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HIV Fusion Inhibitor Peptide T-1249 Is Able To Insert or Adsorb to Lipidic Bilayers. Putative Correlation with Improved Efficiency

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Abstract: T-1249 is a HIV fusion inhibitor peptide under clinical trials. Its interaction with biological membrane models (large unilamellar vesicles) was studied using fluorescence spectroscopy. A gp41 peptide that includes one of the hydrophobic terminals of T-1249 was also studied. Both peptides partition extensively to liquid-crystalline POPC (1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphocholine) ($\Delta G = -7.0$ kcal/mol and -8.7 kcal/mol, for T-1249 and terminal peptide, respectively) and are located at the interface of the membrane. T-1249 is essentially in a random coil conformation in this lipidic medium, although a small α-helix contribution is present. When other lipid compositions are used (DPPC, POPG + POPC, and POPC + cholesterol) (DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine) and POPG (1-palmitoyl-2-oleyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)), partition decreases, the most severe effect being the presence of cholesterol. Partition experiments and fluorescence resonance energy transfer analysis show that T-1249 adsorbs to cholesterol-rich membranes. The improved clinical efficiency of T-1249 relative to enfuvirtide (T20) may be related to its bigger partition coefficient and ability to adsorb to rigid lipidic areas on the cell surface, where most receptors are inserted. Moreover, adsorption to the sterol-rich viral membrane helps to increase the local concentration of the inhibitor peptide at the fusion site.

Introduction

Human immunodeficiency virus type 1 (HIV-1) binding to the target cell and fusion of the membranes of both depend on the viral envelope glycoproteins complex formed by the transmembrane protein gp41, and the surface protein gp120, bounded to the external domain of gp41. The first step in the process of HIV-1 infection of a cell is mediated by gp120 binding to the CD4 receptor, present in the surface of some T-lymphocytes, macrophages, and other immune system cells.[1] This contact induces a conformational change in gp120, facilitating its connection to a second receptor, usually CCR5[2] or CXCR4[3]. The binding of gp120 to CD4 and CCR5 (or CXCR4) triggers a conformational change in gp41, exposing a fusion peptide and allowing its insertion in the membrane of the target cell, leading to the fusion of the two membranes and mixing of the viral and cellular components.[4]

One feature of gp41 is the presence of two heptad repeat (HR1 and HR2) sequences.[5] Enfuvirtide (T20: Fuzeon) is a synthetic 36-amino-acids peptide homologous to the C-terminal region of HR2 of HIV-1 gp41.[6,7] This compound is currently the most advanced clinical drug for inhibiting HIV-1 entry[6] and has recently received approval from the Food and Drug Administration.[8] Despite the therapeutic potency of enfuvirtide, it has met with the emergence of resistant strains.[6,8] T-1249 (a 39-amino-acids peptide) is a second-generation fusion inhibitor, composed of sequences derived from HIV-1, HIV-2, and simian immuno-

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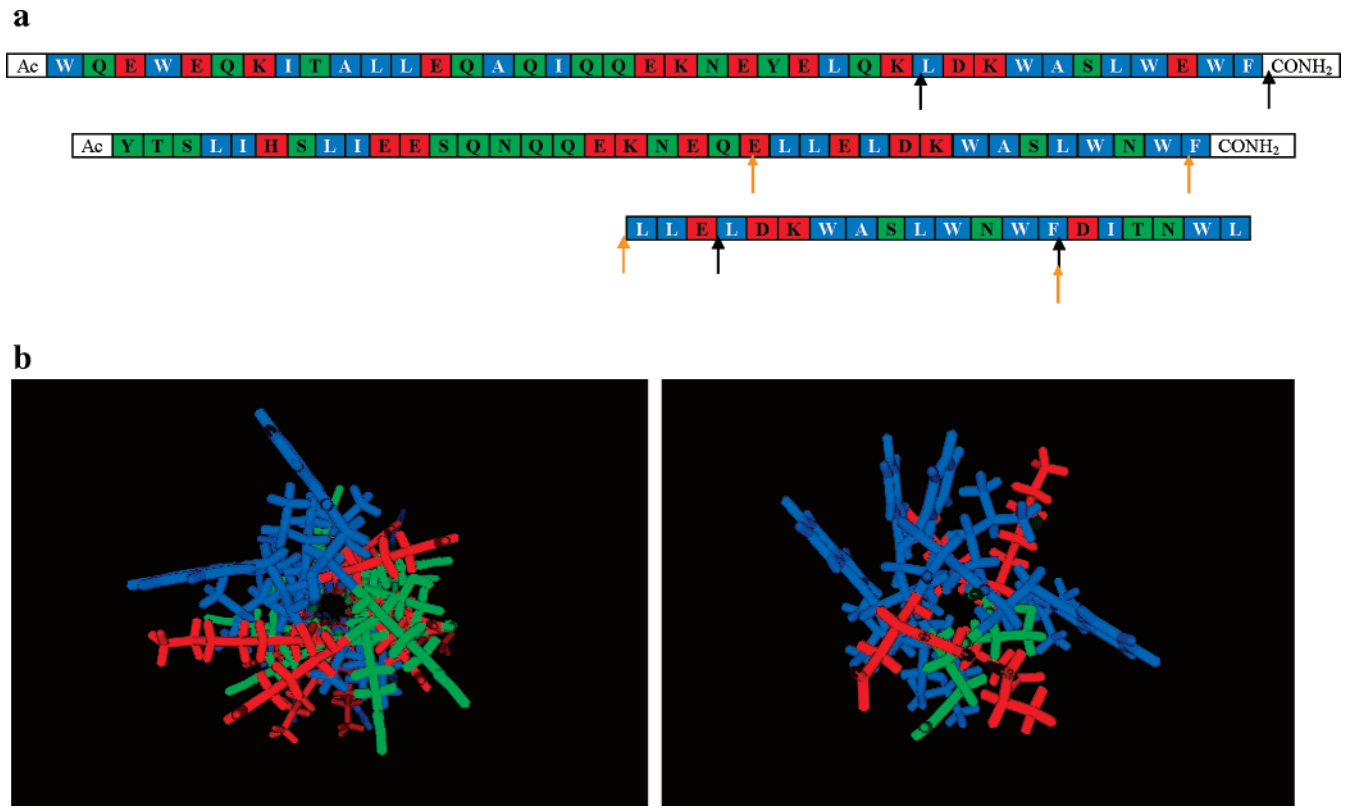

Figure 1. (A) Amino acids sequence of T-1249 (top), enfuvirtide (middle), and CTP (bottom). The arrows indicate the sequence homology between the CTP and the other peptides (black, T-1249; orange, enfuvirtide). (B) Top view of T-1249 (left) and CTP (right) arrangement in an α-helix. Hydrophobic residues are blue, noncharged polar are green, and charged polar are red.

deficiency virus (SIV).[9] Initial phase I/II clinical trials with T-1249 have shown promising results; namely, it is a more potent inhibitor than enfuvirtide (even with a single daily administration instead of the two used for enfuvirtide) and retains activity against most enfuvirtide-resistant strains.[6,7,9,10]

Similarly to enfuvirtide, the T-1249 sequence and presence of amphipathic segments (Figure 1, A and B) suggest that the peptide may interact with biological membranes. Theoretical analysis on the hydrophobicity of the amino acid residues sequence[11,12] of the peptide further confirms this hypothesis. We were prompted to study the interaction of T-1249 with biological membrane models. A gp41 20-amino-acids peptide that includes the hydrophobic C-terminal sequence of enfuvirtide and T-1249 (hereafter named CTP-carboxyterminal peptide) was also studied, to conclude on the mode of insertion of these molecules in lipidic membranes.

Materials and Methods

Materials. Enfuvirtide and T-1249 were a kind gift from Roche (Palo Alto, CA), and the CTP fragment of enfuvirtide was purchased from AnaSpec, Inc. (San Jose, CA). 5NS (5-doxyl-stearic acid) and 16NS (16-doxyl-stearic acid) were from Aldrich Chem. Co. (Milwaukee, WI). L-Tryptophan, acrylamide, Hepes, and NaCl were from Merck (Darmstadt, Germany). POPC (1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphocholine), DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine), and POPG (1-palmitoyl-2-oleyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)]) were purchased from Avanti Polar-Lipids (Alabaster, AL), while cholesterol and dehydroergosterol, DHE (ergosta-5,7,9(11),22-tetraen-3β-ol), were from Sigma (St. Louis, MO). The spectrofluorimeter used was an SLM Aminco 8100 (double monochromators; 450 W Xe lamp), the UV−visible absorption spectrophotometer was a Jasco V-530, and the CD spectropolarimeter was a Jasco J720 (450 W lamp). The time-resolved instrumentation was previously described.[13]

Methods. All studied peptides contain tryptophan residues (Figure 1A), which make fluorescence techniques suitable tools to probe these molecules. In all fluorescence measurements, the excitation wavelength used was 280 nm (except time-resolved fluorescence experiments, 287 nm). 10mM Hepes, pH 7.4/150mM NaCl buffer was used throughout the studies. Enfuvirtide, T-1249, and CTP stock solutions (2.2×10^{-4} M, 1.985×10^{-4} M, and 3.9×10^{-4} M, respectively) in buffer were diluted to the final desired concentration. T-1249 and CTP solubilization required mild sonication. Remaining macroscopic aggregates of CTP were sometimes detected, although in very small quantities. In these cases, a centrifugation step was added to separate the aggregates. Large unilamellar vesicles (LUV) were prepared by extrusion techniques.[14] Pure POPC and DPPC, POPC/POPG 80:20 (mol %), and POPC/cholesterol 67:33, 75:25, and 82:18 (mol %) were used on the studies of the interaction of the peptides with membrane models systems. Average fluorescence lifetimes, $\langle\tau\rangle$, were calculated from triexponential intensity decays (error estimates of the lifetime components ranged from 0.2% to 3.2%).

Membrane partition studies were performed by successive additions of small volumes of LUV (15mM) to the peptide samples (enfuvirtide 1×10^{-5} M, T-1249 6.7×10^{-6} M, or CTP 9.1×10^{-6} M) with a 10

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min incubation in between. The emission wavelength used was 350 nm. Fluorescence intensity data were corrected for dilution effect.

Quenching studies were carried out by successive additions of small amounts of 5NS or 16NS in ethanol to samples of the peptide incubated with LUV (T-1249 4.5×10^{-6} M or CTP 6×10^{-6} M). Ethanol concentration in the sample was kept below 2% (v/v). After each addition of quencher, the sample was incubated for 10 min. The effective quencher concentration in the membrane was calculated from the partition coefficient of the quenchers to lipidic bilayers.[15] The emission wavelength used was 340 nm.

Fluorescence resonance energy transfer studies between the tryptophan (donor) and fluorescently labeled vesicles with DHE (acceptor) were used to study the binding of T1249 to POPC/sterol (67:33 mol %) vesicles. The final concentration of T-1249 used was 2.2×10^{-6} M. The emission wavelength used to prevent interference of DHE emission was 320 nm. The critical distance for energy transfer, R_0, was calculated according to Berberan−Santos and Prieto,[16]

$$R_0 = 0.2108[\kappa^2 \Phi_D n^{-4} \int_0^\infty I(\lambda)\, \epsilon(\lambda)\, \lambda^4\, d\lambda]^{1/6} \quad (1)$$

where κ^2 is the orientation factor (the value $\kappa^2 = {}^2/_3$, relative to the dynamic isotropic limit, was used in this study), Φ_D is the donor quantum yield in the absence of acceptor ($\Phi_D = 0.106$, the value measured in this study for high lipid concentration, was used), n is the refractive index ($n = 1.4$ was used in this study as the refractive index within the vesicles[17]), $I(\lambda)$ is the normalized donor emission spectrum, and $\epsilon(\lambda)$ is the acceptor molar absorption spectrum (expressed as M^{-1} cm^{-1}). If in eq 1 λ is in nm, the calculated R_0 will be in Å. Energy transfer efficiencies, E, were experimentally determined from

$$E = 1 - I_{DA}/I_D \quad (2)$$

where I_{DA} and I_D are the fluorescence intensities of the donor in the presence and absence of acceptor, respectively.

The final concentrations of T-1249 and enfuvirtide in the CD experiments were 6.6×10^{-5} M and 7.3×10^{-5} M, respectively. CD data are represented by the mean residue ellipticity, $[\theta]$, obtained from the observed ellipticity (θ) according to the equation $[\theta] = \theta/(Nlc)$ where l is the path length, c is the molar concentration, and N is the number of amino acid residues in the peptide.[5]

Inner filter effects were corrected[18] in the fluorescence emission experiments.

Results and Discussion

Photophysical Characterization and Partition Coefficient Determination. Red edge excitation shift effects were not detected in the fluorescence emission of T-1249 and CTP tryptophan residues. Linear Stern−Volmer plots were obtained for peptides fluorescence quenching by acrylamide (not shown), revealing that the Trp residues are not localized inside hydrophobic pockets.

Figure 2 shows the result of a theoretical analysis[11,12] of the hydrophobicity of T-1249. Enfuvirtide analysis is also shown for the sake of comparison. $\Delta G_{oct} < 0$ reveals the tendency of peptides to insert in the membrane.

As shown in Figure 3A and B, there is an increase in the fluorescence intensity of T-1249 and CTP in the presence of

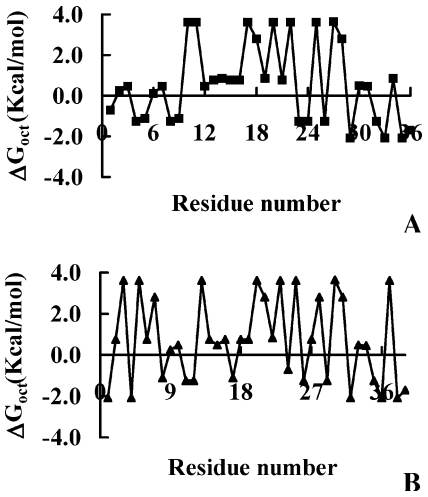

Figure 2. Theoretical analysis of partition into membranes of enfuvirtide (A) and T-1249 (B). Values of $\Delta G_{oct} < 0$ indicate the residues of the peptides with a higher tendency toward insertion in membranes. Figure 1 highlights the match of both peptides with CTP.

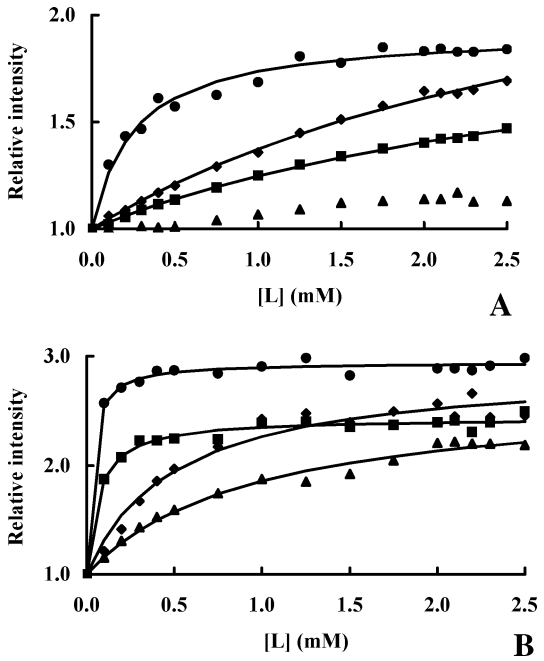

Figure 3. Partition coefficient determinations for T-1249 (A) and CTP (B). For both peptides, the largest increase in fluorescence intensity is detected in the presence of LUV of POPC(●). Although to a lesser extent, there is also an increase in the fluorescence intensity in the presence of gel state vesicles, DPPC (◆), and negatively charged lipid POPG (20% POPG in POPC; ■). The POPC/cholesterol mixture (33% mol cholesterol; ▲) is the least efficient; the increase of T-1249 fluorescence intensity is practically null. The solid lines are fittings of eq 3 to the experimental data. [L] is the concentration of the lipid available in the outer leaflet.

LUV of liquid-crystalline POPC. There is also a blue-shift of the emission spectra (3 and 12 nm for T-1249 and CTP, respectively, when [POPC] = 5 mM). This spectral shift is further evidence of the interaction of the peptides with the membrane model system. The partition coefficient between the lipid and aqueous phases, $K_p = [\text{peptide}]_L/[\text{peptide}]_W$, was determined in order to quantify the extent of interaction of the peptides with the LUV. $[\text{peptide}]_L$ and $[\text{peptide}]_W$ are the peptide concentrations in the lipidic and aqueous environment, respectively. Since there is an increase in the fluorescence quantum

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yield upon membrane incorporation, K_p can be calculated from the fluorescence intensity data, I, by fitting eq 3[19] to the data.

$$\frac{I}{I_W} = \frac{1 + K_p\gamma_L\frac{I_L}{I_W}[L]}{1 + K_p\gamma_L[L]} \qquad (3)$$

(I_W and I_L are the fluorescence intensities expected when all the peptide is in water or in the lipidic phase, respectively; γ_L is the lipidic molar volume;[20] and [L] is the molar concentration of the accessible lipid−outer leaflet of the bilayer.) K_p values of $(5.1 \pm 0.7) \times 10^3$ ($\Delta G = -7.0$ kcal/mol) and $(53 \pm 8.5) \times 10^3$ ($\Delta G = -8.7$ kcal/mol) were obtained for T-1249 and CTP, respectively, in POPC. Thus the K_p obtained for CTP is significantly larger than those obtained for T-1249 and enfuvirtide.[21] This indicates that the hydrophobic segment of enfuvirtide and T-1249, which corresponds to CTP, is of major importance for lipidic membrane partition, while the hydrophilic segments tend to oppose them (theoretical expectations in Figure 2 are thus met). T-1249 has the hydrophobic amino acid moieties more evenly distributed than enfuvirtide, which concentrates the hydrophobic residues at both endings. This may explain the differences found in K_p (1.6×10^3 vs 5.1×10^3 for enfuvirtide and T-1249, respectively). When other lipid compositions are used, the K_p values of CTP and T-1249 decrease (Figure 3A and B). There is a decreased partition into gel phase membranes, due to the higher rigidity of DPPC bilayers. The presence of the 20% POPG-to-POPC ratio mimics the environment of the inner leaflet of mammal biomembranes. T-1249 and CTP have negative net formal charges of −4 and −2, respectively. Decreased K_p in these systems can be related to electrostatic repulsion. However the most severe effect is related to the presence of sterol. For T-1249, the K_p is so small in cholesterol-rich membranes that the partition cannot be quantified ($K_p \approx 0$). At variance with T-1249 and enfuvirtide, CTP partition in these conditions occurs. Thus, the rigidity of the membrane is not the only factor that conditions the partition of the peptides.

Interaction of Enfuvirtide and T-1249 with Cholesterol-Containing Membranes. Lipidic rafts are plasma membrane domains that are enriched in cholesterol and sphingolipid.[22,23] They are organized in a tightly packed, liquid ordered manner,[24] with cholesterol maintaining the rafts in a functional state.[25] It was proposed that rafts may function as platforms for the assembly of membrane-associated macromolecular complexes that are important in biological processes.[26] This prompted us to further investigate the interaction of HIV fusion inhibitor peptides with cholesterol-containing membranes. Vesicles with ~33% of cholesterol (i.e., ~2 phospholipids:1 sterol) are homogeneous[27] (liquid ordered phase). Considering its rigidity, this phase may be regarded as a crude mimic of lipid rafts.

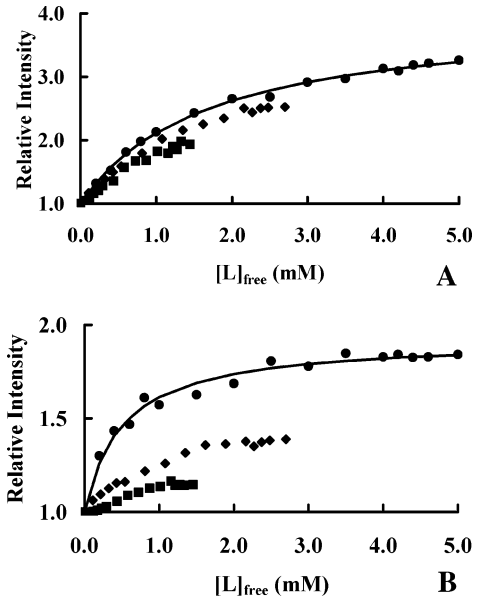

Figure 4. Partition plots of enfuvirtide (A) and T-1249 (B) to LUV of POPC/cholesterol. Fluorescence intensities dependence on $[lipid]_{free}$ (eq 4) for 0% (●), 18% (◆), and 25% (■) cholesterol. Data sets overlap for enfuvirtide (A) but not for T-1249 (B).

Although $K_p \approx 0$, adsorption of the peptides to the rigid bilayers surface cannot be discarded because adsorption can leave Trp residues exposed to the bulk aqueous environment, with unchanged fluorescence quantum yield (i.e., not contributing for K_p calculation). Vesicles with a lower content in cholesterol are heterogeneous, having cholesterol-rich and cholesterol-poor areas.[27] Thus, the liquid disordered phase and raft-like ordered phase coexist. If partition is only occurring to the disordered phase (cholesterol-poor) areas and no adsorption to the ordered phase (cholesterol-rich) is taking place, then one expects partition curves (I/I_W; eq 3) to be only dependent on the lipid concentration which is involved in cholesterol-poor areas; i.e., partition plots against the concentration of POPC external to ordered areas should all coincide, regardless of the cholesterol content of the vesicles. "Free lipid" (i.e., in cholesterol-poor areas) concentration can be calculated from lipid/cholesterol phase diagrams[27] (namely the lower and upper limits of two-phase coexistence) using the lever rule (eq 4):

$$[lipid]_{free} = [lipid + sterol]_{total}\frac{33 - x}{33 - 5} \qquad (4)$$

where x (5−33%) is the cholesterol mol % (18% and 25%, in our study, Figure 4A and B).

Partition curves for enfuvirtide are almost mutually overlapped, regardless of the cholesterol content, showing that enfuvirtide does not significantly adsorb to the cholesterol-rich areas. When K_p is calculated for 18% and 25% cholesterol in the $[lipid]_{free}$ domain, K_p is similar to those obtained with sterol-free membranes. However, T-1249 (Figure 4B) shows a different behavior: partition curves do not superimpose in the $[lipid]_{free}$ domain, and I/I_W decreases with cholesterol content. This may be due to adsorption of the peptide to the cholesterol-rich areas, without significant variation of the fluorescence quantum yield. This additional equilibrium (adsorption) competes with partition, decreasing the molar fraction of molecules embedded in the lipidic bilayers. With the increase of the percentage of choles-

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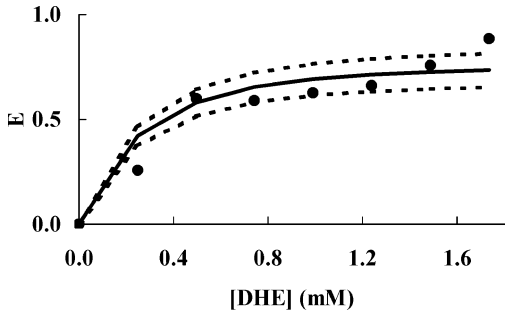

Figure 5. Energy transfer efficiency from Trp residues of T-1249 (donors) to DHE (acceptor) in POPC/cholesterol (33% mol cholesterol) vesicles (5 mM). Nonlinear regression (appendix 2) yields $A_1 = 0.3$ (molar fraction of nonadsorbed peptides). The boundaries $A_1 = 0.2$ and $A_1 = 0.4$ are also represented (dashed lines).

terol, a larger area is occupied by the domains and more adsorption of T-1249 occurs. Appendix 1 quantifies the equilibria balance and rationalizes the altered partition plots. This analysis takes into account partition of T-1249 to POPC and adsorption to the POPC/Cholesterol domains. Data fitting with eq A1.9 (appendix 1; Figure A1.2) leads to $K_a = 3.1 \times 10^3$ and $K_a = 4.6 \times 10^3$ for 18% and 25% of cholesterol, respectively (adsorption partition coefficient).

Interaction of T-1249 with Cholesterol-Rich Area Vesicles. A FRET Study. To further confirm and quantify the adsorption of T-1249 to the cholesterol-rich areas, we used a fluorescence resonance energy transfer (FRET) methodology using DHE as an acceptor for T-1249 tryptophan fluorescence. LUV of POPC/cholesterol/DHE (33−9.9% cholesterol, 0−23.1% DHE) were used. Figure 5 shows an increase in the energy transfer efficiency with DHE content. Thus, the Trp moieties of T-1249 are in the vicinity of the sterol; i.e., adsorption occurs. To quantify the fraction of molecules adsorbed, we followed the methodology described in appendix 2. The fraction of nonadsorbed molecules, A_1, is $A_1 = 0.3 \pm 0.1$, corresponding to 70% of adsorbed molecules when [POPC + cholesterol] = 5 mM (33% molar cholesterol). These values confirm that adsorption of T-1249 to sterol-rich membranes occurs. Equation A1.3 (appendix 1) relates the adsorption partition constant, K_a, lipid and sterol concentrations, and the molar fraction of the adsorbed peptide $(1 - A_1)$. Therefore, $0.4 \times 10^3 < K_a < 1.0 \times 10^3$, which broadly concur to the values calculated in the previous section. K_a was calculated from the volumic concentration of the peptide in the membrane. The partition constant obtained from superficial concentration, $K_{a,sup}$, can be obtained from K_a from $K_{a,sup} = K_a h$, where h is the half-bilayer thickness.

In-Depth Location of the Peptides in Membranes and Secondary Structure Studies. Quenching methodologies were used to evaluate the in-depth location of the Trp residues of the peptides inserted in POPC vesicles. Stearic acid molecules derivatized with doxyl (quencher) groups at carbon-5 (5NS) and −16 (16NS) were used. 5NS is a better quencher for molecules near or at the interface, while 16NS is better for molecules buried deeply in the membrane.[28] Figure 6A shows the Stern−Volmer plots obtained for T-1249 (similar results were obtained with CTP, not shown) using the effective concentration of 5NS and 16NS in the bilayer matrix. The peptides are better quenched by 5NS. So, the Trp residues of the peptides are located near

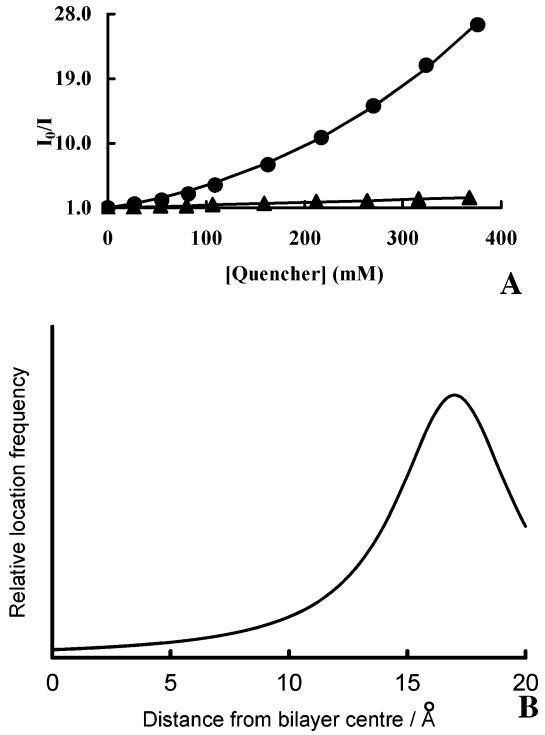

Figure 6. (A) Stern−Volmer plot for the quenching of T-1249 fluorescence by the derivatized lipophilic molecules 5NS (●) and 16NS (▲) in POPC vesicles (5 mM). Effective quencher concentration in the membrane was considered. (B) Using the SIMEXDA method[28] and fluorescence lifetime quenching data (Table 1), it was possible to determine the in-depth distribution of T-1249 Trp residues in the lipidic bilayer. The location is mainly interfacial.

Table 1. Average Fluorescence Lifetimes of T-1249 and CTP[a]

system	⟨τ⟩/ns T-1249	CTP
buffer	2.7	2.2
[POPC] = 5 mM	4.4	4.4
[POPC] = 5 mM, [5NS] = 0.4 mM	2.9	3.2
[POPC] = 5 mM, [16NS] = 0.4 mM	3.6	3.6

[a] The average fluorescence life-time of peptides increases upon incorporation in lipidic membranes. Differential diffusional fluorescence quenching by 5NS and 16NS inside the lipidic matrix enables in-depth location of the Trp residues.

the interface of the membrane. Fluorescence lifetime quenching data (Table 1) enable the application of the SIMEXDA method[28] to obtain the in-depth distribution of the Trp residues of the peptides (Figure 6B; CTP distribution is similar; result not shown). This location distribution does not differ much from the one obtained previously with enfuvirtide.[21] The Trp moieties are mainly located at the membrane interface.

CD experiments show that enfuvirtide and T-1249 are essentially in a random coil conformation in both aqueous and lipidic medium (POPC) (Figure 7). However, enfuvirtide has a negligible α-helix content (∼8% and ∼10% in buffer and POPC, respectively), while a helix component can be detected in T-1249, albeit small (∼30% and ∼25% in buffer and POPC, respectively). The obtained percentages of the α-helix content of the peptides are independent of the method applied to calculate them.[29,30]

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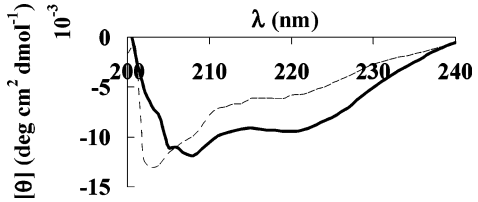

Figure 7. Far-UV CD spectra of enfuvirtide (dashed line) and T-1249 (solid line) in the presence of POPC vesicles ([POPC] = 2 mM; $X_{L,Enfuvirtide}$ = 0.54; $X_{L,T-1249}$ = 0.8). The increased helicity of T-1249 relative to enfuvirtide is noticeable. Spectra in buffer are similar.

Conclusions

T-1249 is a fusion inhibitor peptide under clinical trials but its mode of action is not totally understood. The use of fluorescence spectroscopy-based methodologies to study the interaction of the peptide with model membranes shows that (1) partition to LUV of POPC is very extensive and the peptide is located near the lipids polar heads level, mainly in random coil conformation; (2) in the presence of gel phase membranes (rigid DPPC bilayers), POPG-containing bilayers that mimic the environment of the inner leaflet of mammals biomembranes, and cholesterol-rich membranes, insertion in the lipidic environment is severely decreased; (3) however, there is a very significant adsorption of T-1249 to cholesterol-rich areas. Thus, lipidic membranes may play an important role in the mode of action of T-1249 (Figure 8). Namely, T-1249 can insert in the external layer of cell while it is prevented from translocation due to the repulsion caused by the negatively charged lipids of the inner layer. This way, T-1249 concentrates at the cell surfaces. Unlike enfuvirtide, T-1249 is able to adsorb to cholesterol-rich membranes. This ability raises the concentration of T-1249 in the surface of the cell directly at the fusion site because HIV-1 receptor CD4 and co-receptors CXCR4/CCR5 are considered to be localized within lipid rafts.[31] It is possible that the coalescence of several small rafts, containing these proteins, would be necessary to form an active larger raft domain,[25] enabling receptors oligomerization, viral binding, and membrane fusion. Because the HIV membrane is very rich in cholesterol,[26,31] the local concentration of T-1249 is further raised. Enfuvirtide inhibition occurs when target and viral membranes are near each other ([32] and references therein). The increased extension of partition of T-1249 relative to enfuvirtide,[21] and the possibility to adsorb to cholesterol-rich membranes might be the main cause of its improved efficiency as HIV fusion inhibitor. Hydrophobic derivatives of enfuvirtide proved to be 100× more efficient than enfuvirtide itself in HIV-1 replication inhibition.[33] Gene therapeutic-based strategies are currently being developed with membrane-anchored enfuvirtide.[34] T-1249's longer half-life in circulation (enabling a single daily administration, instead of the two daily administrations of enfuvirtide), together with its promising results in clinical trials, even with patients whose virus have reduced susceptibility to enfuvirtide,[9] can be (at least partially) justified by T-1249 combination of hydrophobicity, membrane partition, and "selective" adsorption, a capability not shared with enfuvirtide.

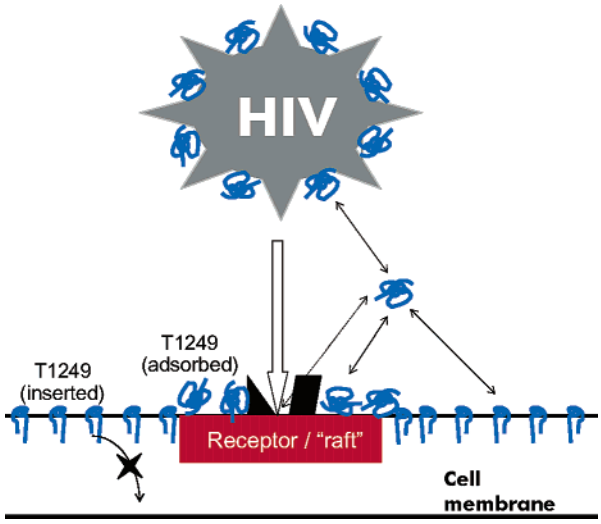

Figure 8. Schematic representation of the proposed involvement of lipidic membranes in T-1249 mode of action. T-1249 attaches to cell membranes in an interfacial position and adsorb to cholesterol-rich domains. Translocation is prevented due to charge effects. The virus outer membrane has loosely bound T-1249, contributing to the rise of the T-1249 local concentration at the fusion site. Moreover, the ability to loosely bind to lipid rafts in the direct vicinity of the receptors improves peptide action at the fusion site. The cell membrane and the virus are a reservoir of the peptide. gp41 fusogenic approach to the target membrane enables direct contact of the peptide with the gp41 C-region, which leads to fusion inhibition.

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Supporting Information Available: Appendix 1: simultaneous partition and adsorption. Appendix 2: fluorescence resonance energy transfer. This material is available free of charge via the Internet at http://pubs.acs.org.

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