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Membrane curvature in flaviviruses

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ABSTRACT

Coordinated interplay between membrane proteins and the lipid bilayer is required for such processes as transporter function and the entrance of enveloped viruses into host cells. In this study, three-dimensional cryo-electron microscopy density maps of mature and immature flaviviruses were analyzed to assess the curvature of the membrane leaflets and its relation to membrane-bound viral glycoproteins. The overall morphology of the viral membrane is determined by the icosahedral scaffold composed of envelope (E) and membrane (M) proteins through interaction of the proteins' stem-anchor regions with the membrane. In localized regions, small membrane areas exhibit convex, concave, flat or saddle-shaped surfaces that are constrained by the specific protein organization within each membrane leaflet. These results suggest that the organization of membrane proteins in small enveloped viruses mediate the formation of membrane curvature.

1. Introduction

Cellular membrane surfaces can assume a variety of different shapes and curvatures, including spherical, flat, cylindrical and saddle-shaped. Membrane remodeling and the resultant changes in surface curvature are key events that enable cells to fulfill important functions such as communication, movement, division, and vesicle fission and fusion (McMahon and Gallop, 2005). Although the chemical properties of the different lipid headgroups and acyl chains can intrinsically favor particular membrane curvatures, the coordinated interplay between the membrane proteins and the lipid membrane has been implicated to be critical for remodeling of the membrane curvature (McMahon and Gallop, 2005; Zimmerberg and Kozlov, 2006).

It has been suggested that membrane proteins influence the curvature through interaction with the headgroups and acyl chains, or through insertion into one or both leaflets of the membrane bilayer (McMahon and Gallop, 2005; Zimmerberg and Kozlov, 2006). For example, an amphipathic α-helix embedded in one membrane leaflet may displace nearby lipid headgroups and reorient the acyl chains to favor the formation of a membrane with a high degree of curvature. Such an event has been implicated in the function of the endocytic accessory protein epsin 1 during membrane invagination associated with clathrin-coated vesicle formation (Ford et al., 2002). In addition, oligomers of peripheral membrane proteins can act as scaffolds meditating dynamic changes in the membrane. For example, dynamin forms a helical structure that enables the production of a cylindrical membrane inside a protein tunnel that can be constricted through structural rearrangements induced by GTP hydrolysis (Chen et al., 2004; Zhang and Hinshaw, 2001). Inevitably, proteins influence membrane curvature through both specific and non-specific interactions between amino acid residues and lipid molecules.

To date, the high-resolution structures of numerous membrane proteins embedded in detergents have been documented; however, only a few high-resolution structures of proteins embedded in lipid membranes have been reported (Gonen et al., 2005; Grigorieff et al., 1996; Hite et al., 2007; Mitsuoka et al., 1999; Reichow and Gonen, 2009; Unwin, 2005). These structures within lipid membranes demonstrate that amino acid residues can influence the positions of the lipid headgroups and the thickness of the bilayer through electrostatic interactions and hydrophobic matching. For example, if a tryptophan residue is positioned near the membrane, its positively charged indole ring can interact with and dictates the positions of the negatively charged headgroups of the lipids in one leaflet (Grigorieff et al., 1996; Mitsuoka et al., 1999). In addition, the hydrophobic transmembrane domains of proteins can influence the positions of the acyl chains of lipid molecules,

Abbreviations: cryo-EM, cryo-electron microscopy; 3D, three-dimensional; WNV, West Nile virus; DENV, dengue virus.

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thereby influencing the thickness of the bilayer. Such structural lipid-protein interplay provides insight into how membrane proteins modulate the positions of lipid molecules.

Studies of these membrane-embedded proteins using electron-crystallography have shed light on the nature of protein-lipid interactions, however they have not addressed the effect of proteins on membrane curvature, since the proteins were crystallized and embedded in a planer membrane. On the other hand, cryo-electron microscopy (cryo-EM) reconstruction maps of enveloped viruses can provide sufficient resolution to study the viral membrane curvature in the context of the protein organization (Laurinmaki et al., 2005). Here we present a study of the curvature of each membrane leaflet in the medium-resolution cryo-EM structures of West Nile virus (WNV, ~10 Å resolution), dengue virus (DENV, 9.5 Å resolution) (W. Zhang et al., 2003) and immature DENV (~16 Å resolution) (Zhang et al., 2004). The overall morphology of the viral membranes is stabilized by icosahedral scaffolding composed of E/M or E/prM glycoproteins through insertion of their stem-anchor regions into the viral membrane. Within these virus particles, each membrane leaflet contains localized regions exhibiting convex, concave, flat or saddle-shaped surfaces. These small membrane regions are constrained by E/M or E/prM proteins arranged in specific oligomerization patterns. The quantitative measurement of membrane curvature in this study demonstrates that the organization of membrane proteins mediates the formation of membrane curvature.

2. Materials and methods

2.1. Cryo-electron microscopy and image processing

Mature WNV was propagated and purified as previously described (Kaufmann et al., 2006). Micrographs of a flash-frozen virus sample were recorded at a magnification of 47,440× using a Philips CM300 field emission gun transmission electron microscope under the low-dose condition (~30 $e^-/Å^2$). The micrographs were digitized on a Zeiss SCAI scanner using a 7 μm interval. Sets of four neighboring pixels in the scanned images were averaged to give an effective sampling step of 2.95 Å at the specimen. A total of 8066 particles were initially selected from 83 micrographs with defocus levels ranging from 1.12 to 3.21 μm. A previously reported (W. Zhang et al., 2003) 24 Å resolution DENV cryo-EM structure was used as an initial model for determining the orientations of the virus particles using the polar Fourier transform method (Baker and Cheng, 1996). Thereafter, the model-based reciprocal space refinement procedure PO^2R (Ji et al., 2006) was used to refine the orientation and origin of each particle. The iteration steps for orientation refinement and 3D reconstruction were handled automatically using the program AUTO3DEM (Yan et al., 2007). A total of 1556 particle images from 68 micrographs were used to compute the final reconstruction. The resolution was determined to be about 10.3 Å, based on where the Fourier shell correlation (FSC) coefficients between the reconstructions using independent half-data sets fell below 0.5.

2.2. Fitting of the atomic structure of the WNV E protein into the cryo-EM density map

The ectodomain of the WNV E protein (PDB accession No. 2HG0) (Nybakken et al., 2006) was divided into two rigid bodies, DI (residues 1–47, 137–194, 286–297)/DIII (residues 298–400) and DII (residues 48–136, 195–285), which were fitted independently into the 3D density map using the program EMfit (Rossmann, 2000). All atoms, excluding hydrogen atoms, were used for the fitting process. The loop residues 256 to 260 in DII, which include three conserved glycine residues and have high temperature factors in the crystal structure of E monomers (Nybakken et al., 2006), were removed to avoid clashes during the fitting procedure. The first E molecule was fitted in the position closest to the icosahedral twofold axis. A "difference" map was then produced by setting the densities within a 3.5 Å sphere of any atom in the fitted E molecule to zero. This map was used to fit the other two independent E molecules in the same icosahedral asymmetric unit. The second molecule to be modeled was nearest the already fitted molecule. The third and final molecule was then placed into the remaining space within the icosahedral asymmetric unit. The structural similarity between WNV and DENV was used to manually position the stem and anchor regions (pdb accession No, 1P58) (W. Zhang et al., 2003) into the appropriate electron densities of the WNV cryo-EM map.

2.3. A 3D map that stores the radii of the membrane leaflets

The radial positions of the membrane leaflets were stored in their respective 3D sampling maps (SAMP-map). A SAMP-map had the same dimension as the cryo-EM map of the virus. The center of the SAMP-map, (x_0, y_0, z_0), represented the center of the virus. The pixels within a 11-pixel-thick shell of the SAMP-map (ranging between 66 and 77 pixel radii) were used as the sampling points. These sampling points were close enough to allow precise measurement of the membrane curvature. The coordinate of each sampling point, (x, y, z), corresponded to a unit direction vector $\bar{v}$, i.e. (u, v, w), pointing from (x_0, y_0, z_0) to (x, y, z). The value of the SAMP-map at (x, y, z) represented the radii of the membrane leaflets along the direction $\bar{v}$.

2.4. Determination of the positions of the membrane leaflets in a spherical enveloped virus cryo-EM map

Radial density plots were calculated using the pixels within 1-pixel-thick spherical shells for cones having a semiangle of 5° radiating from the center of the virus. The wave-shaped radial density plots had two maxima above zero with one less-than-zero peak in between. The two maximum density peaks, located at radial distances of about 150 and 210 Å from the center of the WNV and DENV maps, and 160 and 210 Å from the center of the immature DENV density map, were identified in each cone as the radial positions of the inner (R_{in}) and outer (R_{out}) membrane leaflets, respectively (Fig. 1C). The center of the bilayer (R_{mid}) was defined as the lowest density position between R_{in} and R_{out}. The radial positions of each membrane entity (the inner, outer leaflets and the center of the bilayer) were determined similarly for all sampling points in the respective SAMP-map.

The pixelation effect caused by representing a geometric curve or surface in a discrete space introduces errors into the calculation of curvature. For an example in 2D, if a circle were represented as a set of points and short lines, the curvature at any point on the short lines would be zero, rather than the real curvature of the circle. Similarly, because the radii of the membrane leaflets were calculated based on a discrete 3D density map, the set of sampling points for each membrane entity in a raw SAMP-map convey information of a pixelated surface and was not adequate for calculating the curvature. Therefore, the radii stored in a raw SMAP-map of each membrane entity were fitted in a sextic polynomial function using the Levenberg–Marquardt least square fitting method (Press et al., 1992):

$$R = \sum A_{i,j,k} u^i v^j w^k,$$

where (u, v, w) is the unit direction vector $\bar{v}$ pointing from the center of the SAMP-map, (x_0, y_0, z_0), towards any sampling point