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Review

Structures of vesicular stomatitis virus glycoprotein: membrane fusion revisited

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Abstract. Glycoprotein G of the vesicular stomatitis virus (VSV) is involved in receptor recognition at the host cell surface and then, after endocytosis of the virion, triggers membrane fusion via a low pH-induced structural rearrangement. G is an atypical fusion protein, as there is a pH-dependent equilibrium between its pre- and post-fusion conformations. The atomic structures of these two conformations reveal that it is homologous to glycoprotein gB of herpesviruses and that it combines features of the previously characterized class I and class II fusion proteins. Comparison of the structures of G pre- and post-fusion states shows a dramatic reorganization of the molecule that is reminiscent of that of paramyxovirus fusion protein F. It also allows identification of conserved key residues that constitute pH-sensitive molecular switches. Besides the similarities with other viral fusion machineries, the fusion properties and structures of G also reveal some striking particularities that invite us to reconsider a few dogmas concerning fusion proteins.

Keywords. Vesicular stomatitis virus, rhabdovirus, paramyxovirus, glycoprotein, membrane fusion, viral entry, conformational change.

Introduction

To initiate a productive infection, all viruses must translocate their genome across the cell membrane [1]. For enveloped viruses, this step is mediated by virally encoded glycoproteins that promote both receptor recognition and membrane fusion. Both tasks can be achieved by a single or by separate glycoproteins acting in concert. Activation of the fusion capacity involves large structural rearrangements of the fusogenic glycoproteins upon interaction with specific triggers (e.g. low pH and cellular receptors). These conformational changes result in the exposure of a fusion peptide or fusion loops, which then interact with one or both of the participating membranes, resulting in their destabilization and merger [2]. Triggering of the conformational change in the absence of a target membrane leads to inactivation of the fusion properties of the fusogenic glycoprotein.

Experimental data suggest that the membrane fusion pathway is very similar for all the enveloped viruses studied so far whatever the organization of their fusion machinery [3–5] (Fig. 1). It is generally accepted that fusion proceeds via the formation of intermediate stalks that are local lipidic connections between the outer leaflets of the fusing membranes [6]. Radial expansion of the stalk would induce the

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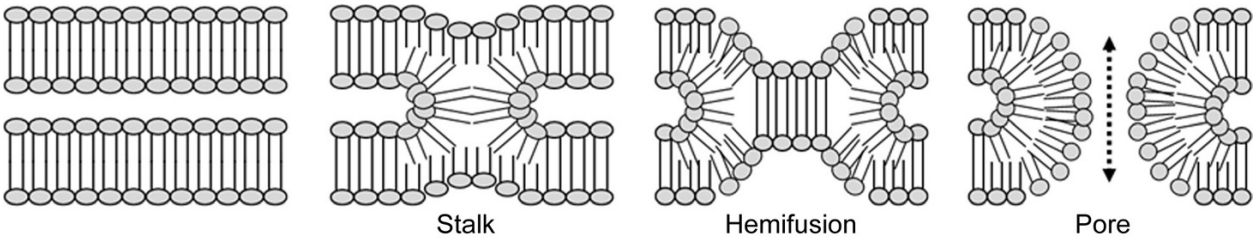

Figure 1. Stages of membrane fusion according to the stalk-pore model [82].

formation of a transient hemifusion diaphragm (i.e. a local bilayer made by the two initial inner leaflets). Depending on the experimental system, hemifusion may be restricted (i.e. without lipid exchange between the two membranes) or unrestricted (i.e. without any restriction of lipid diffusion). Restriction of lipid flux has been proposed to be due to a ringlike aggregate of fusogenic glycoproteins surrounding the hemifusion diaphragm [3, 4]. The next step would be the formation of a pore in the fusion diaphragm. The initial pore is small and is often opening and closing repeatedly (the so-called flickering pore) before its enlargement that leads to complete fusion [7].

Rhabdovirus glycoprotein G

Rhabdoviruses are widespread among a great diversity of organisms (including plants, insects, fishes, mammals, reptiles and crustaceans) [8]. This family includes vesicular stomatitis virus (VSV) as well as significant human pathogens like rabies virus (RV) or Chandipura virus [9]. Rhabdoviruses are enveloped viruses and have in common a bulletlike shape. Their genome is a single RNA molecule of negative polarity. It associates with the nucleoprotein N, the viral polymerase L and the phosphoprotein P to form the nucleocapsid. The nucleocapsid is condensed by the matrix protein M into a tightly coiled helical structure, which is surrounded by a lipid bilayer containing the viral glycoprotein G.

G forms the spikes that protrude from the viral surface. After cleavage of the aminoterminal signal peptide, the complete mature glycoprotein is about 500 amino acids long (495 for VSV Indiana). The bulk of the mass of G is located outside the viral membrane and constitutes the amino-terminal ectodomain. As this ectodomain is the only outer component of the viruses, it is the target of neutralizing antibodies [10–16].

G plays a critical role during the initial steps of virus infection. First, it is responsible for virus attachment to specific receptors. The nature of the receptor remains a matter of debate for both VSV and RV. In the case of VSV, although phosphatidylserine has been considered to be the viral receptor for a long time [17], recent results indicated that it is not [18]. In the case of RV, many molecules, including gangliosides [19], phospholipids [20], the nicotinic acetylcholine receptor [21, 22], neuronal cellular adhesion molecules [23] and the low-affinity nerve growth factor receptor [24], have been proposed to be viral receptors.

After binding, the virions enter the cell by the endocytic pathway. Subsequently, the viral envelope fuses with a cellular membrane within the acidic environment of the endosome [25]. Fusion is triggered by the low pH of the endosomal compartment and is mediated by the viral glycoprotein. The pH dependence is very similar from one rhabdovirus to another and the fusion is optimal around pH 6 [26–28]. Preincubation of the virus at low pH in the absence of a target membrane leads to inhibition of viral fusion. However, this inhibition is reversible, and readjusting the pH to above 7 leads to the complete recovery of the initial fusion activity. This is the main difference between rhabdoviruses and other viruses fusing at low pH, for which low pH-induced fusion inactivation is irreversible [29].

G can assume at least three different conformational states having different biochemical and biophysical characteristics [26, 30]: the native, prefusion state detected at the viral surface above pH 7; the activated hydrophobic state, which interacts with the target membrane as a first step of the fusion [31]; and the post-fusion conformation, which is antigenically distinct from the native and activated states [32]. There is a pH-dependent equilibrium between the different states of G that is shifted toward the post-fusion state at low pH [32]. This indicates that, differently from fusogenic glycoproteins from other viral families, the low-pH induced-conformational change is reversible and thus that the native conformation is not metastable. In fact, the reversibility of the fusogenic low-pH-induced conformational change is essential to allow G to be transported through the acidic compartments of the Golgi apparatus and to recover its native, prefusion state at the viral surface [33].

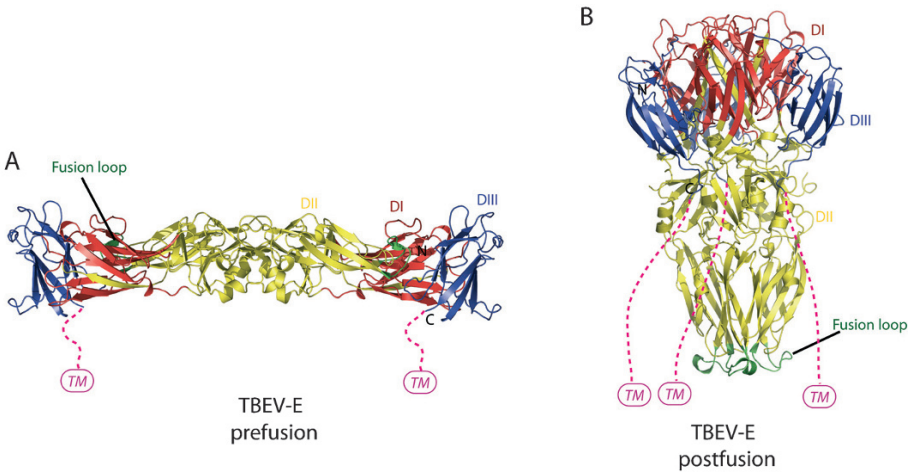

Figure 2. Overall structure of the pre- and post-fusion forms of tick-borne encephalitis virus (TBEV) glycoprotein E, a representative member of class II viral fusion proteins. (*A*) Ribbon diagram of the dimeric pre-fusion structure (PDB: 1SVB [41]). (*B*) Ribbon diagram of the trimeric post-fusion structure (PDB: 1URZ [83]). Domains are coloured as in [41]. The location of the fusion loop is indicated. The magenta dotted lines represent the missing part of the ectodomain that is connected to the transmembrane domain. PDB: Protein Data Bank.

Class I and class II fusion proteins

Before the structure determination of VSV G, two classes of viral fusion proteins had been identified (Fig. 2, 4). The viral fusion proteins belonging to class I, of which the best-characterized members are the influenza virus hemagglutinin (HA) [34, 35] and the fusion protein (F) of the paramyxoviruses [36, 37] but which also include fusion proteins from retroviruses [38] and filoviruses [39], are organized in trimers. Each subunit (or protomer) constituting the trimer results from the proteolytic cleavage of a precursor into two fragments. The C-terminal fragment bears at or near its amino-terminal end (i.e. at or near the cleavage site) a hydrophobic fusion peptide, buried at a trimer interface in the prefusion state. In the post-fusion conformation, this region refolds as a trimeric coiled coil at the N-terminal end of which are displayed the three fusion peptides and against which are packed, in an antiparallel manner, the segments abutting the transmembrane region (Fig. 4B). The protomer shape is thus an elongated hairpin-like structure with the fusion peptide and the transmembrane domain located at the same end, as expected at the end of the fusion process [40].

The class II fusion proteins contain E protein of flaviviruses and E1 of alphaviruses [41–43]. They display a molecular architecture completely different from that of class I proteins (Fig. 2). Their fusion peptide is internal, located in a loop between two β-strands. They are synthesized and folded as a complex with a second viral envelope protein that plays a chaperone role. Proteolytic cleavage of the chaperone primes the fusion protein to trigger membrane merger [44]. In their native conformation (Fig. 2A), they form homo- (flaviviruses) or hetero- (alphaviruses) dimers that are organized in an icosahedral assembly [42, 45]. They lie flat or nearly flat at the viral surface and their fusion loops are buried at a dimer interface. Upon low-pH exposure, dimers dissociate and the protomers reassociate in a trimeric structure [46, 47]. Similar to the structure of post-fusion class I proteins [48], the fusion loops and the transmembrane domains are then located at the same end of an elongated molecule that is now perpendicular to the membrane [49, 50] (Fig. 2B). Thus, even though the structures of class I and class II fusion proteins are unrelated, the mechanisms for refolding share key common features. First, the fusion peptides/loops are exposed and projected toward the top of the glycoprotein, allowing the initial interaction with the target membrane. Second, the folding back of the C-terminal region onto a trimeric N-terminal region leads to the formation of a post-fusion protein structure with the outer regions zipped up against the inner trimeric core [2].

For both class I and class II fusion proteins that trigger membrane merger at low pH, the proteolytic cleavage priming the proteins to undergo their low-pH-induced conformational change occurs in the trans-Golgi network or at the host cell surface [44, 51, 52]. This precludes premature activation of the fusion protein in the acidic compartments of the Golgi apparatus. Thus, reversibility of the low-pH-induced fusogenic transition is not necessary for these proteins.

Biochemical, structural and functional properties of rhabdovirus G suggested that it was distinct from both class I and class II viral fusion proteins that had been already described [29, 53]. Indeed, the pH-dependent equilibrium between the different states of G, the absence of predicted α-helical coiled-coil motif characteristic of class I viral fusion proteins [40] and the absence of activating cleavage (neither in G nor in an accompanying protein) strongly suggested that G could define a new category of fusogenic glycoproteins.

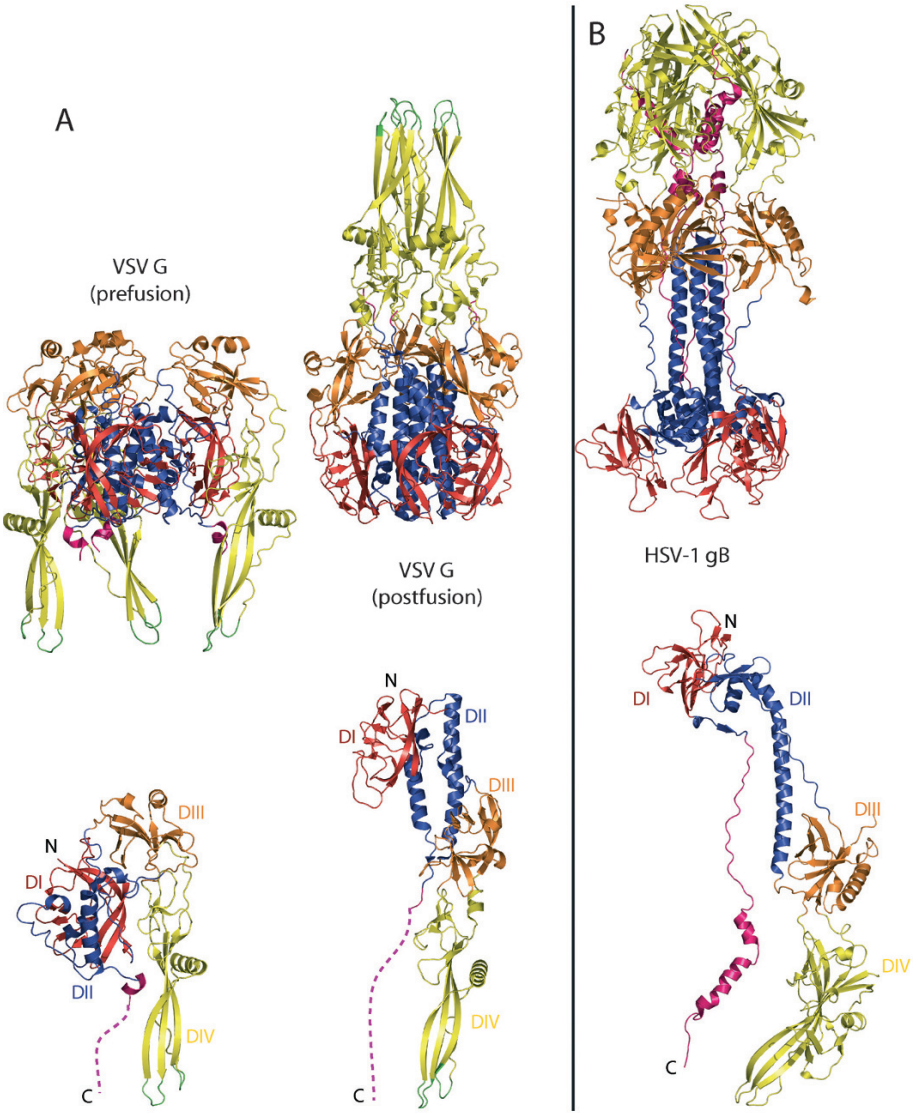

Figure 3. (*A*) Overall structure of the pre- and post-fusion forms of VSV glycoprotein G. Ribbon diagrams of the pre-fusion structure of G trimer (top left) (PDB: 2J6J [55]); of the post-fusion structure of G trimer (top right) (PDB: 2CMZ [54]); of the pre-fusion structure of G protomer (residues 1–413) (bottom left); and of the post-fusion structure of G protomer (residues 1–410) (bottom right). G protein is coloured by domains (domain I: red, domain II: blue, domain III: orange, domain IV: yellow) with the fusion loops in green and the C-terminus in magenta. The protomers are superimposed on their fusion domains (DIV) and the trimers on the rigid blocks made of DI and the invariant part of DII. In the protomer diagrams, the dotted lines represent the missing C-terminal segment of the ectodomain that leads to the transmembrane segment. (*B*) Overall structure of HSV-1 gB. Ribbon diagrams of gB trimer (top) and gB protomer (bottom) (PDB: 2GUM [57]). gB protein is coloured by domains as their homologous counterparts of VSV G.

VSV G structure

We have recently determined the atomic structures of both the pre- and post-fusion forms of the VSV-G ectodomain, generated by limited proteolysis with thermolysin (G_{th}, aa residues 1–422) [54, 55] (Fig. 3A). The dimensions of the two conformations – consistent with the electron microscopy data obtained on RV G [30, 56] – together with the position of the antigenic sites allowed a clear-cut identification of the pre- and post-fusion structures. The structural organization of the two conformations of G is very different from that of other viral fusion proteins described so far. However, amino acid sequence alignment of different G proteins of rhabdoviruses belonging to different genera shows that all these glycoproteins have the same fold.

Four distinct domains of G_{th} have been identified: a β-sheet-rich lateral domain (domain I), a central domain that is involved in the trimerization of the molecule (domain II), a pleckstrin homology domain (domain III) and the fusion domain (domain IV) inserted in a loop of domain III. Major antigenic sites are located in both domains I and III [55].

After the end of the trimerization domain (after amino acid residue 405), there remain 40 amino acids for the polypeptide chain to reach the G transmembrane domain (Fig. 3A, bottom), but their structural organization is unknown after amino acid residue 413 for the pre-fusion conformation and after amino acid residue 410 for the post-fusion conformation.

An unexpected homology

The structure of herpes simplex virus 1 (HSV1, a double-stranded DNA virus) glycoprotein gB [57] was published at the same time as that of G_{th} post-fusion state. Comparison of the two structures revealed that their folds are the same and that they have a common evolutionary origin that could not be detected by looking at the amino acid sequences (Fig. 3B). This was completely unexpected and suggests that rhabdoviruses, and most probably all viruses belonging to the *Mononegavirales* order, have the ability to steal genes from their host (or from another virus during co-infection of a host cell). This might occur when the viral polymerase jumps from the antigenomic template onto an RNA messenger (either of cellular or viral origin) during genomic RNA synthesis. Nevertheless, the exact scenario of G gene acquisition by the rhabdovirus ancestor will still remain a matter of debate for a long time.

The orientation of the central helix relative to the viral membrane in the determined structure of HSV1 gB suggests that gB is in its post-fusion conformation. Nevertheless, as the fusion machinery of herpesviruses is much more complex than that of rhabdoviruses (with four glycoproteins that are essential for virus entry) and as its mode of activation is completely different [58], the extent of gB conformational change cannot be inferred from its homology with VSV G.

The conformational change of VSV G

Comparison of the pre- and post-fusion structures of VSV G reveals a dramatic reorganization of the molecule (Fig. 3A). During the conformational change, domains I, III and IV retain their tertiary structure. Nevertheless, they undergo large rearrangements in their relative orientation due to secondary structure changes in the hinge regions between the fusion and pleckstrin homology domains and major refolding of the central trimerization domain (Fig. 4C). In fact, the pre- and post-fusion states are related by flipping both the fusion domain and the C-terminal segment relative to a rigid block constituted of the lateral domain and the part of the trimerization domain that retains its structure during the molecule refolding.

Global refolding of G from pre- to post-fusion conformation exhibits striking similarities to that of class I proteins such as paramyxovirus fusion protein (F) (Fig. 4A) and influenza virus hemagglutinin subunit 2 (HA2) (Fig. 4C) [35, 37]. Particularly, the reversal of the molecule around the rigid block involves the lengthening of the central helices (that form the trimeric central core of the post-fusion conformation, thus displaying the fusion domains – through the PH domains – at their N-termini) and the refolding of the three carboxy-terminal segments into helices that position themselves in the grooves of the central core in an antiparallel manner to form a six-helix bundle (Fig. 4C). This structural organization is obviously very similar to that of the post-fusion hairpin structure of class I proteins (Fig. 4B), even though, for VSV G, the central helices are not coiled and remain parallel.

Interaction between fusion domains and membranes

The structural organization of the G fusion domain resembles that of class II fusion proteins. The main difference is that the membrane interacting motif of the fusion domain is bipartite (as previously proposed for viral haemorrhagic septicaemia virus, another rhabdovirus [27]), made of two loops, and that the loop sequences are not conserved among rhabdoviruses. However, as in class II fusion proteins, these loops always contain aromatic residues and are located at the tip of an elongated three-stranded β-sheet. Note that in striking contrast to class I and class II viral fusion proteins, the fusion loops are not buried at an oligomeric interface in G pre-fusion conformation (Fig. 3A). Indeed, these loops are much less hydrophobic than the amino-terminal fusion peptides of class I proteins (even when the three fusion domains of G are grouped together in the post-fusion conformation). That these loops are indeed an essential part of the membrane interacting motif is consistent with previous mutagenesis work performed on rhabdoviruses [59, 60] (Table 1) and has since been confirmed for both VSV G [61] (Table 1) and Herpesviruses gB [62, 63].

Although hydrophobic photolabeling experiments have demonstrated the ability of G fusion domain to insert into the target membrane as a first step of the fusion process [31, 64], it is clear, from the presence of charged residues in the vicinity of the loops (as in the fusion domain of class II fusion proteins), that any deep penetration inside the membrane is precluded (Fig. 5). Rather, the tryptophans and tyrosines that are found in the fusion loops of all the rhabdoviral G proteins (Fig. 5) act as sticky fingers by positioning themselves at the interface between the fatty acid chains and head group layers of lipids. It is probable that this interfacial interaction involving only a few residues does not create a strong point of anchoring that can be used to pull the target membrane toward the viral one. Rather, we propose that by perturbating the outer leaflet of the target bilayer, it facilitates the

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