Structure of the Ebola Fusion Peptide in a Membrane-mimetic Environment and the Interaction with Lipid Rafts*§

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The fusion peptide EB16 (GAAIGLAVIPYFGPAA) comprises the fusion domain of an internal sequence located in the envelope fusion glycoprotein (GP2) of the Ebola virus. This region interacts with the cellular membrane of the host and leads to membrane fusion. To gain insight into the mechanism of the peptide-membrane interaction and fusion, insertion of the peptide was modeled by experiments in which the tryptophan fluorescence and 3H NMR were monitored in the presence of sodium dodecyl sulfate micelles or in the presence of detergent-resistant membrane fractions. In the presence of SDS micelles, EBO16 undergoes a random coil-helix transition, showing a tendency to self-associate. The three-dimensional structure displays a 310-helix in the central part of molecule, similar to the fusion peptides of many known membrane fusion proteins. Our results also reveal that EBO16 can interact with detergent-resistant membrane fractions and strongly suggest that Trp-8 and Phe-12 are important for structure maintenance within the membrane bilayer. Replacement of tryptophan 8 with alanine (W8A) resulted in dramatic loss of helical structure, proving the importance of the aromatic ring in stabilizing the helix. Molecular dynamics studies of the interaction between the peptide and the target membrane also corroborated the crucial participation of these aromatic residues. The aromatic-aromatic interaction may provide a mechanism for the free energy coupling between random coil-helical transition and membrane anchoring. Our data shed light on the structural “domains” of fusion peptides and provide a clue for the development of a drug that might block the early steps of viral infection.

The Ebola virus belongs to the Filoviridae family and causes hemorrhagic fever in primates, resulting in high mortality rates (1). Infection by the Ebola virus requires fusion of viral and cellular membranes (1, 2). The membrane fusion process is a common feature of enveloped viruses and is mediated by an envelope glycoprotein that acts as a membrane fusion protein (3–5). The Ebola glycoprotein (GP)2 is responsible for both receptor binding and membrane fusion during entry into the host cell (1). It is comprised of two polypeptides, GP1 and GP2, linked by a disulfide bond. The structure of a fragment of Ebola fusion protein (GP2) was solved at 1.9 Å of resolution (6). However, the fusion peptide (G24GAAIGLAVIPYFGPAA) was not included (6–8). This peptide shares general features with many other viral membrane fusion proteins. As previously predicted, the fusion peptide consists of 16 uncharged residues that are conserved within the virus family (9). The fusion peptide region, which is believed to insert into the cellular membrane, is located at the extreme N terminus of GP2, and the GP2 C-terminal region is adjacent to the transmembrane helix anchored in the viral membrane (10). In most enveloped viruses, when the virus is inactive to fusion, the fusion peptide is buried and protected in a hydrophobic core of the soluble glycoprotein ectodomain, whereas when the virus is in the fusion active state, the fusion peptide is exposed and available for fusion into the target host membrane (10).

To better understand the fusion mechanism, we have determined the structure of the fusion peptide of the GP2 from Ebola virus, utilizing a combination of spectroscopic techniques such as circular dichroism, intrinsic fluorescence, molecular dynamics, and nuclear magnetic resonance. We show the atomic structure and the conformational exchange of the Ebola fusion peptide in the presence of micelles at pH 7 as well as its interaction with detergent-resistant membrane fractions (DRMs), usually considered to correspond to the lipid rafts originated from plasma membrane. The three-dimensional structure reveals how the aromatic residues (Trp-8 and Phe-12) play a crucial role for structure maintenance within the membrane environment. Substitution of Trp-8 causes destabilization of

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2 The abbreviations used are: GP, glycoprotein; NOESY, nuclear Overhauser (NOE) spectroscopy; LUV, large unilamellar vesicle; r.m.s.d., root mean square deviation; DRM, detergent-resistant membrane fraction; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; Cho, cholesterol; wt, wild type.
the helical structure. Knowledge of the EBO16 structure in solution allowed us to characterize the structural changes upon interaction with lipid microdomains. These structural studies are important to determine structural identities among the enveloped viruses, which should help in the rational design of antiviral drugs.

EXPERIMENTAL PROCEDURES

Peptide—The Ebola fusion domain was purchased from Genemed Synthesis, South San Francisco, CA. Its purity and molecular mass were assessed by electrospray mass spectrometry, high performance liquid chromatography, and amino acid analysis. The purity was greater than 95%. Stock solutions were prepared by suspending the peptide at 1 or 2 mM in double-distilled water, where it is fully insoluble. The solubility was acquired after the addition of membrane models or dimethyl sulfoxide. The peptide concentration was calculated from the absorbance at 280 nm using a molar absorption coefficient $\varepsilon_{280}$ of 6970 cm$^{-1}$ M$^{-1}$.

Circular Dichroism—CD data were collected using a Jasco 715 spectropolarimeter. In general, a 2-mm path length cuvette with 100 $\mu$M fusion peptide in 15 mM phosphate, pH 7, was used for CD experiments. Each of the CD spectra was obtained from an average of four scans with a 2-nm bandwidth. The temperature was maintained at 298 K, the scan rate was 50 nm/min, and the step resolution was 1.0; the response time was 8 s. After background subtraction and smoothing, all of the CD data were converted from CD signal (millidegrees) into mean residue molar ellipticity (deg cm$^2$ dmol$^{-1}$) by using the equation $[\theta] = \theta_{10}^{-1} l c^{-1} N^{-1}$, where $l$ is the cell length in cm, $c$ is the molar concentration, and $N$ is the number of amino acid residues in the peptide.

Peptide Binding to SDS Micelles—Peptide binding to SDS micelles was estimated by variation of the fluorescence emitted by Trp, based on the spectral shift that accompanies the change in its environment. Trp was excited at 280 nm, and its fluorescence emission was acquired from 315 to 420 nm. In addition, fluorescence quenching experiments were performed with 10–50 mM acrylamide as an extrinsic quencher. The fluorescence quenching data were analyzed according to the Stern-Volmer relationship (11), $F_0/F = 1 + K[Q]$, where $F_0$ and $F$ are the fluorescence intensities in the absence and in the presence of the quencher, $[Q]$ is the quencher concentration, and $K$ is the quenching constant. Trp fluorescence was recorded at 340 nm ($\lambda_{ex} = 280$ nm). The peptide concentration was 10 $\mu$M. Fluorescence polarization was measured with excitation at 295 nm, and emission recorded through a WG320S filter (50% cut-off at 320 nm). The experiment was carried out at 25 °C in 15 mM phosphate buffer, pH 7. The peptide concentration was 100 $\mu$M.

Three-dimensional Structure—NMR measurements were carried out at 25 °C on a Bruker AMX 600-MHz NMR spectrometer in the phase-sensitive mode using States time-proportional phase incrementation. The sample contained 1 mM peptide, DRMs in phosphate buffer pH 7 (90% H$_2$O, 10% D$_2$O (v/v)). NOESY experiments were recorded with a mixing time of 80 ms using WATERGATE. The spectra were collected in the presence of DRMs with 512 data points in F1, 4096 points in F2, and 32 scans. In the presence of large unilamellar vesicles (LUVs) (PC:PE:PI:Cho) the spectra were collected with 128 data points in F1, 4096 points in F2, and 256 scans. The signal/noise was approximately twice larger in the presence of DRMs than in the presence of LUVs.

RESULTS AND DISCUSSION

Conformational Changes Induced by Membrane-Mimetic Environments—Recent studies have shown the high hydrophobicity of the fusion peptide of Ebola virus (9) and one or two charged amino acids have been added to the peptide sequence to minimize peptide aggregation in solution (18). Because we were interested in the structure of the fusion peptide in a membrane-mimetic environment, we did not add any charged amino acids. To investigate the secondary structure of EBO16,
we examined the far-UV CD spectra. As shown in Fig. 1A, the peptide assumes a random-coil conformation in aqueous buffer and a more defined structure in the presence of SDS micelles, where it displays a typical helical structure. In the presence of 60 mM dodecylphosphocholine, a phospholipid-derived detergent, the fusion peptide displays two conformational states, random coil and α-helix (data not shown). This condition was not suitable for determining the structure by NMR because of the conformational exchange between folded and unfolded states. The CD studies reveal that SDS micelles provide a good model for characterizing and determining the EBO16 structure.

Interactions between the Ebola Fusion Peptide and SDS Micelles—To follow the binding of EBO16 with SDS micelles, we measured tryptophan fluorescence emission. In EBO16 there is only one tryptophan, located in the middle of the sequence. Fluorescence emission in the absence of SDS shows a maximum emission wavelength of 337 nm (Fig. 1B). This maximum reflects the tryptophan accessibility and the peptide aggregation because of its high hydrophobicity and random structure. In fact, when EBO16 was incubated with SDS micelles, the spectral peak was blue-shifted from 337 to 324 nm (Fig. 1B). The shift of about 13 nm suggests that tryptophan enters a hydrophobic environment in the SDS micelles. In addition, the quantum yield was much higher in the presence of SDS micelles (not shown).

To further characterize the peptide insertion into SDS micelles, we carried out acrylamide fluorescence quenching experiments. Stern-Volmer plots provide an indication of solvent accessibility; a steep slope indicates that the tryptophan residue is exposed to the acrylamide, whereas a lower slope indicates that the tryptophan is protected from the acrylamide (19). Fig. 1C shows the Stern-Volmer plots for EBO16 in the absence and in the presence of SDS micelles. In the absence of SDS micelles, a steep slope is observed ($K_{sv} = 15.8 \text{ M}^{-1}$), whereas in the presence of SDS micelles a reduced slope is obtained ($K_{sv} = 10.2 \text{ M}^{-1}$). The fluorescence data clearly indicate that the single Trp of EBO16 is protected by the micelle from quenching. EBO16 in the micellar environment undergoes structural modifications including intramolecular rearrangements and acquisition of helical structure, showing a tendency to self-associate.

NMR Analysis of Secondary Structure—The $^1$H NMR data were obtained using perdeuterated SDS micelles at 298 K and pH 7. The NOESY spectrum of EBO16 displayed numerous well resolved cross-peaks (supplemental Fig. 1), indicating that the peptide is folded and that assignment should be possible. The NOEs were assigned, and differences in the $^1$H chemical shifts ($\Delta$ in ppm) between observed and random-coil values are shown in Fig. 2B. Negative $\Delta$ values >0.1 ppm were observed in the middle of the molecule, which is, therefore, considered to assume a helical conformation (20). In fact, analysis of the NOESY spectra provided similar results when intraresidual, sequential, and medium range connectivities were evaluated, as

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**FIGURE 1.** Interactions between EBO16 and SDS micelles monitored by circular dichroism and tryptophan fluorescence of EBO16. A, circular dichroism of the Ebola fusion peptide in the absence (blue) and in the presence (red) of SDS micelles. The SDS and peptide concentrations were 10 mM and 100 $\mu$M, respectively. B, normalized fluorescence emission spectra of the tryptophan residue of Ebola virus fusion peptide acquired in the absence (solid line) or in the presence (dashed line) of SDS micelles. The inset shows the tryptophan spectra of the peptide in the absence (solid line) or in the presence (dashed line) of SDS micelles. The inset shows the tryptophan spectra of the peptide in the absence (solid line) or in the presence (dashed line) of SDS micelles. The Trp-8 was excited at 280 nm, and fluorescence emission was acquired in the range of 315–420 nm. The peptide and SDS concentrations were 10 $\mu$M and 10 mM, respectively. C, Trp fluorescence quenching experiments of Ebola fusion peptide by acrylamide (Q) in the absence (●) or in the presence (▲) of SDS micelles are shown. Peptide-coupled SDS micelles were preincubated for 10 min in 2 ml of buffer before measurements. Increasing concentrations of acrylamide were added, and Trp fluorescence was recorded at 340 nm ($\lambda_{\text{exc}} = 280$ nm). The peptide concentration was 10 $\mu$M, and the SDS concentration was 10 mM.
summarized in Fig. 2A. The observation of medium $d_{\alpha\alpha}$ (i, i+3) and stronger $d_{NN}$ suggested a stabilized $\alpha$-helix structure. However, the presence of $d_{\alpha\alpha}$ (i, i+2) in the same region indicates a $3_{10}$ helix conformation (21). The data show a continuous pattern of $d_{\alpha\alpha}$ (i, i+3) NOEs between Leu-6 and Phe-12. Furthermore, $d_{NN}$ and $d_{\alpha\alpha}$ NOEs indicate the presence of helical structure in this region. However, the absence of $d_{\alpha\alpha}$ (i, i+4) and the presence of the continuous pattern $d_{NN}$ (i, i+2) and $d_{\alpha\alpha}$ (i, i+2) NOE connectivities suggest the $3_{10}$ helix structure (Fig. 2A). Taken together, the NOE connectivities and chemical shift deviation data support the presence of a helix from Leu-6 to Phe-12 (Fig. 2, A and B). This was also observed in 40, 100, and 200 mM SDS, which confirms a stable interaction between the cates a $3_{10}$ helix conformation (21). The data show a continuous pattern of $d_{\alpha\alpha}$ (i, i+3) NOEs between Leu-6 and Phe-12. Furthermore, $d_{NN}$ and $d_{\alpha\alpha}$ NOEs indicate the presence of helical structure in this region. However, the absence of $d_{\alpha\alpha}$ (i, i+4) and the presence of the continuous pattern $d_{NN}$ (i, i+2) and $d_{\alpha\alpha}$ (i, i+2) NOE connectivities suggest the $3_{10}$ helix structure (Fig. 2A). Taken together, the NOE connectivities and chemical shift deviation data support the presence of a helix from Leu-6 to Phe-12 (Fig. 2, A and B). This was also observed in 40, 100, and 200 mM SDS, which confirms a stable interaction between the peptide and SDS at concentrations above the critical micellar concentration (data not shown).

**NMR Structure Calculation**—From a total of 416 assigned NOE cross-peaks, 198 non-redundant upper-limit constraints were obtained for EBO16 (Table 1). The backbone structures of the lowest energy conformers of EBO16 in SDS micelles at pH 7.0 are shown (Fig. 3B). From the initial 100 structures calculated, 20 structures were selected corresponding to those structures exhibiting the lowest energy (Figs. 3, A and B). They did not violate NOEs and dihedral angle constraints. The overall energy of the selected structures was $23.97 \pm 3.03$ kcal/mol. The root mean square deviation (r.m.s.d.) among all peptide residues was $2.4 \text{ Å}$; however, the best fit was found between Leu-6 and Phe-12 residues, probably because of the proximity of Trp-8 and Phe-12, which stacks the rings edge-to-face (Fig. 3D). The aromatic-aromatic interaction can induce structure stability because of the interaction of partial charges located in the face and in the hydrogen atoms of aromatic rings (22). This region is located on a more hydrophobic face of the EBO16 peptide, as shown in Figs. 3, E and F. Because hydrophobic regions have been related to membrane interaction, this region could play an important role in the lipid bilayer destabilization (9, 23). The Trp-8 could be critical for fusion activity, as observed for dengue virus (24).

To check for the presence of proline isomerization, the connectivities between Ile-9 and Pro-10 residues or Gly-13 and Pro-14 were available. The $d_{\alpha\alpha}$ (i, i+1) connectivity, typical for the cis conformer, and the $d_{\alpha\beta}$ (i, i+1) connectivity, typical for the trans conformer of proline, were evaluated. We found only one spin system for each proline residue, indicating a single conformation for each amino acid (data not shown). The $d_{\alpha\delta}$ (i, i+1) connectivities were observed, indicating that Pro-10 and Pro-14 are in trans conformation. Probably, the high structural convergence in this portion of the molecule justifies the absence of two conformers of Pro-10. In addition, there was a slight kink induced by proline. The proline kink would be expected because of the absence of the amide proton of the proline, which leads to a break in $d_{\gamma\gamma}$ (i, i+1), $d_{\alpha\alpha}$ (i, i+3), $d_{\alpha\alpha}$ (i, i+2), and other connectivities (Fig. 3A). However, the pattern of NOE connectivities of helical structure was maintained by $\alpha$, $\gamma$, $\delta$, and $\beta$ proton connectivities (data not shown).

**Membrane Composition and Peptide Interaction**—The lipid bilayer composition and its curvature should be the limiting step for peptide-membrane interaction (25). EBO16 and EBO18, two fusion peptides of Ebola with charged residues added to decrease their hydrophobicity, were able to fuse with liposomes only in the presence of phosphatidylinositol (10). Phosphatidylinositol usually is present in cellular microdomains (lipid rafts) rich in cholesterol and sphingomyelin (DRMs). These microdomains were shown to interact with Ebola GP protein (26), but the correlation with the fusion mechanism was not investigated.

We used nuclear magnetic resonance to map the interacting amino acid residues with the microdomains. Knowledge of the
EBO16 structure in solution allowed us to follow the conformational changes in the presence of liposomes prepared with mixed lipid composition and from DRMs (Fig. 4). One-dimensional $^1$H spectra in the presence of LUVs containing PC:PE, PC/PE/PI/Cho, and DRMs show sharp lines typical of the peptide EBO16 in fast-intermediate to intermediate exchange between the membrane-bound and free form (Fig. 4B). Chemical shift changes could be observed especially when EBO16 was in the presence of DRMs (see the indolic hydrogen of Trp-8 at ~10.1 ppm). This dynamic interaction enables the observation of the transfer NOEs (Fig. 4A). Because EBO16 is very flexible when free in solution, it did not show NOEs during the 80 ms of mixing time. We observed several NOEs during the same mixing time in the presence of DRMs or LUVs containing PC/PE/PI/Cho (Fig. 4A). These transferred NOEs can be used to map the interacting residues, since these hydrogens will become more rigid upon interaction. The increase in rigidity can be caused by a gain of structure or because of the interaction itself.

The NOESY spectrum of EBO16 in the presence of DRMs showed transfer NOEs for all residues ranging from Leu-6 to Phe-12 (Fig. 4A). We also observed transferred NOEs for the residues ranging from Leu-6 to Ile-9 for EBO16 in the presence of LUVs containing PC/PE/PI/Cho. On the other hand, a lack of these peaks was observed in the absence of DRMs (supplemental Fig. 3). Our data show that the same region that is structured in the presence of SDS interacts with lipid rafts or the LUV containing PC/PE/PI/Cho (Fig. 4, A and C). These data are the first indication that EBO16 can interact with DRMs in an early stage of infection.

**Importance of Aromatic-Aromatic Interaction for Structural Stability**—We hypothesized that the interaction between the aromatic ring of Trp-8 and the side chain of Phe-12 is important for maintenance of EBO16 stability under interaction with mimetic membranes. To show the influence of aromatic-aromatic interaction, we synthesized a mutant W8A. The structural behavior of the mutant peptide was followed by far-UV CD spectra in the presence of SDS micelles. As shown in Fig. 5A, the mutant W8A assumes a random coil conformation in the presence of SDS micelles. On the other hand, the EBO16 wt displays a typical helical structure under the same condition. To identify more specifically the secondary elements of peptide structure, we used $^1$H chemical shift deviation (20). The data indicate a small tendency for the peptide to assume a helical conformation from Ile-4 to Ala-8, because of the smallest values of chemical shift deviation (<0.1 ppm) (Fig. 5B). Indeed, the differences between the chemical shifts of mutant W8A and wild type are more accentuated at Ile-9 (Fig. 5C). A loss of helical content at Ala-7 is also revealed (Fig. 5C). Negative chemical shift difference values show the tendency to increase the helical content, since positive values indicate a decrease. Taken together, CD, chemical shift deviation, and differences show a loss of peptide secondary structure caused by the lack of aromatic-aromatic interaction, which plays an important role to secondary structure maintenance. In addition, Bär and co-workers (27) showed that Phe-12 plays an important role for the fusion process, as observed by cellular assays.

The $^1$H NMR data were obtained using perdeuterated SDS micelles at 298 K and pH 7. The NOESY spectrum of EBO16 W8A displayed numerous well resolved cross-peaks (Fig. 5D), indicating that assignment should be possible. The chemical shift dispersion was more limited than observed with wt,
reflecting the random nature and high degree of backbone mobility of SDS micelles binding structure (Fig. 5D). The interaction between mutant W8A with SDS micelles caused significant chemical shift changes of amide and side-chain hydrogen atoms in relation to the EBO16 wt (Fig. 5D). Ile-9 amide proton was strongly modified for ~375 Hz, Ala-7 had a medium shift of 67.7 Hz, and some amino acids were not changed, such as Ile-4.

Model for the Interaction between Ebola Fusion Peptide and Biological Membranes—The NMR structure and fluorescence data indicate that the peptide preferentially interacts with the hydrophobic sector of micelles and membranes through its central residues. Accordingly, we propose a model in which the middle of the peptide is inserted into the membrane, and both N-terminal and C-terminal residues extend away from the surface of the membrane. Within the membrane, the peptide is more structured because hydrogen bonds must form within and between peptides, whereas outside the membrane greater conformational disorder is allowed because of the formation of peptide-H2O hydrogen bonds. The central portion of EBO16 exhibits more connectivity than the non-structured region (supplemental Fig. 2), suggesting that the central region excludes water. Indeed, the Stern-Volmer constant obtained in the presence of SDS corroborates a shift of Trp-8 from a hydrophilic to a hydrophobic environment, suggesting that the helix containing Trp may be embedded in the bilayer core (Fig. 2B). On the other hand, five N-terminal (GAAIG) and four C-terminal (GPAA) amino acids were not structured, indicating that the environment around these regions may be slightly different from that surrounding the helical portion of the molecule, with a tendency to destabilize the intramolecular connectivities. Glycine is an important residue in fusion peptides present in many enveloped viruses such as influenza, simian immunodeficiency virus, and human immunodeficiency virus 2 (28), although its exact role has not been determined. It has been correlated with the conformational flexibility of fusion peptides, which is necessary for fusion activity (29). In this work we suggest that glycines may be located on the same face before and after the helix, close to polar groups of the lipid bilayer, improving an interface between inner and outer host membrane.

To understand the characteristics of peptide-micelle interactions, molecular dynamics simulations of EBO16 were carried out using coordinates from the lowest energy NMR structure reported here. We run two molecular dynamics simulations, 1) EBO16 in explicit SDS micelles and 2) EBO16 in explicit water-hexane interface. In the first, the peptide was initially placed 1 nm from the interfaces (SDS surface or water:hexane) with the helix oriented toward the interface (supplemental Fig. 4). The peptide structure data were main-
tained frozen until the peptide interaction with the surfaces reached equilibrium (Fig. 6, A and B). A simulation time of 4 ns was sufficient to reveal the interaction between EBO16 and the SDS micelle (Fig. 6 A). The energy necessary for peptide-micelle interaction decreased more as the peptide advanced into the SDS micelle than in the case of the peptide-n-hexane interaction, suggesting that a simple hydrophobic model would favor the peptide interaction (Fig. 6, A and B). In contrast, EBO16 is formed with hydrophobic residues on the surfaces of the helix. Thus, the entry of the entire helix into the cellular membrane should be favored by the difference in dielectric constant between water and membrane. Thus, the interior of the membrane is a more energetically favorable environment than outside, contributing to drive the insertion of EBO16 into the lipid bilayer. For EBO16-SDS micelles, the peptide backbone r.m.s.d. from residues 6–12 stabilized at 2 Å, similar to EBO16-water-n-hexane interface (Figs. 6, C and D). However, the stabilization of residues 1–16 occurs at 4 Å in SDS micelles and at 6 Å in a water-n-hexane interface, suggesting the much higher structural stability of EBO16 in the presence of an amphipathic system (Figs. 6, C and D).

The peptide-membrane interaction has been extensively characterized for influenza virus fusion peptide, which exhibits a small helical structure close to the N terminus at neutral pH (30). In the fusion state, the influenza virus fusion domain undergoes a conformational change that increases the peptide curvature and leads to a transition from an \( \alpha \)-helix to a 3\(_{10}\)-helix localized at the C terminus of the molecule. EBO16 displays a central 3\(_{10}\)-helix structure at neutral pH in the presence of SDS micelles. The 3\(_{10}\)-helix is the fourth most common secondary motif in proteins and has recently attracted the attention of structural biologists, since it may act as a folding intermediate in the formation of an \( \alpha \)-helix (31, 32). In fact, the presence of \( d_{\alpha} \) \((i, i + 2)\) and the absence of \( d_{\alpha} \) \((i, i + 4)\) connectivities illus-
trate the high flexibility and capacity for self-folding of EBO_{16} in the presence of a lipid bilayer (Fig. 2A).

Although EBO_{16} self-folds when it interacts with lipid membranes, the fusion process probably requires the coiled-coil region (present in the GP2 protein), which would help the loop structure of the EBO_{16} peptide to capture the target membrane and bring it in close to the viral membrane so that lipid mixing takes place. This process was described for influenza virus, where the hypothesis of spring loading has been studied (33). Indeed, the similarity in the fusion process among different enveloped viruses points to this process as a potential target for the development of drugs that block virus entry and infection.

**Concluding Remarks**—Here we describe the first structural characterization of the interaction of the fusion peptide of Ebola virus with lipid rafts (DRMs) and membrane-mimetic environments. The Ebola virus causes hemorrhagic fever in pri-
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mates, including humans, resulting in high mortality rates. Because of the difficulty in handling infectious particles, structural studies on the viral domain involved with the fusion with the host cell are crucial to understanding the mechanism of virus entry and developing compounds that might block the early step of viral infection. In this article we have determined the structure of the fusion domain (EBO16) located in the envelope fusion glycoprotein (GP2) of the Ebola virus. This region interacts with the cellular membrane of the host, leading to membrane destabilization and fusion. Fluorescence and NMR experiments as well molecular dynamics simulations allowed us to characterize the interaction with sodium dodecyl sulfate micelles and DRMs. The three-dimensional structure clearly revealed how the aromatic residues (Trp-8 and Phe-12) play a crucial role for structure maintenance within the membrane bilayer. The poor structure of the mutant peptide with a single- amino acid substitution (W8A) clearly demonstrates the key role fusion glycoprotein (GP2) of the Ebola virus. This region interacts with the cellular membrane of the host, leading to membrane destabilization and fusion. Fluorescence and NMR experiments as well molecular dynamics simulations allowed us to characterize the interaction with sodium dodecyl sulfate micelles and DRMs. The three-dimensional structure clearly revealed how the aromatic residues (Trp-8 and Phe-12) play a crucial role for structure maintenance within the membrane bilayer. The poor structure of the mutant peptide with a single-amino acid substitution (W8A) clearly demonstrates the key role of Trp-8 in establishing the interaction with the membrane. It is tempting to propose that the aromatic-aromatic interaction may elicit the random coil-helical transition coupled to membrane anchoring. Knowledge of the EBO16 structure in solution allowed us to characterize the structural changes upon interaction with DRMs, providing the first demonstration that Ebola fusion domain can interact with lipid microdomains in an early stage of infection.

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