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STRUCTURAL FEATURES OF PEPTIDES DERIVED FROM FELINE IMMUNODEFICIENCY VIRUS TM-GLYCOPROTEIN

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Abstract

Feline immunodeficiency virus (FIV) is a pathogen that causes an AIDS-like syndrome in domestic cats and is extensively used as a model system by which criteria for anti-lentiviral vaccines and drugs development can be tested. Despite little homology sequence, the surface and TM gp of FIV and HIV-1 exhibit a common structural framework and appear to play similar roles in Initiation of cell infection mediating the final event of membrane fusion and virus entry. Recently a 20-mer synthetic peptide spanning amino acids ^{767}L-G^{786} of the membrane-proximal ectodomain of FIV (TM) gp - gp41(767-786)- was found to be endowed with potent antiviral activity. Testing deleted or substituted peptides, the 8-mer gp41(770-777), designated C8, including in the sequence three Trp residues was identified as the minimal sequence needed for full antiviral activity. NMR conformational analysis of gp41(770-777) evidenced the presence of a b-turn conformation centered on the residues 773-776. Here we report the conformational analysis of the 20-mer TM-767-786 by means of NMR spectroscopy. A comparison between structural properties of gp41(767-786) and of gp41(770-777) was carried out in order to define the role played by the sequence length and by the Trp side chains on the active fragments stability.

Key words: Feline immunodeficiency virus (FIV), HIV therapy, Transmembrane ectodomain of FIV, Enfuvirtide.

Introduction

Human immunodeficiency virus (HIV) enters the target cell using fusion machinery composed of the non-covalently associated gp120 and gp41 glycoproteins. The formation of the fusion complex induces extensive conformational changes in gp120, resulting in the dissociation of the gp120/gp41 complex and the subsequent release of metastable gp41. Virus-cell fusion is then initiated by the gp41 ectodomain. [1] The gp41 ectodomain contains several characteristic functional domains, including the fusion peptide (FP), N-terminal heptad repeat (NHR), C-terminal heptad repeat (CHR) and membrane proximal extracellular region (MPER). The MPER is located at the C-terminal end of the HIV-1 gp41 ectodomain and is directly followed by the transmembrane domain. In the HIV-1 gp41 pre-hairpin structure, the MPER and the FP are the functional links by which the gp41 pre-hairpin bridges the viral and target cell membranes through the formation of a coiled-coil six-helix bundle (6HB) structure. [2] Fusion inhibitors that target gp41 functional domains to prevent 6HB formation and terminate the HIV-1-cell fusion process can be used as drugs to prevent HIV-1 infection.

In 2003, a 36-amino acid peptide derived from the CHR of the HIV-1 gp41 ectodomain (enfuvirtide T20) was approved by the US FDA for anti-HIV treatment. T20 is the only currently approved drug targeting gp41; however, the high cost and inconvenience of the twice-daily injection of this peptide drug prevents it from being used as a regular anti-HIV drug. [3] Thus, the development of new fusion inhibitors that overcome the limitations of T20 is of great importance. The NHR and CHR are still the most intensively investigated targets in gp41. [4] Other less exploited functional domains, such as the FP [5] and the MPER, [6] are receiving increasing attention as potential targets for fusion inhibitors.

Feline immunodeficiency virus (FIV) is a naturally occurring lentivirus [7] that is studied as a model system for anti-HIV vaccines and anti-HIV drug development. [8, 9] In a manner analogous to that for HIV, FIV enters cells via a mechanism involving a surface glycoprotein named gp36. [10-12] Gp36 has a structural architecture similar to that of gp41. The MPER of gp36 has a crucial role in the membrane fusion process. [13-15] We previously demonstrated that several short synthetic peptides that mimic the MPER of gp36 [16, 17] reduce the infectivity of FIV. [18] In particular, the fragment ^{770}WEDWVGWI^{776}, dubbed C8, elicited antiviral activity as a result of blocking cell entry, as observed for HIV fusion inhibitors. [19, 20] A structure–activity-relationship (SAR) study and preliminary nuclear magnetic resonance (NMR) conformational analysis demonstrated that C8 antiviral activity depends on the presence of regularly spaced Trp residues and that the orientation of the Trp indolyl rings is critical on a turn-shaped backbone conformations. [21] C8 has been studied in different biomimetic conditions and using several spectroscopic techniques and molecular dynamics simulations. [22, 23] Our data show that C8 undertakes a strong interaction with biological membranes [24] requiring a strict interplay among the different lipids. [25] The 20-mer synthetic peptide spanning amino acids ^{767}L-G^{786} of the membrane-proximal ectodomain of FIV (TM) gp, that we name gp41(767-787), was also found to be endowed with potent antiviral activity.

Here we report the conformational analysis of the 20-mer gp41(767-786) by means of NMR spectroscopy. In the attempt to understand the roles played by the sequence length and by the Trp side chains on the stability of the active fragments the structural properties of gp41(767-786) are discussed in comparison with those of gp41(770-777). [26]

Methods

Peptide synthesis

Gp41(770-777) peptide was prepared according to conventional solid-phase synthetic strategy with *N*-α-fluorenylmethoxycarbonyl-protected amino acids and a Rink-amide resin as a solid support. The peptide was N-terminally acetylated and C-terminally amidated to improve enzymatic stability. The crude peptide was isolated in >95% purity, by semipreparative reverse-phase high-pressure liquid chromatography and then lyophilized. The final product was characterized by analytical high-pressure liquid chromatography and electrospray mass spectrometry. [18]

NMR analysis

Sample for NMR experiments were prepared to have gp41(770-777) 1 mM, in DMSO/water 80:20 v/v (pH 6.8 phosphate buffer containing H_2O/D_2O). [27-30] The water signal was suppressed using WATERGATE pulse sequence experiments. [31] Qualitative and quantitative analyses of DQF-COSY, TOCSY, and NOESY spectra were performed using SPARKY software. [32]

All NMR data were recorded at 600MHz (CryoProbe) and 278K

NMR structure calculations

Peak volumes were translated into upper distance bounds with the CALIBA routine from the CYANA software package. [33] After discarding redundant and duplicated constraints, the final list of experimental constraints was used to generate an ensemble of 100 structures with the standard CYANA protocol of simulated annealing in the torsion angle space (using 10000 steps). The best 10 structures after minimization had AMBER energies ranging from -1541.4 to -1391.1 kcal/mol. The final structures were analyzed using the Insight 98.0 program (Molecular Simulations, San Diego, CA, USA).

Results and Discussion

All connectivities of the peptide 8-mer gp41(770-777), C8, sequential and medium-range, were observed in the NOESY spectra DMSO/water 80/20 v:v as shown in fig 1. Due to its high viscosity, DMSO/water solution, at low temperature, limits the conformational freedom of the peptide conformers. A qualitative analysis of NOE sequential and medium range connectivities evidences several diagnostically critical (i, i+2) and α-β(i, i+3) effects in the region 769-780. These effects are typical of regular secondary structures in this region corresponding to incipient turn-helical structures.

On the basis of the these data, structure calculations of gp41(767-786) were carried out using CYANA software. [33]

Figure 2 shows the superposition of the best 20 structures of the 20-mer gp41(767-786). The fit is calculated on the backbone heavy atoms of all residues 770-777. Analysis of the backbone torsion angles according to PROMOTIF algorithms [34] indicated in this region the presence of α-helical conformation.

In figure 3 the side chains of Trp-773 and Trp-776 are also displayed. They have a well-defined orientation and are both protruded on the same side of the helix.

In figure 4 gp41(767-786) NMR model is superimposed on that of the gp41(770-777). The structures showed good agreement at level of residues 772-776. Moreover, the Trp side chains, that play a critical role in the definition of the biological activity, seem to occupy common spatial regions.

The results of the NMR structural investigation of the 20-mer gp41(767-786) showed that the region, endowed with significant structural order along the sequence, is that involving the three Trp amino acid Trp-770, Trp-773 and Trp-776. These data support the hypothesis that the three equally distanced Trp residues determine a remarkable conformational stability that appears to be the key molecular determinant of the inhibitory activity. In this perspective, the orientation of the aromatic side-chains of Trp-773 and Trp-776 on the same side of the gp41(767-786) and gp41(770-777) (Figure 4 in blue and yellow respectively) is consistent with a possible direct involvement of these residues in hydrophobic interaction with target site. Although cooperative molecular interactions outside the active domain providing additional packing forces may be required, a stable helical structure appears to be an essential determinant of binding affinity to the gp41 molecular target as well as of antiviral activity.

Accordingly, C8 antiviral activity depends on the presence of regularly spaced Trp residues and the orientation of the Trp indolyl rings is critical on a turn-shaped backbone conformations.

This C8 interesting fragment should be considered as a lead skeleton, which can undergo optimization process for the development of new fusion inhibitors that overcome the limitations of T20 in HIV therapy.

Since it has been noticed that using a peptide based on such a domain a certain delay in infection following FIV exposure occurs, the achievements of this study should be further evaluated also for the design of FIV immunoprophylactic agents and cat immunization. [35] In this contest the small size and structural features of peptide C8 make it an ideal lead compound for the development of novel peptide-mimetic anti-FIV drugs and vaccines first and anti-HIV soon after.

Concluison

With this study we have defined the role played by the sequence length and by the Trp side chains on the active fragments of gp41 ectodomain by means the complete superimposing of the two sequence characterized by three Trp residues of 20-mer and 8-mer gp41. C8 showed to retain full antiviral activity ensured by the minimal amino acids sequence required. A considerable antiviral potency, in spite of small size, may be attributed to C8, compared with that possessed by 20-mer synthetic peptide of the membrane-proximal ectodomain of FIV transmembrane glycoprotein, which potently inhibits

the growth of tissue culture-adapted FIV in feline fibroblastoid CrFK cells. This C8 interesting fragment should be considered as a lead skeleton, which can undergo optimization process for the development of new fusion inhibitors that overcome the limitations of T20 in HIV therapy as well as of novel peptide-mimetic anti-FIV/HIV drugs and vaccines.
FIV-inhibitory activity of innovative peptide-mimetics, related to C8 structure on primary isolates contain viral variants FIV-GL8, FIV-M2 and FIV-Pet are currently underway.

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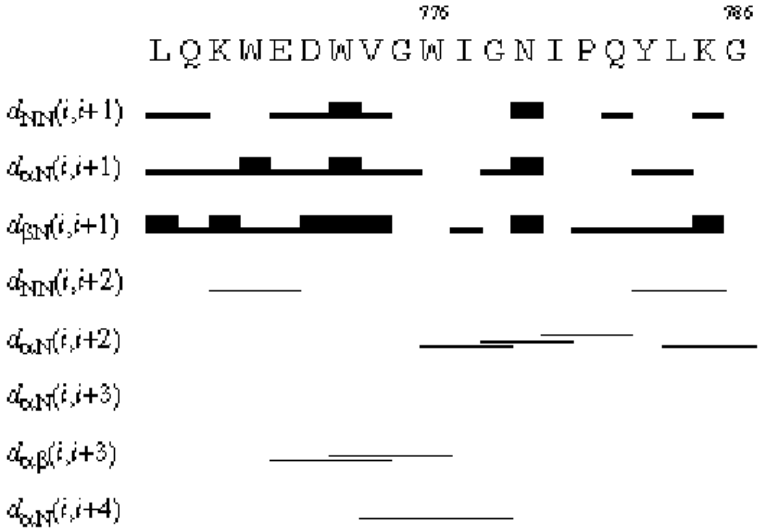
Figure 1. Sequential and medium range NOEs for gp41(767-786) in water/DMSO mixture.



Data were obtained from a 600 MHz NOESY experiments with a mixing time of 150 ms, at 278K.

Figure 2. The best CYANA calculated structures of gp41(767-786) as derived from DMSO/water NMR data at 600MHz and 278K.

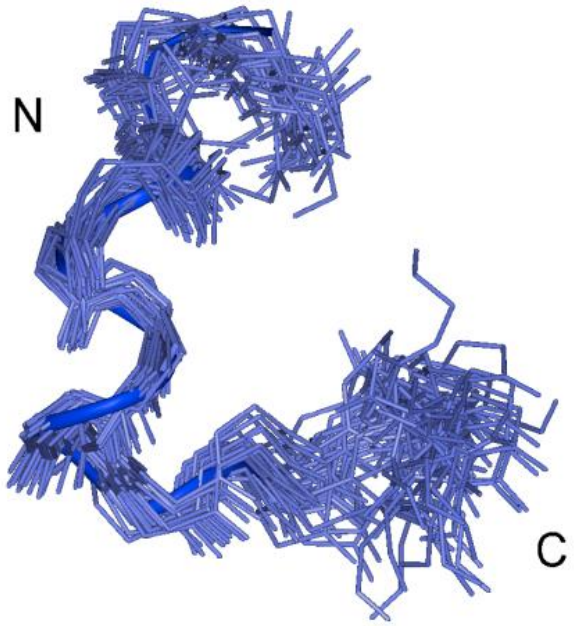


Figure 3. Best gp41(767-786) NMR structure (CYANA). The side chains of Trp 770, Trp 773 and Trp776 are shown.

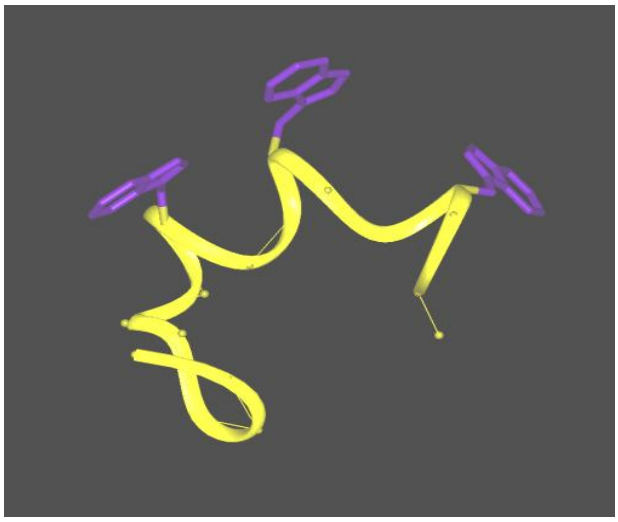


Figure 4. Superposition of NMR model of gp41(767-786) (blue) with the corresponding NMR structure of gp41(770-777) (yellow). Trp rings are shown for both the models.