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Real-time monitoring of interactions between Ebola fusion peptide and solid-supported phospholipid membranes: Effect of peptide concentration and layer geometry

M. Khorshid [a, b, **, 1], P. Losada-Pérez [b, c, *, 1], G. Wackers [a], D. Yongabi [a], F.U. Renner [b, c], R. Thoelen [b, c], P. Wagner [a]

[a] Soft-Matter Physics and Biophysics Section, Department of Physics and Astronomy, KU Leuven, Celestijnenlaan 200D, B-3001, Leuven, Belgium
[b] Institute for Materials Research IMO, Hasselt University, Wetenschapspark 1, B-3590, Diepenbeek, Belgium
[c] IMEC vzw, Division IMOMEC, Wetenschapspark 1, B-3590, Diepenbeek, Belgium

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ABSTRACT

The pathogenesis of the Ebola virus which leads to a severe hemorrhagic fever in hosts is a very complex process which is not completely understood. Glycoproteins of the viral envelope are believed to play a crucial role in receptor binding and subsequently in fusion of the virus with the target cells of the host. As a result, the virus enters the cells and replicates. This process causes further cytopathic, and pathological reactions in the host's body. To gain further insights into the fusogenic interactions of the virus with cell membranes, we used well-controlled simple biomimetic systems, consisting of solid-supported phospholipid layers together with a small sequence of the viral glycoprotein (EBO17), which is believed to be the most important part responsible for viral pathogenesis. We monitor the real-time interaction of a EBO17 peptide sequence from the Ebola virus with dipalmitoylphosphatidylcholine (DMPC) phospholipid membranes using quartz crystal microbalance with dissipation monitoring (QCM-D) as a label-free method. In particular, we focus on the influence of the concentration of the peptide and the lipid layer geometry on the disrupting mechanism of the EBO17 peptide. Results indicate that for 2D supported lipid bilayers, low peptide concentrations induce a small, but detectable change in layer stability due to the presence of an α-helix configuration of the peptide. With large peptide concentrations, the peptide acquires a β-sheet configuration and no significant layer changes can be observed. A different mechanism is responsible for the interaction of the EBO17 peptides with the more complex 3D supported vesicle layers, for which a concentration-dependent trend can be observed leading to thicker lipid layers. Complementary analysis of the lipids' main phase transition evidences the differences induced in layer organization on the two layer geometries. These results confirm the importance of the interplay between lipid layer geometry and related peptide organization as an essential marker in peptide activity.

1. Introduction

Fusion peptides (FPs) are hydrophobic domains required for fusogenic activity of glycoproteins from different viruses such as the Ebola virus. The Ebola virus is a part of the *Filoviridae* family, which is a highly virulent pathogen in primates and human [1]. This virus causes epidemics of African hemorrhagic fever with a high mortality rate in humans and with no specific efficient treatment so far, partly because the precise mechanisms of viral action are poorly understood.

In this respect, fusion of the viral envelope with target cell-membranes plays a major role in the infection mechanism [2]. The fusion process is directed by a glycoprotein (GP) on the virion surface which is a class I fusion protein [3]. As the virion matures, this glycoprotein is activated resulting in GP1 and GP2 subunits [4,5]. Binding to the cell's receptors is mediated by the GP1 subunit

* Corresponding author. Institute for Materials Research IMO, Hasselt University, Wetenschapspark 1, B-3590 Diepenbeek, Belgium.
** Corresponding author. Soft-Matter Physics and Biophysics Section, KU Leuven, Celestijnenlaan 200 D, B-3001 Leuven, Belgium.
E-mail addresses: mehran.khorshid@kuleuven.be (M. Khorshid), patricia.losadaperez@uhasselt.be (P. Losada-Pérez).
[1] Both authors contributed equally.

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of the class I fusion protein while the GP2 subunit with its highly hydrophobic sequence of fusion protein at its N-terminus accelerates the fusion process between viral and host cell membranes [4,6]. Exposure of the fusion peptide to the host cell membrane induces conformational changes in the GP2 subunit and are believed to be essential for viral fusion [7]. In this context, the interaction pathways of Ebola fusion peptide with lipid bilayers are of major interest since lipids especially phospholipids constitute the major building blocks of cell membranes. Despite its relevance as a fusogenic peptide, the study of Ebola peptide-lipid bilayer interactions is restricted to few experimental and simulation studies. Fluorescence microscopy, Circular dicroism and Infrared Spectroscopy revealed the importance of calcium divalent ions in the fusion mechanism with phosphatidylinositol-containing biomimetic membranes [8,9]. In the absence of Ca^{2+}, the Ebola fusion peptide partitions in membranes containing phosphatidylinositol, and stabilizes an α-helical conformation without vesicle fusion. In the presence of millimolar Ca^{2+}, in turn, the membrane-bound peptide adopts a β-sheet configuration and induces inter-vesicle mixing of lipids [9]. The presence of lipid rafts, cholesterol-enriched domains with enhanced order within the lipid bilayer, act as a target for virus entry into cells, as revealed by structural nuclear magnetic resonance NMR characterization [10,11]. Nevertheless, a β-structure does not seem to be the unique feature needed for fusion in raft-containing liposomes [11]. A recent study on model lipid monolayers using ellipsometry showed reversible structural polymorphism between α-helix and β-sheets conformations in relation to the concentration of both the lipids and the peptide [12]. Therefore, the correlation between peptide structure and fusion does not seem to follow a simple general rule and further studies on more model lipids and geometries different from monolayers, i.e., lipid bilayers or vesicle layers are desirable.

In this context, quartz crystal microbalance with dissipation monitoring (QCM-D) is a powerful surface-sensitive and label-free technique which enables to monitor the interactions as well as the conformational changes between adsorbed lipid layers and peptides in real time. QCM-D has been successfully applied in studying supported lipid layers, which are useful for biotechnology applications [13] and their interactions with peptides, in particular, with antimicrobial pore forming peptides [14–19]. In addition, phase transitions, a useful indicator of membrane organization and structural changes upon interaction with peptides can be also probed by QCM-D [20–26].

In the present study, we examine the interactions of the Ebola fusion peptide on different supported layer geometries, to get an in-depth understanding of the interaction mechanism. Both supported lipid vesicles (SLV) and supported lipid bilayers (SLB) offer specific advantages and represent the effects of geometry changes in between extended flat layers and living cells. Therefore, SLV and SLB are interesting layers for basic biophysical studies to understand the pathological peptide pathways. For simplicity, we have chosen dipalmitoylphosphatidylcholine (DMPC) as a simple saturated phospholipid which shows its melting transition in the working range of QCM-D set up, and the peptide sequence "GAAIGLAWIPYFGPAAE", which we will henceforth denote as EBO17. EBO17 is a hydrophobic peptide sequence which is responsible for the fusogenic activity of the Ebola virus. The peptide sequence consists of 17 amino acids with the hydrophobic part near the N-terminus. We have monitored the interactions of the EBO17 with DMPC SLVs and SLBs formed onto different substrates, gold and silicon oxide, respectively, at different peptide concentrations.

2. Materials and methods

DMPC powder was purchased from Avanti Polar Lipids (Alabaster, AL). Spectroscopic grade chloroform assay 99.3% (stabilized with about 0.6% ethanol) was obtained from Analar (Normapur, Leuven, Belgium). HEPES buffer (pH 7.4) composed of 10 mM HEPES from Fisher Scientific (assay 99%) and 150 mM NaCl from Sigma-Aldrich, Diegem, Belgium (assay ≥ 99.5%) was used for hydration of the dried DMPC. DMSO (assay ≥ 99.9% was purchased from Sigma-Aldrich). The quantity of lipid was determined gravimetrically using a Sartorius balance yielding a maximal mole fraction uncertainty of ±0.002. The Ebola fusion peptide sequence "EBO17: GAAIGLAWIPYFGPAAE" with amidation at the C-terminus was purchased from Eurogentec, Seraing, Belgium. The peptide was dissolved in HEPES buffer with a small amount of DMSO to make a stock solution of 1 mg/ml.

2.1. Vesicle preparation

DMPC was dissolved in spectroscopic grade chloroform in a round bottomed flask and the afterwards the solvent was evaporated under a gently flow of nitrogen. As a result, a lipid film on the flask bottom was formed and kept under vacuum overnight. Subsequently, the lipid was hydrated with HEPES buffer. Hydration to 0.2 mg/ml was performed under continuous stirring in a temperature-controlled water bath at 45 °C (above the melting temperature of DMPC, T_m ~ 24 °C [23,24]). Small unilamellar vesicles were fabricated extruding 25 times through a polycarbonate membrane (Avanti Polar Lipids) with a pore size of 100 nm. The effective vesicle sizes and the polydispersities of the DMPC-vesicle dispersions were measured by dynamic light scattering, DLS (Zeta Pals, Brookhaven Instruments Corporation) yielding an average diameter of ~140 nm and a polydispersity index of 0.08.

2.2. Quartz crystal microbalance with dissipation monitoring

Quartz crystal microbalance with dissipation monitoring (QCM-D) is a label-free surface-sensitive technique which monitors in real-time the frequency (f) and the energy dissipation factor (D) shifts of different overtones upon the addition of mass on a solid quartz surface coated with a given material, for instance gold [27]. Absorption of molecules to an oscillating quartz crystal results in shifts in the resonance frequency and dissipation which can be related to the adsorbed mass and to the viscoelastic properties of the layer. The layer is sensed as a hydrogel and is described by a complex shear modulus, defined as [28,29].

$$G = G' + iG'' = \mu_f + 2\pi i f \eta_f = \mu_f(1 + 2\pi i \chi), \quad (1)$$

where G' and G'' stand for energy storage and dissipation, respectively, f is the oscillation frequency, μ_f is the elastic shear storage modulus, η_f is the shear viscosity, and $\chi = \eta_f/\mu_f$, is the relaxation time of the layer.

For our measurements, we have used QCM-D on a Q-sense E4 instrument (Gothenborg, Sweden) monitoring the frequency shift Δf and the dissipation change ΔD. AT-cut quartz crystals with Au and SiO_2 coatings (diameter 14 mm, thickness 0.3 mm, surface roughness 3 nm and resonant frequency 4.95 MHz) were used. The Au-coated quartz sensors were cleaned with a 5:1:1 mixture of Milli-Q water (conductivity of 0.055 S cm^{-1} at 25 °C), ammonia and hydrogen peroxide. They were exposed to UV-ozone for 15 min using a Digital PSD series UV-ozone system from Novascan. Afterwards, the sensors were rinsed with Milli-Q water and dried with N_2. The SiO_2-coated quartz sensors were cleaned in a sodium dodecyl sulfate solution (2% SDS) for 30 min and UV-ozone exposed for 1 h, followed by rinsing in water and drying with N_2. Oxidized Au is well-known to promote layers of intact vesicle layers, while hydrophilic and negatively charged SiO_2 typically induces the