Biochimica et Biophysica Acta 1808 (2011) 2534–2543

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Influence of the lipid composition of biomimetic monolayers on the structure and orientation of the gp41 tryptophan-rich peptide from HIV-1

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ARTICLE INFO

Article history:
Received 22 October 2010
Received in revised form 3 June 2011
Accepted 6 June 2011
Available online 13 June 2011

Keywords:
Brewster angle microscopy
Langmuir monolayer
Membrane proximal external region of gp41
Peptide orientation
PM-IRRAS

ABSTRACT

The tryptophan-rich peptide of gp41 (so-called gp41W), one of the two envelope glycoproteins of HIV-1, is known to play a crucial role in the fusion between this virus and the host cell membranes. The influence of lipids on this role was investigated using different lipid monolayers at the air–water interface. Gp41W affinity for the lipid monolayer was measured by following the peptide-induced variation in the lateral surface pressure and we demonstrated that gp41W binds to monolayers containing the saturated zwitterionic dipalmitoylphosphatidylcholine (DPPC) as well as to the anionic dipalmitoylphosphatidylglycerol (DPPG) and to mixed monolayers containing DPPC and cholesterol (Chol). The secondary structure of gp41W in the presence of these lipid monolayers was determined by polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS). The data showed that gp41W was an oriented α-helix in the presence of DPPG. However this spectroscopic method was unable to detect the gp41W structure in the presence of DPPC and DPPC/Chol monolayer. The peptide-induced modifications of the DPPC/Chol, DPPC and DPPG monolayer morphology were analyzed by Brewster angle microscopy (BAM). The peptide-induced changes in the DPPG monolayer morphology suggest that gp41W disturbed the lipid intermolecular interactions. Furthermore the peptide delayed the condensed state of DPPC and DPPC/Chol, indicating that, although gp41W was not detected by PM-IRRAS, it was present in these lipid monolayers.

1. Introduction

The first step of the HIV-1 infection is its binding on the host cell receptors. This promotes the fusion between the virus membrane and the host cell plasma membrane, in order to release the virus genomic RNA into the host cell. These steps involve the virus envelop glycoproteins gp120 and gp41, which are non-covalently linked [1,2]. Gp120 promotes the binding step, while gp41 is involved in the fusion step. Gp120 fixes on the host cell CD4 receptors as well as on the CCR5 or CXCR4 coreceptors [3], inducing successive conformational changes in gp120 and gp41, which lead to the fusion step [4].

Gp41 is an anchored protein containing an ectodomain, a transmembrane domain (TM) and a cytoplamic domain (CD). The ectodomain is constituted by the fusion peptide (FP), the N-terminal helical heptad repeat (N-Helix), the C-terminal helical heptad repeat (C-Helix), and the membrane proximal external region (MPER) which contain the gp41 tryptophan-rich peptide, so-called gp41W [5] (Fig. 1). Indeed gp41W contains five tryptophan (Trp), phenylalanine, tyrosine and two positively charged lysine (Lys) residues at each end.

Each gp41 ectodomain undergoes conformational changes to make closer the virus and the host cell plasma membranes. More precisely, FP inserts the host cell membrane, while the N-Helix and C-Helix form a hairpin-structure [6], approaching the viral and the host cell membranes in order to move closer the gp41W and the two membranes [7]. Thus gp41W, a highly conserved region located near the TM domain, plays a critical role in the fusion between the target cell and the virus membranes [8–14].

Furthermore, both the virus gp41 and the host cell receptors are located in the lipid raft region, a specific membrane domain [15–18], which is a well-organized subdomain with high concentration in cholesterol (Chol), sphingolipids and highly saturated phospholipids [19]. This is the reason why many research groups have investigated the gp41W role in the viral fusion using vesicular and liposomal systems mimicking lipid rafts. It was shown that gp41W has a strong affinity to zwitterionic vesicles containing Chol [20] and that sphingomyelin is important for the pore formation, while Chol seems to be essential for vesicle fusion [21]. Indeed, Chol promotes self aggregation of gp41W at the lipid interface, and this seems to be dependent on the coexistence of lipid/liquid disordered state and solid-like state induced by the addition of Chol to the membranes [21,22]. Furthermore, Trp has been reported to interact preferentially with membrane Chol [23,24].

Abbreviations: BAM, Brewster angle microscopy; Chol, cholesterol; DPPC, dipalmitoylphosphatidylcholine; DPPG, dipalmitoylphosphatidylglycerol; ET, exposure time; GL, grey level; Π/A, surface pressure/area; Π_i, initial surface pressure; PM-IRRAS, polarization modulation infrared reflection absorption spectroscopy

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doi:10.1016/j.bbamem.2011.06.003

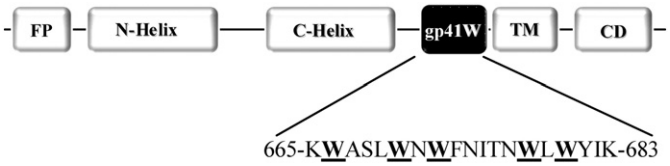

Fig. 1. Different domains of gp41W from HIV-1. FP: fusion peptide; N-Helix: N-terminal helical heptad repeat; C-Helix: C-terminal helical heptad repeat; gp41W: Trp-rich sequence; TM: transmembrane domain; CD: cytoplasmic domain.

In this work, we chose to mimic the interactions between gp41W and membranes by using interfacial lipid monolayers. Since the lipid raft is enriched in highly saturated phospholipids, we investigated the interaction of gp41W with dipalmitoylphosphatidylcholine (DPPC) monolayer at the air–water interface, in order to define the influence of the saturated fatty acyl chains on the Trp-rich peptide. Then, since the viral membrane contains high level of cholesterol [16], the influence of Chol on this interaction, if any, was investigated by mixing this sterol with DPPC. Furthermore, since Lys-containing peptides interact with negatively charged phospholipids [25], we have investigated whether there are electrostatic interactions between positively charged Lys residues of gp41W and anionic phospholipids using dipalmitoylphosphatidylglycerol (DPPG) monolayer at the air–water interface.

The gp41W affinity for different lipid monolayers was followed as peptide-induced increase in lateral surface pressure at a constant area. Peptide adsorption and interaction with the lipid monolayer were analyzed by measuring isotherm compression, before and after peptide adsorption. Changes in the lipid monolayer morphology, and possible peptide aggregation were followed by Brewster angle microscope (BAM) at the air–water interface during the monolayer compression. In a recent work, we have demonstrated that gp41W is α-helical at the air–water interface [26], whatever was the surface pressure. In this work, we looked for possible structure changes of gp41W due to its interaction with different lipid monolayers. This was investigated using Polarization Modulation Infrared Reflection Absorption Spectroscopy (PM-IRRAS), an IR spectroscopy adapted to the air–water interface.

2. Materials and methods

2.1. Chemicals

Cholesterol (Chol), dimethylsulfoxide (DMSO), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-dipalmitoyl-sn-glycero-3-phospho-rac-(1-glycerol) (DPPG) and 1,2-dipalmitoyl-rac-glycero-3-phospho-L-serine were purchased from Sigma Chemical (St. Louis, MO) and Tris from Boeringer (Mannheim, Germany). Gp41W was synthesized by Genecust Europe (Dudelange, Luxembourg) and its identity and purity (95.33%) was checked by high-performance liquid chromatography and mass spectrometry. All organic solvents were analytical grade. The ultra pure water, purified with a Millipore filtering system (Bedford, MA), had a resistivity of 18.2 MΩ cm. Stock solutions were prepared as follows. Gp41W was dissolved at 0.545 mM in DMSO, while Chol and DPPC were dissolved at the same molarity in hexane-ethanol (9/1, v/v) and DPPG in chloroform-methanol (4/1, v/v) and DPPS in chloroform-methanol (2/1, v/v). Stock solution of DPPC/Chol (70/30, mol/mol), at 0.545 mM, was obtained by mixing the individual lipids.

2.2. Interfacial film formation and surface pressure measurements

All experiments were performed at 21 °C. The film balance was built by Riegler & Kirstein (Wiesbaden, Germany) and equipped with a Wilhemy-type surface pressure measuring system. The subphase was a 10 mM Tris buffer (further named Tris buffer) at pH 8.5 or at pH 7.4.

2.3. Peptide adsorption at constant area

Peptide adsorption experiments were performed on a small Teflon Langmuir trough (diameter, 4 cm) with a subphase volume of 12 mL. Different amounts of lipids were spread at the air–water interface in order to reach the desired initial surface pressure (Π_i). After lipid monolayer stabilization (corresponding to about 30 min), gp41W was injected into the subphase at a final concentration of 0.68 μM. The subphase was stirred with a magnetic stirrer for 1 min, and it was then stopped during the rest of the experiment. The peptide adsorption to the lipid monolayer was followed as an increase in surface pressure during at least 150 min. The same volume (15 μL) of free DMSO was injected in the subphase, underneath the lipid monolayers, and no change in the surface pressure was observed.

2.4. Isotherm measurements

Surface pressure/area (Π/A) isotherms were measured after spreading the lipids at the air–water interface of a Langmuir trough (surface, 165 cm^2; subphase volume, 120 mL). After 30 min, corresponding to the solvent evaporation, the lipid monolayer was compressed at a 6 cm^2/min rate until the lateral pressure reached about 30 mN/m. This isotherm was considered as a control Π/A isotherm of the lipid alone. Then, the lipid monolayer was decompressed, leading to zero surface pressure; 25 min later, gp41W was injected into the subphase at a final concentration of 68 nM. The subphase was continuously stirred with a magnetic stirrer. After 90 min, leading to the peptide adsorption, the monolayer was compressed and the isotherm was recorded.

2.5. BAM measurements

Interfacial film morphology was observed with a Brewster angle microscope (NFT iElli-2000, Göttingen, Germany) mounted on an R&K Langmuir trough as described in Ref. [27]. Surface pressure and grey level (GL) were measured simultaneously during the interface compression at 3 $Å^2$/molecule/min, corresponding to 2 cm^2/min. BAM images were acquired at different shutter speeds, corresponding to different exposure times (ET), with a spatial resolution of ~2 μm and a size of 430 × 320 μm.

2.6. PM-IRRAS measurements

PM-IRRAS is an IR spectroscopic method adapted to the air–water interface [28–30]. PM-IRRAS measurements were performed using a Nicolet 850 spectrometer (Thermo Electron, Nicolet Instrument, Madison, WI). The infrared beam was reflected by a mirror towards an optical bench. The reflected beam was polarized by a ZnSe polarizer and modulated between parallel (p) and perpendicular (s) polarizations by a photoelastic modulator. The polarized beam was then directed toward the air–water interface onto a Langmuir balance (Nima Technology, UK) (surface, 90 cm^2; subphase volume, 50 mL). Afterwards, the beam was reflected on a photovoltaic HgCdTe detector cooled at 77 K. The optimal angle of incidence at the air–water interface was 75° to the interface normal. The detected signal was then processed to obtain the differential reflectivity spectrum:

$$\Delta R / R = J_2\left(R_p - R_s\right) \Big/ \left(R_p + R_s\right)$$

where J_2 is the Bessel function depending only on the photoelastic modulator, while R_s and R_p are the parallel and perpendicular reflectivity. To remove the Bessel function contribution as well as that of the water absorption, the monolayer spectrum was divided by that of the pure subphase. PM-IRRAS spectra were measured before and after injection of gp41W (at a final concentration of 1.63 μM)