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Dengue Virus and its Structure - A Promising Target for Drug Discovery

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Abstract

Dengue is the most endemic disease of last decade; this disease is caused by the infection of Dengue Virus (DENV). This virus completes its lifecycle in *Aides* mosquito and human. There are several vaccines against this virus but they only prevent from disease and reduce the risk of death. DENV have three serotypes all with minor difference in structure. Immunity against one serotype does not impart immunity against all serotypes in case of infection, it may cause disease extremity. Many researchers believe that it is difficult to develop a vaccine against all serotypes because of difference in structure. A potent inhibitor is required for the disease to be treated. In this study we considered its (DENV) structure and mode of infection so that we can develop or search a promising target and compound for treatment of this disease.

Key-Words: Dengu, Drug Discovery, DENV

Introduction

Dengue is a serious disease which has become a global health saddle in the last decade. Currently, there are no approved vaccines or antiviral therapies to combat the disease. This pathogenic dengue virus (DV) is a growing global threat for which there is no specific treatment and prevention. Several vaccines have been developed against the disease, but they only prevent the disease and reduce the risk of death. Consequently, the antiviral drug becomes the most powerful treatment to solve this problem. Dengue is the most common arthropod-born viral infection in the world (Gubler DJ., 2006; WHO, 2010). This disease is prevalent in more than 100 through Africa, the Americans, the Esetrn Mediterranen, Soth Asia, and the Western Pacific (Izabela et al., 2010), The U.S. Center for Disease Control and Prevention (CDC) estimated that over 2.5 billion peoples at threat for epidemic transmission (Kee et al., 2007; Frimatanti et al., 2011; Ilyas et al., 2011). It is estimated that 100 million cases of dengue fever (DF) and about half a million cases of dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) occur worldwide which cause 25,000 death, annually (WHO, 2002).

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Dengue fever is an acute febrile disease which is characterized by-

1. Sudden onset fever of 3-5 days.
2. Intense headache.
3. Myglia.
4. Anthragic retro-ornital pain.
5. Anorexia.
6. GI disturbance and rash (Tambunan et al., 2009).

Dengue haemorrhagic fever is characterized by-

1. Increased vascular permeability.
2. Hypovolaemia.
3. Abnormal blood clotting machenism (Tambunan et al., 2009).

This disease is caused by dengue virus. Dengue virus infection occurs by *Aedes* mosquito and maintained in a cycle that involves human and *Aedes* mosquito *(Aedes aegypthi)*. The geographical distribution of dengue virus has exceedingly extended over the last 30 years, because of increased breeding potential of Aedes aegypti which is the vector species for virus. This is impelled by demographic explosion, rapid growth of urban centers with a strain on public services, such as potable water. Breeding of Aedes aegypti was escalating rapidly due to rainwater storing in diverse types of containers *Aedes aegypthi* is a domestic mosquito which prefers day-biting and feed on human (Irie, K., et al 1989). Infections of dengue virus exhibit a variety of clinical illness ranging from a nonspecific viral syndrome to severe and fatal hemorrhagic disease. Important hazardous factors for DHF comprise the

strain of the infecting virus as well as age, and especially the prior dengue infection history of the patient.

1. Each serotype provides specific lifetime immunity, and short—immunity.
2. All serotypes can cause severe and fatal disease.
3. Genetic variation within serotypes.
4. Some genetic variants within each serotype appear to be more virulent or have greater epidemic potential.

Dengue virus belongs to flavivirus family. They include four serotypes-

1. DENV 1
2. DENV 2
3. DENV 3
4. DENV 4

The incubation period of this virus is 4-7 days (ranges 3-14 days). This in an ssRNA positive-strand virus consist of 11,000 bases in its genome that code for three structural proteins-

1. C
2. prM
3. E

Seven non structural proteins-

1. NS 1
2. NS 2a
3. NS 2b
4. NS 3
5. NS 4a
6. NS 4b
7. NS 5

And short non coding regions on both the 5' and 3' end (Mackenzie J., 2004). For the maturation of the dengue virus optimal activity of the NS3 serine protease is required and for the optimal catalytic activity of NS3 the presence of NS2 is required (Bianchi E. and Pessi A., 2002). NS3, the second larger protein that is encoded by the dengue virus that contains a serine proteinase catalytic triad within the terminal region of 180 amino acid residues and it requires the 40 amino acid residues of NS2B for protease activity (Chambers et al., 1991). This virus contains a type I cap structure at the 5'-end and codes for single polyprotein precursor (3391 amino acid residues for DEN2) which arranged in order NH2-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-COOH (WHO., 2007).

The processing of this polyprotein precursor occurs co-translationally as well as post translationally and this is performed either by host signalase is association with the membranes of the endoplasmic reticulum or the viral protease. The NS2B/NS3 component of the protease activates the cleavage in the non-structural region of the viral polyprotein at NS2A/NS2B, NS2B/NS3, NS3/NS4A and NS4B/NS5 junctions (Arias, 1993). These sites have a common pair of dibasic amino acids, Lys-Arg, Arg-Arg, Arg-Lys and intermittently, Gln-Arg at the P1 and P2 positions, followed by the short chain amino acid, Gly, Ser or Ala at the P1(Schechter et. al, 1967). The viral protease cleave internally within NS2A (Bazan et. al., 1989) and NS3 (Nestorowicz, 1994). The *N*-terminal region of the non-structural 3 protein (NS3) of dengue virus is a serine protease (Falgout et al., 1991; Chambers, 1993). This serine protease binds to an NS2B cofactor which is required to cleave the polyprotein. That NS2B-NS3 protease complex is requisite for replication of virus (Chambers, 1993). Thus, it serves as a promising target for antiviral drug development against the infection of dengue virus (Leyssen et. al., 2003; Sampath et. al.,2009**)**.

There are no prophylactic vaccine is currently available to prevent infection of Dengue virus. The most efficient measures to protect from infection are those that avoid mosquito bites. There are many ongoing vaccine development programs. Among them is the Pediatric Dengue Vaccine Initiative set up in 2003 with the aim of accelerating the development and introduction of dengue vaccine(s) that are affordable and accessible to poor children in endemic countries (Halstead SB et. al., 2002). Increased efforts are therefore needed in the development of an effective vaccine against DENV. The E glycoprotein having 495 amino acids which is responsible for envelope formation of virus was reported to play an important role in the DENV attachment to the host cell receptors and entry into the target cells (Roehrig JT., 1997). Therefore, it is one of the most valuable candidate proteins for the development of DENV vaccine.

According to Indonesian Department of Health fact sheet, DF is on the rank 8 of 10 infection diseases, which are considered important in the budget priority and political commitment. All of the serotype of dengue virus (DENV) are prevalent in many of the big cities and occurred at yearly basis (Kusriastuti et. al., 2005; IVI Team, 2007). The vaccine of dengue fever is not yet available. The devastating obstacles for the dengue vaccine development are the unknown pathogenesis of dengue virus in human host and the difficulty of virus growth in the culture medium. Immunity against one serotype does not meant immunity against the rest of other serotypes of dengue virus it may cause dieses severity in case of further infection by another serotype of dengue virus. The animal testing was done for immunogenic and disease prevention purpose only (Chatuverdi et. al., 2005;

Guglan et. al., 2005; Elgert, 1996). An attenuated vaccine is under clinical trial on human subject now. The vaccine is developed by Mahidol University at Bangkok and Walter Reed Army Institute of Research at the United States. There are risks that occur by misusing attenuated vaccine. If the virus in the vaccine is not attenuated adequate then the vaccine will attack the human host like the DENV infection. Moreover, if the vaccine contains over attenuated virus, the vaccine would not induce the body immune response (Halstead et. al., 2002; Chatuverdi et. al., 2005).

Genome of Dengue Virus

Dengue virus is a small virus carrying a single strand of RNA as its genome. Its genome encodes only ten proteins. Three of these are structural proteins that form the coat of the virus and deliver the RNA to target cells, and seven of them are nonstructural proteins that orchestrate the production of new viruses once the virus gets inside the cell. The outermost structural protein, termed the envelope protein, PDB entry 1k4r (Mukhopadhyay et. al., 2005). The virus is enveloped with a lipid membrane, and 180 identical copies of the envelope protein are attached to the surface of the membrane by a short transmembrane segment. The job of the envelope protein is to attach to a cell surface and begin the process of infection.

Infection of Dengue Virus

In the infectious form of the virus, the envelope protein lays flat on the surface of the virus, forming a smooth coat with icosahedral symmetry. However, when the virus is carried into the cell and into lysozomes, the acidic environment causes the protein to snap into a different shape, assembling into trimeric spike (Modis et al., 2004). Several hydrophobic amino acids at the tip of this spike, insert into the lysozomal membrane and cause the virus membrane to fuse with lysozome. This releases the RNA into the cell and infection starts. The hemagglutinin protein on the surface of influenza virus plays a similar role, but the two proteins use entirely different mechanisms to perform a similar task.

Vaccine Development

A dengue vaccine has proven difficult to develop, in part because there are four major subtypes of dengue virus, each with slightly different viral proteins. Many researchers currently believe that the deadly dengue hemorrhagic disease is caused when a person is infected with one subtype, and then infected later by a second subtype. The antibodies, and immunity, gained from the first infection appear to assist with the infection by the second subtype, instead of providing a general immunity to all subtypes. This means that an effective vaccine will have to stimulate protective antibodies against all four types at once, a feat that has not yet been achieved.

Propagation

Dengue virus also makes several proteins that create new viruses once it is inside a cell. Two of the major ones are shown on the reverse. Both are multifunctional proteins with several enzymes strung together. The one on the left, NS5 from PDB entries 1l9k (Egloff et al., 2002) and 2j7w (Yap et al., 2007), contains a methyltransferase and a polymerase, and the one on the right, NS3 from PDB entry 2vbc (Luo et al., 2008), contains a protease and a helicase. Each of these enzymes performs a different part of the life cycle. The polymerase builds new RNA strands based on the viral RNA, the helicase helps to separate these strands, and the methyltransferase adds methyl groups to the end of them, protecting the RNA strands and coaxing the cell's ribosomes to create viral proteins based on them. The viral proteins are created in one long polyprotein chain, which is finally clipped into the functional units by the protease. The little chain colored blue is a portion of another viral protein, NS2B that assists with the protease activity.

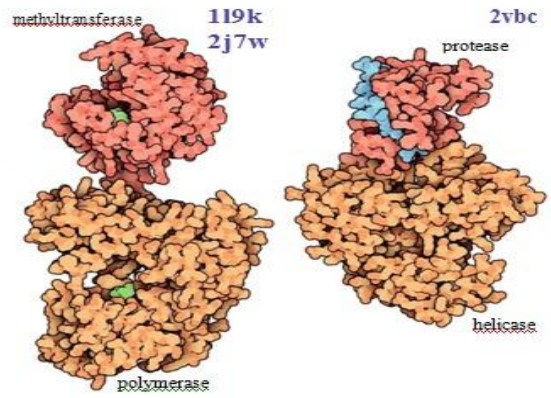

Fig. 1: Structure of dengue virus Polymerase, Protease and Helicase (Modies *et al 2003*)

Dengue Virus Structure

Cryoelectron microscopy has been used to study many aspects of the life cycle of the dengue virus. In these structures, a low resolution image of virus, not quite detailed enough to see atoms is obtained by the electron microscope, and then atomic structures of the individual pieces are fit into the image to generate the final model. PDB entry 2r6p (Lok et. al.), the envelope protein on the surface of the virus with many antibody Fab fragments bound to the viral proteins. Researchers have discovered that the antibodies distort the arrangement of the envelope proteins, blocking their

normal action in infection. Other dengue virus structures in the PDB include immature forms of the virus (for instance, in PDB entry 1n6g (Zhang et. al., 2003)) and structures that include the membrane-spanning portions of the viral coat (PDB entry 1p58 (Zhang W. et al., 2003)).

Structure of Virion

Dengue virus completes its life cycle in *aedes* mosquito and human. Infection with one serotype confers protective immunity against that serotype but not against other serotypes. In fact, several retrospective and prospective studies have revealed that secondary infection with another serotype is a risk factor for developing DHF (Halstead, 1970; Thein et. al., 1997). Infants born to dengueimmune mothers are at risk to develop more severe dengue during a primary infection (Halstead et. al., 2002; Kliks et. al., 1988). Antibodies can play an important role in controlling the outcome of an infection, they specifically direct the virus particles to cells carrying Fc-receptors (FcR), for instance monocytes, macrophages, and dendritic cells, that are the natural targets for the virus and permissive for DENV infection. This leads to enhanced infection of these cells and thus, to high viral loads, resulting in extensive T cell activation early in the infection process. High amounts of cytokines and chemical mediators are released, as consequences resulting in endothelial cell damage and subsequent plasma leakage. Other factors that are implicated in disease pathogenesis include viral virulence, the ethnic background and age of the individual and specific epidemiological conditions (Gubler, 2002; Thomas et al., 2003).

Mature virions contain three structural proteins- The capsid protein C, Membrane protein M and Envelope protein E.

Multiple copies of the C protein (11 kDa) encapsulate the RNA genome to form the viral nucleocapsid (Chang et. al., 2001; Ma et. al., 2004). This nucleocapsid is delimited by a host-cell-derived lipid bilayer. In this host-cell-derived lipid bilayer 180 copies of M and E are anchored. The M protein is a small (approx. 8 kDa) proteolytic fragment of its precursor form prM (approx. 21 kDa). The E protein is 53 kDa and has three distinct structural domains (Fig.-1) (Rey et. al., 1995; Modis et. al., 2005). Domain I is structurally positioned between domain II, the homodimerization domain, and the immunoglobulin like domain III.

Domain I in red, domain II in yellow with fusion loop in green, and domain III in blue. The image was prepared using the program PyMol Molecular Graphics System

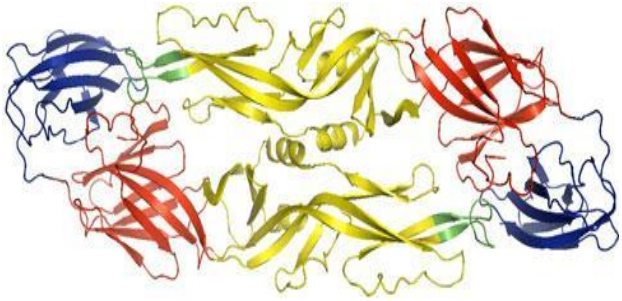

Fig. 2: Dengue E protein dimer with three defined domains within each monomer (Modies *et al 2003*).

The structural analysis of mature DENV virions revealed that the virus possesses an icosahedral envelope organization and a spherical nucleocapsid core (Kuhn, 2002). In mature virions, envelop protein E is organized as 90 head-to-tail orientated homodimers, which lie in sets of three nearly parallel to each other and to the viral surface, forming a smooth ‘‘herringbone’’ configuration. As a result, DENV virions lack a true T = 3 symmetry, which means that the three E monomers present in each icosahedral asymmetric unit exist in three chemically distinct environments and may therefore play a distinct role in different stages of the infection (Kuhn, 2002; Mukhopadhyay et. al., 2005).

Envelope Glycoprotein E epitopes of Dengue Virus

The E glycoprotein of dengue virus is responsible for the viral binding to the receptor. The crystal structure of envelope glycoprotein has already been determined. However, where the well-defined B- cell and T-cell epitopes are located is still a question. The conserved regions were found in more than 600 DENV E glycoprotein sequences. Both the B-cell and T-cell epitopes were predicted and the hydrophobicity, antigenicity, accessibility and flexibility of the highly conserved E glycoprotein were further predicted by using different bioinformatics algorithms. The secondary structure was obtained and the predicted epitopes were pointed out in it. Binding sites on glycoprotein of DENV-3 for attachment of virus to the receptor was identified, while keeping those attachments in which new drugs for dengue related infections could not be designed.

B-cell epitopes of E DENV

B cell epitopes prediction was done by using Antigen Prediction server, which can be accessed freely in their website at http://bio.dfci.harvard.edu/Tool. Antigenic peptides are determined (table-1) using the method of Kolaskar and Tongaonkar (Kolaskar et. al., 1990). Antigen Prediction server needs protein sequence data as its input for B cell epitope prediction. The prediction

result was peptide sequences with their start and end position in the E DENV-3 protein sequences.

Table 1: Predicted B-cell epitopes of DENV-3 glycoprotein E

S/No.	Start Position	Sequence	End Position
1	17	GATWVDVVLEHGGCVT	32
2	50	ATQLATLRKLCIE	62
3	87	DQNYVCKHTY	96
4	210	WFFDLPLPW	218
5	232	KELLVTF	238
6	244	KKQEVVVLG	252
7	315	HGTILIKV	322
8	350	ITANPVVT	357
9	422	SVGGVLN	428

T-cell epitopes of E DENV-3 protein prediction (Modies *et al 2003*.)

T cell epitopes prediction was done with a neural network based MHC Class-I Binding Peptide Prediction Server (nHLAPred) (Bhasin et. al., 2005). The server website is http://www.imtech.res.in/raghava/nhlapred/.nHLAPred needs data input of E DENV-3 protein sequences in order to predict the epitope

Table 2: Predicted T-cell epitopes of DENV-3 glycoprotein E

HLA sites	Peptides position		
HLA-A2	106-114	171-179	291-299
HLA-A11	30-38	237-245	274-282
HLA-A24	298-306	388-396	479-487
HLA-B51	204-212	210-218	389-397
HLA-B60	48-56	181-189	254-262
HLA-B62	412-420	45-53	268-276
HLA-A2	106-114	171-179	291-299
HLA-A11	30-38	237-245	274-282
HLA-A24	298-306	388-396	479-487

Structure of the dengue virus envelope protein (E protein) after membrane fusion (Modies *et al 2003*)

Dengue virus enters a host cell when the viral envelope glycoprotein, E, binds to a receptor and responds by conformational luxation to the reduced pH of an endosome. The conformational change induces fusion of viral and host-cell membranes. A three-dimensional structure of the soluble E ectodomain (sE) in its trimeric, postfusion state reveals striking differences from the dimeric, prefusion form. The elongated trimer bears three 'fusion loops' at one end, to insert into the host-cell membrane. Their structure allows us to model directly how these fusion loops interact with a lipid bilayer. The protein folds back on itself, directing its carboxy terminus towards the fusion loops. We propose a fusion mechanism driven by essentially irreversible conformational changes in E and facilitated by fusion-loop insertion into the outer bilayer leaflet. Specific features of the folded-back structure suggest strategies for inhibiting flavivirus entry.

Membrane fusion is the central molecular event during the entry of enveloped viruses into cells. The critical agents of this process are viral surface proteins, primed to facilitate bilayer fusion and triggered to do so by the conditions of viral interaction with the target cell. The best-studied example is the influenza virus haemagglutinin (HA), synthesized as a single-chain precursor and then cleaved into two chains, known as HA_1 and HA_2, during transport of the trimeric glycoprotein to the cell surface. The binding of HA_1 to a cell-surface receptor leads to endocytic uptake; acidification of the endosome triggers dramatic conformational rearrangement of HA_2. The latter is a two-stage process. Exposure of the amino-terminal 'fusion peptide' of HA_2 first allows it to insert into the endosomal membrane; subsequent folding-over of the entire HA_2 polypeptide chain brings together its N and C termini and thus forces the target-cell membrane (held by the fusion peptide) and the viral membrane (held by the C-terminal transmembrane anchor of HA) against each other.

HA is the prototype of a large class of viral fusion proteins—for example, those of other myxo- and paramyxoviruses such as measles virus, retroviruses such as HIV, and filoviruses such as Ebola virus (Skehel et. al., 2000). All of these 'class I' viral fusion proteins are two chain products of a cleaved, single-chain precursor, and all bear a hydrophobic fusion peptide at or near the N terminus created by the cleavage (Wilson et al., 1981). Moreover, in all class I fusion proteins, a three-chain, *a*-helical, coiled-coil assembles during the conformational change, drives the fusion peptide towards the target-cell membrane (Baker et. al., 1999; Melikyan et. al., 2000; Russell et. al., 2001), and creates the central structural element of the fusion machinery.

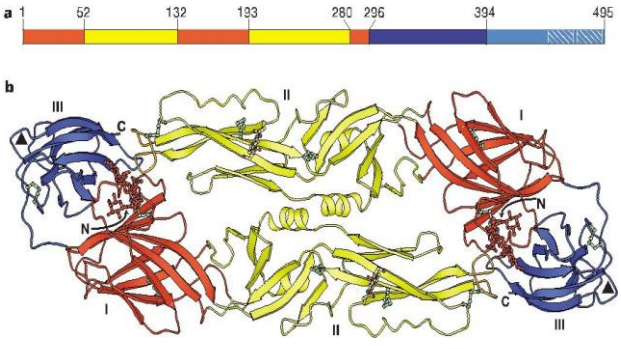

Fig 3: Structure of the dimer of dengue E soluble fragment (sE) in the mature virus particle (Modies *et al 2003*).

a, The three domains of dengue sE. Domain I is red, domain II is yellow, domain III is blue. A 53-residue 'stem' segment links the stably folded sE fragment with the C-terminal transmembrane anchor. b, The sE dimer (Modis et. al., 2003).

Membrane insertion and trimer formation

Like its TBE homologue, the dimer formed by dengue sE (residues 1–395 of E) dissociates reversibly (Allison et. al., 1995; Modis et. al., 2003). At acidic pH, dissociation is essentially complete at protein concentrations of 1 mg ml (Zhang et al., 2003); at neutral pH, the dissociation constant is one to two orders of magnitude smaller. The fusion loop at the tip of domain II would be exposed in the monomer (Allison et. al., 1995; Rey et. al., 1995), but exposure does not cause non-specific aggregation of the protein (Wimley et. al., 1992). Liposome co-flotation experiments show that the fusion loop of monomeric TBE sE allows association with lipid membranes and that this membrane associ-ation catalyses irreversible formation of sE trimers at low pH (Wimley et. al., 1992). Dengue E exhibits an identical behaviour: on acidification, sE dimers dissociate, bind liposomes and trimerize. Membrane-associated sE is readily detected by electron microscopy of negatively stained preparations; chemical cross-linking confirms that the protein has trimerized. The trimers are tapered rods, about 70–80 A long and 30–50 A in diameter, with the long axis perpendicular to the membrane and their wide end distal to it. They tend to cluster on the liposome surface, often forming a continuous layer. These heavily decorated areas appear to have a greater than average membrane curvature, resulting in smaller vesicles. This observation suggests that E trimers can induce curvature, a property that may help promote fusion.

Domain rearrangements in the trimer

Crystals of the detergent-solubilized dengue sE trimers diffract to high resolution (2.0 A); the structure was determined by molecular replacement. The three domains of sE retain most of their folded structures, but undergo major rearrangements in their relative orientations, through flexion of the interdomain linkers. Domain II rotates approximately 308 with respect to domain I, about a hinge near residue 191 and the kl hairpin (residues 270–279), where mutations that affect the pH threshold of fusion are concentrated. As a result of the rotation, the base of the kl hairpin is pulled apart, and the l strand forms a new set of hydrogen bonds with the D_0 strand of domain I, shifted by two residues from the hydrogen-bonding pattern in the dimer. Although detergent is present, the kl hairpin does not adopt the open conformation seen in the dimer with bound b-OG (Modis et. al., 2003). The small hydrophobic core beneath this hairpin acts as a 'greased hinge' for the rotation between domains I and II. Domain III undergoes the most significant displacement in the dimer-to-trimer transition. It rotates by about 708, and its centre of mass shifts by 36 A towards domain II. This folding-over brings the C terminus of domain III (residue 395) 39 A closer to the fusion loop.

The fusion loop

The fusion loops in the sE trimer have the same conformation as in the dimer (Modis et. al., 2003). Because the trimers are obtained by detergent extraction from liposomes, we conclude that this conformation is also present when the loop inserts into a membrane. Furthermore, because dimers can dissociate reversibly, the fusion loop is stable when fully exposed. It thus appears that the fusion loop retains essentially the same conformation, whether buried against another subunit, inserted into a lipid membrane, or exposed to aqueous solvent.

In the trimer, the three hydrophobic residues in the fusion loop conserved among all flaviviruses—Trp 101, Leu 107 and Phe 108— are fully exposed on the molecular surface, near the three-fold axis. They form a bowl-like concavity at the trimer tip, with a hydro-phobic rim. Indeed, in the P321 crystal form, there can be no detergent micelle covering the fusion loop, as this region is involved in close crystal contacts with residues in domain III of a symmetry-related molecule. We conclude that detergent is required to dissolve away the liposome on which the trimer formed, and hence to solubilize the protein, but that once the protein has been extracted from the membrane, the three-fold-clustered fusion loops do not retain a tightly associated detergent micelle.

Tryptophans tend to appear in membrane proteins at the interface between the hydrocarbon and head-group layers of the lipid (Allison et. al., 1999), but if the indole amine participates in a hydrogen bond, as is the case for Trp 101, the side chain may be completely buried in the hydro-carbon layer. The E trimers penetrate about 6 A into the hydrocarbon layer of the target membrane. They cannot penetrate further, because of exposed carbonyls and charged residues on the outside rim of the fusion-loop bowl. Thus, the fusion loop is held in the membrane mainly by an 'aromatic anchor' formed by Trp 101 and Phe 108. The bowl is lined by the hydrophobic side chains of Leu 107 and Phe 108, so that it cannot accommodate lipid

headgroups. We expect that fatty-acid chains from the inner leaflet of the membrane may extend across to contact the base of the fusion-loop bowl, or that fatty-acid chains from the outer leaflet may bend over to fill it. In either case, insertion will produce a distortion in the bilayer, which could be important for the fusion process.

A postfusion conformation

The folding back of domain III and the rearrangement of *b*-strands at the trimer interface projects the C terminus of sE towards the fusion loop, and positions it at the entrance of a channel, which extends towards the fusion loops along the intersubunit contact between domains II. The 53-residue 'stem' connecting the end of the sE fragment with the viral transmembrane anchor could easily span the length of this channel, even if the stem were entirely *a*-helical. By binding in the channel, the stem would contribute additional trimer contacts with domain II of another subunit. The stem does indeed promote trimer assembly even in the absence of liposomes (Stiasny et. al., 2002). In the virion, the stem forms two *a*-helical segments, which lie near the outer surface of the lipid bilayer and contact the subunit from which they emanate (Zhang et. al., 2003). The proposed stem conformation places the viral transmembrane domain in the immediate vicinity of the fusion loop, just as in the postfusion conformations of class I viral fusion proteins. We there-fore believe that the trimer we have crystallized represents a postfusion state of the protein.

Mechanism of membrane fusion

1. E associates with a cell-surface receptor, probably through domain III (Holzmann et. al., 1990; Lobigs et. al., 1990; Cecilia et. al., 1991; Jiang et. al., 1993; Gao et.. al., 1994; Jennings et. al., 1994; Crill et. al., 2001); there is evidence for glycan-mediated interactions as well (Chen et. al., 1997; Navarro et. al., 2003; Tassaneetrithep et. al., 2003). Receptor binding leads to endosomal uptake.
2. Reduced pH in the endosome causes E dimers on the virion surface to dissociate (Stiasny et. al., 1996), exposing their fusion loops and allowing domains I and II to flex relative to one another.
3. Outward projection of domain II will destroy tight packing interactions on the virion outer surface, allowing lateral rearrangement of E monomers. Thus, the absence of trimer clustering in the virion (Mukhopadhyay, 2005) is not, in principle, a barrier to trimer formation
4. Formation of trimer contacts spreads from the fusion loops at the trimer tip to domain I at the base. Domain III shifts and rotates, folding the C terminus of sE back towards the fusion loop. The length of the interdomain linker permits independent rotation of individual domains III, allowing for the spontaneous symmetry-breaking required at this point.
5. Formation of a 'hemifusion stalk', with proximal leaflets fused and distal leaflets unfused, is thought to be an essential intermediate in the membrane fusion reaction (Kozlov et. al., 1998; Razinkov et. al., 1999; Kuzmin et. al., 2001). Hemifusion could occur at any point during the conformational changes, depending on the length of the hemifusion stalk.

In the final state, the trimer has reached the conformation seen in our crystal structure, with the stems (not present in our current crystals) docked along the surface of domains II and with the fusion loops and transmembrane anchors now next to each other in the fused membrane.

Conclusion

Above study shows that the membrane protein M and two nonstructural proteins *i.e.* NS2b and NS3 are plays major role in infection of this virus (DENV). NS2b/NS3 is serine protease complex which assist the maturation of dengue virus, a potent inhibitor against this complex can be use for treatment of this disease. Further studies can be done on this serine protease.

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