The minimal fusion peptide of simian immunodeficiency virus corresponds to the 11 first residues of gp32[‡]

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Abstract: We had previously predicted successfully the minimal fusion peptides (FPs) of the human immunodeficiency virus 1 (HIV-1) gp41 and the bovine leukemia virus (BLV) gp30 using an original approach based on the obliquity/fusogenicity relationship of tilted peptides. In this paper, we have used the same method to predict the shortest FP capable of inducing optimal fusion *in vitro* of the simian immunodeficiency virus (SIV) mac isolate and of other SIVs and human immunodeficiency virus (HIV-2) isolates. In each case, the 11-residue-long peptide was predicted as the minimal FP. For the SIV mac isolate, liposome lipid-mixing and leakage assays confirmed that this peptide is the shortest peptide inducing optimal fusion *in vitro*, being therefore the minimal FP. These results are another piece of evidence that the tilted properties of FPs are important for the fusion process and that our method can be used to predict the minimal FPs of other viruses. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

INTRODUCTION

The entry of enveloped viruses into cells is mediated by fusion glycoproteins located at the viral envelope [1,2]. Mutagenesis studies showed that a region called *fusion peptide* (FP) is essential for fusion [1,2]. Depending on the virus type, this region is located at the *N*-terminal extremity or in the interior of glycoproteins. Many studies strongly suggest that FPs insert into target membranes and destabilise them to promote fusion [2–5]. Synthetic peptides corresponding to the FP of many glycoproteins have membrane perturbing effects and induce fusion *in vitro*, confirming the fusogenic properties of the FPs [1,5].

By molecular modelling, we predicted that the FPs of many viruses such as those of human immunodeficiency virus 1 (HIV-1), bovine leukemia virus (BLV), Ebola, influenza A virus and simian immunodeficiency virus (SIV) are tilted peptides [6–9]. A tilted peptide is a peptide with a hydrophobicity gradient when helical [7,10]. Because of this gradient, tilted peptides adopt an angle into the membrane between 30 and 60° with respect to the membrane plane [7]. In the case of *N*-terminal FPs of HIV-1, SIV and influenza A virus, infrared (IR) and/or neutron diffraction analyses have confirmed the tilted orientation [11–14]. The existence of a correlation between the tilt of the FPs into membranes and their fusogenicity was demonstrated by combining molecular modelling and experimental approaches. Indeed, mutations that modify the tilted orientation of SIV, HIV and BLV FPs reduce the fusogenic properties of the corresponding peptides [11,12,15] and of the whole glycoproteins [15–18]. The relationship between the tilt of a peptide and its fusogenicity has also been observed for other peptides [19–21].

Previous studies showed an effect of the length of the FP on its fusogenic properties [22,23]. For example, a peptide corresponding to the 12 first residues of the gp32 SIV protein induces more lipid mixing than a peptide corresponding to the 16 or 24 first residues [22]. However, the minimal FP, i.e. the shortest peptide capable of inducing significant membrane fusion, has not yet been determined. Recently, we used the tilted peptide theory to develop an original method that allowed the prediction successfully of the minimal FPs of HIV-1 and BLV [15,18]. According to this theory, a helical peptide that inserts obliquely into the membrane induces fusion. Moreover, the more tilted the peptide, the more important the fusion. So, we postulated that the minimal FPs of HIV-1 and BLV correspond to the shortest helical fragments of their glycoproteins capable of inserting into the membrane with an angle close to 45° with respect to the membrane [15,18]. Experimental assays on liposomes confirmed that the predicted peptides are the minimal FPs.

In this paper, we used the same method to predict the minimal FP of the SIV gp32. The minimal FP was predicted with the Integral Membrane Protein and Lipid

Association (IMPALA) algorithm [24] for the SIV mac as well as other SIV and HIV-2 strains. Liposome lipid-mixing and leakage assays were carried out with peptides corresponding to the SIV mac isolate to check the prediction.

MATERIAL AND METHODS

Materials

Egg phosphatidylcholine (PC), egg phosphatidylglycerol (PG), egg phosphatidylethanolamine (PE), cholesterol (CHOL), bovine brain sphingomyelin (SM), Hepes, Triton X-100 and dimethyl sulfoxide (DMSO) were purchased from Sigma (St Louis MO, USA). Octadecylrhodamine chloride B (R18), N-N'-p-xylenebis(pyridinium bromide) (DPX) and 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS) were from Molecular Probes (Eugene, Oregon, USA). NaCl was from Merck Eurolab (Leuven, Belgium).

The 9–12- and 16-residue-long FPs from the mac isolate were synthesised by conventional solid phase peptide synthesis, using Fmoc for transient NH_2-terminal protection, and were characterised using mass spectrometry. These peptides have free N-termini and amidated C-termini. The peptide purity was higher than 85%, as indicated by analytical HPLC. For lipid-mixing and leakage assays, peptides were dissolved in DMSO.

Methods

Peptide sequence. The sequence of the gp32 from the SIV mac isolate corresponds to the Swissprot entry P05884. The sequences from the gp32 of other SIV and HIV-2 strains were extracted from the HIV sequence database of the Los Alamos National Laboratory (http://www.hiv.lanl.gov/). Sequences were aligned with Clustalw [25].

Insertion of the peptides into the membrane. All peptides were constructed as α-helices using Hyperchem 6.0 (Hypercube Inc.) assigning phi/psi values of -58 and $-47°$, which correspond to the classical α-helical structure [26]. Backbone and side chain conformation was then optimised with Hyperchem by a Polak–Ribiere conjugate gradient procedure using the AMBER force field. The termination conditions were fixed to an RMS gradient of 0.01 kcal/(Å.mol).

The insertion of the peptides into an implicit bilayer was predicted using the IMPALA algorithm developed by Ducarme *et al.* [24]. The parameters of lipid bilayer and of the calculations were the same as previously described [15,18,27]. The position of the structure with the lowest energy is the most stable in the bilayer. Calculations were repeated three times for each peptide. All repeats gave similar results.

Liposome preparation. *In vitro* assays were performed with lipid unilamellar vesicles (LUVs) composed of PC/PE/SM/CHOL (26.3/26.3/26.3/21% w/w), as previously described [22]. Lipids were dissolved in a chloroform/methanol (2/1 v/v) solution. The lipid film was obtained after evaporation under vacuum obtained with a rotovapour (Van Der Heyden Büchi, Switzerland). The lipid film, dried for one night, was then dispersed in 2 ml of lipid-mixing buffer (see 'Materials') and was incubated for 1 h at 37 °C.

To obtain LUVs, the hydrated lipid dispersion was exposed to five freeze–thaw cycles (-180 °C/$+25$ °C) and extruded 10 times through a polycarbonate membrane (0.1 nm) under 20 bars pressure (Extruder Lipex Biomembranes, Vancouver, Canada).

Liposome concentration was determined by phosphorous analysis [28].

Lipid-mixing assays. Mixing of liposome membranes was followed by measuring the fluorescence increase of R18, a lipid-soluble probe, after the fusion of labelled and unlabelled liposomes. Labelled liposomes were obtained by incorporating R18 in the dry lipid film at a concentration 5% of the total lipid weight. Labelled and unlabelled liposomes were mixed at a weight ratio 1 : 4, and a final concentration of 12.5 μM in the lipid-mixing buffer. The 100% fusion is determined by adding Triton X-100 at 2% to labelled/unlabelled (1 : 4 w/w) LUVs. Fluorescence was recorded at room temperature (λ_{exc} : 560 nm, λ_{em} : 590 nm) on an LS-50B Perkin Elmer fluorimeter. The pH value was fixed at 7.4.

Leakage assays. Vesicle leakage was monitored using an assay based on the quenching of HPTS by DPX [29]. HPTS and DPX are encapsulated together in liposomes. Leakage of vesicles was followed by the dequenching of HPTS released into the medium. Fluorescence was recorded at room temperature (λ_{exc} : 360 nm, λ_{em} : 520 nm) on an LS-50B Perkin Elmer fluorimeter. LUVs were prepared as described above in 12.5 mM HPTS (45 mM NaCl), 45 mM DPX (20 mM NaCl), 10 mM HEPES buffer at pH 7.4. Vesicles containing encapsulated HPTS and DPX were eluted in the void volume of a Sephadex G-75 column, with 10 mM HEPES buffer (pH 7.4).

For lipid-mixing and leakage experiments, assays were repeated three times with different batches of peptides. The peptide/lipid ratio varied from 0.01 to 0.25.

RESULTS

In Silico Prediction of the Minimal FP for the SIV Mac

To predict the minimal FP of SIV, we evaluated with IMPALA the membrane interaction abilities of N-terminal helical peptides with length from 9 to 16 residues from the SIV mac isolate. Figures 1 and 2(A) show the results of the simulations. All peptides have their N-terminus in the membrane hydrophobic core and their C-terminus around the lipid headgroups (Figure 1). They insert into the implicit membrane with an angle between 55 and 80° with respect to the membrane plane (Figure 2(A)). Tilted peptides are defined as having an angle between 30 and 60° [7]. Only two peptides have a tilted insertion: the 11- and 12-residue-long peptides. They adopt an angle of 57 and 59° with respect to the membrane plane, respectively. The 11-residue-long peptide is the shortest peptide that inserts into the membrane with an optimal tilted orientation. It is therefore the minimal FP predicted.

In Vitro Validation

To check the prediction, we carried out lipid-mixing and leakage assays of PC/PE/SM/CHOL
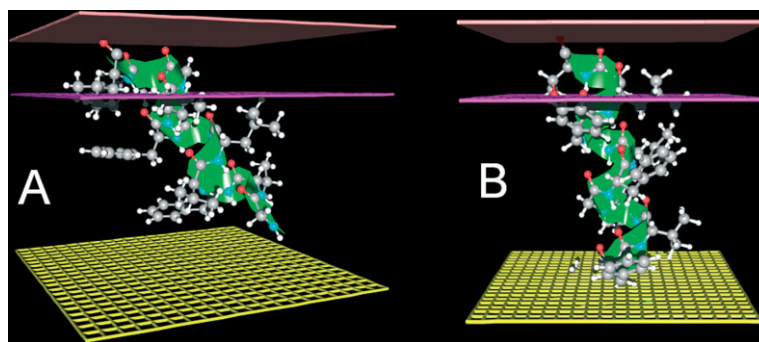
Figure 1 Best position in the IMPALA membrane for (A) 11-residue FP and (B) 13-residue FP. Only one layer of the membrane is represented. Bottom plane (yellow), bilayer centre; first upper plane (mauve), lipid chain/polar headgroups interface at 13.5 Å from the centre; second upper plane (pink), lipid/water interface at 18 Å from the centre.

(26.3/26.3/26.3/21% w/w) LUVs with 9–12- and 16-residue-long peptides corresponding to the sequence of the gp32 mac isolate. This lipid composition was chosen because it contains the major neutral lipids of a typical mammalian plasma cell and it has further been shown that the lipid mixing induced by SIV FP of different lengths is maximal with this composition [25]. Large vesicles (LUVs) were preferred to small vesicles (SUVs) in order to better mimic the lipid membrane curvature and stability of cells.

In the lipid-mixing assays, R18-labelled and R18-free liposomes were mixed. When there is fusion between
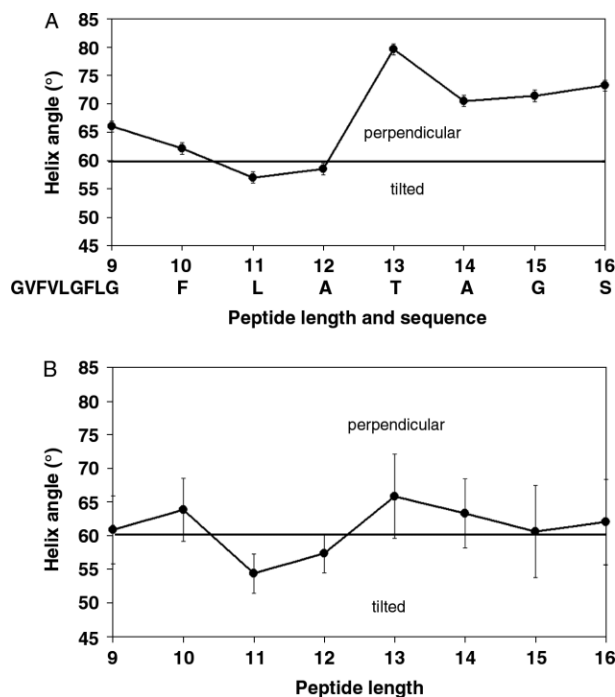
Figure 2 (A) Optimal angle with respect to the membrane plane predicted by IMPALA for *N*-terminal FPs of different length. (B) Optimal angle with respect to the membrane plane predicted by IMPALA for FPs of SIV and HIV-2 as a function of the peptide length. Standard deviation for the different sequences is represented.
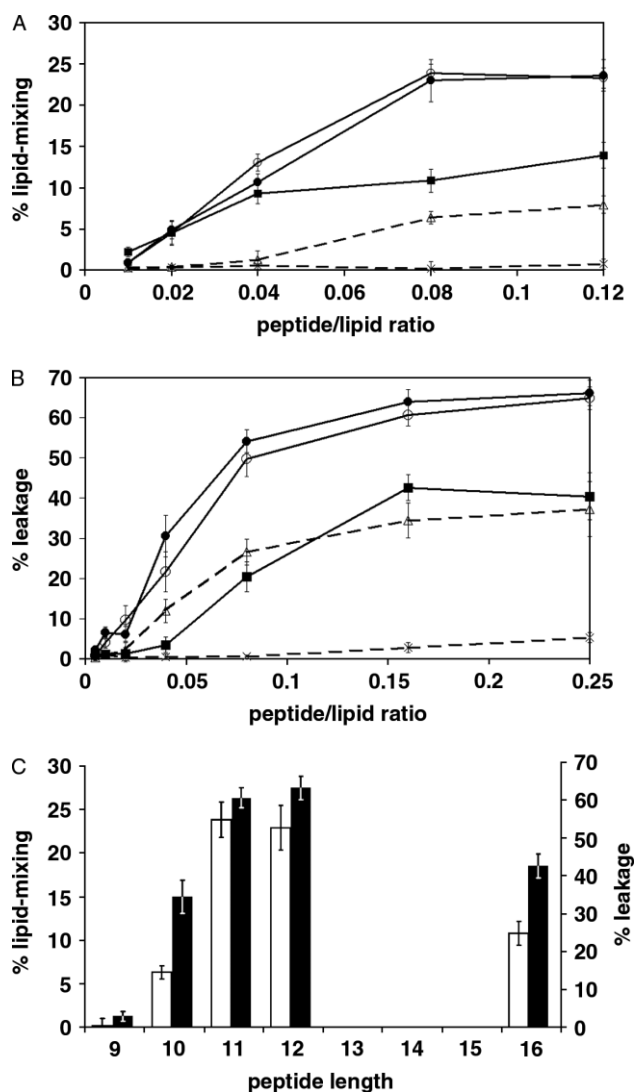
Figure 3 Percentage of lipid-mixing (A) and of leakage (B) of LUVs induced by the 9-residue FP (X), 10-residue FP (Δ), 11-residue FP (○), 12-residue FP (●), and 16-residue long FPs (■) as a function of peptide/lipid molar ratio. (C) : Percentage of lipid-mixing (white) and of leakage (black) of LUVs induced by FPs of different length at a peptide/lipid ratio of 0.08 and 0.16, respectively.

the two populations, R18 is subsequently diluted and dequenched, inducing a fluorescence increase. Figure 3(A) shows that the 11-residue-long peptide induces lipid mixing to the same extent as the 12-residue-long peptide (Figure 3(A)). Lipid mixing induced by these two peptides increases with the peptide/lipid ratio to reach a maximum of 24% at a peptide/lipid ratio of 0.08 (Figure 3(A)). The 10- and 16-residue-long peptides induce less lipid mixing, while the 9-residue-long peptide does not induce significant lipid mixing, whatever the peptide/lipid ratio.

To further characterise the membrane destabilizing activity of the 11-residue FP, we performed leakage assays. In these assays, HPTS and its quencher DPX are encapsulated together in liposomes. The destabilisation of liposomes induces the release of HPTS and DPX in the medium. The distance between HPTS and its quencher increases causing an increase in HPTS fluorescence. The 11-residue FP induces as much leakage as the 12-residue FP (Figure 3(B)). Leakage induced by the two peptides is dose dependent, as in the lipid-mixing assays. The maximal response of 62% of leakage is obtained at a peptide/lipid ratio of 0.16. The 9-, 10- and 16-residue-long peptides induce less leakage than the 11- and 12-residue-long peptides.

Figure 3(C) shows the maximum of liposome lipid mixing and leakage induced by the SIV mac FP as a function of the peptide length. In summary, the predicted minimal FP, the 11-residue FP, is the shortest peptide that induces optimal membrane destabilisation and fusion. It is therefore the minimal FP.

Sequence Analysis of FPs of HIV-2 and SIV

We then analysed the sequence of FPs from other SIV and HIV-2 strains. HIV-2 glycoproteins have FPs closely similar to those of SIV glycoproteins [30,31]. This is why HIV-2 FPs were also included in our analysis. The SIV and HIV-2 glycoprotein sequences were downloaded from the HIV sequence database of the Los Alamos National Laboratory (http://www.hiv.lanl.gov/). The glycoproteins with an FP sequence similar to that of the BRU HIV-1 isolate (AVGIGALFLGFLGAAG) were removed from the dataset because they were already analysed in a previous study [18].

Table 1 shows the consensus sequence of the 141 remaining sequences. It corresponds to the mac sequence and is found for 73 strains (51.8%). The analysis of the alignment shows that the regions 4–11 and 14–15 are highly conserved (>95% of identity). In these regions, only conservative mutations are observed. Indeed, SIV mac residues are substituted with the same hydrophobicity. Only three mutations modify the hydrophobicity: L5Q, F7S and A14R. The FLGFL motif, included in the highly conserved 4–11 region, is also observed in the FP sequence of HIV-1. It was previously shown that substitutions in the FLGFL motif of

SIV, HIV-2 and HIV-1 FP drastically decrease the fusogenicity of the corresponding glycoprotein [16,30–32]. This confirms the importance of the motif for the fusion process. The 1st–3rd, 11th, 12th and 16th residues are less conserved (between 65 and 85% of identity). The two first residues GV are mainly substituted by another hydrophobic motif, VP (15.6%). The 3rd residue is always hydrophobic. The 1–11 region is therefore hydrophobic, while the 12–16 region is less so. The consensus sequence contains indeed two polar residues (T13, S16) in the latter and the alanine located at the 12th position is substituted for 15% by a hydrophilic residue (S or T). The SIV and HIV-2 FPs can therefore be divided into two regions: a 1–11 region, which is highly hydrophobic and conserved, followed by a less hydrophobic and less conserved *C*-terminal region.

In Silico Prediction of the Minimal FP for HIV-2 and SIV Strains

The interaction with membrane of all non-redundant FPs of SIV and HIV-2 from 9- to 16-residues in the alignment was analysed using IMPALA. Table 2 gives the number of the peptides analysed with respect to the peptide length. It shows that respectively 100% and around 90% of 11- and 12-residue-long peptides are tilted. Figure 2(B), corresponding to the average insertion angle of all non-redundant FPs of SIV and HIV-2 with respect to the length, is similar to that obtained for the SIV mac isolate (Figure 2(A)). Only the 11- and 12-residue-long peptides are tilted, with an angle of 54 and 57° (±3°), respectively, with respect to the membrane plane. Compared to shorter and longer peptides, the standard deviation of these two peptides is less important, indicating that whatever the strain, the 11- and 12-residue-long peptides adopt an insertion angle near the average angle. For other peptide lengths, the insertion angle is dependent of the strain studied. The 11-residue-long peptide is the shortest peptide with an angle near the optimal angle (54° ± 3° with respect to the membrane plane) and with the smallest standard deviation. It is therefore the minimal FP predicted, as for the SIV mac isolate.

DISCUSSION

It was previously predicted that a peptide corresponding to the 12 first residues of SIV gp32 adopts a tilted orientation in membranes when helical [7,8]. This peculiar orientation was afterwards confirmed by IR and neutron diffraction assays [11,14]. The ability of this peptide to induce *in vitro* fusion is related to its ability to adopt a tilted orientation [11]. A helical structure is assumed for the SIV peptide. Indeed, by using non-tilted SIV mutants, it has previously been shown that there is a correlation between helicity, tilt and *in vitro* fusogenicity (11). Our *in silico* results show that the

Table 1 Consensus sequence and percentage of identity of the 16 first residues of the SIV and HIV-2 glycoproteins. The percentage of substituted amino acids (% sub.) is indicated. Pho res. = hydrophobic residues. Phi res = hydrophilic residues

Position			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Consensus sequence			G	V	F	V	L	G	F	L	G	F	L	A	T	A	G	S
% identity			83	76.6	80.9	95.3	98.6	98.6	95.1	99.3	99.3	96.2	100	69.5	67.5	98.6	100	73.9
% substitution	Pho res.	I	1.4	—	1.4	—	0.7	—	0.7	—	—	—	—	—	—	—	—	—
		F	—	—	—	—	—	—	—	0.7	—	—	—	—	—	—	—	—
		L	—	—	7.8	2.1	—	0.7	0.7	—	—	2.8	—	—	2.8	—	—	—
		V	15.6	—	2.8	—	—	—	0.7	—	—	—	—	—	0.7	—	—	1.4
		M	—	—	5	0.7	—	—	2.1	—	—	—	—	—	9.9	—	—	—
		A	—	6.4	—	—	—	—	—	—	0.7	—	—	—	17	—	—	13
		G	—	0.7	—	1.4	—	—	—	—	—	—	—	16	—	—	—	0.7
		P	—	15.6	2.1	—	—	—	—	—	—	—	—	—	—	—	—	—
	Phi res.	T	—	—	—	—	—	0.7	—	—	—	—	—	11	—	—	—	11
		S	—	0.7	—	—	—	—	0.7	—	—	—	—	3.5	—	—	—	—
		Q	—	—	—	—	0.7	—	—	—	—	—	—	—	—	—	—	—
		R	—	—	—	—	—	—	—	—	—	—	—	—	2.1	0.7	—	—

Table 2 Analysis of non-redundant FPs of SIV and HIV-2 strains. Results are presented with respect to the length of peptides

	Length	9	10	11	12	13	14	15	16
Ref. seq.	GVFVLGFL	G	F	L	A	T	A	G	S
Number of peptides		21	21	21	27	30	31	31	33
Tilted peptides (%)[a]		57.1	9.5	100	88.9	23.3	25.8	35.5	24.2
Average angle (°)		61	64	54	57	66	63	61	62
Standard deviation		6	5	3	3	6	5	7	6

[a] Peptides are considered tilted when their angle is between 30 and 60° with respect to the membrane. Ref. seq. = sequence from the SIV mac isolate.

11-residue-long peptide is tilted in the membrane (57°). The *in vitro* results show that the 11- and 12-residue-long peptides induce more fusion and leakage than shorter peptides. As previously shown [22], the 16-residue-long peptide induces less fusion and leakage. The 11-residue-long peptide induces as much liposome fusion and leakage as the 12-residue-long peptide. It is therefore the minimal FP. Interestingly, the minimal FP contains the highly conserved FLGFL motif which is essential for the fusion activity not only of HIV-2 and SIV glycoproteins but also of HIV-1 [16,30–32].

The method described in this paper was also previously used to successfully predict the minimal FPs of HIV-1 gp41 and BLV gp30 [15,18]. They represent respectively the 12 first (AVGIGALFLGFL) and 15 first (SPVAALTLGLALSVG) residues of the corresponding glycoproteins. The minimal FPs of HIV-1 and BLV adopt an angle to the lipids of 55 and 48°, respectively, with respect to the membrane plane. So, despite differences in the sequence and length, FPs of SIV, HIV-1, HIV-2 and BLV are predicted to be tilted in the membrane. The fusogenicity of HIV-1, SIV and BLV glycoproteins was shown to be correlated with the ability of their FPs to adopt a tilted orientation in membranes [15–18]. Therefore, obliquity seems to be more important than length or sequence. This is further reinforced by the fact that tilted peptides have also been predicted for FPs of other viruses such as NDV, influenza A and Ebola viruses, whose lengths and sequences are different from those of HIV-1, BLV and SIV [7–9]. It should be noted that the tilted orientations of the FPs of influenza A and avian sarcoma leukosis viruses have been measured by nuclear magnetic resonance (NMR), electron paramagnetic resonance (EPR) and IR [13,33,34].

The method used in this paper, based on the obliquity/fusogenicity relationship, provided the minimal FPs of HIV-1, HIV-2, BLV and SIV. Therefore, it could be applied to other viral glycoproteins to determine their

minimal FPs. The knowledge of the minimal fusogenic region of viral glycoproteins should help in developing new molecules targeted against it to inhibit entry of viruses into cells.

Acknowledgements

REFERENCES

1. Martin I, Ruysschaert J, Epand RM. Role of the N-terminal peptides of viral envelope proteins in membrane fusion. *Adv. Drug Deliv. Rev.* 1999; **38**: 233–255.

2. Shai Y. Functional domains within fusion proteins: prospectives for development of peptide inhibitors of viral cell fusion. *Biosci. Rep.* 2000; **20**: 535–555.

3. Brasseur R, Vandenbranden M, Cornet B, Burny A, Ruysschaert JM. Orientation into the lipid bilayer of an asymmetric amphipathic helical peptide located at the N-terminus of viral fusion proteins. *Biochim. Biophys. Acta* 1990; **1029**: 267–273.

4. Nieva JL, Agirre A. Are fusion peptides a good model to study viral cell fusion? *Biochim. Biophys. Acta* 2003; **1614**: 104–115.

5. Epand RM. Fusion peptides and the mechanism of viral fusion. *Biochim. Biophys. Acta* 2003; **1614**: 116–121.

6. Brasseur R, Pillot T, Lins L, Vandekerckhove J, Rosseneu M. Peptides in membranes: tipping the balance of membrane stability. *Trends Biochem. Sci.* 1997; **22**: 167–171.

7. Brasseur R. Tilted peptides: a motif for membrane destabilization (hypothesis). *Mol. Membr. Biol.* 2000; **17**: 31–40.

8. Lins L, Charloteaux B, Thomas A, Brasseur R. Computational study of lipid-destabilizing protein fragments: towards a comprehensive view of tilted peptides. *Proteins* 2001; **44**: 435–447.

9. Adam B, Lins L, Stroobant V, Thomas A, Brasseur R. Distribution of hydrophobic residues is crucial for the fusogenic properties of the Ebola virus GP2 fusion peptide. *J. Virol.* 2004; **78**: 2131–2136.

10. Thomas A, Brasseur R. Tilted peptides: the history. *Curr. Protein Pept. Sci.* 2006; **7**: 523–527.

11. Martin I, Dubois MC, Defrisequertain F, Saermark T, Burny A, Brasseur R, Ruysschaert JM. Correlation between fusogenicity of synthetic modified peptides corresponding to the Nh2-terminal extremity of Simian immunodeficiency virus Gp32 and their mode of insertion into the lipid bilayer – an infrared-spectroscopy study. *J. Virol.* 1994; **68**: 1139–1148.

12. Martin I, Schaal H, Scheid A, Ruysschaert JM. Lipid membrane fusion induced by the human immunodeficiency virus type 1 gp41 N-terminal extremity is determined by its orientation in the lipid bilayer. *J. Virol.* 1996; **70**: 298–304.

13. Luneberg J, Martin I, Nussler F, Ruysschaert JM, Herrmann A. Structure and topology of the influenza virus fusion peptide in lipid bilayers. *J. Biol. Chem.* 1995; **270**: 27606–27614.

14. Bradshaw JP, Darkes MJM, Katsaras J, Epand RM. Neutron diffraction studies of viral fusion peptides. *Physica B* 2000; **276**: 495–498.

15. Lorin A, Lins L, Stroobant V, Brasseur R, Charloteaux B. Determination of the minimal fusion peptide of bovine leukemia virus gp30. *Biochim. Biophys. Res. Commun.* 2007; **355**: 649–653.

16. Horth M, Lambrecht B, Khim MCL, Bex F, Thiriart C, Ruysschaert JM, Burny A, Brasseur R. Theoretical and functional-analysis of the Siv fusion peptide. *EMBO J.* 1991; **10**: 2747–2755.

17. Voneche V, Portetelle D, Kettmann R, Willems L, Limbach K, Paoletti E, Ruysschaert JM, Burny A, Brasseur R. Fusogenic segments of bovine leukemia virus and simian immunodeficiency virus are interchangeable and mediate fusion by means of oblique insertion in the lipid bilayer of their target cells. *Proc. Natl. Acad. Sci. U.S.A.* 1992; **89**: 3810–3814.

18. Charloteaux B, Lorin A, Crowet JM, Stroobant V, Lins L, Thomas A, Brasseur R. The N-terminal 12 residue long peptide of HIV gp41 is the minimal peptide sufficient to induce significant T-cell-like membrane destabilization in vitro. *J. Mol. Biol.* 2006; **359**: 597–609.

19. Pillot T, Goethals M, Vanloo B, Talussot C, Brasseur R, Vandekerckhove J, Rosseneu M, Lins L. Fusogenic properties of the C-terminal domain of the Alzheimer beta-amyloid peptide. *J. Biol. Chem.* 1996; **271**: 28757–28765.

20. Pillot T, Goethals M, Vanloo B, Lins L, Brasseur R, Vandekerckhove J, Rosseneu M. Specific modulation of the fusogenic properties of the Alzheimer beta-amyloid peptide by apolipoprotein E isoforms. *Eur. J. Biochem.* 1997; **243**: 650–659.

21. Lins L, Flore C, Chapelle L, Talmud PJ, Thomas A, Brasseur R. Lipid-interacting properties of the N-terminal domain of human apolipoprotein C-III. *Protein Eng.* 2002; **15**: 513–520.

22. Martin I, Defrisequertain F, Mandieau V, Nielsen NM, Saermark T, Burny A, Brasseur R, Ruysschaert JM, Vandenbranden M. Fusogenic activity of Siv (Simian Immunodeficiency Virus) peptides located in the Gp32 Nh2 terminal domain. *Biochem. Biophys. Res. Commun.* 1991; **175**: 872–879.

23. Duzgunes N, Shavnin SA. Membrane destabilization by N-terminal peptides of viral envelope proteins. *J. Membr. Biol.* 1992; **128**: 71–80.

24. Ducarme P, Rahman M, Brasseur R. IMPALA: a simple restraint field to simulate the biological membrane in molecular structure studies. *Proteins* 1998; **30**: 357–371.

25. Higgins DG. CLUSTAL W: multiple alignment of DNA and protein sequences. *Methods Mol. Biol.* 1994; **25**: 307–318.

26. Creighton TE. In *Proteins: Structures and Molecular Properties*, Creighton TE (ed.). W. H. Freeman and Company: New York, 1992.

27. Lorin A, Thomas A, Stroobant V, Brasseur R, Lins L. Lipid-destabilising properties of a peptide with structural plasticity. *Chem. Phys. Lipids* 2006; **141**: 185–196.

28. Mrsny RJ, Volwerk JJ, Griffith OH. A simplified procedure for lipid phosphorus analysis shows that digestion rates vary with phospholipid structure. *Chem. Phys. Lipids* 1986; **39**: 185–191.

29. Lorin A, Charloteaux B, Fridmann-Sirkis Y, Thomas A, Shai Y, Brasseur R. Mode of membrane interaction and fusogenic properties of a de novo transmembrane model peptide depends on the length of the hydrophobic core. *J. Biol. Chem.* 2007; **282**: 18388–18396.

30. Bosch ML, Earl PL, Fargnoli K, Picciafuoco S, Giombini F, Wongstaal F, Franchini G. Identification of the fusion peptide of primate immunodeficiency viruses. *Science* 1989; **244**: 694–697.

31. Steffy KR, Kraus G, Looney DJ, Wongstaal F. Role of the fusogenic peptide sequence in syncytium induction and infectivity of human-immunodeficiency-virus type-2. *J. Virol.* 1992; **66**: 4532–4535.

32. Delahunty MD, Rhee I, Freed EO, Bonifacino JS. Mutational analysis of the fusion peptide of the human immunodeficiency virus type 1: identification of critical glycine residues. *Virology* 1996; **218**: 94–102.

33. Tamm LK. Hypothesis: spring-loaded boomerang mechanism of influenza hemagglutinin-mediated membrane fusion. *Biochim. Biophys. Acta* 2003; **1614**: 14–23.

34. Cheng SF, Wu CW, Kantchev EA, Chang DK. Structure and membrane interaction of the internal fusion peptide of avian sarcoma leukosis virus. *Eur. J. Biochem.* 2004; **271**: 4725–4736.