Changes in Lipid Bilayer Structure caused by the Helix-to-Sheet Transition of an HIV-1 gp41 Fusion Peptide Derivative

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Abstract

HIV-1, like other enveloped viruses, undergoes fusion with the cell membrane to infect it. Viral coat proteins are thought to bind the virus to the membrane and actively fuse the viral and cellular membranes together. The actual molecular mechanism of fusion is challenging to visualize, resulting in the use of model systems. Here, the bilayer curvature modifying properties of a synthetic variant of the HIV-1 gp41 fusion peptide with lipid bilayer vesicles composed of a mixture of dimyristoyl phosphatidylcholine (DMPC) and dimyristoyl phosphatidylserine (DMPS) were studied. In 7:3 DMPC:DMPS vesicles made with deuterium-labeled DMPC, the peptide was observed to undergo a concentration-dependent conformational transition between an α -helix and an antiparallel β -sheet. Through the use of small-angle neutron scattering (SANS) and selective deuterium labeling, it was revealed that conformational transition of the peptide is also accompanied by a transition in the structure of the lipid bilayer. In addition to changes in the distribution of the lipid between the leaflets of the vesicle, the SANS data are consistent with two regions having different thicknesses. Of the two different bilayer structures, the one corresponding to the smaller area fraction, being ~8% of the vesicle area, is much thicker than the remainder of the vesicle, which suggests that there are regions of localized negative curvature similar to what takes place at the point of contact between two membranes immediately preceding fusion.

Keywords: HIV-1 fusion peptide, small-angle neutron scattering, intrinsic curvature.

1. Introduction

Infection by HIV-1 involves fusion of the viral envelope with the cell membrane. The process involves two glycoproteins from HIV-1: gp120, which binds to a receptor on the cell and gp41, which interacts with the target membrane (Freed and Martin, 1995). The N-terminal 23-residue sequence of HIV-1 glycoprotein gp41, which is termed the fusion peptide (FP¹), plays a key role in the fusion of the virus with a cell by anchoring the virus to the target membrane (Freed and Martin, 1995). Introducing mutations into this region of gp41 degrades the ability of the virus to fuse with a cell. For example, replacing hydrophobic amino acids with polar ones results in a virus that does not fuse with cells as well as the wild-type virus (Bergeron et al., 1992; Felser et al., 1989; Freed et al., 1992; Freed et al., 1990).

Biophysical studies using synthetic peptides derived from the FP domain have been used as surrogates for obtaining a molecular-level understanding of how the FP domain interacts with membranes (Castano and Desbat, 2005; Curtain et al., 1999; Martin et al., 1996; Mobley et al., 1999; Nieva et al., 1994; Pereira et al., 1997; Rafalski et al., 1990; Saez-Cirion and Nieva, 2002; Slepishkin et al., 1990). While wild-type peptides and some point mutations promote fusion, point mutations that produce less functional full-length proteins also result in less effective peptides. Fusion also depends on a variety of factors including the composition of the vesicle and the peptide

¹ Abbreviations: FP, fusion peptide; SANS, small-angle neutron scattering; DMPC, dimyristoyl phosphatidylcholine; DMPS, dimyristoyl phosphatidylserine; P/L, peptide-to-lipid ratio; d54-DMPC, chain perdeuterated dimyristoyl phosphatidylcholine; CD, circular dichroism spectroscopy; DLS, dynamic light scattering; SLD, scattering length density; TFE, 2,2,2-trifluoroethanol; HG, head group; HC hydrocarbon chain; POPC, 1-palmitoyl-2-oleoyl phosphatidylglycerol; POPG, 1-palmitoyl-2-oleoyl phosphatidylglycerol; POPS, 1-palmitoyl-2-oleoyl phosphatidylserine.

concentration. The concentration of divalent cations can also impact peptide-driven fusion (Nieva et al., 1994).

Fusion correlates with what is perhaps the most interesting feature of the HIV-1 gp41 FP, namely its lipid- and concentration-dependent conformation. The gp41 FP and variants adopt either an α -helical or a β -sheet structure when associated with lipid bilayers. Broadly speaking, neutral phospholipids and lower peptide concentrations promote the α -helical conformation (Martin et al., 1996; Nieva et al., 1994; Pereira et al., 1997; Rafalski et al., 1990). The α -helical form of the wild-type peptide is inserted into the membrane at an oblique angle relative to the bilayer normal (Castano and Desbat, 2005), much like the simian immunodeficiency virus fusion peptide (Bradshaw et al., 2000). Mutant gp41 FPs have been observed to lie with their helical axis more parallel to the membrane surface (Martin et al., 1996). The α -helical conformation forms ion-conducting channels in black lipid membranes (Slepushkin et al., 1990). In contrast, charged phospholipids and higher peptide concentrations correlate with the β -sheet conformation responsible for enabling fusion (Martin et al., 1996; Mobley et al., 1999; Nieva et al., 1994; Rafalski et al., 1990), and divalent cations promote this structure (Nieva et al., 1994; Pereira et al., 1997; Saez-Cirion and Nieva, 2002). Other viral fusion peptides display similar helix-to-sheet transitions (Agopian and Castano, 2014; Yao and Hong, 2013), suggesting that the β -sheet conformation impacts the lipid bilayer structure in a way that promotes fusion.

Here, conformational changes of a derivative of HIV-1 gp41, having the sequence RKGIGALFLGFLGAAGSTMKR and hereafter referred to as gp41rk, interacting with

lipid bilayers composed of dimyristoyl phosphatidylcholine (DMPC) and dimyristoyl phosphatidylserine (DMPS) lipid were correlated with changes in the structure of lipid bilayer vesicles using circular dichroism spectroscopy (CD), dynamic light scattering (DLS) and small-angle neutron scattering (SANS) with contrast variation and selective deuteration (Heller, 2010). Importantly, the SANS data, which were collected using deuterium-labeled DMPC and unlabeled DMPS, show that higher concentrations of gp41rk, where the peptide adopts a β -sheet structure, also resulted in a structural transition in the lipid bilayer vesicle. The thickness of the bilayer did not change for concentrations up to and including $P/L = 1/200$, when the peptide is α -helical, although there were changes to the internal structure and scattering length density (SLD) profile of the bilayer with increasing peptide concentration. The transition of the peptide to a β -sheet structure created a vesicle in which two different bilayer structures were present. The dominant region, being $\sim 92\%$ of the vesicle, was slightly thinner than the peptide-free bilayer, while the minor area fraction was much thicker. The results suggest a localization of the gp41rk that induces a negative curvature that would promote fusion.

2. Materials and Methods

2.1. Materials. The amidated form of the gp41rk peptide, having the sequence shown in Table 1, was synthesized by GenScript (Piscataway, NJ, USA). The modifications to the gp41 fusion peptide, which left the central 17 amino acids identical to residues 521-537 of the wild-type gp41 sequence, were made with the intent of improving solubility for the peptide synthesis and reducing the propensity of the peptide

for causing fusion, which would complicate the interpretation of the SANS data. The peptide was provided at 91.2% purity, as determined by HPLC, and was used without further purification. An 11 mg/mL stock solution of gp41rk was prepared in 2,2,2-trifluoroethanol (TFE) for preparing the lipid vesicle solutions with peptide. Chain perdeuterated DMPC (d54-DMPC; product 860345) and DMPS (product 840033) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). Chloroform was obtained from Amresco, LLC (Solon, OH, USA; purchased from VWR International, LLC, Radnor, PA, USA), TFE was obtained from Fisher Scientific USA (Pittsburg, PA, USA), and D₂O (99.8% D) was obtained from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA). These chemicals were also used without further purification. Deionized water (18.2 MΩ) was obtained from a Barnstead DI water system (Thermo Fisher Scientific, Inc.; Waltham, MA, USA).

2.2. Vesicle Solution Preparation. Vesicle solutions were prepared as described previously (Qian and Heller, 2011, 2015; Qian et al., 2014; Rai et al., 2016). Briefly, appropriate amounts of lipid and peptide stock solutions, being in chloroform (d54-DMPC), TFE (gp41rk) or a chloroform:TFE mixture (DMPS), were mixed to the appropriate molar ratios. The d54-DMPC:DMPS molar ratio was 7:3 and the P/L were 1/50, 1/200, 1/500 and 1/1000. A peptide-free vesicle sample was also prepared. The organic solvent was blown off under a stream of dry nitrogen and the samples were freeze dried overnight to remove any remaining organic solvent. Then, D₂O was added to the samples to achieve a lipid concentration of 20 mg/mL. The samples were subjected to

thorough vortexing and three freeze-thaw cycles between -80 °C and 40 °C. After the final thawing, the unilamellar vesicles were prepared *via* extrusion with an Avanti Polar Lipids, Inc. (Alabaster, AL, USA) mini-extruder fit with a polycarbonate membrane having 100 nm diameter pores. The final samples in 50% D₂O/50% H₂O, 70% D₂O/30% H₂O and 100% D₂O were made from the extruded vesicle stock solutions by a 50% dilution with appropriate amounts of H₂O and D₂O. Samples not used immediately for SANS measurements were stored up to 24 hours in an incubator set at 12 °C to avoid freezing the samples (D₂O freezes at ~4 °C).

2.3. Solution Circular Dichroism. All spectra were collected using a Jasco J-810 CD spectropolarimeter (Tokyo, Japan) having a Jasco PFD-425S Pelletier temperature controller maintained at 37 °C. A lipid-free aqueous solution of gp41rk, prepared from the TFE stock by blowing off the organic solvent, freeze drying the peptide for 4 hours and then re-suspending it in H₂O at 11 mg/mL, was measured using a 1 mm path length quartz cuvette and the data were corrected for the water background. Vesicle solutions were measured in 0.1 mm path length quartz sample cells having detachable windows from Hellma (Müllheim, Germany). By using a very short path length, it was possible to measure the samples that were prepared for the SANS experiments while minimizing artefacts resulting from light scattering by the vesicles. Depending on the P/L of the sample, CD data were collected from 180 nm to 260 nm using a step size of either 0.2 nm or 0.5 nm and up to 20 spectra were averaged. Data collected with gp41rk were corrected for the CD background due to the vesicles.

2.4. *Dynamic Light Scattering.* Dynamic light scattering (DLS) data were collected using a Wyatt Technology Corporation (Santa Barbara, CA, USA) DynaPro Nanostar instrument. Samples were maintained at 37 °C during the measurements and were stored in a 37 °C oven in between measurements to enable tracking the behavior of the vesicle size over a period of time similar to the maximum allocation of time for a SANS experiment (~3 days maximum, although the 37 °C measurements presented here were finished in ~1 day). The values presented are the averages and standard deviations taken from 10 measurements.

2.5. *Small-Angle Neutron Scattering Measurements.* SANS data were collected using the EQ-SANS instrument of the Spallation Neutron Source at Oak Ridge National Laboratory (Zhao et al., 2010). Vesicle solutions and the associated vesicle-free backgrounds were measured. A single configuration of the instrument was used for all of the measurements. The sample-to-detector distance was set to 4m, while the choppers were set to run at 30 Hz to provide two bands of neutrons, one from 2.5 Å to 6.1 Å and the second from 9.4 Å to 13.1 Å. The configuration covers $q = 4\pi\sin(2\theta)/\lambda$, where 2θ is the scattering angle and λ is the neutron wavelength, from $\sim 0.005 \text{ Å}^{-1}$ to $\sim 0.45 \text{ Å}^{-1}$. The sample temperature was maintained at 37 °C. All data reduction into $I(q)$ vs. q and merging the data from the two wavelength bands employed the standard procedures that are implemented in the MANTID software package (Arnold et al., 2014). The data were

scaled into units of 1/cm during the data reduction by using a calibrated standard as a reference (Wignall and Bates, 1987).

2.6. SANS Data Analysis. Analysis of the SANS data was built around the polydisperse core, multishell spherical model developed for previous work (Qian and Heller, 2011, 2015; Qian et al., 2014; Rai et al., 2016). The specific model for the bilayer profile utilized is described below, and it should be noted that the material composition approach for defining the SLD profile of the bilayer used for melittin in the same lipid composition (Rai et al., 2016) was not employed for the results presented here due to the need to use the two bilayer profile model (described below) for the data collected at P/L = 1/50. The q -range used for the modeling was $0.01 \text{ \AA}^{-1}$ to $0.35 \text{ \AA}^{-1}$, which spans the length scales of interest for the structure of the bilayer and avoids the high and low ends of the range measured (down to $0.004 \text{ \AA}^{-1}$ and up to $0.45 \text{ \AA}^{-1}$) that have the greatest potential to be impacted by inelastic neutron scattering processes in the sample that the instrument resolves (Do et al., 2014). One model profile was calculated from the model structure for each solvent D₂O content, thereby creating a contrast variation series of model SANS profiles. The model profiles were convoluted with the resolution function of the instrument that was output by the data reduction (i.e. ‘smeared’) prior to comparison against the SANS data using the fitting parameter described previously for comparing a set of model profiles against contrast series of SANS data (Heller, 2006; Tjioe and Heller, 2007).

The bilayer profile model employed for the lower concentrations of gp41rk divides the structure into 4 layers that roughly correspond to the inner head group (HG), inner hydrocarbon chain (HC), outer HC, and outer HG regions. The thicknesses and scattering length densities of the four layers were variables for the fitting. A Gaussian polydispersity model for the inner core radius of the vesicles was employed for the calculation of the model intensity profile. Both the inner core radius and the polydispersity were variables used for the fitting.

Due to poor convergence of the fitting for $P/L = 1/50$ with the single bilayer profile model, a second model was employed for fitting the data that utilized two different bilayer profiles, which is a method used previously (Qian and Heller, 2015; Qian et al., 2014). In the present work, two different 4-layer structures were employed that correspond to two regions of different composition. The regions were assumed to be structurally uncorrelated. The thicknesses and scattering length densities of the layers were variables used for the fitting. Here, the bilayer mid-plane radius, being the inner HC/outer HC boundary, and its polydispersity were also variables used for the fitting. The additional parameters, while complicating the search through parameter space, do not represent an unreasonable number of parameters for the three SANS data sets being simultaneously fit over the aforementioned q -range (Moore, 1980).

Regardless of the model of the structure of the vesicle employed, the data fitting software employed a modified simulated annealing approach to find the best fit to the data in which 50 temperature steps involved testing 2500 models, followed by another 50 temperature steps in which 200 models were tested at each step. Convergence and

reproducibility of the fitting was done by performing 20 independent fitting runs for all P/L except 1/50, for which 80 independent fitting runs were performed due to the increased complexity of the search space. The random number generator was seeded at the start of program execution with the system time plus the system process ID, which ensured sampling of different models. Initial runs of the modeling employed broad constraints on the layer thicknesses and number of waters associated with the HG regions. The results of the initial runs were used for additional refinement runs based on what appeared to be convergent solutions. The averages and standard deviations from the resulting set of parameters from the converged models from the refinement runs were calculated and are reported herein.

3. Results and Discussion

3.1. gp41rk Undergoes a Concentration-Dependent Helix-to-Sheet Transition.

The conformation of gp41rk in the 7:3 d54-DMPC:DMPS lipid bilayers was characterized using CD. The data, along with the spectrum collected from the peptide in lipid-free aqueous solution, are presented in Figure 1. Without vesicles, the peptide adopts a random coil conformation. When associated with vesicles, the CD results clearly demonstrate that the peptide becomes better-structured. Further, it undergoes a concentration-dependent conformational transition. At the three lowest concentrations studied ($P/L = 1/1000$, $1/500$ and $1/200$), the CD data are consistent with a mixture of α -helix and random coil structures (Brahms and Brahms, 1980). While the data at $P/L = 1/1000$ and $1/500$ are considerably noisier than the $P/L = 1/200$ data, the shape of the

spectra and the relative strength of the positive and negative bands suggests that the secondary structure content is very similar across this range of concentrations and points to little or no change in the fraction of peptides present that are bound to the vesicles. The CD data collected at P/L = 1/50 are strikingly different, and indicate that the peptide has transitioned to a predominantly anti-parallel β -sheet structure (Brahms and Brahms, 1980).

Other studies have observed that gp41 and variants adopt different secondary structures depending on both the lipid composition and the peptide concentration. Rafalski and coworkers studied two 23 AA-long variants of gp41, with the distinction between the being either arginine (the LAV_{1a} strain of HIV-1) or alanine (the LAV_{mal} strain of HIV-1) at position 22, in the presence of 1-palmitoyl-2-oleoyl phosphatidylglycerol (POPG) or 1-palmitoyl-2-oleoyl phosphatidylglycerol (POPC) lipid bilayers (Rafalski et al., 1990). Both peptides adopted an anti-parallel β -sheet structure when associated with POPC at P/L = 1/200 or 1/30, but the alanine variant was α -helical in POPG regardless of concentration, while the arginine variant underwent a helix-to-sheet transition between P/L = 1/200 and 1/30 (Rafalski et al., 1990). A later study by Nieva and coworkers of the same LAV_{1a} gp41 variant in POPG lipid bilayer membranes found divalent cations to be important for promoting the anti-parallel β -sheet structure, and found that the conformational transition correlated with vesicle fusion (Nieva et al., 1994). Similarly, Sáez-Ciri3n and Nieva found that the LAV_{1a} gp41 with an F8W substitution was α -helical in POPG vesicles. In 4:1 mixtures of POPC and either POPG or 1-palmitoyl-2-oleoyl phosphatidylserine (POPS), Lai and coworkers found LAV_{1a}

gp41 at P/L = 1/100 to be α -helical when cholesterol was absent, but 30 mol % cholesterol resulted in a β -sheet structure (Lai et al., 2012). The present results of a helix-to-sheet transition between P/L = 1/200 and 1/50 are consistent with previous studies when one considers the different amino acid sequence of gp41rk and the specific lipids employed.

3.2. β -Sheet Conformation of gp41rk does not Promote Vesicle Fusion

The results of the DLS characterization of the samples are presented in Table 2. Over times consistent with a SANS experiment (up to 3 days), the sizes of the vesicles do not change dramatically, indicating that the samples are suitably stable to allow for characterization of the structure of the bilayer by SANS. The two highest P/L measured (1/50 and 1/200) show an increase in size over the 3-day maximum measurement window, but are unchanged over the first day, which was sufficient to collect the SANS data presented here. The results demonstrate that the modifications to the sequence produced the desired reduction in fusogenicity, even though the β -sheet conformation has been identified as the more fusogenic conformation (Lai and Freed, 2014). A similar decrease in fusogenicity was observed for the V2E mutant of the full-length, WT gp41 peptide, which adds a charged residue near the end terminal similar to gp41rk (Dimitrov et al., 2003; Kliger et al., 1997; Lai and Freed, 2014). The sample preparation procedure, described above, may also play a role in the observed behavior. In the present work, the peptide is not introduced to an existing vesicle solution, unlike other studies of gp41-induced fusion (Curtain et al., 1999; Haque et al., 2005; Lai et al., 2012; Li and Tamm,

2007; Martin et al., 1996; Nieva et al., 1994; Pereira et al., 1997; Rafalski et al., 1990; Slepushkin et al., 1990; Yang and Weliky, 2003).

3.3. The Helix-to-Sheet Transition Correlates with a Change in the Structure of the Bilayer.

The SANS data collected for the concentration series of gp41rk in the 7:3 d54-DMPC:DMPS vesicles are shown in Figure 2. The data appear quite similar, suggesting that the peptide does not greatly alter the structure of the bilayer. Close inspection of the region of the data that arises from the structure of the bilayer, which is highlighted for the 100% D₂O data presented in Figure 3, reveals that the P/L = 1/50 data subtly differ from the data collected from the samples having lower peptide concentration for $q > 0.10 \text{ \AA}^{-1}$. Specifically, the first minimum is less shallow than the first minima in the other data sets, rather than the position of it being different. The differences between the curves in this region are statistically significant.

A better understanding of how the structure of the bilayer responds to increasing peptide concentration was obtained by modeling the data. The model intensity profiles obtained as described in Section 2.6 are presented in Figure 2 with the data. Data from samples having $P/L \leq 1/200$ were well-fit by the simple 4-shell model and displayed good convergence. The structures found are presented schematically in Figure 4 and all fitting parameters including χ^2 are summarized in detail in Table S1. The results for P/L = 1/50, obtained using the two bilayer profile model, are also presented in Figures 2 and 4 and

Table S1. All sets of the model intensity profiles are also presented with the data in Figure 2.

In light of the recently published study using the same lipid system in which the SANS data was interpreted using a model built from the material compositions (Rai et al., 2016), it is instructive to compare the peptide-free results with the present study. The structures of the bilayers (Table S2 of (Rai et al., 2016), which also presents SLDs and layer thicknesses that permits direct comparison with the present work and Table S1 presented here) differ in that the inner leaflet of the present profile is thinner than found previously (~ 17.4 Å versus ~ 20.8 Å, respectively). Similarly, the outer leaflet of the present structure is thicker than was found previously (~ 21.9 Å versus ~ 18.8 Å, respectively). The SLD profiles also differ somewhat, which might be expected in light of the different layer thicknesses and the way in which the SLD was generated from the material compositions in the previous work (Rai et al., 2016), rather than being found in random and then refinement searches from within ranges of SLDs bound by the materials (see Table S2). In the present work, the inner HG has an SLD very nearly that of a PC HG, and the SLD of the inner HC region shows a high content of deuterated lipid chains, also consistent with a high PC content. The SLD of the outer HC region is the same as was found previously. The outer HG region was previously found to have a lower SLD than that in the present work, which presently has nearly the same SLD as the outer HC region. Interestingly, the radii and polydispersity are very different, being ~ 360 Å with a polydispersity of 0.36 previously and ~ 480 Å with a polydispersity of 0.26 in the present

work. In both cases, the SANS results are consistent with DLS measurements of the samples.

The differences between the results in the two studies merit discussion before presenting the modeling results for the samples with gp41rk. First and foremost, the use of different modeling methods has the potential to result in different structures. However, the exact same modeling approach that was employed in the previous study was attempted with the present data prior to the realization that the $P/L = 1/50$ data could not be well-modeled in that manner, which prompted the change in approach. The results of the material composition modeling are presented in Table S3. The structures of the peptide-free bilayers found are not identical. The data fitting invariably fits the noise, which will not be identical between two different measurements. Further, any systematic errors, such as may arise in the instrument hardware, the neutron source, or even background arising from the experiments running on adjacent beamlines, that may be present in the data will contribute to the fitting results. Such artefacts will be subtle, but the region of data that gives rise to the structure of the bilayer lacks strong features. Another possibility for the differences between the current and previous results lies in the sample preparation. All samples in the previous study were prepared by DKR, while all of those in the present study were prepared by WTH. The steps in the process were nominally the same. However, one known difference was that WTH applied considerably more pressure during the extrusion of the vesicles than DKR. The previous and present studies are self-consistent as the amount of peptide present increases, making the results useful for understanding how the peptides interact with the bilayers when

viewed independently, but care must be taken when comparing the peptide-driven changes from the two studies.

When gp41rk is present at $P/L \leq 1/200$, the structure of the bilayer does not change greatly from the peptide-free bilayer, as can be seen in Figure 4 and Table S1. In all cases, the SLD profile of the bilayer indicates that the outer leaflet of the bilayer is enriched in DMPS, and that the low concentrations of the peptide do not alter this distribution greatly, as was the case when melittin was present at $P/L \leq 1/500$ (Rai et al., 2016). The inner HG region does not change with increasing P/L . Similarly, the inner HC region remains unchanged at the level of the uncertainty of the experimental technique. In contrast gp41rk produces changes in the outer leaflet of the bilayer. The outer HC region first thickens and the SLD increases at $P/L = 1/500$ before returning to the peptide-free thickness and SLD at $P/L = 1/200$. In parallel over this concentration range, the outer HG region responds in an opposite sense by becoming thinner with a lower SLD at $P/L = 1/500$, creating a more distinct layer than is present at other peptide concentrations. The changes in the thicknesses of the outer HC and HG regions balance, resulting in a bilayer near-constant thickness. It is possible that an initial transition of some form takes place in the system near $P/L = 1/500$, but the CD results in Figure 1 do not provide evidence supporting or refuting such a transition. The SANS data and modeling results are not sufficiently different to support the existence of a structural transition in the vesicles for $P/L \leq 1/200$. The low-concentration results are consistent with the idea that the α -helical, non-fusogenic mutant forms of gp41 associate with the HG region of the bilayer in a peripheral manner (Lai and Freed, 2014; Lai et al., 2012).

At $P/L = 1/50$, where gp41rk adopts a β -sheet conformation, the bilayer had to be modeled using two different SLD profiles to obtain good fits to the data (Figure 4 and Table S1). The dominant region, which comprised $\sim 92\%$ of the area of the vesicle, was a $\sim 4 \text{ \AA}$ thinner structure than the lower concentrations of peptide produced. The bulk of the differences between the structures are localized to the HC regions. The SLD profile in this fraction of the vesicle is more uniform, indicating a more uniform distribution of d54-DMPC and DMPS than in the peptide-free vesicles, and the SLD of the HG region is lower than the peptide-free outer HG SLD. The other region, which comprises $\sim 8\%$ of the area of the vesicle, is a much thicker than either the peptide-free structure or the thin region of the $P/L = 1/50$ vesicle. While others have observed an increase in lipid ordering with gp41 (Haque et al., 2005; Lai and Freed, 2014), which is consistent with a thicker bilayer, x-ray diffraction and scattering indicates that the peptide actually thins the bilayer (Tristram-Nagle et al., 2010). The present work suggests that both structural effects are taking place. The bulk of the bilayer in the vesicle becomes thinner, while regions adopt a thicker structure consistent with more ordered acyl chains. The outer HC and HG regions now have a lower SLD than the peptide-free vesicle, indicating that this leaflet of the bilayer is more enriched in DMPS than the peptide-free bilayer. As can be seen in Table S2, the peptide is also capable of lowering the SLD of a layer with which it is associated.

It is possible to roughly estimate how large of a feature could form within the thicker region of the bilayer. The area of the average vesicle found by the fitting is $\sim 3.37E6 \text{ \AA}^2$, which contains $\sim 5.56E4$ lipids if we assume that the area per lipid is that of

DMPC in the L_α phase (Kucerka et al., 2005). While it is possible that the area per lipid in the mixture is actually lower than this value due to the DMPS (Petrache et al., 2004), the fact that the dominant region of the structure is thinner than the peptide-free bilayer (Table S1) makes the DMPC area per lipid acceptable for this exercise. Under these assumptions, the thicker, minor area fraction of the bilayer has an area of $\sim 2.70 \times 10^5 \text{ \AA}^2$. We further assume that gp41rk is associated with this thicker region of the bilayer because it has been shown to increase lipid ordering (Haque et al., 2005; Lai and Freed, 2014). Such a localization is not unambiguous because x-ray diffraction found that gp41 thinned membranes (Tristram-Nagle et al., 2010). At P/L = 1/50, the vesicle has ~ 1130 peptides, or ~ 370 of the predicted trimers of the peptide (Mao et al., 2012). Then, under the assumption that the peptide is associating with the thicker region of the vesicle, each trimer would be associated with an area of $\sim 730 \text{ \AA}^2$, or a disk with a $\sim 15 \text{ \AA}$ radius. For reference, this area is equivalent to ~ 12 lipids but would necessarily be fewer if the gp41rk is present in the structure. It is not a large structure, but it could accommodate a gp41rk trimer and a few DMPS lipids that could give rise to the decreased SLD in the outer HC region and some of the DMPS depletion subsequently induced could account for the increase in the SLD of the outer HC region in the dominant area fraction of the vesicle. In light of the information content of the SANS data, attempting to develop a more detailed model of the structures of the thicker regions that comprises the minor area fraction of the vesicle, particularly because it accounts for $\sim 8\%$ of the area, is not productive.

The thickness distortions produced when the peptide is present at $P/L = 1/50$ can be thought of as defects in the local curvature of the membrane. Either the edge of the outer HC region of the thicker, minor area fraction is encircled by the gp41rk to shield it from the surrounding water, or there is a continuous transition in thickness at the edge that is guided by the peptide. The latter seems more likely as the HC chains would also have to sharply transition between ordered and disordered if the transition between domains were sharp. In either case, the vesicle would have an appearance reminiscent of a balloon being gently stretched outward at a great many points on its surface. Such a structure would be suggestive of an isolated bilayer participating in the structures that immediately precede stalk formation when two bilayers are nearly in contact (Yang and Huang, 2002), even though the peptide is not fusogenic (Table 2).

4. Conclusions

The results presented here show how a lipid bilayer membrane responds to a variant of the gp41 fusion peptide from HIV-1. While the peptide is nonfusogenic, it does display the helix-to-sheet transition observed for the native, full-length peptide. The transition creates a biphasic vesicle in which the dominant structure is thinner than the peptide free bilayer and those with which lower concentrations of the peptide in an α -helical conformation is present. The structure in the minor area fraction is considerably thicker, suggesting that the structure may consist of regions where the intrinsic curvature is pinched outward in a manner that would be consistent with the structural rearrangements that take place during the fusion of two bilayers.

5. Acknowledgements

D.K.R. was supported by the Laboratory Directed Research and Development program of Oak Ridge National Laboratory (LDRD-7428). Research at the Spallation Neutron Source of Oak Ridge National Laboratory and W.T.H. were sponsored by the Scientific User Facilities Division, Office of Basic Energy Sciences, US Department of Energy under contract number DE-AC05-00OR22725.

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Table 1. Sequence of the HIV1 LAV1a gp41 fusion peptide and the gp41rk variant used in the present study. The differences between the parent sequence and the sequence from the present work are highlighted in bold text.

Peptide	Sequence
HIV1 LAV _{1a} gp41	AVGIG ALFLG FLGAA GSTMG ARS
gp41rk	RKGIG ALFLG FLGAA GSTMK R

Table 2. Hydrodynamic radii of vesicles as a function of time after extrusion.

Sample	Day of Extrusion	+1 day	+2 days	+3 days
P/L = 0	45.2 ± 0.9 nm	47.9 ± 1.1 nm	46.4 ± 1.3 nm	46.9 ± 1.4 nm
P/L = 1/1000	48.2 ± 1.3 nm	42.9 ± 1.6 nm	46.2 ± 0.9 nm	47.0 ± 0.7 nm
P/L = 1/500	40.5 ± 1.3 nm	43.4 ± 1.7 nm	43.2 ± 1.2 nm	41.9 ± 1.3 nm
P/L = 1/200	46.6 ± 1.2 nm	49.1 ± 0.8 nm	52.0 ± 0.9 nm	51.1 ± 0.8 nm
P/L = 1/50	51.8 ± 1.1 nm	51.2 ± 0.8 nm	53.9 ± 0.7 nm	54.6 ± 0.9 nm

Figure Captions

Figure 1. Solution CD spectra of gp41rk in aqueous solution (cyan) and in the presence of 7:3 d54-DMPC:DMPS vesicles at P/L = 1/1000 (blue), 1/500 (green), 1/200 (red) and 1/50 (black).

Figure 2. SANS profiles of 7:3 d54-DMPC:DMPS vesicles collected in 100% D₂O (A), 70% D₂O (B) and 50% D₂O (C) at P/L = 0 (black open squares), P/L = 1/1000 (red circles), P/L = 1/500 (green up triangles), P/L = 1/200 (blue down triangles) and P/L = 1/50 (cyan diamonds). The solid lines are the sets of model intensity profiles found during the data fitting.

Figure 3. SANS data collected in 100% D₂O for P/L = 0 (black), P/L = 1/1000 (red), P/L = 1/500 (green), P/L = 1/200 (blue) and P/L = 1/50 (cyan).

Figure 4. Schematics of the bilayer structures found by modeling the SANS data for (A) P/L = 0, (B) P/L = 1/1000, (C) P/L = 1/500, (D) P/L = 1/200 and (E) P/L = 1/50. The HG regions are orange, while the HC regions are blue. In the case of P/L = 1/50, which required two bilayer profiles to model the SANS data, the orange and blue correspond to the major area fraction structure, while the minor area fraction HG and HC are semi-transparent red and cyan, respectively.