Influenza fusion peptides

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Abstract

The 'fusion peptides' of a group of enveloped viruses that includes influenza, paramyxovirus-, retro- and filo-viruses are N-terminal regions of their membrane fusion proteins generated by cleavage of non-functional precursors. For the influenza membrane fusion protein, haemagglutinin (HA), the three-dimensional structures of precursor HA, cleaved HA and fusion-activated HA show that the fusion peptides are located in different positions in all three forms and adopt different structures. Analyses of mutant HAs with changes in fusion peptide sequence indicate the importance of specific residues for membrane-fusion activity and suggest a structure for the fusion peptide in a fusion-active molecule.

Enveloped viruses infect cells by binding to cell-surface receptors and fusing their membranes with cellular membranes. For influenza, the haemagglutinin (HA) surface glycoprotein is both a receptor-binding and membrane fusion protein [1]. It is a trimer of identical subunits, each containing two disulphide-linked polypeptides, HA1 and HA2, that are derived by proteolytic cleavage of a precursor, HA0, that has a signal sequence at its N-terminus and a membrane anchor sequence at its C-terminus. Cleavage to form HA1 and HA2 generates the N-terminus of the smaller polypeptide, HA2, which has the membrane anchor sequence at its C-terminus. Cleavage is required for membrane fusion activity. The HA2 N-terminal sequence is called the 'fusion peptide' because cleavage at similar hydrophobic sequences is also required for the activity of other virus fusion proteins, and because 20-residue synthetic peptide analogues of the sequence fuse membranes in vitro.

Comparison of the three-dimensional structures of cleaved HA and uncleaved HA0 indicates their close similarity except for 19 residues at the site of cleavage (Figure 1). The site is an almost circular surface loop with the membrane-proximal

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Abbreviations used: HA, haemagglutinin.

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section formed by 12 residues that following cleavage becomes the fusion peptide and re-folds into an adjacent pocket of charged residues. Receptor-bound influenza viruses are taken into endosomes and the fusion potential of cleaved HA is activated at endosomal pH, between pH 5 and 6 depending on the particular influenza virus. Activation involves extensive changes in HA structure that result in extrusion of the fusion peptide to the N-terminus of a 100 Å-long coiled-coil located centrally in a newly formed rod-shaped structure (Figure 1). In this position the HA N-terminal fusion peptide is at the same end of the molecule as the HA C-terminal membrane anchor. The structure of the fusion peptide in this molecule is not known because it was removed in the preparation of a soluble form for crystallization. In the three structures shown in Figure 1, therefore, the fusion peptide occupies three different locations and assumes at least two distinct structures. In combination, these structural requirements place particular constraints on its sequence, which is highly conserved.

It is generally assumed that during infection, as a consequence of the structural rearrangements in HA at fusion pH, the fusion peptide is inserted into the target endosomal membrane. Experiments with photactivatable hydrophobic reagents indicate that in vitro the fusion peptide uniquely associates with liposomes [2], and models based on the distribution of hydrophobic and relatively hydrophilic residues in the peptide suggest that it inserts into liposomes at an angle of about 35° to the plane of the membrane [3].

At least two consequences of these interactions for the mechanism of fusion are commonly suggested; the target membrane containing the fusion peptide and the viral membrane containing the HA membrane anchor are bridged and drawn together [4] and the bilayer structure of the membranes is destabilized to rearrange into the highly curved stalk structures proposed as intermediates in fusion pore formation [5]. Co-location of the fusion peptide and the membrane anchor at one end of the fusion-pH HA structure is consistent with the first suggestion, particularly if the rod-shaped molecule lies parallel between the two membranes. Differential scanning calorimetry has shown that synthetic fusion peptide analogues from a variety of viruses, including influenza,

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**Figure 1**

The three conformations adopted by influenza HA during virus replication

HA0 [10], the biosynthetic precursor of the HA [11] of infectious viruses, is cleaved at residue 329 in a prominent membrane-proximal surface loop, to generate the fusion peptide at the N-terminus of HA (grey). In HA the fusion peptide has re-folded into the trimer interphase. At fusion pH the membrane distal (black) receptor-binding domains retain their structures but de-trimerize, and the central structure formed by HA re-folds into a 100 Å-long trimeric coiled-coil and rearranges the positions of the fusion peptide and the C-terminal membrane-anchor region to the same end of the coil. The fusion-pH HA diagram is a composite of the HA monomer (black) structure [12] and the structure of HA residues 23−185, which lacks both the fusion peptide and the membrane anchor, for solubility, expressed in Escherichia coli [13].

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lower the bilayer to hexagonal phase-transition temperature of target liposomes, and this is consistent with proposals of their induction of bilayer destabilization during fusion.

The use of synthetic fusion peptide analogues has been valuable in this way in indicating possible roles for this region of HA in fusion and also in direct analyses of fusion peptide structure. CD and ATR (attenuated total reflection)-Fourier-transform infrared spectroscopy, for example, indicate about 45% α-helical content for fusion peptides in liposomes [6]. In estimates of fusion activity also, correspondence between the activity of synthetic peptides and intact HAs has been observed and this is particularly remarkable for a number of site-specific mutations which affect fusion by peptides and HAs similarly [7]. On the other hand, the low solubility of the peptides and the lack of correlation in certain instances between particular properties, such as angle of insertion into liposomes and fusion activity, are less useful; and the dependence on low pH of their membrane association and fusion activities is contrary to observations that the pH of fusion by HA correlates with the pH of its conformational change.

Attention has, therefore, also focused on defining important features of the fusion peptide for the fusion properties of intact HA, by site-specific mutagenesis [8,9]. Our studies of vaccinia recombinant expressed fusion peptide mutant HAs have confirmed the preference for glycine at the N-terminus and at residue 8 and shown the importance of fusion peptide length for fusion. Uncharged residues other than glycine are tolerated at positions 2, 6 and 10, and no functional significance was shown for the charged residues at positions 11 and 15. Within the 10 N-terminal residues, substitutions in selected or site-specific mutants invariably caused increases of about 0.3 in the pH of fusion or, if the mutant HA was negative for fusion, in the pH of its conformational change. Recent experiments in which mutant HAs are incorporated into infectious viruses to increase the sensitivity of functional HA detection have indicated no absolute requirement for glycine at the N-terminus, with leucine, phenylalanine and serine also being accepted. As in the in vitro fusion assays, however, glycines at residues 2, 6 or 10 were not accepted in infectious virus and, importantly, alanine substitutions at these positions, while yielding virus, were selected against on passage with larger side-chain hydrophobic residues being preferred. On the basis of these observations the fusion peptide in fusion-active HA can be modelled as a helical structure in which residues 2, 6 and 10 form one face of the helix with glycines at positions 1, 4 and 8 on the opposite face. If the fusion peptides are trimeric, the relatively polar glycine residues may form the trimer interphase and the large hydrophobic side chains would then be on the surface of a coiled-coil (Figure 2).

Figure 2

The fusion glycoproteins of influenza viruses, paramyxoviruses, retroviruses and filoviruses are all synthesized as precursors that require proteolytic cleavage to prime their membrane-fusion potential. The sequences at the N-termini generated by cleavage are the fusion peptides (for influenza [14], paramyxoviruses Sendai [15] and Rous Sarcoma virus [16], and retrovirus HIV [17]), except in the case of filovirus fusion glycoproteins in which, for Ebola virus [18], the N-terminus of the peptide shown is 23 residues from the site of cleavage. Major points of similarity include the lack of charged residues, the number of large-side-chain hydrophobic residues and the number and distribution of glycine residues.

**Fusion Peptide Sequences**

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References


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