Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochimica et Biophysica Acta 1614 (2003) 116–121
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.bba-direct.com

Review

Fusion peptides and the mechanism of viral fusion

Richard M. Epand*

Health Science Centre, Department of Biochemistry, McMaster University, 1200 Main Street West, Hamilton, ON, Canada L8N 3Z5

Received 28 April 2003; accepted 15 May 2003

Abstract

Segments of viral fusion proteins play an important role in viral fusion. They are defined by a number of criteria, including the sensitivity of this region of the viral fusion protein to loss of function as a consequence of mutation. In addition, small model peptides designed to mimic this segment of viral fusion proteins often have some membrane perturbing activity. The properties of viral fusion peptides are quite varied. Many are found at the amino terminus of viral fusion proteins. As isolated peptides, they have been found to form both α-helical as well as β-structure. In addition, some viruses have internal fusion peptides. Just as there are several structural motifs for viral fusion peptides, there are also several mechanisms by which they accelerate the process of membrane fusion. These include the promotion of negative curvature, lowering the rupture tension of the lipid monolayer, acting as an anchor to join the fusion membranes, transmitting a force to the membrane or imparting energy to the system by other means. It is not likely that the fusion peptide can fulfill all of these diverse roles and future studies will elucidate which of these mechanisms is most important for the action of individual viral fusion peptides.

Keywords: Viral fusion; Fusion peptide; Peptide–membrane interaction

1. What is a "fusion peptide"?

Although the term fusion peptide has been widely used in the literature, there is not a precise definition as to what this term means. Furthermore, the term has been used both for a short model peptide mimicking a segment of a fusion protein as well as for the segment of the fusion protein itself. In this review, we will use the term fusion peptide only to refer to the small model peptide and we will call the corresponding region of the fusion protein the fusion peptide segment. This review will also focus on fusion proteins from viruses, since more is known about these systems and, in general, fewer proteins are involved in the acceleration of viral fusion.

Despite the fact that there is no precise definition of the term fusion peptide, for many viruses, there is little disagreement about which region of the fusion protein corresponds to the fusion peptide segment. There are a number of criteria that characterize fusion peptide segments. None of these criteria are absolute in defining a fusion peptide segment, but in combination, they are sufficiently restrictive to often allow the identification of one region of the protein as being the fusion peptide segment. The fusion peptide segment is often found to be hydrophobic, rich in Gly residues and at the N-terminal end of the fusion protein. Although this helps to identify fusion peptide segments that meet these criteria, there are examples of other segments that have been identified as fusion peptide segments that do not meet these criteria. Another criterion is that mutation in the fusion peptide segment of the fusion protein leads to loss of activity because of the essential nature of the fusion peptide segment for membrane fusion. Again, while this is a good generality, it is not an absolute rule. For example, mutation of the amino terminal Gly of the fusion peptide segment of influenza virus can result in a variety of phenotypes, depending on the nature of the amino acid substituted [1]. In addition, there are many examples of mutations outside of the region of the fusion peptide segment that result in loss of fusogenic activity. The amino acid residues that are involved in mutations leading to new strains of infectious virus must be outside of the fusion peptide segment. Hence, although some regions can be excluded as being

Abbreviations: ATR-FTIR, Fourier transform infrared spectroscopy; SIV, simian immunodeficiency virus; NMR, nuclear magnetic resonance; T_H, bilayer to hexagonal phase transition temperature

* Tel.: +1-905-525-9140x22073; fax: +1-905-521-1397.
E-mail address: epand@mcmaster.ca (R.M. Epand).

0005-2736/03/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0005-2736(03)00169-X

part of the fusion peptide segment, there is no single criterion that can be used to positively identify a fusion peptide.

In addition to properties of segments of the intact viral fusion protein, a small synthetic peptide having a sequence corresponding to the fusion peptide segment will have membrane-perturbing activity. These fusion peptides rarely support non-leaky fusion with the mixing of the aqueous contents of the fusing particles. They do, however, generally promote lipid mixing between liposomes and more generally they destabilize membrane bilayers. Because the action of these fusion peptides on membranes is not very specific and there is no quantitative criterion for the degree of membrane destabilization expected for a fusion peptide, this property also does not provide a clear criterion for defining a fusion peptide. Despite the fact that there is no absolute criterion to define what is and what is not a fusion peptide segment, it is clear that certain segments of viral fusion proteins have a more important role in the fusion process than other segments.

2. Conformational properties of fusion peptides

The information that one would really like to know is the conformation of the fusion peptide segment of a viral fusion protein in the presence of membranes. In general, however, one is able to either obtain a detailed X-ray or nuclear magnetic resonance (NMR) structure of a segment of the ectodomain of a viral fusion protein as a crystal in the absence of membranes or to study by NMR the conformation of a small synthetic fusion peptide, either in the presence or absence of membranes.

A general assessment of the secondary structure of a fusion peptide can be made using less specific spectroscopic techniques, based on the properties of the amide chromophores, such as circular dichroism or infrared spectroscopy. Many viral fusion peptides have been found to be α-helical when inserted into a membrane. This is not unanticipated, since the insertion of amide groups into membranes is energetically highly unfavorable [2], unless the amide groups are hydrogen bonded, such as when they have a high secondary structure content. In addition to the secondary structure, polarized attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) can be employed to determine the orientation of a helical peptide in a membrane. It has been found that many viral fusion peptides are inserted into a membrane at an oblique angle [3–5].

There have been attempts to obtain more specific structural information using small angle neutron scattering from oriented membranes with an inserted simian immunodeficiency virus (SIV) viral fusion peptide containing substituted deuterium atoms [6]. This work also suggested that the insertion of the peptide was at an oblique angle. A more detailed structure of the influenza fusion peptide was obtained using a combination of NMR of the fusion peptide in micelles and spin label quenching of the peptide in a membrane as a function of pH [7]. That work demonstrated that the peptide had a conformation that was not a simple helical structure and that a kink in the peptide played an important role in changing the depth of insertion of the peptide as a function of pH [7,8].

In addition to fusion peptides being α-helices or partially α-helical, there is also some evidence for viral fusion peptides having β-structure. Most proteins that insert into membranes as β-structures do so as β-barrels, in which there is no "edge" with unfulfilled hydrogen bonding. There is also the possibility of the model fusion peptides aggregating as β-structures on the surface of the membrane. In the case of the intact fusion protein, it is difficult to see a priori how a sufficient number of fusion proteins can cluster together to have their fusion peptide segments associate to form a β-barrel. Nevertheless, there is evidence that the fusion peptide of HIV can form a β-structure at the amino terminus [9,10].

In addition to these two types of fusion peptides, there are also internal fusion peptides, whose sequence suggests that they do not form extensive secondary structures. These include an internal peptide of avian leukosis and sarcoma virus [11–13], vesicular stomatitis virus G-protein [14] and the non-enveloped fusogenic avian and Nelson Bay reoviruses [15].

3. Curvature modulation by fusion peptides

A common property of a number of fusion peptides is that they lower the bilayer to hexagonal phase transition temperature (T_H) of phosphatidylethanolamine, indicating that they promote negative curvature [16]. This property is well correlated with conditions that lead to membrane fusion. Thus for example, the fusion peptide from influenza virus lowers T_H at acidic pH where the virus is fusogenic, but not at neutral pH where the rate of fusion is slow [17]. In addition, there is a correlation between the fusion activity of viral mutants and the ability of their fusion peptide to lower T_H, both with influenza virus [18] as well as with SIV [19,20]. The promotion of negative curvature by fusion peptides is in accord with the requirement to increase the negative curvature of the contacting monolayers to form the hemifusion intermediate [21,22]. It also may help to explain the common finding that fusion peptides insert into a membrane as a tilted helix. Such an orientation of the inserted helical segment is consistent with the observation that the peptide promotes negative curvature, since it would be anticipated that such an angle of insertion would have a greater effect in expanding the region in the center of the bilayer than at the membrane interface.

4. Role of the fusion peptide as a membrane anchor

The fusion peptide has been shown to insert into target membranes, at least in some stage of the fusion process [23]. This may be an important component in bridging the two fusing structures. In addition, it has been suggested that the inserted fusion peptide segment may be an attachment to the membrane that allows a conformational rearrangement (see below) of the protein to transmit a force to the membrane [24].

5. Membrane rupture tension

In order for two bilayers to merge into one, each monolayer must rupture and reform. The energy required to break the membrane will be dependent on its rupture tension. It has been shown that low concentrations of the order of 1% of hydrophobic peptides, including Lys–Leu_{16}–Lys [25] and the influenza fusion peptide [26], lower the rupture tension of membranes. This property has been used to explain the ability of the influenza virus to promote the formation of cubic phases [27]. This proposed role for the fusion peptide to lower the rupture tension is independent of membrane curvature. It would require the fusion peptide to access the *trans* monolayer to rupture it so as to form a fusion pore. It is possible that both the promotion of negative curvature and the lowering of membrane rupture tension are important for accelerating the rate of membrane fusion at different stages of the process.

6. Fusogenic activity of model fusion peptides

In general, synthetic viral fusion peptides are much less effective in accelerating membrane fusion compared with the intact fusion protein. It of course is not surprising that a small peptide, generally less than 25 residues in length, does not reproduce all of the functions of an intact protein of tens of kilodaltons. There are at least two factors that are likely to contribute to the greater activity of the intact protein. One is that viral fusion proteins form stable multimers and in addition they tend to self-associate in membranes to form higher order complexes [28,29]. Thus, the fusion peptide segment does not function as an isolated unit, but is rather assembled into an organized complex in which several fusion peptide segments can act in a coordinated fashion.

In addition, there are other regions of the fusion protein that our outside of the fusion peptide but can also participate in accelerating fusion. Evidence for a role of the transmembrane segment and the cytoplasmic tail of the fusion protein has recently been summarized [30]. In this article, we will focus on the role of other regions of the ectodomain of the fusion protein.

Isolated fusion peptides promote contents leakage of vesicles [31] and therefore true fusion, or mixing of aqueous contents cannot be measured [32]. Other regions of the viral proteins, apart from the fusion peptide, are required for binding to allow the two fusing structures to become juxtaposed. In addition, other segments of the viral protein are important for the evolution of the hemifusion intermediate into a fusion pore. Even a large 127-amino-acid ectodomain fragment of the HA2 chain of the influenza virus hemagglutinin does not efficiently promote full fusion [33]. To monitor the mixing of aqueous contents, the rate of formation of a fusion pore has to be more rapid than the rate of leakage. However, an agent that will destabilize membrane bilayers will induce leakage. Even many intact viruses induce hemolysis and it has been found that influenza virus causes the lysis of liposomes [34]. It is therefore not surprising that a small fragment of the viral fusion protein, the fusion peptide, causes sufficient leakage to prevent measuring the mixing of aqueous contents.

7. Other regions of the viral fusion protein

It has been shown that a construct corresponding to the major portion of the ectodomain of the HA2 chain of the influenza hemagglutinin protein, is many fold more active in inducing lipid mixing than is the fusion peptide of this same protein. This construct maintains the pH dependence of fusion rate found for the native virus [35]. There are also other cases of viral proteins that have N-terminal fusion peptides, but also contain other segments of the ectodomain that contribute to the fusion mechanism [36]. One example is Sendai virus that has a segment adjacent to, but independent of, the N-terminal fusion peptide. This is based on the orientation of the proximal segment along the plane of the membrane, rather than being inserted into the membrane as is the fusion peptide [37,38]. This internal peptide is part of the heptad repeat motif. A similar arrangement has been shown for another paramyxovirus, measles [39], suggesting the possibility that this is a general characteristic of this class of viruses. In addition, HIV-1, a retrovirus, has a segment of the heptad repeat that binds to membranes in addition to stabilizing a trimeric structure [40]. These peptides do not insert deeply into the membrane, as do the fusion peptides. The presence of aromatic residues is important for stabilizing the peptide in a membrane region close to the interface [2]. This property has been used to identify regions of the ectodomain of viral fusion proteins that interact with the membrane surface and facilitate fusion [41,42].

8. Energy that drives the fusion reaction

There has been discussion in the literature about the "source of energy for fusion". It should be remembered,

however, that the fusion process is one in which the energy of the starting structures and of the products is similar. There is a similar morphology of both reactant and end product, in which the bilayer is essentially flat on a molecular scale. The fusion protein may undergo some change in structure, but it is not initially in a metastable high-energy state, at least in the case of the influenza hemagglutinin protein [43,44]. The function of the fusion protein is to lower the activation energy for membrane fusion. The intermediate structures as well as the transition states in the fusion process are of higher energy than either the starting structures or the products since these intermediates will include the bending and transient rupture of monolayers, processes that do not occur spontaneously. The fusion protein may contribute energy to allow more rapid formation of the intermediates that are formed in the pathway to fusion. In this sense, the fusion protein may participate in a process that yields energy that is coupled with the reorganization of membrane lipids to facilitate fusion. There are several alternative explanations that have been proposed for the process that gives rise to this energy. Since there is such a large conformational change of the influenza hemagglutinin protein upon acidification [45], an attractive hypothesis is that this conformational change is coupled with the release of energy that can facilitate the formation of fusion intermediates. Although there is no high energy, metastable state as a consequence of the constraints placed on HA2 by the presence of the surrounding HA1 subunits, there could be an increase in the energy of the protein following acidification. Electrostatic calculations have shown that there is increased repulsion among HA1 subunits as a consequence of acidification [46]. If the release of this electrostatic repulsion as a consequence of the loosening of the oligomeric structure of the hemagglutinin protein could be coupled with changes in the organization of the lipid, this could impart an energy that could accelerate the fusion process.

The fusion peptide segment is sequestered within the structure of the hemagglutinin protein before fusion [47]. When this segment becomes exposed during the conformational rearrangement of the protein, a subsequent insertion of the hydrophobic fusion peptide segment into a membrane would be expected to liberate energy that could be coupled with membrane fusion. A less direct coupling of the insertion of the fusion peptide segment into a membrane with a resulting facilitation of membrane fusion has been proposed. In this hypothesis, the fusion peptide is initially inserted into the viral membrane. As a consequence of the conformational rearrangement of the ectodomain, there is a force transmitted to the membrane by pulling on the fusion peptide [24]. This process creates a dimpling of the membrane that would destabilize the bilayer and be a site for the initiation of membrane fusion. It has also been pointed out that the fusion peptide segment does not function as an isolated monomeric structure, but rather a cooperative interaction among fusion proteins occurs [28].

9. Conclusions

The mechanism by which proteins facilitate the formation of fusion intermediates is a complex process involving several segments of fusion proteins (Table 1). The best understood fusion mechanism is that of the fusion of enveloped viruses with target membranes. However, even this process is not simple and there are significant differences in the mechanism among different viruses.

A component of the acceleration of fusion by these fusion proteins is the interaction with the membrane of the fusion peptide segment. This segment is not the sole factor in promoting fusion, but in several cases, it has been shown to be an essential component. There are several hypotheses for the mechanism by which this segment of the fusion protein accelerates the formation of fusion intermediates. One of the most developed examples is that of the fusion peptide segment of influenza virus that has the additional feature that its action is strongly pH dependent. This can be a combined consequence of the change in membrane insertion as a function of pH [7] or as a result of the formation of an extended coiled-coil as a result of the conformational rearrangement of the hemagglutinin protein. This later conformational change is a requirement for fusion with the intact protein to make the fusion peptide segment accessible. As a consequence, mutations that alter the pH dependence of the fusion peptide may have no discernable effects on the kinetic properties of fusion catalyzed by the intact protein [48]. However, this conformational rearrangement is required only to expose the segments of the fusion protein required for rapid fusion, but not for the fusion process itself, since a fragment of HA2, already in the extended coiled-coil form at neutral pH, does not promote fusion until the pH is lowered to close to 5 [33,35]. Thus,

Table 1
Segments of class I viral fusion proteins

Segment	Properties	Putative role
Fusion peptide	Frequently found at amino terminus. Maybe α-helical or β-structure. Hydrophobic. High content of Gly.	Inserts into target membrane. Destabilizes bilayer. Promotes negative curvature to facilitate stalk formation. Lowers bilayer rupture tension to promote formation of fusion pore.
Heptad repeat	Triple helical coiled-coil structure	Contributes to cooperativity among fusion peptides.
Other specific regions of ectodomain	Various. Maybe surface seeking helix.	Greatly potentiates action of fusion peptide, possibly dehydrates membrane surface.
Transmembrane helix	Hydrophobic helix	Ties viral membrane to fusion apparatus in ectodomain. Promotes self-association of viral protein. May facilitate partitioning of fusion protein into membrane domains.
Cytoplasmic tail	Variable	Differing importance, according to specific example.

many factors contribute to the potency and regulation of the activity of fusion proteins. Although the detailed structure of major portions of the ectodomain of influenza virus have been known for several years, there are still many questions to be answered regarding the mode of action of this protein and others in accelerating membrane fusion reactions.

References

[1] H. Qiao, R.T. Armstrong, G.B. Melikyan, F.S. Cohen, J.M. White, A specific point mutant at position 1 of the influenza hemagglutinin fusion peptide displays a hemifusion phenotype, Mol. Biol. Cell 10 (1999) 2759–2769.

[2] W.C. Wimley, S.H. White, Experimentally determined hydrophobicity scale for proteins at membrane interfaces, Nat. Struct. Biol. 3 (1996) 842–848.

[3] J. Luneberg, I. Martin, F. Nussler, J.M. Ruysschaert, A. Herrmann, Structure and topology of the influenza virus fusion peptide in lipid bilayers, J. Biol. Chem. 270 (1995) 27606–27614.

[4] M. Horth, B. Lambrecht, M.C. Khim, F. Bex, C. Thiriart, J.M. Ruysschaert, A. Burny, R. Brasseur, Theoretical and functional analysis of the SIV fusion peptide, EMBO J. 10 (1991) 2747–2755.

[5] I. Martin, M.C. Dubois, F. Defrise-Quertain, T. Saermark, A. Burny, R. Brasseur, J.M. Ruysschaert, Correlation between fusogenicity of synthetic modified peptides corresponding to the NH2-terminal extremity of simian immunodeficiency virus gp32 and their mode of insertion into the lipid bilayer: an infrared spectroscopy study, J. Virol. 68 (1994) 1139–1148.

[6] J.P. Bradshaw, M.J. Darkes, T.A. Harroun, J. Katsaras, R.M. Epand, Oblique membrane insertion of viral fusion peptide probed by neutron diffraction, Biochemistry 39 (2000) 6581–6585.

[7] X. Han, J.H. Bushweller, D.S. Cafiso, L.K. Tamm, Membrane structure and fusion-triggering conformational change of the fusion domain from influenza hemagglutinin, Nat. Struct. Biol. 8 (2001) 715–720.

[8] C.H. Hsu, S.H. Wu, D.K. Chang, C. Chen, Structural characterizations of fusion peptide analogs of influenza virus hemagglutinin. Implication of the necessity of a helix–hinge–helix motif in fusion activity, J. Biol. Chem. 277 (2002) 22725–22733.

[9] J. Yang, C.M. Gabrys, D.P. Weliky, Solid-state nuclear magnetic resonance evidence for an extended beta strand conformation of the membrane-bound HIV-1 fusion peptide, Biochemistry 40 (2001) 8126–8137.

[10] S.G. Peisajovich, R.F. Epand, M. Pritsker, Y. Shai, R.M. Epand, The polar region consecutive to the HIV fusion peptide participates in membrane fusion, Biochemistry 39 (2000) 1826–1833.

[11] S.E. Delos, J.M. Gilbert, J.M. White, The central proline of an internal viral fusion peptide serves two important roles, J. Virol. 74 (2000) 1686–1693.

[12] S.E. Delos, J.M. White, Critical role for the cysteines flanking the internal fusion peptide of avian sarcoma/leukosis virus envelope glycoprotein, J. Virol. 74 (2000) 9738–9741.

[13] L.D. Hernandez, J.M. White, Mutational analysis of the candidate internal fusion peptide of the avian leukosis and sarcoma virus subgroup A envelope glycoprotein, J. Virol. 72 (1998) 3259–3267.

[14] S. Shokralla, R. Chernish, H.P. Ghosh, Effects of double-site mutations of vesicular stomatitis virus glycoprotein G on membrane fusion activity, Virology 256 (1999) 119–129.

[15] M. Shmulevitz, R. Duncan, A new class of fusion-associated small transmembrane (FAST) proteins encoded by the non-enveloped fusogenic reoviruses, EMBO J. 19 (2000) 902–912.

[16] R.M. Epand, Lipid polymorphism and protein–lipid interactions, Biochim. Biophys. Acta 1376 (1998) 353–368.

[17] R.M. Epand, R.F. Epand, Relationship between the infectivity of influenza virus and the ability of its fusion peptide to perturb bilayers, Biochem. Biophys. Res. Commun. 202 (1994) 1420–1425.

[18] R.M. Epand, R.F. Epand, I. Martin, J.M. Ruysschaert, Membrane interactions of mutated forms of the influenza fusion peptide, Biochemistry 40 (2001) 8800–8807.

[19] A. Colotto, I. Martin, J.M. Ruysschaert, A. Sen, S.W. Hui, R.M. Epand, Structural study of the interaction between the SIV fusion peptide and model membranes, Biochemistry 35 (1996) 980–989.

[20] R.F. Epand, I. Martin, J.M. Ruysschaert, R.M. Epand, Membrane orientation of the SIV fusion peptide determines its effect on bilayer stability and ability to promote membrane fusion, Biochem. Biophys. Res. Commun. 205 (1994) 1938–1943.

[21] D.P. Siegel, The modified stalk mechanism of lamellar/inverted phase transitions and its implications for membrane fusion, Biophys. J. 76 (1999) 291–313.

[22] L. Chernomordik, Non-bilayer lipids and biological fusion intermediates, Chem. Phys. Lipids 81 (1996) 203–213.

[23] P. Durrer, C. Galli, S. Hoenke, C. Corti, R. Gluck, T. Vorherr, J. Brunner, H+-induced membrane insertion of influenza virus hemagglutinin involves the HA2 amino-terminal fusion peptide but not the coiled coil region, J. Biol. Chem. 271 (1996) 13417–13421.

[24] M.M. Kozlov, L.V. Chernomordik, A mechanism of protein-mediated fusion: coupling between refolding of the influenza hemagglutinin and lipid rearrangements, Biophys. J. 75 (1998) 1384–1396.

[25] E. Evans, D. Needham, Physical properties of surfactant bilayer membranes: thermal transitions, elasticity, rigidity, cohesion and colloidal interactions, J. Phys. Chem. 91 (1987) 4219–4228.

[26] M.L. Longo, A.J. Waring, D.A. Hammer, Interaction of the influenza hemagglutinin fusion peptide with lipid bilayers: area expansion and permeation, Biophys. J. 73 (1997) 1430–1439.

[27] D.P. Siegel, R.M. Epand, Effect of influenza hemagglutinin fusion peptide on lamellar/inverted phase transition in dipalmitoleoylphosphatidylethanolamine: implications for membrane fusion mechanisms, Biochim. Biophys. Acta 1468 (2000) 87–98.

[28] I. Markovic, E. Leikina, M. Zhukovsky, J. Zimmerberg, L.V. Chernomordik, Synchronized activation and refolding of influenza hemagglutinin in multimeric fusion machines, J. Cell Biol. 155 (2001) 833–844.

[29] L.D. Hernandez, L.R. Hoffman, T.G. Wolfsberg, J.M. White, Virus–cell and cell–cell fusion, Annu. Rev. Cell Dev. Biol. 12 (1996) 627–661.

[30] B. Schroth-Diez, K. Ludwig, B. Baljinnyam, C. Kozerski, Q. Huang, A. Herrmann, The role of the transmembrane and of the intraviral domain of glycoproteins in membrane fusion of enveloped viruses, Biosci. Rep. 20 (2000) 571–595.

[31] J.D. Lear, W.F. DeGrado, Membrane binding and conformational properties of peptides representing the NH2 terminus of influenza HA-2, J. Biol. Chem. 262 (1987) 6500–6505.

[32] M.E. Haque, A.J. McCoy, J. Glenn, J. Lee, B.R. Lentz, Effects of hemagglutinin fusion peptide on poly(ethylene glycol)-mediated fusion of phosphatidylcholine vesicles, Biochemistry 40 (2001) 14243–14251.

[33] E. Leikina, D.L. LeDuc, J.C. Macosko, R. Epand, R. Epand, Y.K. Shin, L.V. Chernomordik, The 1–127 HA2 construct of influenza virus hemagglutinin induces cell–cell hemifusion, Biochemistry 40 (2001) 8378–8386.

[34] T. Shangguan, D. Alford, J. Bentz, Influenza-virus–liposome lipid mixing is leaky and largely insensitive to the material properties of the target membrane, Biochemistry 35 (1996) 4956–4965.

[35] R.F. Epand, J.C. Macosko, C.J. Russell, Y.K. Shin, R.M. Epand, The ectodomain of HA2 of influenza virus promotes rapid pH dependent membrane fusion, J. Mol. Biol. 286 (1999) 489–503.

[36] S.G. Peisajovich, Y. Shai, New insights into the mechanism of virus-induced membrane fusion, Trends Biochem. Sci. 27 (2002) 183–190.

[37] S.G. Peisajovich, O. Samuel, Y. Shai, Paramyxovirus F1 protein has two fusion peptides: implications for the mechanism of membrane fusion, J. Mol. Biol. 296 (2000) 1353–1365.

[38] J.K. Ghosh, S.G. Peisajovich, Y. Shai, Sendai virus internal fusion peptide: structural and functional characterization and a plausible mode of viral entry inhibition, Biochemistry 39 (2000) 11581–11592.
[39] O. Samuel, Y. Shai, Participation of two fusion peptides in measles virus-induced membrane fusion: emerging similarity with other paramyxoviruses, Biochemistry 40 (2001) 1340–1349.
[40] M. Rabenstein, Y.K. Shin, A peptide from the heptad repeat of human immunodeficiency virus gp41 shows both membrane binding and coiled-coil formation, Biochemistry 34 (1995) 13390–13397.
[41] J.L. Nieva, T. Suarez, Hydrophobic-at-interface regions in viral fusion protein ectodomains, Biosci. Rep. 20 (2000) 519–533.
[42] T. Suarez, W.R. Gallaher, A. Agirre, F.M. Goni, J.L. Nieva, Membrane interface-interacting sequences within the ectodomain of the human immunodeficiency virus type 1 envelope glycoprotein: putative role during viral fusion, J. Virol. 74 (2000) 8038–8047.
[43] R.M. Epand, R.F. Epand, The thermal denaturation of influenza virus and its relationship to membrane fusion, Biochem. J. 365 (2002) 841–848.
[44] R.F. Epand, R.M. Epand, Irreversible unfolding of the neutral pH form influenza hemagglutinin demonstrates it is not in a metastable state, Biochemistry 42 (2003) 5052–5057.
[45] P.A. Bullough, F.M. Hughson, J.J. Skehel, D.C. Wiley, Structure of influenza haemagglutinin at the pH of membrane fusion [see comments], Nature 371 (1994) 37–43.
[46] Q. Huang, R. Opitz, E.W. Knapp, A. Herrmann, Protonation and stability of the globular domain of influenza virus hemagglutinin, Biophys. J. 82 (2002) 1050–1058.
[47] I.A. Wilson, J.J. Skehel, D.C. Wiley, Structure of the haemagglutinin membrane glycoprotein of influenza virus at 3 A resolution, Nature 289 (1981) 366–373.
[48] T. Korte, R.F. Epand, R.M. Epand, R. Blumenthal, Role of the Glu residues of the influenza hemagglutinin fusion peptide in the pH dependence of fusion activity, Virology 289 (2001) 353–361.