Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 355 (2007) 649–653
BBRC
www.elsevier.com/locate/ybbrc

Determination of the minimal fusion peptide of bovine leukemia virus gp30

Aurélien Lorin [a], Laurence Lins [a], Vincent Stroobant [b], Robert Brasseur [a,*], Benoit Charloteaux [a]

[a] Centre de Biophysique Moléculaire Numérique, Faculté Universitaire des Sciences Agronomiques, 2 Passage des déportés, B-5030 Gembloux, Belgium
[b] Ludwig Institute for Cancer Research—Brussels Branch, 74 Avenue Hippocrate, B-1200 Brussels, Belgium

Received 26 January 2007
Available online 8 February 2007

Abstract

In this study, we determined the minimal N-terminal fusion peptide of the gp30 of the bovine leukemia virus on the basis of the tilted peptide theory. We first used molecular modelling to predict that the gp30 minimal fusion peptide corresponds to the 15 first residues. Liposome lipid-mixing and leakage assays confirmed that the 15-residue long peptide induces fusion *in vitro* and that it is the shortest peptide inducing optimal fusion since longer peptides destabilize liposomes to the same extent but not shorter ones. The 15-residue long peptide can thus be considered as the minimal fusion peptide. The effect of mutations reported in the literature was also investigated. Interestingly, mutations related to glycoproteins unable to induce syncytia in cell–cell fusion assays correspond to peptides predicted as non-tilted. The relationship between obliquity and fusogenicity was also confirmed *in vitro* for one tilted and one non-tilted mutant peptide.

Keywords: BLV; Membrane fusion; Fusion peptide; Tilted peptide; Molecular modelling; HIV-1; Infrared spectroscopy; ATR-FTIR; Lipid-mixing; Leakage

The bovine leukemia virus (BLV) is a retrovirus resembling the Human T-cell lymphotropic virus (HTLV). Similarly to other retroviruses like HIV, the envelope of BLV contains a protein anchored in the membrane by its C-terminal domain. This protein, named gp30, is involved in the fusion process taking place during the early stages of target cell infection. Like for many other enveloped viruses, the N-terminal extremity of gp30 corresponds to a fusion peptide (FP) [1]. The implication of this region in the fusogenicity of the whole glycoprotein has been shown by mutagenesis [1]. To date, peptides equivalent to the gp30 FP were not shown to induce lipomose fusion *in vitro*. Anyway, molecular modelling indicated that a helical peptide corresponding to the 12 N-terminal residues adopts a tilted orientation at a hydrophilic/hydrophobic interface [1]. A correlation between FP obliquity and gp30 fusogenicity was also shown. Mutations that suppress the tilt of the FP drastically decrease gp30 fusogenicity [1]. These observations were at the starting point of the "tilted peptide" theory [2–4]. According to this theory, tilted peptides are peptides that present a hydrophobicity gradient when they fold into an α-helix structure. Due to this gradient, they insert into a membrane with a tilted orientation that induces the destabilization of the phospholipid acyl chains and catalyses fusion.

Recently, we used molecular modelling simulations in the context of an implicit membrane model to predict successfully the minimal FP of HIV gp41 [4]. The minimal FP corresponds to the shortest peptide able to induce significant membrane fusion. It was predicted as the shortest peptide with an optimal tilted angle in the membrane. Here, we used the same strategy to predict the region of the BLV FP necessary and sufficient to induce optimal fusion. We searched for the shortest N-terminal peptide tilted into an

* Corresponding author. Fax: +32 81 62 25 22.
E-mail address: brasseur.r@fsagx.ac.be (R. Brasseur).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.01.182

implicit membrane using the IMPALA algorithm. Liposome lipid-mixing and leakage assays were then carried out to check the validity of the prediction. Furthermore, IMPALA was used to evaluate the impact of the mutations reported in the literature [1] and to predict retrospectively their effect on fusogenicity. One functional and one defective mutant peptides were assayed for lipid-mixing and leakage of liposomes mimicking the B-cell membrane.

Materials and methods

Materials

1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC), cardiolipin (CL) from bovine heart, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol (CHOL), and bovine brain sphingomyelin (SM) were purchased from Sigma (St. Louis MO, USA).

Trifluoroethanol (TFE) was 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) came from Molecular Probes (Eugene, Oregon, USA). Hepes, Triton X-100 came from Sigma (St. Louis MO, USA). NaCl came from Merck Eurolab (Leuven, Belgium).

The wild-type (WT) 12- to 16- and 21-residue long FPs, and the MU0 and MU4 mutant peptides were synthesized by conventional solid phase peptide synthesis, using Fmoc for transient NH_2-terminal protection, and were characterized using mass spectrometry. These peptides have free N-termini and amidated C-termini. Peptide purity is more than 75%, as indicated by analytical HPLC.

In silico analysis

Peptide sequence and structure. The sequence of the FP corresponds to the American VDM isolate (SwissProt Accession Code: P25505). Peptides were constructed as α-helices using Hyperchem 6.0 (Hypercube Inc.) assigning phi/psi values of −58° and −47° that correspond to the classical α-helical structure [5]. Backbone and side chains conformation was then optimized with Hyperchem by a Polak–Ribiere conjugate gradient procedure using the AMBER force field. The termination conditions were fixed to a RMS gradient of 0.01 kcal/(Å mol).

Insertion of the peptides into membrane. The insertion of the peptides into an implicit bilayer was predicted using the IMPALA algorithm (Integral Membrane Protein and Lipid Association) developed by Ducarme et al. [6]. The parameters of the lipid bilayer and of the calculations were the same as previously [4]. 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.

In vitro analysis

Liposome preparation. DOPC/DOPE/CHOL/SM/CL (34/27/20/13/6 m/m) LUVs were prepared as described previously [4] to mimic B-cell composition [7]. LUVs used in lipid-mixing experiments were prepared in 5 mM Hepes and 100 mM NaCl at pH 7.4. LUVs used in leakage

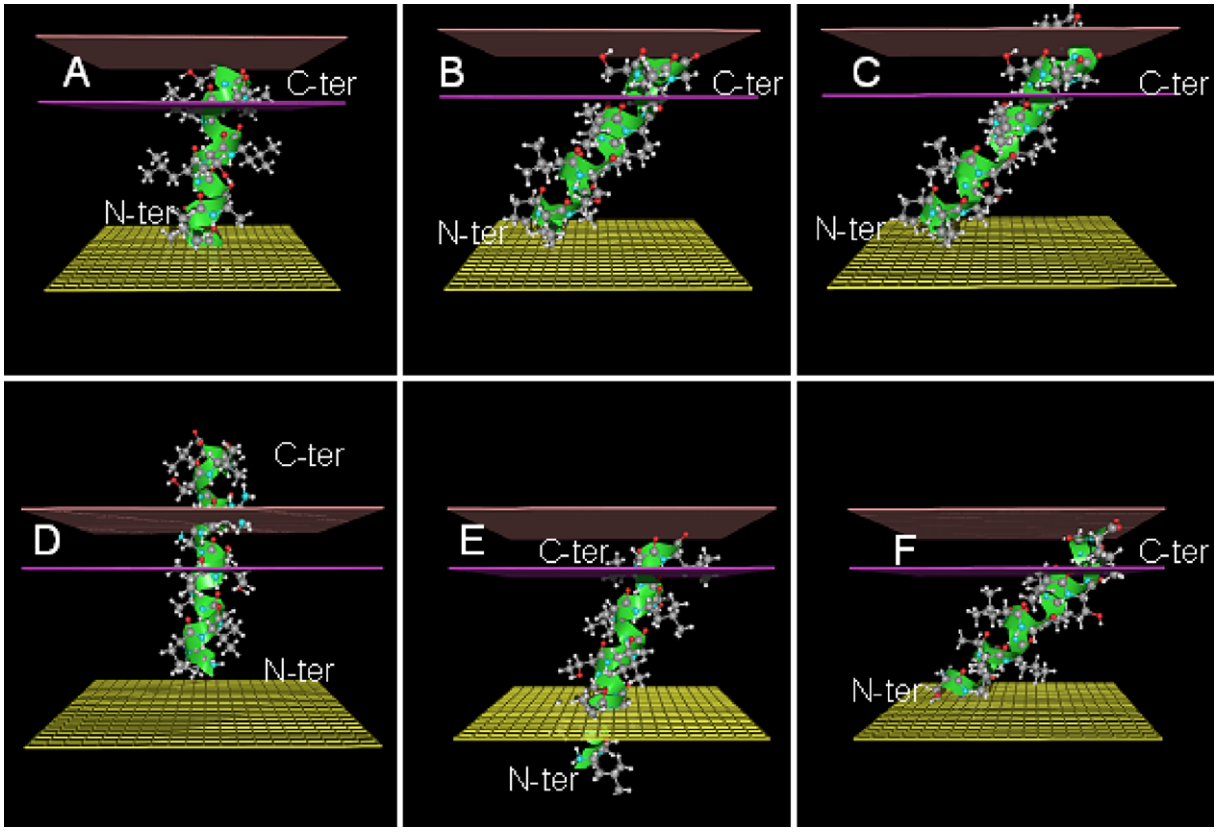

Fig. 1. Best position in the IMPALA membrane for (A) 13-residue FP, (B) 15-residue FP, (C) 16-residue FP, (D) 16-residue peptide MU0 mutant, (E) 16-residue peptide MU1 mutant, (F) 16-residue peptide MU4 mutant. N- and C-terminal extremities are indicated. Only one layer of the membrane is represented. 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)