Biochimica et Biophysica Acta xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Structure and orientation study of Ebola fusion peptide inserted in lipid membrane models

Audrey Agopian, Sabine Castano *

CBMN-UMR 5248 CNRS, Université de Bordeaux, IPB, Allée Geoffroy Saint Hilaire, 33600 Pessac, France

ARTICLE INFO

Article history:
Received 17 May 2013
Received in revised form 4 September 2013
Accepted 9 September 2013
Available online xxxx

Keywords:
Ebola fusion peptide
Lipid/peptide interaction
Secondary structure
Viral fusion mechanism
IR spectroscopy
Ellipsometry

ABSTRACT

The fusion peptide of Ebola virus comprises a highly hydrophobic sequence located downstream from the N-terminus of the glycoprotein GP2 responsible for virus–host membrane fusion. The internal fusion peptide of GP2 inserts into membranes of infected cell to mediate the viral and the host cell membrane fusion. Since the sequence length of Ebola fusion peptide is still not clear, we study in the present work the behavior of two fusion peptides of different lengths which were named EBO17 and EBO24 referring to their amino acid length. The secondary structure and orientation of both peptides in lipid model systems made of DMPC:DMPG:cholesterol:DMPE (6:2:5:3) were investigated using PMIRRAS and polarized ATR spectroscopy coupled with Brewster angle microscopy. The infrared results showed a structural flexibility of both fusion peptides which are able to transit reversibly from an α-helix to antiparallel β-sheets. Ellipsometry results corroborate together with isotherm measurements that EBO peptides interacting with lipid monolayer highly affected the lipid organization. When interacting with a single lipid bilayer, at low peptide content, EBO peptides insert as mostly α-helices mainly perpendicular into the lipid membrane thus tend to organize the lipid acyl chains. Inserted in multilamellar vesicles at higher peptide content, EBO peptides are mostly in β-sheet structures and induce a disorganization of the lipid chain order. In this paper, we show that the secondary structure of the Ebola fusion peptide is reversibly flexible between α-helical and β-sheet conformations, this feature being dependent on its concentration in lipids, eventually inducing membrane fusion.

1. Introduction

The Ebola virus is one of the most virulent pathogens for humans and primates. It has caused several outbreaks in central Africa in the past decade and currently no treatments or vaccines are approved yet. Ebola virus is an enveloped virus that belongs to the *filoviridae* family. It causes severe hemorrhagic fever that is often lethal in humans. Early target cells are monocytes, macrophages, dendritic cells and hepatocytes then the infection rapidly spreads to the viral organs of the host. Infection of a target cell by enveloped virus requires fusion between the viral enveloped and host cell membrane. The membrane fusion process is a common feature of enveloped viruses and is mediated by a glycoprotein (GP) that acts as a membrane fusion protein. The Ebola GP is a class I fusion protein that forms trimeric spikes on the virion surface [1]. During the maturation of the virion, GP is activated by proteolytic cleavage into two parts: GP1 and GP2, connected by a disulfide bond. In all the class I fusion protein, the first subunit binds to the cell receptors while the second subunit mediates the fusion between viral and host cell membranes. For Ebola, the fusion process is mediated by the virally encoded glycoprotein GP2 that contains a highly hydrophobic sequence generally referred as the fusion peptide which initiates the fusion event by anchoring glycoprotein GP2 to the cell membrane surface. Unlike other class I fusion proteins, the Ebola fusion peptide is located within the GP2 structure downstream from the N-terminus. This conserved peptide sequence is absolutely required for the fusogenic activity of the glycoprotein GP2 but its structural and functional determinants remain unknown. Previous studies showed that endosome allows pH activating cathepsin L and B activities that is required for viral fusion to occur where GP2 undergoes a subsequent conformational change exposing the fusion peptide to the host membrane [2–4]. However, how Ebola fusion peptide binds to the membranes and induces fusion remains poorly understood.

Several studies about Ebola fusion peptide have been done in different conditions and different mimetic membrane environments, partly leading to inconsistent results. In general, Ebola fusion peptide can adopt different structures depending on its environment. Moreover, the exact peptide sequence involved in the fusion, which was initially predicted by Gallaher et al. [5] is not well defined to date, as numerous studies used different fusion peptide lengths. Freitas et al. [6] utilized Ebola fusion peptide composed of 16 amino acids, EBO16 (524–539

Abbreviations: DMPC, dimyristoyl-phosphatidylcholine; DMPG, dimyristoyl-phosphatidylglycerol; DMPE, dimyristoyl-phosphatidylethanolamine; PMIRRAS, polarization modulation infra-red reflection absorption spectroscopy; ATR, attenuated total reflection spectroscopy; BAM, Brewster angle microscopy; Ri, lipid to peptide ratio

* Corresponding author at: CBMN/University Bordeaux 1, Allée Geoffroy Saint Hilaire, F-33600 Pessac, France. Tel.: +33 5 40 00 68 48; fax: +33 5 40 00 22 00.
E-mail address: s.castano@cbmn.u-bordeaux.fr (S. Castano).

0005-2736/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.bbamem.2013.09.003

from GP2) and determined its secondary structure by circular dichroïsm (CD) and nuclear magnetic resonance (NMR). They showed a random coil–helix transition when they added SDS micelles to an insoluble water-diluted EBO16 solution. The NMR structure of EBO16 showed that in the presence of mimetic membranes, the peptide adopts a central 3_{10}-helix structure stabilized by aromatic interaction between amino acids Trp8 and Phe12. Suárez et al. [7] used a 17 amino acid sequence as fusion peptide (EBO_{GE}: 524–540 from GP2) and also showed by UV CD spectroscopy structural transition between random-coil in aqueous buffer and an α-helix structure induced by increasing concentration of TFE. Moreover, they demonstrated by IR spectroscopy that in association to PC/PI (1:2) vesicles, the fusion peptide undergoes a conformational change from an α-helix to an anti-parallel β-structure, presumably induced by the presence of calcium. In fact, the α-helical structure seems to be related to the ability of the peptide to induce destabilization of the membrane while the presence of Ca^{2+} provocated a conformational transition of the peptide to a β-sheet structure [7–9]. In a more recent study [10], Freitas et al. suggested that although their EBO16 fusion peptide adopts a β-sheet structure, it was only able to induce PC:PE:SPM:Chol liposome aggregation but not fusion. They also showed by experiments of raft aggregation and cell–cell fusion that EBO16 fusion peptide is able to use lipid raft domains enriched in cholesterol [11] as a target for virus entry into cells. This suggests an important role of cholesterol since cholesterol depleted cells have impaired EBO virus glycoprotein-pseudotype-virion entry and fusion [12]. They also demonstrated that the fusion peptide EBO16 can induce lipid mixing of the living Vero cells even in absence of Ca^{2+}. In the same study, EBO16 was proposed to lie parallel to the membrane anchored by the aromatic amino acids, Trp8 and Phe12. In another study conducted by Adam et al. [13], they used a shorter peptide of 11 amino acid long (524 to 534) capable of liposome destabilization and fusion. They also analyzed its behavior in a hydrophobic/hydrophilic environment by molecular modeling. They proposed a mechanism where the peptide is obliquely inserted in the host membrane and further able to destabilize it.

The aim of the present work was to clarify the inconsistencies between the previously published data and to improve our understanding of the fusion mechanism by which the Ebola fusion peptide inserts into lipid membrane. The structural behavior of the individual Ebola fusion peptide as well as that bound to a lipid membrane model has thus been investigated using several infrared (IR) spectroscopy techniques: PMIRRAS at the air/water interface of the peptides alone and inserted into lipid monolayers and ATR spectroscopy of peptides inserted in bi- and multi-bilayers. Investigations at the air/water interface were combined with ellipsometric measurements to analyze the morphology of the layers. The peptide orientation given by IR experimental results were analyzed using spectral simulation. Two peptide sequences were studied: the 17 (residues 524–540, EBO17) and the 24 (residues 524–547, EBO24) amino acid consensus sequences.

2. Materials and methods

2.1. Lipids and peptides

Lipids (DMPC, DMPG, cholesterol and DMPE) were purchased from Sigma. The Ebola fusion peptides EBO17: GAAIGLAWIPYFGPAAE and EBO24: GAAIGLAWIPYFGPAAEGIYTEGL were purchased from GenScript (Piscataway, USA). EBO17 and EBO24 were solubilized in hexafluoroisopropanol (HFIP) from Sigma at micromolar concentrations. Peptide concentrations were calculated from the absorbance at 280 nm using a molar extinction coefficient ε_{280} of 7000 $M^{-1}\cdot cm^{-1}$ for EBO17 and ε_{280} of 8400 $M^{-1}\cdot cm^{-1}$ for EBO24. The molar extinction coefficient of the peptides has been determined using the formula $\varepsilon_{280nm} = 5600 \times n_W + 1400 \times n_Y$ which takes into account the individual extinction coefficient of the amino acids W and Y.

2.2. Film formation and surface pressure measurements

Monolayer experiments were performed on a computer-controlled Langmuir film balance (Nima Technology, Coventry, England). The rectangular trough and the barrier were made of Teflon. The surface pressure (π) was measured by the Wilhelmy method using a filter paper plate. The trough was filled with a saline solution (150 mM NaCl, pH 5.6) using ultra pure water (MilliQ, Millipore). The experiments were carried out at 22 ± 2 °C. Pure peptide films were obtained by spreading of few μl of HFIP peptide stock solutions at the air/water interface. The mixed lipids/peptides at the defined lipid to peptide ratio, Ri was obtained by co-spreading of the lipid/peptide mixture at the water surface. The monolayer lipid composition used was DMPC:DMPG:cholesterol:DMPE (6:2:5:3). After ~15 min of stabilization, the film was slowly compressed up to 40 $mN\cdot m^{-1}$ and decompressed at the same rate speed than during the compression (8 $Å^2\cdot molecule^{-1}\cdot min^{-1}$).

2.3. PMIRRAS spectroscopy

PMIRRAS spectra were recorded on a Nicolet Nexus 870 spectrometer equipped with a photovoltaic HgCdTe detector cooled at 77 K. Spectra were obtained by performing 800 scans at a resolution of 8 cm^{-1}. Briefly, PMIRRAS combines FT-IR reflection spectroscopy with fast polarization modulation of the incident beam between parallel (p) and perpendicular (s) polarizations. The differential reflectivity spectrum is obtained using a two-channel processing signal. To remove the contribution of liquid water absorption and the dependence on Bessel functions, the monolayer spectra are divided by that of the subphase. With an incidence angle of 75°, transition moments preferentially oriented in the plane of the interface give intense and upward oriented bands, while perpendicular ones give weaker and downward oriented bands [14].

2.4. ATR spectroscopy of the fusion peptide inserted in a lipid bilayer

Small unilamellar vesicles (SUV) composed of DMPC:DMPG: cholesterol:DMPE (6:2:5:3) were prepared pure or mixed with the fusion peptide at a defined molar ratio, Ri. For pure lipid SUV, lipids were first dissolved in chloroform and mixed at the desired ratio. The solvent was then evaporated giving a lipid film. SUV were then prepared by tip sonication after direct hydration of the lipid film with a solution of D_2O containing 150 mM NaCl. For the preparation of the SUV containing the mixed fusion peptide/lipids, the fusion peptide powder is first dissolved in HFIP then mixed with the lipids in chloroform at the desired ratio. Solvents were evaporated before hydration by D_2O and tip sonication for SUV formation. SUV were then burst on a germanium ATR crystal to form a single bilayer which is controlled by the measurement of the absolute IR intensity.

ATR spectra were recorded on a Nicolet 6700 spectrometer Thermo Scientific equipped with a MCT detector cooled at 77 K. Since ATR spectroscopy is sensitive to the orientation of the structures [15–17], spectra were recorded with parallel (p) and perpendicular (s) polarizations of the incident light with respect to the ATR plate. 400 scans were recorded at a resolution of 8 cm^{-1}. All the orientation information is then contained in the dichroïc ratio $R_{ATR} = Ap/As$, where Ai respectively represents the absorbance of the considered band at p or s polarization of the incident light.

2.5. Ellipsometric measurements

The morphology of pure peptides or mixed lipids with the peptides was observed at the air/water interface using an ellipsometer iElli2000 microscope (NFT, Göttinger, Germany) mounted on a Langmuir trough. The microscope was equipped with a doubled frequency Nd:Yag laser (532 nm, 50 mW), a polarizer, a compensator, an analyzer and a CCD camera. The spatial resolution of the pictures with the 10× magnification lens was about 2 μm and the image size was 600 × 450 μm. For

Please cite this article as: A. Agopian, S. Castano, Structure and orientation study of Ebola fusion peptide inserted in lipid membrane models, Biochim. Biophys. Acta (2013), http://dx.doi.org/10.1016/j.bbamem.2013.09.003