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Characterization and lipid phase effect on the interaction of GBV-C E2-derived peptide, P6-2VIR576, with lipid membranes relating it with the HIV-1 FP inhibition

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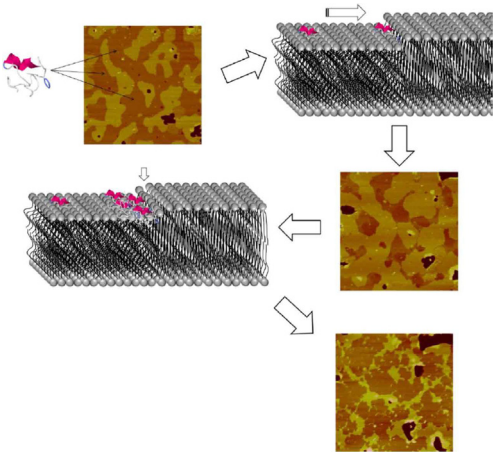
GRAPHICAL ABSTRACT



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ABSTRACT

This study is an extension of our previous paper on the interaction of AcP6-2 and the VIR576 peptides with DPPC: DPPS (3:2) and DMPC: DMPS (3:2) model membranes [*Colloids and Surfaces A*. 532 (**2017**) 483–492]. In the present contribution, the temperature effect and the role of the lipid phase in the lipid-peptide interaction were investigated. Moreover at the same time, relating them to HIV-1 FP inhibition.

Several biophysics experiments as lipid-peptide binding, Trp fluorescence quenching and Atomic Force Microscopy (AFM) visualization were used to evidence the different interaction of the peptide depending on the physical state of the lipids. In addition, the inhibition effect of HIV-1 FP by P6-2 VIR576 peptide was conducted by fluorescence resonance energy transfer (FRET) and also by AFM microscopy. P6-2VIR576 showed a preference to the liquid crystalline phases from where the peptide can diffuse and interact with the gel phases. Firstly, P6-2VIR576 induces a rigidifying of the membrane to finally, promote the vanishing of these gel phases.

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Concerning to the inhibition of HIV-1 FP peptide by P6-2VIR576 peptide, FRET and AFM results evidencing P6-2VIR576 peptide is a promising structure to be in mind in the development of new or improved drugs in HIV therapies.

1. Introduction

Human immunodeficiency virus type 1 (HIV-1) is a pathogenic agent that causes acquired immunodeficiency syndrome (AIDS). Since its discovery [1], research has continued to develop and improve therapy against the virus, resulting in the development of many antiviral drugs [2]. The majority of these drugs act on the enzymes required for the HIV-1 life cycle, while others, such as HIV-1 fusion peptide (HIV-1 FP) inhibitors, target HIV penetration and the release of viral contents into the host cell [3–5]. Blocking HIV ability to penetrate cells has therapeutic advantages over other therapies because avoids the entry of virus and consequently the release of its genetic material to the host cell.

GB virus C (GBV-C) is an enveloped virus belonging to the Flaviviridae family and has been investigated in the context of HIV-1 infection. Coinfection of HIV with GBV-C has been linked to the prolonged survival of AIDS patients [6–8]. Since both are enveloped viruses that share transmission routes and entry into the host cell through a fusion mechanism, it is possible that certain GBV-C components might inhibit HIV-1 FP. Over the last two decades, some research groups have focused on studying GBV-C peptides to determine any possible inhibitory effect on the fusion process leading to HIV infection of a host cell [9–11].

P6-2VIR576 is derived by combining the AcP6-2 and VIR576 peptides. AcP6-2 is a GBV-C peptide corresponding to the amino acid sequence 45–64 of the E2 structural protein. It has been demonstrated to be active in cell cultures in a study investigating the HIV-1 fusion process [10–12]. VIR576 is a synthetic variant of a virus-inhibitory peptide (VIRIP) that blocks HIV-1 invasion by binding to the hydrophobic HIV-1 FP [13].

A recent study [14] evaluated the interaction of AcP6-2 and P6-2VIR576 with membrane models and their role in inhibiting HIV-1 FP at 25 °C. P6-2VIR576 showed higher surface activity than its parent peptide AcP6-2, with an area per molecule value in the π-A compression isotherms approximately twice that of AcP6-2. Interaction studies were performed using penetration experiments in lipid monolayers and lipid bilayer binding assays. The effects of ionic strength and changes in membrane dipole potential were also considered. The results demonstrated that P6-2VIR576 interacted with both DPPC: DPPS (3:2) membrane and DMPC: DMPS (3:2). Results, also indicated that the lipid-peptide interaction was driven by electrostatic forces followed by hydrophobic interactions. Koedel and colleagues [10] demonstrated the ability of P6-2VIR576 to inhibit HIV-1 FP activity in cell cultures. Morphological surface analysis of Langmuir-Blodgett monolayers by fluorescence microscopy showed that P6-2VIR576 suppressed the interaction between HIV-1 FP and DPPC: DPPS (3:2) membranes [14]. However, P6-2VIR576 was also seen to interact with more fluid membrane models, yielding a new intermediate phase and thus suggesting a distinct interaction between the peptide and the membrane depending on the lipid phase of the membrane where the peptide is first adsorbed. All these data indicate that the peptide interacts differently with the diverse lipid phases present in the membrane models studied. Therefore, further research is required to gain more knowledge on the lipid-peptide interaction between the membrane and P6-2VIR576.

This study aimed to further explore the interaction between P6-2VIR576 and membrane models by first evaluating the effect of temperature on the peptide-lipid interaction by peptide-lipid binding assays at 37.0 ± 0.1 °C. To elucidate the position of the peptide in the membrane, quenching assays were performed at 25.0 ± 0.1 °C and 37.0 ± 0.1 °C. Fluorescence resonance energy transfer (FRET) assays and topographic analysis of lipid bilayers by atomic force microscopy (AFM) were also undertaken to assess the interaction between P6-2VIR576 and DMPC: DMPS (3:2) membrane models with or without HIV-1 FP. Large unilamellar vesicles (LUVs) and supported lipid bilayers (SPBs) were used as membrane models. A 3:2 M ratio of phosphatidylcholines (PCs) to phosphatidylserines (PSs) was used in accordance with Lev et al. [15] who have studied the thermal stability, expression, purification and structure of the entire ectodomain of HIV-1 gp41 in solution and at membranes. HIV-1 FP disrupts and induces the lipid mixing of negatively-charged phospholipid membranes. Moreover, PS has been reported to appear on the outer surface of the cell during apoptosis and is thus expressed at high levels in HIV-1-infected cells [16. 17] DPPC: DPPS (3:2) membranes used at 25 °C and 37 °C presented a lipid gel phase, while DMPC: DMPS (3:2) membranes used at 25 °C showed both the gel and liquid crystalline phases because of the lipid transition temperatures

2. Materials and methods

2.1. Materials

Peptides HIV-1 FP (AVGIGALFLGFLGAAGSTMGAAS), AcP6-2 (Ac-LCDCPNGPWVWVPAVCQAVG) and P6-2VIR576 (LCDCPNGPWVW-VPAVCQAVG–LEAIPCSIPPEFLFGKPFVF were synthesized in the Department of Medical Chemistry at the CSIC (Barcelona, Spain) [14]. The dry lipid 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dimyristoyl-sn-glycero-3-phospho-L-serine (DMPS), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dipalmitoylsn-glycero-3-phospho-L-serine (DPPS), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) ammonium salt (Rho-PE) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) ammonium salt (NBD-PE) were purchased from Avanti Polar Lipids (Alabaster, AL). Acrylamide (AC), promethazine hydrochloride (PMT), acetronitrile and 2-Amino-2-(hydroxymethyl) propane-1, 3-diol (TRIS) were obtained from Sigma (St Louis, MO), while sodium chloride (NaCl), chloroform and methanol (both HPLC grade) were acquired from Merck (Darmstadt, Germany). Ultrapure water (18.2 MΩ cm) was produced by deionization with a Milli-Q purification system (Millipore Corp.). For supported lipid bilayers (SPBs) formation, mica disk slides (0.71 cm^2) were used as solid substrates.

2.2. Sample preparation

2.2.1. Preparation of peptide solutions

Peptide stock solutions were obtained dissolving lyophilised peptide powder in water /acetonitrile (1:1) to a final concentration of 1 $mg{\cdot}mL^{-1}$, stirring them to complete homogenization. Measured volumes of this solution were mixed with 10 mM Tris pH 7.40 to obtain peptide samples of desired final concentration. Acetonitrile concentration in the samples was always below 0.5 $\mu g{\cdot}mL^{-1}$. Blank samples with the same concentration of acetonitrile were evaluated with no change observed in the physicochemical magnitude analysed.

2.2.2. Preparation of large unilamellar vesicles (LUVs)

The method for obtaining multillamelar vesicles (MLVs) has been previously described [18]. Briefly, lipids were dissolved in chloroform: methanol (2:1, v/v) and different proportions of DMPC and DMPS or DPPC and DPPS were mixed to obtain the desired composition in a conical tube. The solvent was evaporated under a stream of nitrogen. The lipid films were maintained under reduced pressure for at least 2 h.