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Molecular dynamics simulations of T-2410 and T-2429 HIV fusion inhibitors interacting with model membranes: Insight into peptide behavior, structure and dynamics



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HIGHLIGHTS

G R A P H I C A L A B S T R A C T

- Both T-2410 and T-2429 interact with model membranes.
- T-2410 and T-2429 interaction with membranes is stronger which correlates to their lower IC50.
- FI interaction with rigid bilayers has a low dependence on FI-Chol interaction.



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ABSTRACT

T-2410 and T-2429 are HIV fusion inhibitor peptides (FI) designed to present a higher efficiency even against HIV strains that developed resistance against other FIs. Similar peptides were shown to interact with model membranes both in the liquid disordered phase and in the liquid ordered state. Those results indicated that such interaction is important to function and could be correlated with their effectiveness. Extensive molecular dynamics simulations were carried out to investigate the interactions between both T-2410 and T-2429 with bilayers of pure 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) and a mixture of POPC/cholesterol (Chol) (1:1). It was observed that both peptides interact strongly with both membrane systems, especially with the POPC/ Chol systems, where these peptides show the highest number of H-bonds observed so far. T-2410 and T-2429 showed higher extent of interaction with bilayers when compared to T-20 or T-1249 in previous studies. This is most notable in POPC/Chol membranes where, although able to form H-bonds with Chol, they do so to a lesser extent than T-1249 does, the latter being the only FI peptide so far that was observed to form H-bonds with Chol. This behavior suggests that interaction of FI peptides with rigid Chol rich membranes may not be as dependent from peptide/Chol H-bond formation as previous results of T-1249 behavior led to believe. As in other similar peptides, the higher ability to interact with membranes shown by T-2410 and T2429 is probably correlated with its higher inhibitory efficiency.

Abbreviations: FI-HIV, fusion inhibitor peptides; Chol, cholesterol; POPC, 1-palmitoyl-2-oleoyl-phosphatidylcholine

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1. Introduction

Membrane fusion between enveloped viruses and host cell membranes is a mandatory process of viral infection mediated by viral glycoproteins [1-3]. The initial events of HIV-1 infection are mediated by the viral envelope glycoprotein complex formed by the transmembrane protein gp41 (responsible for membrane fusion) and the surface protein gp120 (responsible for host recognition), the latter being bound to the external domain of gp41 [4]. Entry of HIV into a target cell is performed in three steps: (i) binding of the viral gp120 to the target cell surface protein CD4 [5]; (ii) conformation change of gp120 enabling this protein to bind to vet another receptor on the immune system cell's surface, typically CCR5 [6] or CXCR4 [7]; and (iii) gp41-mediated membrane fusion [4]. The process, mediated by gp120, induces a conformational change in gp41, which exposes a fusion peptide and allows it to insert into the membrane of the target cell, thus leading to the fusion of the two membranes and the entry of the viral components into the target cell [4,8].

Several peptides based on the C-terminal region of HIV's gp41 have been synthesized as possible HIV fusion inhibitors (FI) (reviewed in [9-10]). Among these is T-20 (also known as enfuvirtide). T-20 is a HIV FI approved for clinical use [11] (Fig. 1). It is a 36-amino-acid peptide, homologous to the C-terminal region of HR2 of HIV-1's gp41 [12-15]. The elucidation of the core structure of gp41 has helped to understand the inhibitory activity of FIs such as T-20 [14]. The peptide sequence (sequence 643–678 of HIV- 1_{LAI} [12]) partially corresponds to the CHR region of gp41 and it can bind to the opposite NHR region, thus preventing the formation of a hairpin structure and ultimately, blocking the membranes fusion [13]. Despite the therapeutic potency of T-20, it has met the emergence of resistant HIV strains. Later generation peptides, such as the ones studied here, T-2410 and T-2429 (Fig. 1), have been designed to be effective against these strains and at lower concentrations [10,16]. T-2410 is a peptide that is derived from a gp41 HR2 region approximately N-terminal to that from which T-20 is derived [10,16] (Fig. 1). This peptide (see Fig. 1A for amino acid (aa) sequence) has exhibited potent antiviral activity in the ng/mL concentration range against several HIV strains (some of which are resistant to T-20 and T-1249) [10,16]. T-2429 (Fig. 1) was designed having T-2410 as a template, with modifications/mutations aimed at further stabilizing the helical structure of the peptide, namely by introducing charged Glu and Arg residues into non-core positions so that the spacing (i, i + 4) favored the formation of an ion pair in the helical conformation [10,16]. The N-terminal Met was also changed to Thr to eliminate complications caused by oxidation [10,16].

Both T-2410 and T2429 are molecules optimized for function, when compared with previous peptides and are being used as templates for further optimizations [10,16]. As such, we address their behavior because the detailed picture of the inhibitory mechanism induced by these FIs is still incomplete and the reasons for the differences in the effectiveness of these peptides are still a matter of debate [17–23].

It has been observed that both T-20 and T-1249 showed an efficient partition to zwitterionic bilayers. However, only T-1249 is able to interact/adsorb effectively to cholesterol-rich membranes, which may be the main cause of its improved efficiency (see [17,18] for a detailed discussion). Both fluorescence spectroscopy data [17,18] and molecular simulation [19–23] studies have shown that peptides such as these have the capacity to adsorb to and interact with the bilayer surface and

suggest this as, at least, part of its mechanism of action. Adsorption of FI peptides to the membrane surface would increase their local concentration at the site were everything happens. Amphipathic FI peptides such as the ones studied here and others studied elsewhere [19–23], designed to be capable of interacting with both the membrane surface and the bulk water, would thus be more effective in the inhibition of HIV fusion.

In this work, we study the interaction of both T-2410 and T-2429 with 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) and POPC/cholesterol (Chol) membranes using molecular dynamics (MD) simulations in the 200–400 ns time scale. Structure and behavior of all intervening molecular species is addressed. Our results mostly agree with the model of Veiga et al. [17,18] for the role of lipid bilayers in the mode of action of FI peptides and may explain the relatively more effective action of these peptides against HIV fusion when compared with that of T-20 [21,23] or of T-1249 [20,22,23], since higher affinity to the bilayers implies higher local concentrations of the peptides and hence the bilayer surface becomes a richer reservoir of anti-fusion peptides.

2. Methods

The initial α -helix models of both T-2410 and T-2429 (see Fig. 1 for primary structure) were built with the Arguslab 4.01 package [24] and solvated in cubic simulation boxes with SPC water [25], allowing for a distance between each peptide and the box walls of at least 1 nm.

A POPC molecule model (Fig. 1A) and its bonded and non-bonded interactions parameters were downloaded from Tieleman's group web page [26]. A cholesterol molecule model (Fig. 1B) and its bonded and non-bonded parameters were taken from [27] and were downloaded from the GROMACS web page [28]. For further details about the lipid models see Martins do Canto et al. [23]. Initial models of both membranes (POPC and POPC/Chol (1:1); see Fig. 1C and D) were built. To this purpose, one POPC molecule (with mostly stretched and parallel acyl chains) from the downloaded POPC bilayer PDB file (together with one Chol molecule in the case of the POPC/Chol, T-2410 + POPC/Chol and T-2429 + POPC/Chol system) was replicated to build custom sized bilayers models using GROMACS 5.1 model preparation packages [29-33] (for snapshots of the final structures of the peptide + membrane systems see Fig. 1E-H, corresponding respectively to the systems T-2410 + POPC, T-2410 + POPC/Chol, T-2429 + POPC and T-2429 + POPC/Chol). The latter software was also used to perform all simulations. The GROMACS force field (which is a modified GROMOS87 force field) was used to describe all the interactions (see the GROMACS manual for details) [29]. Preliminary MD simulations of the solvated peptides, solvated POPC (Ld) bilayer and solvated POPC/ Chol (Lo) bilayer were performed under the same conditions as the final MD runs for the above systems, for at least 200 ns, in the first two cases and for 400 ns in the latter. These preliminary MD simulations were performed to ensure that the bilayers were equilibrated prior to the peptide inclusion in the system, thereby losing memory of their initial structure during the equilibration process and, also to serve as a baseline for comparison with the peptide + bilayer systems. From these systems, with no added peptide, the main structural and dynamical lipid properties were successfully calculated and verified for validation purposes, as described below.

Peptide and bilayer models were then combined, using the final peptide structures obtained after 200 ns simulation of each peptide in



Fig. 1. Sequence alignment of the peptides of interest: T-20, T-1249, T-2410 and T-2429. In red are depicted the aa residues common to T-20, in yellow the aa residues common with T1249 and in green the aa residues only common to T-2410 and T-2429. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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