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Changes in lipid bilayer structure caused by the helix-to-sheet transition of an HIV-1 gp41 fusion peptide derivative☆

William T. Heller*, Durgesh K. Rai[1]

Biology and Soft Matter Division, Oak Ridge National Laboratory, Oak Ridge, TN, 37831, USA

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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.

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 (FP), 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, 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

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.

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* Corresponding author at: Oak Ridge National Laboratory, PO Box 2008, MS-6473, Oak Ridge, TN 37831, USA.
E-mail addresses: hellerwt@ornl.gov (W.T. Heller), dkrai@mit.edu (D.K. Rai).
[1] Present address: Nuclear Reactor Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.

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et al., 1997; Rafalski et al., 1990; Saez-Cirion and Nieva, 2002; Slepushkin 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 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 phospahtidylcholine (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 ∼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 D_2O (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).

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	**AV**GIG ALFLG FLGAA GSTM**G ARS**
gp41rk	**RK**GIG ALFLG FLGAA GSTM**K R**

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, D_2O 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% D_2O/50% H_2O, 70% D_2O/30% H_2O and 100% D_2O were made from the extruded vesicle stock solutions by a 50% dilution with appropriate amounts of H_2O and D_2O. Samples not used immediately for SANS measurements were stored up to 24 h in an incubator set at 12 °C to avoid freezing the samples (D_2O 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 h and then re-suspending it in H_2O 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