MINI REVIEW

Common Properties of Fusion Peptides from Diverse Systems

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Although membrane fusion occurs ubiquitously and continuously in all eukaroytic cells, little is known about the mechanism that governs lipid bilayer fusion associated with any intracellular fusion reactions. Recent studies of the fusion of enveloped viruses with host cell membranes have helped to define the fusion process. The identification and characterization of key proteins involved in fusion reactions have mainly driven recent advances in our understanding of membrane fusion. The most important denominator among the fusion proteins is the fusion peptide. In this review, work done in the last few years on the molecular mechanism of viral membrane fusion will be highlighted, focusing in particular on the role of the fusion peptide and the modification of the lipid bilayer structure. Much of what is known regarding the molecular mechanism of viral membrane fusion has been gained using liposomes as model systems in which the molecular components of the membrane and the environment are strictly controlled. Many amphilphilic peptides have a high affinity for lipid bilayers, but only a few sequences are able to induce membrane fusion. The presence of α-helical structure in at least part of the fusion peptide is strongly correlated with activity whereas, β-structure tends to be less prevalent, associated with non-native experimental conditions, and more related to vesicle aggregation than fusion. The specific angle of insertion of the peptides into the membrane plane is also found to be an important characteristic for the fusion process. A shallow penetration, extending only to the central aliphatic core region, is likely responsible for the destabilization of the lipids required for coalescence of the apposing membranes and fusion.

INTRODUCTION

The mechanism of membrane fusion involved in biological processes such as endo- or exocytosis, membrane recycling, fertilization and enveloped virus infection is still unclear. Given the vast diversity of membrane fusion events in eukaryotes, the question arises whether these fusion reactions share common features (Hernandez *et al.*, 1996; Hughson, 1995b). The aim of this review is to summarize and integrate the recent significant studies in this area.

Membrane fusion implies that two phospholipid bilayers merge in an aqueous environment. Before fusion, the participating membranes are separate but must be closely apposed. During fusion, an aqueous fusion pore forms (Almers and Tse,

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1990), requiring non-bilayer transition states of the fusing membranes (Chizmadzhev *et al.*, 1999, 2000). Then the pore expands and thereby completes the fusion reaction (Bentz *et al.*, 1990; 1993; 2000a, b). Transition states in which the phospholipids are not arranged in bilayers and in which the monolayers are highly curved (Burger, 1997; Chernomordik and Zimmerberg, 1995; Epand, 1990) are energetically unfavorable since biological membranes are submitted to strong repulsive hydration electrostatic and steric barriers. These barriers can be overcome by membrane proteins, which facilitate local dehydration and are thought to induce local perturbations in the lipid bilayer through their insertion into membranes (Bonnafous and Stegmann, 2000). Among the few well-characterized fusion proteins are viral spike glycoproteins responsible for penetration of enveloped virus into their host cells (Marsh and Helenius, 1989) and more particularly the hemagglutinin of influenza virus. In spite of similar functions, viral fusion proteins are diverse with little sequence similarities among various viruses (Gallaher *et al.*, 1989; Gething *et al.*, 1986; Gonzalez-Scanaro *et al.*, 1987). However, in their sequences, these proteins possess a "fusion peptide", a short segment (up to 20 amino acids) of relatively hydrophobic residues, commonly found in a membrane-anchored polypeptide chain (Bosch *et al.*, 1989). In recent years, candidate fusion proteins containing a fusion peptide have been identified, which are involved in gamete fusion (Blobel *et al.*, 1992), in myoblast fusion (White, 1992), in vesicular fusion in neurons (Mayer, 1999). . . . Considerable experimental evidence implicates the fusion peptide as playing an indispensable role in the fusion process. In general, the exposed fusion peptides are believed to insert into the target cell membrane, and cause a local destabilization of the lipid bilayer necessary to catalyze fusion. To simulate protein-mediated fusion, many studies on peptide-induced membrane fusion have been conducted on model membranes such as liposomes and have employed synthetic peptides corresponding to the putative fusion sequences of viral proteins (Durell *et al.*, 1997, Martin *et al.*, 1999; Pécheur *et al.*, 1999a). The purpose of this review is to cover the wide range of studies conducted on fusion peptides, and examine what this reveals about the molecular mechanism of the fusion process.

FUSION PROTEINS

To date, spike glycoproteins of enveloped viruses are by far the most studied and best-characterized fusion proteins with a particular attention for the HA fusion protein of influenza virus. This is due, at least in part, to the success in determining the crystal structure of nearly the entire ectodomain in a preactivated neutral pH state (Wilson *et al.*, 1981; Bullough *et al.*, 1994). The viral fusion glycoproteins share several common features, which will be briefly discussed. For more comprehensive overviews, the reader is referred to recent reviews specifically devoted to this topic (Blumenthal and Dimitrov, 1997; Baker *et al.*, 1999; Bentz 2000b).

Despite this diversity, all of the known fusion proteins have been found to be class 1 integral membrane proteins: with the *C*-terminal domain inside the viral envelope, and the larger *N*-terminal domain (80–95% of the protein) on the outside. These are also often referred to as "spike proteins" due to their appearance in electron micrographs, where they project like spikes from the viral surface (Booy, 1993).

Most of the studied viral proteins are homo- or hetero-oligomers, which are associated by non-covalent interactions. In many cases, these envelope proteins must first be activated by a specific environmental stimulus before fusion can start. Such processing results in the exposure of the fusion peptide at this N-terminal position. Further activation, probably to position the fusion peptide near the target membrane, is accomplished through conformational changes in the fusion protein, induced either by exposure to low pH (for example: hemagglutinin protein, (Gray and Tamm, 1998) or as a consequence of virus-cell binding (for example: gp160 of HIV (Berger et al., 1999; Damico et al., 1998)). The insights gained from the crystal structure of HA can be applied to other viral fusion proteins including those of the paramyxovirus, coronavirus and retrovirus families. The recently obtained 3-dimensional structures of portions of the HIV gp41 protein (Chan et al., 1997; Weissenhorn et al., 1997), the 44 kDa ectodomain of SIV gp32 (Caffrey et al., 1998) and a fragment of the Moloney murine leukemia virus transmembrane protein (Fass et al., 1996) have confirmed a structural model for retroviral fusion proteins. For influenza hemagglutinin, the key difference between the "native" structure of HA and the low-pH structure is the formation of an extended coiled coil and helix-turn motif occurring with the C-terminal end of the native coiled-coil, near the transmembrane domain (Carr and Kim, 1993; Bullough et al., 1994, Bentz 2000a). In the activated form, the N-terminally located fusion peptides reside on top of an extended, triple stranded coiled-coil. Once activated, the fusion proteins are no longer fusogenic and are unusually stable. Thus, the conformational changes during activation have been suggested to drive the fusion reaction (Bollough et al., 1994; Bron et al., 1993; Doms et al., 1993). Probably, the protein inserts the fusion peptide into the target membrane during activation and subsequently forces a reorientation of the two-phospholipid bilayers. Reorientation may be mediated by an alignment of oligomers of the fusion peptides and transmembrane regions that finally induce the formation of a fusion pore. The presence of a trimer-of-hairpins structure is emerging as a general feature of many viral membrane fusion proteins (Fass et al., 1996; Weissenhorn et al., 1999, 1997; Kobe et al., 1999; Bullough et al., 1994; Chan et al., 1997; Malashkevich et al., 1998; Caffrey et al., 1998).

Conformational studies (Körte et al., 1997, 1999; Shangguan et al., 1998) suggest that the formation of extended coiled coil appeared not to be a precursor of fusion but creates a high-energy hydrophobic membrane defect in the viral envelope allowing fusion to occur. However, as underlined by Bentz (2000a), it remains to be proven that the formation of the extended coiled coil is the "essential" conformational change for the formation of the first fusion pore. As yet, no structural technique has succeeded in showing a rigorous correlation between the kinetics of fusion intermediates formation and the kinetics of extended coiled coil or helix turn formation (Bentz, 2000a).

Cell-cell fusion reactions are critical in development. Fusion of gametes to form the zygote, myoblasts to form myotubes and monocytes to form osteoclasts set off new developmental pathways (White, 1992; Hernandez et al., 1996). Membrane fusion is also a key event in intracellular membrane traffic (Jahn and Südhof, 1999; Jahn and Hanson, 1998; Kondo et al., 1997). Compared with our knowledge of

virus cell-fusion, information about cell-cell fusion is at a rudimentary level. However, current efforts are focused on identifying cell-cell fusion proteins. Recently, a candidate fusion protein called fertilin, involved in mammalian sperm-egg fusion has been identified. (Snell and White, 1996; Evans *et al.*, 1995; Primakoff *et al.*, 1987; Almeida *et al.*, 1995). Fertilin α/β are the prototypes of the newly described ADAM (proteins containing A Disintegrin and Metalloprotease) gene family (Wolfsberg *et al.*, 1995; 1993). This protein shares several biochemical characteristics with viral fusion glycoproteins: it is synthesized as a precursor which is proteolytically processed into α and β subunits, form high-order oligomers and the α-subunit contains a relatively hydrophobic internal sequence that fulfills the criteria of a candidate fusion peptide (Myles *et al.*, 1994; Blobel *et al.*, 1990; 1992).

Work in recent years has also identified a conserved framework of proteins that appear to be involved in all intracellular fusion reactions (Jahn and Südhof, 1999; Mayer, 1999). The "fusion machinery" is composed of three elements: the ATPase NSF (for NEM sensitive fusion protein), SNAP (for soluble NSF attachment proteins) and SNARES (for SNAP receptors). Actually, there is no obvious reminiscence between this fusion complex and that of viral or other cellular fusion proteins but some of the viral fusion principles also apply to fusion proteins operating in intracellular fusion in eukaryotic cells. In particular, the presence of a fusion peptide in synaptobrevin (SNARE of SV) has been suggested but not proven (Jahn and Südhof, 1994) and the presence of a coiled coil structure similar to that of the virus has been shown (Weber *et al.*, 1998; McNew *et al.*, 1999).

FUSION PEPTIDES

Viral Fusion Peptides

Sequence and mutational analysis of the cloned genes have been used to identify and characterize the fusion peptides of a number of different viral fusion proteins (Gething *et al.*, 1986; Bosch *et al.*, 1989; Freed *et al.*, 1990; White, 1990; Horth *et al.*, 1991; Horvath and Lamb, 1992; Steffy *et al.*, 1992). On a basic level, the fusion peptides can be distinguished by whether they occur at the *N*-terminal of the cleavage site or at an "internal" location in the sequence. Examples of fusion proteins with *N*-terminal fusion peptides include influenza HA, Sendai F (paramyxovirus family), and HIV *env* (retrovirus family); whereas, viruses with fusion proteins which are postulated to have internal fusion peptides include Semliki Forest Virus and Sindbis Virus (both togavirus family), Rous Sarcoma Virus (retrovirus family), Vesicular Stomatitis Virus (rhabodovirus family), and Uukenemi virus (bunyavirus family) (White, 1990, 1992). The existence of internal fusion peptides in the fusion glycoproteins of Rabies Virus and Vesicular Stomatitis Virus has been confirmed by site-directed mutagenesis and photolabeling studies (Zhang and Ghosh, 1994; Whitt *et al.*, 1990; Fredericksen and Whitt, 1995; Durrer *et al.*, 1995, 1996).

It is believed that the fusion peptide is the domain of the viral transmembrane protein that interacts with the target and/or virus membrane bilayer to trigger fusion. Such a membrane interaction has been directly demonstrated in the case of the Influenza virus fusion peptide (Harter *et al.*, 1989; Stegmann *et al.*, 1991;

Brunner and Tsurudome, 1993). Photolabeling experiments using the bromelain-solubilized ectodomain of HA (BHA) show that the *N*-terminal 21 residues of BHA2 are all photolabeled. The distribution of photolabeled amino acids among these 21 residues suggest an amphipathic α-helical structure (Harter *et al.*, 1988; Brunner *et al.*, 1991).

N-terminal fusion peptides range in length from ca. 20 to 36 amino acids, with the *N*–terminal amino acid often identified as the first positively charged residue. While the *N*-terminal fusion peptides of paramyxoviruses and most retroviruses are exclusively apolar, the fusion peptides of inluenza HA's contain 2 to 3 negatively-charged amino acid residues. In contrast, the internal fusion peptides are defined as stretches of 16–20 apolar amino acids bounded by charged amino acids residues: positively-charged on the *N*-terminal end and either positively or negatively-charged on the *C*-terminal end (Ohnishi, 1988). These peptides tend to be rich in alanine and glycine residues. In many cases, they can be modeled as sided helices with the bulky apolar amino acids on one face of the helix (White, 1990, 1992). This representation of the fusion peptide as a α-helix predicts that most glycine residues would be found on one face of the helix. The role of the glycine strip in fusion is still hypothetical (Gray *et al.*, 1996). It could mediate interaction between several fusion peptides or interaction with host cell factors (such as binding to a putative second receptor (Henderson and Qureshi, 1993). Oligomerization of fusion peptides may facilitate in the formation of a ''fusion pore'' between the viral and target membranes that would expand and allow entry of the internal contents of the virus into the cell. Another explanation would be that the glycine residues provide the proper balance of amphipathicity necessary for merging viral and cell membrane. Finally, conservation of the glycine residues may be important for oblique insertion of the fusogenic peptide into the target membrane as suggested by Horth *et al.* (1991). More recently, Pritsker *et al.* (1999) suggest that the FLGFL motif, a prominent feature of the viral fusion peptide, contributes to the functional organization of the HIV-1 fusion peptide and that the *C*-terminal domain following the fusion peptide contributes to the membrane fusion process. Based on the NMR signal attenuation data, Chang *et al.* (2000) suggest a role of highly conserved Glu11 of HA fusion peptides, in promoting oligomerization of the fusion peptide.

To better understand the mechanistic, structural roles that the fusion peptide plays in the fusion process, many studies have been conducted with synthetic peptide analogs. These studies using liposomes and synthetic peptides corresponding to the fusion regions of enveloped virus proteins have revealed that the secondary structure and the orientation of the peptides when inserted into a lipid bilayer determine their fusogenic and lytic activities (Pécheur *et al.*, 1999; Martin *et al.*, 1999; Durrell *et al.*, 1998).

Synthetic peptides corresponding to the *N*-terminal domain of the transmembrane glycoprotein of influenza (Wharton *et al.*, 1988; Rafalski *et al.*, 1991), HIV (Rafalski *et al.*, 1990; Martin *et al.*, 1996; Nieva *et al.*, 1994; Kliger *et al.*, 1997; Chang *et al.*, 1999; Cladera *et al.*, 1999), VSV (Duzgunes and Shavnin, 1992), Sendai virus (Rapaport and Shai, 1994) Feline Leukemia Virus (Davies *et al.*, 1998a, b) and other viral fusion proteins are able to promote fusion of lipid bilayers, giving further experimental support for the role of *N*-terminal fusogenic sequences in viral fusion.

The biologically activate state of fusogenic peptides appears to involve oligomeric forms, according to studies with model membrane (Cladera *et al.*, 1999; Prisker *et al.*, 1999) as well as biological systems (Chang *et al.*, 2000, 1999). The structural changes that occur when viral fusion peptides interact with target membranes, or when the pH is changed, has been characterized mainly by circular dichroism (CD) and Fourier transform infrared (FTIR) spectroscopy. CD studies of synthesized HA fusion peptides indicated that sequences of at least the first 20 *N*-terminal residues adopt a helical conformation when in contact with liposome membranes (Lear and DeGrado, 1987; Wharton *et al.*, 1988; Takahashi, 1990; Burger *et al.*, 1991; Rafalski *et al.*, 1991; Murata *et al.*, 1992, 1987). The extent of the helical structure was estimated to range from 45 to 100%, depending on environmental conditions. Studies evaluating the requirements for liposome fusion identified a loose correlation between helical content of the HA peptides and fusogenic ability, suggesting that other factors such as membrane penetration and tertiary structure likely play a role (Lear and DeGrado, 1987; Wharton *et al.*, 1988; Burger *et al.*, 1991). A lack of change in α-helical structure between HIV and SIV wild-type peptides and non-fusogenic mutants was also observed in separate studies using neutrally charged liposomes (Martin *et al.*, 1994, 1996; Kliger *et al.*, 1997), or for wild-type and non-fusogenic forms of the Sendai (Rapaport and Shai, 1994) fusion peptides, where the secondary structure was determined in the membrane-mimetic environments of 40% trifluoroethanol and 1% sodium dodecylsulfate aqueous solutions. Differing proportions of α-helic and β-sheet conformations are observed depending on environmental conditions (incl. lipid composition, ionic strength and pH) (Rafalski *et al.*, 1990; Gordon *et al.*, 1992; Nieva *et al.*, 1994; Pereira *et al.*, 1995, 1997; Martin *et al.*, 1991, 1993, 1994, 1996). For example, Rafalski *et al.* (1990) found that with negatively charged liposomes, the HIV fusion peptide binds in a α-helical conformation and induces fusion; whereas, with neutrally charged liposomes, the bound peptide adopts a β-structure and doesn't induce fusion. In contrast, also using negatively charged liposomes, Nieva *et al.* (1994) concluded that membrane-bound α-helical conformations are associated with leakage, while β-sheet conformations are associated with fusion.

More recently, FTIR spectroscopy methods have been developed to examine the orientation of membrane-bound peptides and proteins with respect to the bilayer plane (Goormaghtigh *et al.*, 1999; Tamm and Tatulian, 1997). For the HA-fusion peptide bound to the bilayer, Ishiguro *et al.* (1993, 1996) and Lüneberg *et al.* (1995) found major proportions of the peptide adopting an α-helical conformation, with the exact percentage depending on the environmental conditions. Additionally, the orientation of the helical portion was found to range from parallel to oblique; also depending on the pH, peptide concentration and other environmental factors. Similar oblique insertion angles were found for the HIV/SIV fusion peptides when they are inserted into the membrane (Martin *et al.*, 1993, 1994 and 1996). The oblique membrane insertion of SIV fusion peptides has also recently been demonstrated using neutro diffraction (Bradshaw *et al.*, 2000). Mutation studies to deliberately alter the oblique angle of insertion were found to disrupt fusion efficiency in both intact virus (Horth *et al.*, 1991; Vonèche *et al.*, 1992) and synthetic fusion peptide systems utilizing HIV/SIV (Martin *et al.*, 1994, 1996; Colotto *et al.*, 1996; Kliger

et al., 1997) and Bovine Leukaemia Virus (Vonèche *et al.*, 1992). Similar insertion modes have not been demonstrated experimentally for other types of small membrane-associating peptides, and may be specific to the fusogenic peptides. This orientation was originally predicted by calculation of the directions of the hydrophobic moments, assuming that the sequence adopt a helical conformation (Brasseur, 1990, 2000). Support for the importance of the orientation comes from studies in which the *N*-terminus of the fusion peptides is labeled with the NBD fluorophore, the emission properties of which are sensitive to the polarity of the environment. Fluorescently labeled peptide analogues can be used to determine the peptide-membrane partition coefficient and the position of the *N*-terminus or *C* terminus in the membrane. Using this technique for the fusion protein of Sendal virus, Rapaport and Shai (1994) show that a 33 residue peptide corresponding to the NH_2 terminus of the wild-type fusion protein is located within the lipid bilayer and that the peptide tends to self-associate in the membrane-bound state, while an ''non fusogenic'' mutant peptide lies on the surface of the bilayer but has the same tendency to self-associate. The observed differences in the peptide fusogenic activities are hypothesized to result from the difference in the degree of penetration of the peptide into the membrane (Rapaport and Shai, 1994). For the Sendai fusion peptide, the *N*-terminal of the more fusogenic mutant (G12A) was found to be closer to the surface of the membrane than the wild-type: thus indicating a more oblique (closer to parallel) angle of insertion (Rapaport and Shai, 1994). Similar results were found for the HIV fusion peptide, in which the *N*-terminal of the inactive mutant (V2E) was inserted deeper than the wild-type (Kliger *et al.*, 1997). In addition, both the membrane-bound Sendai and HIV fusion peptides were found to be efficiently cleaved by proteinase K, thus indicating only a shallow penetration into the bilayer. This is in contrast to transmembrane-bopund peptides, which are typically protected from enzymatic cleavage (Gazit and Shai, 1993). During the last few years, effort has been made to determine the exact position and the insertion depth of the viral fusion peptide in the lipid bilayer. Using EPR technique, Macosko *et al.* (1997) demonstrated that the *N*-terminal fusion peptide of the HA2 fragment inserted into the membrane bilayer with a maximum depth of 15 Å form the phosphate group, suggesting that the fusion peptide did not transverse both leaflets of the bilayer. Chang *et al.* (2000) go further in the determination of the fusion peptide position, using fluorescence quenching and NMR experiments. The Trp14 residue of the HA fusion peptide is inside the vesicular interior and residue 16–18 are at the micellar boundary. To explain these spectroscopic measurements, they made the hypothesis that there is an equilibrium between helix and non helix forms for the segment in the membrane interior. Indeed, with residues 16–18 located at the boundary, it is impossible for the penetrating segment to span both monolayers of the membrane with purely helical form (Chang *et al.*, 2000).

The structural effects of viral fusion peptides on lipid polymorphism were largely investigated with several biophysical techniques. A major achievement was to show that the oblique angle of the inserted helix causes a significant disruption of the alignment of the phospholipid alkyl chains, which does not occur for the parallel or perpendicular orientations (Epand and Epand, 1994; Epand *et al.*, 1994). It is postulated that this gives rise to the lipid structures which are associated with the

initial events of membrane fusion. Since the oblique orientation prevents the helix from fully transversing the bilayer, it has also been suggested that this causes an expansion of the membrane core; which in turn increases negative curvature strain, destabilizes the bilayer, favors the formation of inverted phases and facilitates membrane fusion (Colotto *et al.*, 1996). The SIV fusion peptide was shown to lower the hexagonal transition temperature of palmitdeoylphosphatidylethanolamine (Epand *et al.*, 1994), suggesting that insertion of the fusion peptide into the target bilayer disrupts bilayer packing. Moreover, ^{31}P n.m.r. studies demonstrated the presence of H_{II} phase structure for MeDOPE vesicles at 37°C in the presence of the SIV fusion peptide (Epand *et al.*, 1994). Cryo-transmission electron microscopy (cryoTEM) of diluted vesicles to which HIV fusion peptides has been externally added reveals a morphology that is compatible with the formation of nonlamellar lipidic aggregates during lipid mixing (Pereira *et al.*, 1999). The HIV, SIV and Influenza fusion peptides are able to modulate the lipid polymorphism in pelletted membrane by promoting the thermotropic formation of inverted phases and driving the lamellar-to-nonlamellar transition towards the formation of isotropic phases (Pereira *et al.*, 1999; Colotto *et al.*, 1996; Colotto and Epand, 1997). The fusion peptide of feline leukamia virus (FeLV), the hydrophobic amino-terminus of the fusion protein (p15E), has also been proven to be fusogenic and to promote the formation of highly curved, intermediate structures on the lamellar liquid-crystal to inverse hexagonal phase transition pathway. The peptide is also shown to reduce the lamellar repeat distance of the membrane prior to the onset of an inverted cubic phase suggesting that membrane thinning may play a role in peptide-induced membrane fusion and strengthens the link between the fusion pathway and inverted cubic phase formation (Darkes *et al.*, 1999).

Amphiphiles like lysophosphatidylcholine (LPC) or lipophosglycan (LPG), which raise the bilayer to hexagonal phase transition temperature of model membranes, inhibit the rate of viral fusion (Chernomordik, 1998; Miao *et al.*, 1995; Easterbrook *et al.*, 1995). LPC, a naturally occurring phospholipid, inhibits fusion between cell membranes, organelles and between organelle and plasma membrane (Günther-Ausborn *et al.*, 1995; Pécheur *et al.*, 2000). Fusion was arrested at a stage preceding pore formation, suggesting that biological fusion involves the formation of a highly bent intermediate between membranes: the fusion stalk (Chernomordik *et al.*, 1993). LPG was shown to inhibit Sendai virus-induced hemolysis as well as the fusion of Sendal virus and influenza virus with erythrocyte membranes and liposomes (Miao *et al.*, 1995; Easterbrook *et al.*, 1995; Razinkov *et al.*, 1999a, b). Regarding the fusion peptides and model membrane vesicles, it has been demonstrated a direct correlation between membrane lipid composition and fusogenic activity of the peptides. Exogenous lysoPC in a dose dependent and temperature dependent way inhibits the fusogenic activity (lipid mixing) of the SIV/HIV fusion peptides (Martin *et al.*, 1997). LPG, inserted in lipid bilayer, inhibits lipid mixing and the structural rearrangement of the viral fusion peptides. FTIR experiments reveal that LPG facilitates the formation of β-sheet structure instead of α-helical structure. This β-sheet is associated to the membrane but probably not inserted. This topology is less disturbing for the lipid bilayer than the oblique α-helix, and has less effect of membrane spontaneous curvature (Martin *et al.*, 1999; Razinkov

et al., 1999a). The ability of these amphiphiles to inhibit the fusion occurring between cell membrane organelles and between organelles and plasma membrane induced by several distinct fusogenic agents like Ca^{2+}, GTP or viral fusion peptide (Chernomordik *et al.*, 1993) suggests that these molecules act by altering a common motif of membrane fusion, and more particularly the ability of membranes to form highly curved "stalk" intermediates.

Fusion Peptide of Sperm-Egg Fusion Protein

Fertilin is a guinea pig sperm surface membrane protein that seems to play a role in sperm-egg membrane fusion. The two subunits α and β of fertilin share membrane topologies and other characteristics with viral binding and fusion proteins (Primakoff *et al.*, 1987; Blobel *et al.*, 1990). The β subunit contains a potential receptor binding domain and the α subunit contains a putative fusion peptide (Blobel *et al.*, 1992; White, 1992; Wolfsberg *et al.*, 1993), suggesting that fusion events which occur during fertilization may share a common mechanism with penetration of enveloped viruses into host cells. The sequence suggested to be involved in fusion comprises residues 89–111 of the α-subunit of fertilin (Blobel *et al.*, 1992). This sequence fulfills all three criteria of an internal fusion peptide: (a) located in a membrane anchored subunit, (b) relatively hydrophobic and (c) able to be modeled as a "sided" α helix with most of the bulky hydrophobic residues on one face and charged amino acids on the other face (White, 1992). This peptide has been shown to cause membrane lysis and fusion (Martin and Ruysschaert, 1997; Niidome *et al.*, 1997; Muga *et al.*, 1994). Study with FPE, as spectroscopic membrane probe, has revealed that, following a rapid binding reaction, the peptide becomes inserted into the body of the membrane and represent the first direct demonstration that such a process may take place during fertilization (Wolfe *et al.*, 1999). The fertilin-peptide membrane interaction appears to be sensitive to the presence of negative surface charge in the form of a small percentage of PS. FPE studies have also revealed that the initial binding reaction of the peptide exhibits a cooperative nature. This implies that more than one molecule of peptide is involved in achieving a fruitful binding reaction, suggesting also that once bound to the surface, the fertilin peptides penetrate the body of the membrane in a trimeric or dimeric complex (Wolfe *et al.*, 1999). The ATR-FTIR study supports a model where the peptide, upon binding to the lipid phase, adopts an almost helical conformation with unordered structures only at the ends of the helix. The contribution of the β-structure was weak suggesting that the β-sheet is not the main structure of membrane bound fertilin peptide. There appears to be a strong correlation between the insertion of fertilin fusion peptide into the membrane, its fusogenic properties and its α-helical structure as shown for the viral fusion peptide (Martin *et al.*, 1998).

The existence of another fusion peptide B18, derived from the membrane associated sea urchin sperm bindin, has been demonstrated. This acrosomal protein plays a key role in fertilization by mediating the adhesion and fusion between sperm and egg (Hoffman and Glabe, 1994; Vacquier *et al.*, 1995; Glabe, 1985). The peptide sequence B18 exhibits many similarities to viral fusion peptides and may have a corresponding function in fertilization. Indeed, the B18 peptide can imitate several

functions of the much larger parent protein (Ulrich *et al.*, 1998), which include the binding, aggregation and fusion of neutral lipid vesicles (Ulrich *et al.*, 1999; Glaser *et al.*, 1999). Membrane fusion involves an α-helical peptide conformation, which oligomerize further in the membrane.

Model Fusion Peptide

To closely simulate membrane fusion induced by a membrane-bound protein and to obtain a truly fusion-mimicking system, recent studies have investigated the fusogenic activity of an 11-mer synthetic peptide, called WAE 11, in a liposomal system (Pécheur *et al.*, 1997, 1998, 1999a and 2000). It was found that, in close agreement with the behavior of viral fusion-inducing peptides in their membrane-bound environment, anchorage of this short peptide via a disulfide bond to a liposo-mal surface was an essential requirement to exert its fusogenic properties (Pécheur *et al.*, 1997). Moreover, the WAE peptide shows an enhanced ability to engage in hydrophobic interactions when lipid headgroup spacing increases, suggesting that this latter parameter most likely regulates the degree of penetration of the peptide into the target membrane (Pécheur *et al.*, 1999b). The combination of infrared spectroscopy and tryptophan fluorescence structural data with fluorescence assays for the functional analysis of peptide-induced fusion demonstrate that anchorage is a prerequisite to bring about efficient fusion, and that the α helical structure was a necessary but not sufficient condition for fusion to take place (Martin *et al.*, 1999). Indeed, relative hydrophobicity, certain requirements for the amino acid composition, and possibly the ratio α helix/βstructure appears of importance as well, as revealed by comparing structural and functional properties of several ''mutants'' peptides (Pécheur *et al.*, 1998). Pécheur *et al.* (2000) also demonstrate that fusion can be regulated by a switch of the secondary structure of the peptide from a non-fusogenic β-sheet to a fusion-permissive α-helix, and *vice-versa*. The positioning of the switch is governed by the presence of distinct lipids in the target membrane. Insertion of LPC, inhibits WAE-induced fusion in a dose-dependent manner. Secondary structure determination by FTIR, indicate that at conditions where WAE-induced fusion is abolished, the secondary structure of the (coupled) fusion peptide shifts from a fusion-permissive α-helix to a fusion-inhibiting β-sheet. Importantly, removal of the interfering lipid reverses the process. As holds for the model system, the fusion activity of influenza virus with erythrocytes can be modulated similarly by the action of distinct target membrane lipids, suggesting that distinct lipids in target membranes may act as a (reversible) secondary structure switch for fusion peptides, thus providing an elegant means for regulating membrane fusion activity. Still, the underlying mechanism that controls the switch remains to be elucidated (Pécheur *et al.*, 2000).

RELEVANCE OF MODEL SYSTEMS TO STUDY MEMBRANE FUSION

Working with small peptides has the obvious advantages of being able to obtain a large quantity of a pure sample, and not having measurements overshadowed by larger portions of the protein. A major disadvantage is that one does not always

know whether the behavior of the isolated peptide is truly reflective of the intact system, or simply artifactual. The conformation adopted by the isolated peptide segment may not be the same as the conformation of that segment when present in the intact protein. Moreover, it should be noted that the fusion peptide is not always sufficient for fusion. Indeed, the soluble part of HA (BHA, which contains the fusion peptide of hemagglutinin, does not promote liposome–liposome fusion even at low pH. Fusion fails to occur despite the fact that the fusion peptide of BHA clearly enters the target membrane. The protein containing the exposed fusion peptide should be specifically anchored to the viral membrane perhaps requiring trans-membrane domains. However, this is not enough for fusion to occur: with mutant hemagglutinin which the transmembrane domain is replaced by a glycosylphos-phatidylinositol (GPI) anchor, membrane fusion is arrested in a stable hemifusion state (Bailey *et al.*, 1997; Kemble *et al.*, 1994; Melikyan *et al.*, 1995). However, model fusogenic peptides can still provide information about some of the early events that trigger viral fusion (Epand *et al.*, 1992). The molecular nature of these steps may be more difficult to elucidate using the more complex system of an intact virus. Fortunately, several studies have revealed a direct correlation between the properties of the isolated and intact systems (especially regarding the effect of mutations on fusion), and thus support the judicious use of the peptides in those cases as models for investigating the full protein-mediated fusion process (Martin *et al.*, 1996, 1994; Horth *et al.*, 1991; Schaal *et al.*, 1995).

The exact manner in which the fusion peptide associates with target membranes poses some pressing questions. Many different techniques have being applied to the general problem of determining the structure-function relationship of membrane interactive peptides. The different results suggest that: (1) the position and orien-tation of the viral fusion peptide within the fusion site are at least as important as its hydrophobicity and secondary structure, (2) the existence of a direct correlation between the ability of the fusion peptide to induce lipid mixing and its capacity to modulate lipid polymorphism in stacked bilayers. However, several questions remain unanswered. Does the fusion peptide assume different structures at different stages of interaction with the target membrane? Is the interaction of the fusion peptide with the target membrane complete before, during or at the end of the lag phase that precedes fusion reaction? What is the aggregation state of the peptide at the fusion site? Actually, there is a large debate about the position of the viral fusion peptide. On the one hand, there has been along literature proposing that the exposed *N*-terminal of HA inserts the target membrane to start fusion (reviewed in Durrell *et al.*, 1997; Martin *et al.*, 1999 and Pécheur *et al.*, 2000) and on the other hand there is a new literature suggesting that HAs can have their fusion peptides either suspended between the membranes or embedded in the target bilayer or in their own bilayer (Bentz, 200a; Shangguan *et al.*, 1998; Kozlov and Chernomordik, 1998). Moreover, Kozlov and Chernomordik (1998) and Bentz (2000a) argue that those HAs whose fusion peptide embed into the viral/HA expressing cell envelope are on the fusion pathway. Further studies will be needed before any conclusions can be reached. Several different experimental approaches will be required to elucidate the architecture of the fusion site. However, these experiments will not be simple to perform or to analyze. The current period may prove to be particularly auspicious,

with the recent release of crystallographically determined structures of fragments of the fusion protein of moloney murine leukemia virus (Fass *et al.*, 1996) and gp41 from HIV-1 (Binley and Moore, 1997; Chan *et al.*, 1997; Weissenhorn *et al.*, 1997).

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