45.0	

Table 1. Summary of the Gaussian fit parameters for the calculated peptide (difference SLD) profiles in reciprocal space. Five orders of diffraction were used in the fitting procedure. The position of TAT peptide is expressed as the distance from the center of the bilayer. The width is the full width at half height.

Furthermore, at the lowest peptide concentration (0.1 mol%), 45% of TAT peptide already penetrates deeply into the hydrophobic core region of the lipid bilayer at a position close to the double bond of fatty acyl chain 5.8 Å. With the introduction of more peptides into the lipid bilayer, the peptide distribution at hydrophobic core region completely disappeared. From a physicochemical point of view, it is quite surprising that a highly positively charged peptide carrying a net charge of +8 could penetrate so deeply into the hydrophobic core region. It is conceivable that the peptide distribution in the hydrophobic core region is due to an artifact of the sample preparation or the analysis procedure.

Water distribution in DOPC/DOPS lipid bilayer

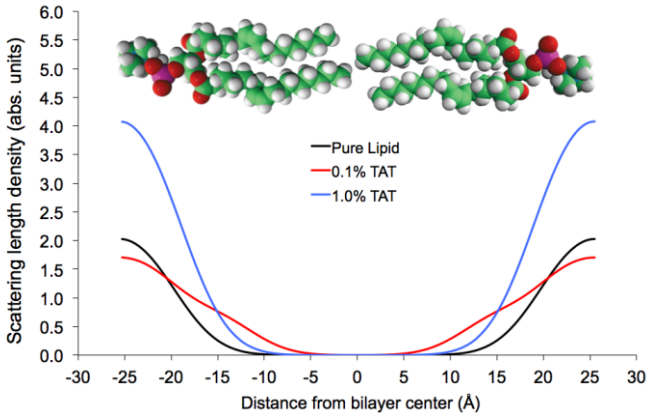

Figure 2. Summary of Gaussian fitting results to observed water (difference SLD) profiles at a series of TAT peptide concentrations in neutral DOPC bilayers. The interbilayer water compartment is at the two edges of the graph. A pair of phospholipid molecules is shown above the graph to illustrate the orientation of the lipid bilayer. All comparisons are set on the same *D*-repeat.

Further support for the deep penetration of the peptide into the hydrophobic core region is given by the water distribution across the bilayer. The dependence of the SLD profile on the D_2O content in water vapor enabled the calculation of the water distribution across the bilayer by subtracting the structure factors 8.06% D_2O from those at high D_2O concentration (25%). All fitted water profiles are presented in Figure 2 for easy comparison and the resultant parameters from the Gaussian fitting are summarized in Table 2.

Usually, the distribution of water can be accurately described as a single Gaussian peak centered near the edge of the crystallographic unit cell. Comparing the water distribution profiles in the absence and presence of different amounts of peptide, a markedly different distribution pattern was found in the sample with 0.1 mol% peptide. Notably, the best fit to this water profile could only be modeled by two discrete Gaussian peaks, representing two water populations across the bilayer. One is located in the aqueous bulk between adjacent bilayers at 22.5 Å from the center of the bilayer and the second is located in the glycerol backbone region at 15.0 Å from the center of the bilayer. Uniquely two discrete water distributions originate from the intercalation of TAT peptide into the hydrophobic core region of bilayer, which requires more water penetrates into the bilayer to hydrate the peptide.

All the above data supports the previous assumption that the electrostatic interaction between the negatively-charged headgroups of phospholipids and the positively charged TAT peptides to some extent is an obstacle rather than a facilitator for the intrinsic deep location of TAT peptide into the glycerol backbone region of the lipid bilayers.