Molecular basis of endosomal-membrane association for the dengue virus envelope protein

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ABSTRACT

Dengue virus is coated by an icosahedral shell of 90 envelope protein dimers that convert to trimers at low pH and promote fusion of its membrane with the membrane of the host endosome. We provide the first estimates for the free energy barrier and minimum for two key steps in this process: host membrane bending and protein–membrane binding. Both are studied using complementary membrane elastic, continuum electrostatics and all-atom molecular dynamics simulations. The predicted host membrane bending required to form an initial fusion stalk presents a 22–30 kcal/mol free energy barrier according to a constrained membrane elastic model. Combined continuum and molecular dynamics results predict a 15 kcal/mol free energy decrease on binding of each trimer of dengue envelope protein to a membrane with 30% anionic phosphatidylglycerol lipid. The bending cost depends on the preferred curvature of the lipids composing the host membrane leaflets, while the free energy gained for protein binding depends on the surface charge density of the host membrane. The fusion loop of the envelope protein inserts exactly at the level of the interface between the membrane's hydrophobic and head-group regions. The methods used in this work provide a means for further characterization of the structures and free energies of protein-assisted membrane fusion.

1. Introduction

Dengue virus (DV) is a flavivirus borne by mosquitos that causes flu-like symptoms and, in cases of secondary infection with a heterologous serotype, can lead to hemorrhagic fever. The virus is endemic to tropical regions, where it accounts for approximately 50 to 100 million infections and 500,000 hospitalizations annually [1]. The icosahedral envelope of the virus is made up of 180 identical copies of a single envelope (E) protein [2–5]. Two alpha helices anchored in the viral membrane attach to E through a 53-residue C-terminal stem [6]. Domain III, at E's C-terminus, helps the virus target cell receptors, leading to endocytosis [7–14]. Once inside the endosome, a low pH-driven conformational change of E results in exposure of hydrophobic residues at the tip of the beta-structured Domain II that attach E to the host endosomal membrane and promote virus–membrane fusion (Fig. 1) [15,16].

Recent experiments report that DV fusion with host endosomal membranes depends on the lipid composition of the endosome. The presence of cholesterol, on the one hand, substantially increases the fusion efficiency of viruses and virus-like particles with liposomes comprised of neutral lipids for tick-borne encephalitis [17,18] and West Nile flaviviruses [19], as well as Semliki forest virus (SFV), an alphavirus with an envelope protein homologous to E [20]. On the other hand, fusion of DV with the plasma membrane of insect cells (rich in anionic lipid) is independent of cholesterol [21]. Others report that fusion of DV is strongly promoted by the presence of anionic lipids in liposomes or host membranes [22]. These results raise questions about the factors that regulate E protein's binding and fusion efficiency, and in particular, the relative importance of anionic lipids and cholesterol.

Structural information for the E protein reveals that activation by low pH involves outward rotation of a primarily beta-structured Domain II relative to a 'base' Domain I/III located at the viral membrane surface [23]. This rotation exposes a large portion of Domain II to solvent, and triggers a conformational rearrangement from the 'smooth' dimeric shell of the mature virus (Fig. 1a) to 'spiky' trimeric assemblies of E protein on the virus surface (formed stepwise as in Fig. 1b and c). The rotation also leaves a fusion peptide (magenta in Fig. 1) exposed at the outer tip of the trimeric E protein assembly [5,6]. The E protein contains several positively charged residues on Domain II, resulting in substantial electrostatic attraction with negatively-charged membranes. The E protein fusion peptide consists of a short hydrophobic amino acid segment comprising residues 100–108. As confirmed by NMR and molecular simulation studies [24,25], hydrophobic residues, including tryptophan (Trp101) and phenylalanine (Phe108), promote

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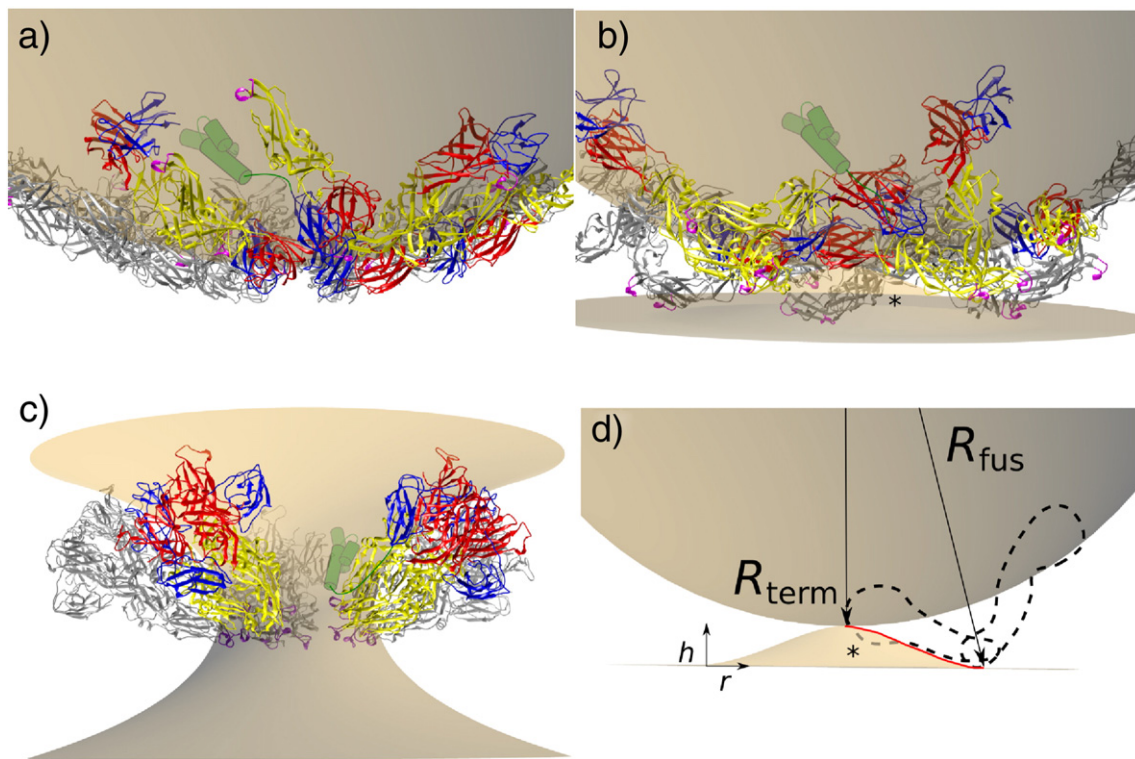
Fig. 1. Possible hemifusion route to virus (upper)–host (lower) membrane fusion, illustrated through alignment of E protein to: a) dimeric, mature viral assembly (3C6R [101]); b) an intermediate structure during trimerization approximated by the two cryo-EM structures with exposed fusion loops, 3C6D [101] and 3IXY [9]; c) target, fused state with trimeric form (1OK8 [6]) as proposed in earlier works [6,77], arbitrarily positioned to interact with a catenoid-shaped, zero mean curvature, membrane. Panels (b) and (d) are marked by * to illustrate the state defining the free energy barrier for this process. Panel (d) shows a red outline for the minimal energy dimple shape of the host membrane, $h(r)$, explained further in Fig. 2, and an outline of the 3IXY E protein structure used to constrain the host membrane shape. Two radii measured from the virus center identify the distance to the E protein fusion loop (R_{fus}) and N-terminal alpha-carbon (R_{term}). The actual conformation of the protein at steps (b)–(c), and the mechanism promoting the membrane dimple, are unknown. For clarity, only five trimers (i.e. from one pentagon in Fig. 2) are shown in (a)–(c), and the far three are colored gray. Protein domains I, II, III are colored (red, yellow, blue). Although not modeled in this work, the C-terminal stem and the perimembrane part of its anchor [5] are shown for reference (green) for one E monomer in (a)–(c). This stem region would sit between the E protein and the viral membrane. All E protein fusion peptides are colored magenta. Binding and conformational transitions of the fusion envelope protein may assist in lipid rearrangement or curvature formation during membrane fusion.

insertion of the E protein fusion peptide into the host endosomal membrane [26]. These structural insights do not contain energetic information required for comparison with existing models of the hemifusion process [27]. This study describes a method of obtaining reliable binding free energies that will be helpful for establishing the relative importance of cholesterol and anionic lipids.

In this work, we use atomistic and continuum-level simulations to present the first results on the membrane binding free energy of the E protein trimer. The potential of mean force (PMF) shows a broad minimum for viral protein–membrane association. Anionic lipids at 30 mol% concentration present a sufficiently strong attractive force on E protein to make this surface-associated protein–membrane contact irreversible. We also propose a transition state for the host membrane shape that puts an upper bound on the activation barrier to membrane bending needed to achieve membrane–membrane fusion. The host membrane composition can have a large influence on this barrier through its intrinsic curvature. The results reported here can be tested against experimental measurements of the protein-membrane binding free energy, E protein insertion depth, and dependence of binding and fusion on host membrane curvature.

The free energy barrier reported for fusion is an upper bound based on the geometry of initial host/virus contact. When attached at the largest membrane-facing face of the icosahedral viral envelope, the host endosomal membrane will simultaneously contact the fusion loops of five E protein trimers (Fig. 1b). The height and width of the E trimers present geometric bounds on this contact complex that are used to obtain energetic information. Because the barrier is determined

by mechanical constraints on the membrane, it specifies the amount of work that must be supplied by E to initiate fusion.

2. Theory

Building a detailed energetic picture of viral membrane fusion with endosomal membranes requires a combination of membrane elastic, dielectric continuum, and all-atom free energy methods. Membrane bending free energy models provide details on lipid rearrangements that take place on time-scales much larger than currently accessible with atomistic dynamics. Protein–membrane binding models provide details on atomistic rearrangements that take place locally and on short time scales. The E protein trimer measures roughly 10 nm in height and 7 nm wide at its base, on the viral membrane side, while the endosomal membrane adds an additional 4 nm in height, making full atomistic simulation challenging. By matching the all-atom and dielectric continuum potential of mean force curves for water-mediated protein-membrane interaction, we extend the all-atom results to complete separation, 3 nm from protein–membrane contact. The PMF value at complete separation establishes an absolute energy scale for the protein–membrane binding free energy.

2.1. Membrane bending free energy

The most widely accepted mechanism of spontaneous membrane fusion involves three major steps [27–30]. At first contact, the two membranes form an initial point connection (Fig. 1b and d). Next the