Structure of the uncleaved ectodomain of the paramyxovirus (hPIV3) fusion protein

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Class I viral fusion proteins share common mechanistic and structural features but little sequence similarity. Structural insights into the protein conformational changes associated with membrane fusion are based largely on studies of the influenza virus hemagglutinin in pre- and postfusion conformations. Here, we present the crystal structure of the secreted, uncleaved ectodomain of the paramyxovirus, human parainfluenza virus 3 fusion (F) protein, a member of the class I viral fusion protein group. The secreted human parainfluenza virus 3 F forms a trimer with distinct head, neck, and stalk regions. Unexpectedly, the structure reveals a six-helix bundle associated with the postfusion form of F, suggesting that the anchor-minus ectodomain adopts a conformation largely similar to the postfusion state. The transmembrane anchor domains of F may therefore profoundly influence the folding energetics that establish and maintain a metastable, prefusion state.

The entry of paramyxoviruses into cells requires the merger of viral and cellular membranes and formation of an entry pore, which is catalyzed by the viral fusion (F) protein (1–3). The paramyxovirus F proteins belong to the class I viral fusion protein type, of which the best characterized member is the influenza virus hemagglutinin (HA), but which also includes the fusion proteins from retroviruses, coronaviruses, Ebola virus, and others (2, 4–6). Studies of the structure and mechanism of HA-mediated membrane fusion have led to a general mechanism for class I fusion proteins, based on similarities in the oligomerization, processing, and structures of fragments of a number of family members. In general, class I viral fusion proteins follow the following paradigm. Three identical polypeptide chains assemble into trimers, which are subsequently proteolytically cleaved into two fragments at a specific site N-terminal to an internal hydrophobic domain of ∼25 aa, commonly referred to as the fusion peptide (Fig. 1A). Sequences adjacent to the fusion peptide and the transmembrane (TM) anchor domain typically reveal a 4-3 (heptad) pattern of hydrophobic repeats that correspond to helical regions of the protein critical for fusogenic activity. The structures of the heptad-repeat regions of many class I viral fusion proteins have been solved by x-ray crystallographic or NMR methods and reveal common fold characteristics (1, 4, 5). Although the structural details vary, all reveal a trimeric, coiled coil beginning near the C-terminal end of the hydrophobic fusion peptide. The C-terminal segment abutting the TM domain is also often helical and packs in an antiparallel direction along the outside of the N-terminal coiled coil, placing the fusion peptides and TM anchors at the same end of a rod-like structure. These helical hairpin or six-helix bundles (6HB) typically represent a relatively small fraction of the intact fusion protein, yet their structures are generally highly thermostable, with melting temperatures near 100°C. Intermediates along the pathway of membrane fusion can be trapped by the addition of peptides derived from either the N-terminal or C-terminal heptad repeat (HR) region for many class I fusion proteins (4, 7, 8), indicating that the intact protein undergoes conformational changes that expose both HR regions, before refolding to the final 6HB. The intermediates are thought to represent partially refolded forms of the fusion protein, with hydrophobic fusion peptides anchored in the target cell membrane and the TM domains anchored to the viral membrane. The formation of the 6HB is tightly linked to the merger of lipid bilayers, probably coupling the free energy released on protein refolding to membrane fusion (9, 10).

The general mechanism for class I viral fusion proteins posits the folding of the uncleaved protein to a metastable state, which can be activated to undergo large conformational changes leading to a more stable fusogenic or postfusion state. For the influenza HA protein, crystal structures of three forms of the protein have been determined that support this model and provide the structural basis for the general class I mechanism (11–14). Influenza HA structures have been determined for the uncleaved (HA0) form (12), the cleaved/activated form (HA1/HA2) (11), and the postfusion form activated by low pH or revealed by the recombinant expression of the C-terminal HA2 fragment (13, 14). Minor conformational changes are observed between HA0 and HA1/HA2 structures, which reveal the fusion peptide, initially at the protein surface in HA0, tucked into the interior of the HA2 coiled coil after proteolysis. The low-pH-induced, postfusion form shows a dramatic refolding of the HA2 fragment, bringing the fusion peptide and TM anchors into close proximity. For HA, it is thought that proteolytic cleavage primes the protein for membrane fusion (5), potentially influencing the stability of the prefusion state. Thus, the attainment of the prefusion conformation, its regulation, and its relative free energy as compared with the postfusion form are all key to the process by which class I viral fusion proteins function.

To understand better how protein folding and structure have evolved to catalyze membrane fusion in viral entry, we have undertaken structural studies of the paramyxovirus F proteins. Previous studies have shown that the HR regions of two paramyxovirus F proteins, from parainfluenza virus 5 (SV5) and human respiratory syncytial virus (hRSV), form 6HBs (15, 16). A partially proteolyzed structure of a soluble form of the Newcastle disease virus (NDV) F protein has also been determined (17), revealing the fold of much of the extracellular region of F but raising many questions regarding the conformational state of the protein and its relationship to postulated prefusion and postfusion forms. We now report the crystal structure of the uncleaved, extracellular domain of the human parainfluenza virus 3 (hPIV3) F protein. Unexpectedly, the structure contains a complete 6HB not observed in the proteolyzed NDV F structure but with good correspondence to the SV5 and hRSV F1 core structures, which are thought to represent the postfusion

Abbreviations: 6HB, six-helix bundle; HA, hemagglutinin; HN, hemagglutinin-neuraminidase; hPIV3, human parainfluenza virus 3; HR, heptad repeat; NDV, Newcastle disease virus; RSV, respiratory syncytial virus; hRSV, human RSV; SV5, parainfluenza virus 5; TM, transmembrane.

Data deposition: The atomic coordinates and structure factors have been deposited in the Protein Data Bank, www.pdb.org (PDB ID code 1ZTM).

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conformation of the protein. The implications of the hPIV3 F structure for the folding and function of class I viral fusion proteins are discussed.

Materials and Methods

Cloning, Expression, and Purification of Soluble hPIV3 F. The hPIV3 (strain 47885) F protein cDNA was mutated to ablate intracellular processing of the F0 precursor (R.G.P., unpublished work). By using standard PCR protocols, a soluble form of F0 (solF0) was generated that contained a baculovirus gp64 signal sequence, in place of the F signal sequence, and contained a thrombin cleavage site, His6-tag, and termination codon immediately upstream of the hPIV3 F TM domain (Fig. 1A). The nucleotide sequence of the construct was obtained by using a 3100-Avant sequencer (Applied Biosystems). Recombinant baculovirus was generated by using a BacVector 3000 transfection kit (Novagen). The production of high-titer virus stocks and protein expression experiments were carried out by using Sf9 cells and High Five cells, respectively. The virus titer was estimated by using plaque assays. The soluble hPIV3 F protein containing a His6-tag was purified by Co2+ affinity chromatography (TALON Resin, BD Biosciences).

hPIV3 solF0 Protein Crystallization. Crystals were grown at 20°C by hanging-drop vapor diffusion methods, mixing equal volumes of protein solution (10 mg/ml) and precipitant [10% (wt/vol) polyethylene glycol 3000/0.1 M sodium citrate, pH 6.5], and crystals were obtained after 48 h. Crystals were harvested and subsequently transferred into 15% polyethylene glycol 3000, 0.1 M sodium citrate, and 15% propylene glycol for flash freezing in liquid nitrogen. The crystals belong to space group P2_12_1, with one hPIV3 solF0 trimer in the asymmetric unit with a noncrystallographic threefold axis.

Structure Determination and Refinement. An initial hPIV3 data set was collected to 3.5 Å from crystals soaked in 1 mM K2Pt(CN)4 for 24 h; this was used in molecular replacement and the initial rounds of model refinement. Subsequently, data from an untreated crystal were collected to 3.05 Å for the final stages of refinement. Diffraction data were processed with MOSFLM and scaled and reduced to structure factor amplitudes by using the CCP4 suite of programs (18). A molecular replacement solution for the structure was identified by using the program PHASER (19), using a partial model derived from stalk and neck regions of the NDV F structure (PDB ID code 1G5G). The resultant model phases and overall molecular envelope from the NDV F were used as input to the program DM (18) and improved by using threefold noncrystallographic symmetry averaging, density modification, and phase extension from 8 to 3.5 Å resolution in 1,500 steps. The resultant electron-density maps revealed features not present in the starting model and extended beyond the boundaries of the initial NDV F envelope, in particular at the base of the stalk.

The hPIV3 solF0 structure was refined by using the program CNS (20), followed by manual rebuilding with the program O (21). Simulated annealing omit maps and DM-averaged electron density maps were used for model building. After several rounds of manual model rebuilding and refinement using the 3.05-Å data, the final structure has an Rwork of 21.80% and an Rfree of 26.49% (PDB ID code 1ZTM). A Ramachandran plot shows that 99.2% of the residues lie in the most favorable or additionally allowed regions. PROCHECK (22) was used to assess the quality of the final model. The final refinement statistics are summarized in Table 1, which is published as supporting information on the PNAS web site.

Results

Expression and Crystallization of the hPIV3 F Protein. The hPIV3 F protein was expressed in insect cells by using the baculovirus system after appending a secretion signal sequence to the mature N terminus, truncating the protein just before the membrane anchor domain, and appending to the C terminus a cleavable His6-tag for purification (Fig. 1A). This construct is designated solF0 protein. Purified solF0 migrates as a single band on
regions and an axial channel penetrating from the top (Fig. 1). The head region of the hPIV3 solF0 structure has three electron density was observed in the interior of the coiled coil and core (T154, T204, S208, and T211). At one of these sites (T211), positions in the solF0 coiled coil, serine or threonine packs into the helix breaks and residues 136–140 reverse direction toward residue HRB extending to the end of the HRA coiled coil, where the HRA B presence of a 6HB formed by the HRA and HRB regions (Fig. 1). Cleavage sites are located on the exterior of the structure, exterior of the hPIV3 stalk. Modeling shows that the missing 41 defined structure in solution but are flexibly draped along the neck region of the structure (Fig. 1 E). The most unexpected observation in the solF0 structure is the globular, predominantly β-sheet-containing head domain, a neck region formed by both β-sheet and α-helices, and a stalk region that is predominantly α-helical (Fig. 1). Electron density is observed for residues 22–94 and 142–484 in monomer A, for residues 22–94 and 141–485 in monomer B, and for residues 20–94 and 136–484 for monomer C. Thus, residues 22–94 and 142–484 are observed in all three chains. In chain C, residues 136–140 are observed at the base of the stalk (Fig. 1 B and C) and will be discussed below. No electron density is observed for residues 486–494 and the C-terminal tag region that was retained during crystallization. The missing amino acids (95–135) begin 15 residues N-terminal to the internal cleavage site (see Fig. 6, which is published as supporting information on the PNAS web site), extending through the fusion peptide (starting at residue 110) and the N-terminal part of HRA (residues 129–192). Residue 142 defines the bottom of the stalk region three-helix coiled coil. In one monomer, residues 136–140 form an extended chain, which turns sharply at the base of the stalk and heads upwards toward residue 94, which is located ~122 Å away in the neck region of the structure (Fig. 1 B, D, and E). Residues 136–140 of this chain are likely visible because of contacts to a neighboring trimer in the crystal, consistent with the interpretation that, for all of the monomers, residues 95–140 do not adopt a defined structure in solution but are flexibly draped along the exterior of the hPIV3 stalk. Modeling shows that the missing 41 aa are sufficient to span the distance between residues 95 and 140, with most of the chain in an extended conformation. Thus, in the solF0 protein structure, the fusion peptide and protease cleavage sites are located on the exterior of the structure, analogous to influenza HA0 (12).

The most unexpected observation in the solF0 structure is the presence of a 6HB formed by the HRA and HRB regions (Fig. 1 B–E). The 6HB forms the base of the stalk region, with residues of HRB extending to the end of the HRA coiled coil, where the HRA helix breaks and residues 136–140 reverse direction toward residue 94. The coiled coil of the hPIV3 stalk contains a 3-4-4-4-3 stutter (residues 172–187), also observed in the SV5 and hRSV F1 core structures (15, 16) but absent in the NDV F fragment (17). At four positions in the solF0 coiled coil, serine or threonine packs into the core (T154, T204, S208, and T211). At one of these sites (T211), electron density was observed in the interior of the coiled coil and modeled as water but which may represent bound halide ion (15, 24). The head region of the hPIV3 solF0 structure has three prominent radial channels at the border of the neck and head regions and an axial channel penetrating from the top (Fig. 1 C).

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These channels have dimensions of ~7 × 50 Å and ~15 × 22 Å for the axial and radial channels, respectively, and meet at the center of the trimer. Similar channels have been observed in the NDV F crystal structure (12) and in cryo-EM reconstructions of Sendai virus F (25).

Overall, the solF0 structure is formed by three narrow and elongated monomers, which wrap their C-terminal regions (residues 428–484) across the trimer axis to interact with the adjacent two monomers (Fig. 1). HRB segments do not contact HRA residues within the same peptide chain but only interact with HRA segments in the other two monomers. The solF0 protein monomer dimensions are ~27 × 160 Å, generating a narrow projection profile (Fig. 1 E), with potential points of flexibility between distinct globular regions. The hPIV3 solF0 head region is formed by two β-sheet domains (domains I and II in Fig. 1 D and E), which are similar to those in NDV F. The neck region of the structure is formed by part of the central coiled coil, decorated by β-sheet and an α-helical (HRC) segment that ends at residue 94, forming domain III (Fig. 1 D and E). At the top of the coiled coil, a partly helical assembly packs into the interior of the trimer in the neck region.

The hPIV3 F 6HB Is Similar to Other Paramyxovirus 6HB Fragments. Peptides corresponding to the HRA and HRB regions from SV5 (15) and hRSV (16) F proteins assemble into stable 6HBs (the F1 core), and their structures have been determined. Superposition of the hPIV3 solF0 structure with these 6HB fragments reveals only minor differences (Fig. 2).

The N1 (HRA) peptide in the SV5 F1 core forms a longer coiled coil with three additional helical turns at its N terminus compared with hPIV3 solF0 (Fig. 2 A). The additional helical turns involve amino acids corresponding to residues 136–140 in solF0, which in the hPIV3 structure are not helical and instead reverse direction along the outside of the 6HB. The hRSV N57 peptide (HRA) is shorter at its N-terminal end than in SV5 and matches the hPIV3 solF0 coiled coil. The SV5 N1 peptide was generated by design from the F protein sequence (26, 27), whereas the RSV N57 peptide was identified after proteolytic digestion of a longer engineered fragment (16), potentially contributing to the observed structural differences in the three-helix bundle. The start of the helical regions in both the hRSV and hPIV3 structures corresponds to an ion-binding site at the core of the SV5 N1 bundle (15). Although the lengths of the isolated HRA regions differ at the
N-terminal end of the coiled coil, there are otherwise only minor structural differences observed.

For the C-terminal HRB regions, the hPIV3 structure corresponds more closely to the SV5 F1 core, in particular at the N-terminal end of the segment. For all structures, the C-terminal endpoints of the HRB helices are similar, indicating that the full 6HB structure is formed in hPIV3 solF0. The N-terminal end of the HRB region is characterized by an extended, nonhelical polypeptide segment, which contains residues important for both 6HB formation and for the activation of F-mediated membrane fusion (28, 29). Two residues, hPIV3 1454 and 1456, fit into a hydrophobic pocket formed by two adjacent HRA helices (Fig. 2B). The backbone conformations and positions of these hPIV3 residues overlay closely with those of the corresponding SV5 residues L447 and I449, which both affect the fusogenic activity of SV5 F (28). These residues correspond to F483 and F488 in hRSV F, which forms a unique additional turn of helix, enabling the two phenylalanines to interact with a hydrophobic pocket in the HRA coiled coil (Fig. 3B). The similarity in the positions and interactions of these key residues in the hPIV3, hRSV, and SV5 structures further suggests that the 6HB structure is well formed in the solF0 structure.

Charged Residues Line the hPIV3 Radial and Axial Channels. The head region of hPIV3 solF0 is formed by lateral interactions between domains I and II, with domain III below (Fig. 1). There are three radial channels that merge with the central axial channel, forming a large solvent-exposed cavity in the center of the head (Fig. 3 A and B). It has previously been proposed that the radial channels, also observed in the NDV F protein fragment structure, could provide a “docking” site for the hydrophobic fusion peptide (6, 17).

In the hPIV3 solF0 structure, the radial and axial channels are lined with charged residues forming an extensive network of salt bridges (Fig. 3 A and B). The network includes many residues at the base of the central cavity and radial channels, such as D216, E223, K224, K227, E330, E333, E260, K263, R265, and R281. These highly charged surfaces differ from the more hydrophobic radial channels observed in the NDV F structure (6, 17). The predominance of charges in the hPIV3 solF0 channels suggests that this region is not well suited to forming interactions with the hydrophobic fusion peptide. In addition, when the conformation of hPIV3 F residues 110–142 is modeled as a completely extended β-sheet, the N terminus of the fusion peptide does not reach the entrance to the radial channel but only the midpoint of the exterior of the neck region. Therefore, substantial shortening of the F stalk region would be necessary for the fusion peptide to enter the radial channels, and it is not clear how the highly charged surface of the channels would accommodate the hydrophobic fusion peptides.

In the head region of solF0 (Figs. 1 and 3), domain I of one chain contacts residues in domain II (375–428) and the linker between domains I and II (residues 369–374) in an adjacent chain. A gap between these two contact surfaces generates a secondary radial channel between monomers (Fig. 3C) located above the prominent radial channels described above. Domain II residues bury only 272 Å² of surface area on the adjacent monomer, but including the linker residues (369–374) raises this value to 576 Å². Thus, six residues in the linker provide as much, or more, contact surface as the entire domain II interaction. This suggests that the intermonomer interactions in the head domain may not be particularly stable.

Mapping of Mutations That Affect F-Mediated Membrane Fusion. A number of studies have identified F protein residues that regulate fusion activity and the dependence of F-mediated fusion on the coexpression of the homotypic hemagglutinin-neuraminidase (HN) protein (6, 17, 28, 30) (see Table 2, which is published as supporting information on the PNAS web site). Fig. 3D shows the mapping of the majority of these residues onto the hPIV3 solF0 structure; two of the sites are not visible in the protein structure but correspond to conserved glycines in the fusion peptide, located on the outside of the stalk region.

The fusion mutants are found in both the head and stalk regions of the solF0 structure. For those residues located in the head region, the majority lies in or near the radial channels of the trimer or at the interface of domains I and III. These mutations include residues corresponding to hPIV3 amino acids 261, 282, and 297. Residue 22, near the N terminus of the hPIV3 ectodomain, is located above the radial channel and distant from any domain-domain interfaces.

In the stalk region, one of the sites is located in HRA, at hPIV3 residue 139, that is disordered in two of the three monomers and connects the base of the stalk through the flexible fusion peptide to the neck domain. The corresponding residue is also present in the SV5 F1 core structure (E132) and is solvent-exposed within the helical coiled coil below the C terminus of HRB. Three additional residues are located adjacent to HHR (residues 450, 454, and 456). These residues have been identified as a potential “switch” region, which likely adopts distinct interactions in the prefusion and postfusion states (28). Mutations of 454 and 456 to aromatic residues generate an HN-independent, hyperfusogenic SV5 F protein, whereas mutations

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Comparison with the Proteolyzed NDV F Structure. The hPIV3 solF0 structure shows overall similarity to the previously determined NDV F crystal structure (6, 17), which did not include the 6HB. The alignment of the two structures based on superposition of the homologous HRA coiled-coil regions is shown in Fig. 4. The hPIV3 HRA coiled coil extends further down the stalk by about six additional helical turns (29 residues). The 6HB between HRA and HRB is not observed in the NDV F structure, presumably because of partial proteolysis mapped to a residue downstream of the fusion peptide and within HRA (17, 23). A top view of this hPIV3 and NDV alignment shows a significant rotation of the hPIV3 head compared with NDV F (Fig. 4B). Superimposition of individual domains also reveals significant differences in the relative orientations of domains I–III to each other and to the overall neck and stalk regions of the structure. In particular, the orientations of domains I and II relative to the coiled-coil stalk region are strikingly different. Differences in the connections between domains I–III and the linker to HRB highlight these as points of structural variation that could be related to conformational transitions important for membrane fusion.

Discussion

The crystal structure of the uncleaved extracellular domain of the hPIV3 F protein reveals the unanticipated formation of the 6HB bundle that is thought to represent the postfusion conformation of the protein. Many lines of evidence suggest that the observed conformation primarily represents the postfusion form, although the polypeptide chains are intact in the crystal, and the fusion peptide is not located at the appropriate end of the 6HB. The 6HB is well formed and undistorted, similar to the previously determined SV5 and hRSV F 6HB structures (15, 16).

The hPIV3 structure is inconsistent with peptide inhibition data that show, at least for SV5 F, that the HRA and HRB peptides are exposed at distinct steps of the membrane fusion reaction (9). In the current structure, the release of HRB from 6HB would simultaneously expose HRA sites to inhibition, which conflicts with the inhibition data. Instead, the hPIV3 structure would predict that both HRA and HRB peptides would acquire the ability to inhibit membrane fusion simultaneously, requiring the observed 6HB to dissociate to be consistent with the previous mechanistic studies. From the current structure, it is also not clear how membrane fusion could be achieved. Cleavage of the hPIV3 solF0 protein would most likely allow repositioning of the fusion peptide to the N terminus of the HRA coiled coil and concomitant insertion into the anchoring membrane for the TM domain. Finally, the current structure cannot explain the behavior of F protein mutants that destabilize the prefusion conformation of F and significantly enhance its membrane fusion activity (6, 28–30). Mutation of two hydrophobic residues of HRB in SV5 (447 and 449 in SV5 corresponding to 454 and 456 in hPIV3) to larger aromatic residues leads to a hyperfusion phenotype of the SV5 F protein, which is most easily interpreted as leading to a destabilization of the prefusion conformation (28). The mutation of conserved glycines in the fusion peptide leads to similar behavior (30).

The structure of hPIV3 solF0 is similar to that determined for a fragment of the secreted NDV F protein (17) and to a cryo-EM structure of Sendai virus F (25). Although we favor the interpretation that both the hPIV3 and NDV F protein structures represent the postfusion conformation, the cryo-EM reconstruction was done with full-length Sendai F protein that was also shown to have some fusion activity after reconstitution into virosomes. It may be that the extent of the conformational changes that distinguish the prefusion and postfusion conformations is relatively small and cannot be discerned at low resolution or, alternatively, that the biochemical procedures associated with the solubilization and reconstitution of the intact F protein may influence the F conformational state. There is some evidence from monoclonal antibody binding studies that detergent solubilization of intact F affects antibody recognition, potentially by inducing F protein conformational changes (31). Finally, it has been suggested that the related hRSV F protein is activated by proteolytic cleavage to cause membrane fusion (32, 33). Studies of the stability and conformation of soluble and membrane-bound hRSV F suggest that the overall characteristics (antibody binding, thermal stability, secondary structure) of the F protein do not change upon membrane fusion (33). However, for SV5 F, it is now well established that monoclonal antibodies show distinct reactivity to F fusion mutants, consistent with their ability to distinguish different conformational states of F (9, 28–30, 34, 35).

The comparison of the hPIV3 solF0 structure with that of NDV F shows differing conformational arrangements of their respective domain regions. This comparison suggests at least three points of conformational diversity that could be important “hinge” regions for potential structural changes accompanying membrane fusion. These potential hinge points include the linkage between domains I and II, the linkage between domains I and III, and the linkage between domain II and HRB. Although the stalk and the individual domains generally superimpose well between the two protein structures, the relative arrangements of these rigid domains is significantly different. Although some of this conformational diversity is almost certainly associated with...
sequence differences between the two F proteins, these linkage points are clearly adaptable and variable. Further study of the flexibility of domain arrangements within a single paramyxovirus F protein, for example by the elucidation of the structures in multiple crystal forms, may allow one to further identify key regions of F protein structural changes.

Finally, the structure of hPIV3 solF0 raises questions regarding the formation and stability of the prefusion conformation of the paramyxovirus F protein. If the soluble, secreted hPIV3 and NDV F proteins, and by inference other paramyxovirus family members as well, primarily adopt a postfusion conformation, there are at least two possible explanations for this behavior. First, the TM anchor (and potentially the cytoplasmic tail) could be an important determinant of the stability of the prefusion conformation, providing a significant fraction of the energy barrier that traps the protein in a metastable state. In this case, the secreted protein may fold to the prefusion form transiently but then refold to the postfusion form. A second possible explanation for the structural results is that the TM domain is important for the protein to attain the prefusion metastable state and that in the absence of this region, the soluble F protein folds directly to the final, most stable postfusion conformation. In either case, it appears that the amino acids comprising the intact F protein ectodomain are not sufficient for the protein to fold to and maintain a metastable conformation. It is interesting that the structures of the prefusion forms of the influenza HA protein, HA0 and HA1/HA2, were both initially obtained from protein produced in the membrane-bound form before proteolytic removal of the TM domains (11, 12). Truncated, secreted HA protein does not trimerize efficiently, suggesting that the folding of the HA ectodomain is not as robust as for the paramyxovirus F proteins but may also require membrane anchor domains (36). In contrast, the folding of the isolated HA2 region to the postfusion form, when produced recombinantly in bacteria, is robust (37, 38). Thus, the secreted paramyxovirus F proteins may act more similarly to the isolated influenza HA2 domain in terms of their ability to adopt the more stable, postfusion conformation.

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