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Coiled Coils in Both Intracellular Vesicle and Viral Membrane Fusion

Minireview

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Introduction

Recent structural studies of protein complexes involved in neurotransmitter release and intracellular vesicle trafficking reveal similarities with the structures of a group of virus membrane fusion proteins. In both cases hydrophobic sequences, embedded in the membranes to be fused, are located at the same end of a rod-shaped complex composed of a bundle of long α helices. This molecular arrangement is proposed to cause close membrane apposition as the complexes are assembled for membrane fusion.

Molecular Rods with One Sticky End

Vesicle Fusion. Membrane fusion processes within cells are utilized to transport material between specialized cellular compartments and for export. In the last 10 years it has become generally accepted that fusion between the membranes of vesicles and their targets in trafficking events such as exocytosis involves three groups of membrane receptors collectively called SNAREs (abbreviations are detailed in the Figure 1 legend) (Sollner et al., 1993). A combination of electron microscopic, spectroscopic, and X-ray crystallographic data shows that these receptors form a rod-shaped complex that is a coiled coil of four α helices, one contributed by the vesicle SNARE, synaptobrevin, and three by the plasma membrane SNAREs syntaxin, which provides one α helix, and SNAP-25, which provides two. In the electron microscope this core complex is a 14 nm × 4 nm rod, which antibody probes and mannose-binding protein labels showed has the membrane anchor regions of both syntaxin and synaptobrevin at one end (Hanson et al., 1997). A similar orientation of site specifically labeled syntaxin and synaptobrevin was inferred from fluorescence resonance energy transfer experiments (Lin and Scheller, 1997). Circular dichroism (CD) spectroscopy of the SNAREs shows that on forming the core complex, synaptobrevin and SNAP-25 convert from comparatively unstructured, toxin protease-sensitive forms into α helices, and that the helical content of syntaxin also increases. These highly α-helical core SNARE complexes are extremely stable, melting at over 95°C.

Most recently, X-ray crystallography of a soluble synaptic fusion complex formed between two helical segments of SNAP-25 and the C-terminal regions of synaptobrevin and syntaxin, expressed without their membrane

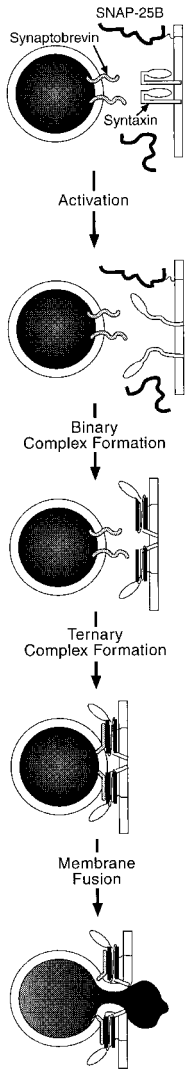

Figure 1. Schematic Diagram of the Formation of the SNARE Complex and Its Possible Role in Vesicle Fusion

The synaptic vesicle membrane protein synaptobrevin, a vesicle (v-) SNARE, and the plasma membrane proteins syntaxin and the 25 kDa synaptosome-associated protein SNAP-25, two target (t-) SNAREs, interact to form the SNARE core complex (Sollner et al., 1993). Two soluble proteins, N-ethylmaleimide sensitive factor (NSF), an ATPase, and soluble NSF attachment protein (α-SNAP), are proposed to disassemble the core complex in vesicle recycling. In this process, α-SNAP binds to the SNAP receptors (SNAREs) and then binds NSF. SNARE complex formation, which is preceded by vesicle docking, is followed by membrane fusion. Electrophysiological, biochemical, and genetic data indicate that docking, the process of core SNARE complex formation, and efficient fusion are dependent on additional factors that are not included in this diagram. Figure modified from Figure 8 of Nicholson et al. (1998).

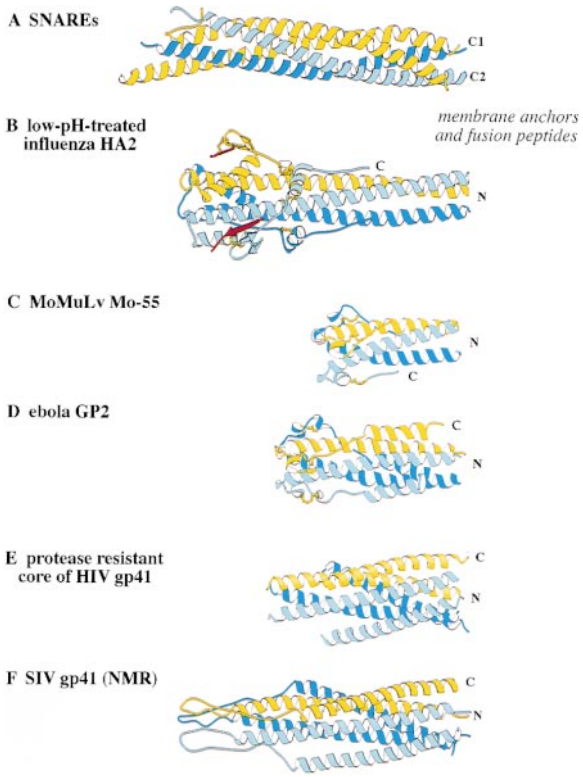

Figure 2. Rod-Shaped α-Helical Bundles of the Ectodomains of Membrane Fusion Proteins, with the Regions that Insert in the Participating Membranes at One End (Right Side)

(A) Recombinant synaptic fusion complex with the v-SNARE synaptobrevin ectodomain (light blue), t-SNARE syntaxin C-terminal ectodomain (dark blue), and the two helical regions of the t-SNARE SNAP-25B (yellow) (Sutton et al., 1998). C1 and C2 label the C termini that are inserted, prefusion, in the plasma membrane and vesicle membrane, respectively.

(B) Low-pH-treated influenza virus HA after proteolytic cleavage at HA1 residue 27 and HA2 residue 38, which removes the bulk of HA1 (28–328) and the fusion peptide region HA2 (1–38) (Bullough et al., 1994). HA1 1–27 is in red.

(C) Fifty-five-residue recombinant fragment of Moloney murine leukemia virus TM subunit (Fass et al., 1996).

(D) Recombinant Ebola virus GP2 (Weissenhorn et al., 1998).

(E) Recombinant, proteolysis resistant core of HIV-1 gp41 (Weissenhorn et al., 1997, and references therein).

(F) Recombinant SIV gp41 (Caffrey et al., 1998). This figure, modified from Weissenhorn et al. (1998), was generated with RIBBONS.

anchors, shows that they are bundles of four α helices in which the α helices are parallel (Sutton et al., 1998). This conclusion has also been drawn from electron paramagnetic resonance spectroscopic analysis (Poirier et al., 1998). The transmembrane anchors that would allow association with membranes are colocated at one end of the complex.

Virus Fusion. Enveloped viruses fuse their membranes with cellular membranes to transfer their genomes into cells at the beginning of infection. Fusion is mediated by virus surface glycoproteins, and, like SNAREs, the C-terminal membrane-proximal domains of a number of these fusion proteins form rod-like structures (Figure 2). The group of viruses for which this information is available includes the myxoviruses, influenza and parainfluenza viruses; the filovirus, Ebola; and the retroviruses, HIV, SIV, and Moloney murine leukemia virus (Mo). A common feature of these viruses is that their fusion glycoproteins are synthesized as precursors that are primed for their function in membrane fusion by proteolytic cleavage. Cleavage results in the formation of influenza HA1 and HA2, parainfluenza F2 and F1, Ebola Gp1 and GP2, HIV and SIV gp120 and gp41, and Mo SU and TM. The hydrophobic N-terminal sequences of HA2, F1, GP2, gp41, and TM that are generated by cleavage have become known as fusion peptides; their C-terminal sequences contain their viral membrane anchors. Electron microscopy of ectodomains of these subunits from a number of viruses (Weissenhorn et al., 1998, and references therein) shows rod-like structures and combined with immunoelectron microscopy, in the case of HA2, indicates the colocation of the membrane anchor and the fusion peptide at one end of the rod. CD studies indicate that the rods are highly α helical and melt at high temperatures like SNARE complexes (Weissenhorn et al., 1998, and references therein). X-ray studies of HA2, GP2, Mo-TM, and HIV gp41 (Bullough et al., 1994, and references therein; Fass et al., 1996; Chan et al., 1997; Weissenhorn et al., 1997, 1998, and references therein) and NMR analyses of SIV gp41 (Caffrey et al., 1998) have used soluble fragments of the proteins, from which the hydrophobic N-terminal fusion peptides and C-terminal membrane anchors have been removed. These studies show that the rods are trimers in which an N-terminal central coiled coil is surrounded by a sheath of antiparallel chains that terminate with their C termini near the N termini of the central helices (Figure 2).

Results from a range of similar analytical procedures, therefore, lead to the view that the virus fusion proteins and the SNARE complexes, while clearly different in subunit composition, in the derivation of their membrane associated regions, and in their detailed structures, share two major features: they are α-helical rods, and the helices are oriented to colocate the membrane-associated regions at one end of the rod. Recognition of the latter feature in particular has suggested a common role for these structures in which membranes to be fused are brought together at the fusion site by the juxtaposition of the membrane anchors in the two participating membranes in the case of the SNARE complex and the membrane anchor and fusion peptide in the case of the virus glycoproteins.

Activation and Refolding of Fusion Proteins. For the virus proteins to mediate membrane fusion, specific activation is required. In the case of influenza, for example, the activating signal is the low pH of endosomes, between pH 5.0 and 6.0; for HIV, activation involves binding to CD4 and to chemokine receptors at the T cell surface. The structural consequences of activation are only known in detail for HA, but a number of features that HA2 shares with HIV gp41 (Chan et al., 1997; Weissenhorn et al., 1997) and with Ebola GP2 (Weissenhorn et al., 1998), and experiments on the inhibition of HIV infectivity by specific peptides suggest that similar events may also occur in their activation. Comparison of X-ray crystallographic data for HA at neutral pH and fusion pH shows that activation involves extensive rearrangement and refolding of the molecule. Two features of the structural change are noteworthy. In the first, the fusion peptide, which at neutral pH is buried in the trimer interface only 30 Å from the virus membrane, is extruded at fusion pH

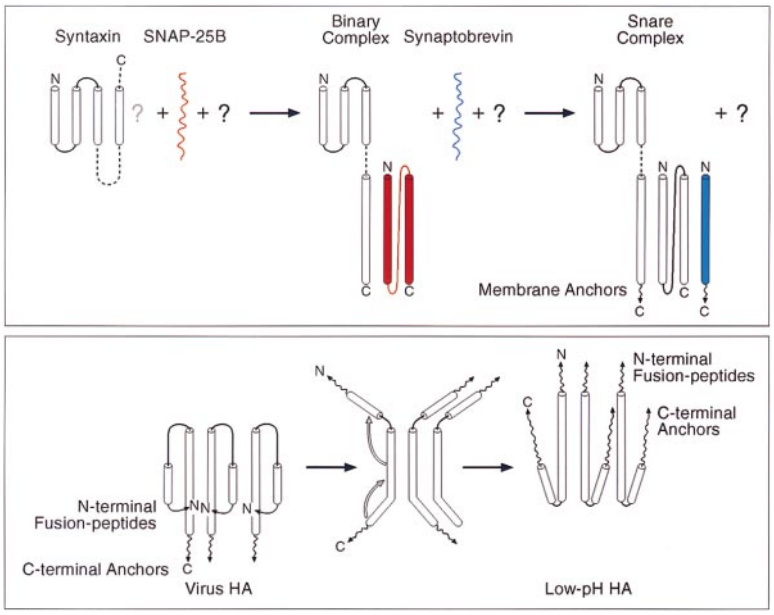

Figure 3. Activation and Refolding of Cellular and Viral Fusion Proteins

In vitro refolding is as observed with molecules (syntaxin and synaptobrevin) without their C-terminal membrane anchors, or the palmitolyted connecting loop in SNAP-25B. SNAP-25B and synaptobrevin are shown unfolded before assembly; syntaxin is modeled intramolecularly inhibited by the N-terminal domain (see text). The stochiometry of the binary complex has been reported as 2:1 or 1:1 syntaxin:SNAP depending on the system studied. Question marks indicate uncertainty in the structure of the C-terminal half of uncomplexed syntaxin and the presence of other known or suspected proteins that are outside the scope of this minireview. The C-terminal anchors of HA are inserted in the viral membrane. The N-terminal fusion peptides, after relocating to the top of the coiled coil, apparently enter the cellular membrane.

to the N-terminal tip of the 100 Å trimeric coiled coil that is newly formed at this low pH; its formation involves relocation of a short α helix and refolding of a segment of extended chain into an α helix (Carr and Kim, 1993; Bullough et al., 1994; Figure 3). In the second, four residues toward the C terminus of the coiled-coil region that are α-helical at neutral pH refold into a 180° turn that reorients the remaining C-terminal parts of the molecule antiparallel and toward the fusion peptide end of the coiled coil (Bullough et al., 1994). These changes in conformation produce the thermostable form of HA2 that is analogous to the structures of fusion peptide-and membrane anchor-less Mo TM, HIV and SIV gp41, and Ebola GP2. Evidence obtained from analyses of mutant HAs indicates that these changes in structure are required for membrane fusion (Bullough et al., 1994, and references therein); the similarities of the structures formed by the fusion proteins of this highly diverse group of viruses provide additional support for the proposed functional significance of the fusion pH-induced structure.

The recruitment of nonhelical segments of protein into helical bundles as occurs in HA2 at fusion pH is also a feature of the assembly of the rod-shaped SNARE complexes. By CD, both synaptobrevin and SNAP-25 appear to be nonhelical before entering the complex; the C-terminal region of syntaxin increases in α helical content to 70% on assembly.

Before assembly of the core complex, in vitro, the C-terminal and N-terminal regions of syntaxin appear to be associated (Nicholson et al., 1998, and references therein). The three-dimensional structure of the N-terminal domain has recently been determined by NMR (Fernandez et al., 1998). The structure is an up-down-up three-helix bundle with a left-handed twist. Typically, helical bundles with this twist contain four or more helices, and in fact, a groove of highly conserved residues exists on the surface of the three-helix bundle that appears capable of accommodating a fourth α helix. Fernandez et al. (1998) suggest that this groove may bind an α helix from another component of the exocytic machinery and/or a section of the C-terminal α helix of syntaxin in a preassembly conformation. The length of the N-terminal three-helix bundle, about 33 residues, would only provide a binding site for about half of the C-terminal region in helical conformation, suggesting that the remainder may adopt a nonhelical preassembly conformation (Figure 3). From examining the X-ray structure of the SNARE complex, Sutton et al. (1998) noted that the syntaxin and SNAP-25 helices are knitted together by many more nonpolar interactions than hold the v-SNARE, synaptobrevin, into the four helix coiled coil; the binary t-SNARE complex forms a sort of cradle into which the v-SNARE appears to bind. This description, coupled with the preassembly association of the C-terminal helix of syntaxin with its helical N-terminal domain, suggests the possibility that formation of the SNARE complex may involve a helix exchange step analogous to that which occurs in the fusion pH-induced reorganization of HA (Figure 3). α-helical coiled coils may offer a particularly apt architecture for the control and refolding required to juxtapose molecular segments in such structures.

Reversibility of Fusion Complexes. Disassembly of SNARE complexes is an important component of the recycling process proposed to be required in the formation of new vesicles. It is catalyzed by the NSF ATPase, a hexameric ring-shaped molecule that binds to SNARE-associated α-SNAPs (see Figure 1 legend). Electron microscopy has shown that NSF binds to one end of the SNARE rod where it catalyses the dissociation of the SNARE helical bundle (Hanson et al., 1997). The crystal structure of the oligomerization domain of NSF has recently been determined (Lenzen et al., 1998; Yu et al., 1998) and may contribute to an understanding of the structural basis of the disassembly process, currently thought to provide dissociated, primed SNARE molecules for subsequent prefusion reassembly (Nichols et al., 1997).

In contrast, formation of the stable virus fusion protein

structures discussed here is irreversible; disassembly is not a stage in virus protein-mediated membrane fusion. Triggered refolding in the absence of target membranes results in inactivation of their membrane fusion capacity and loss of virus infectivity. Prevention of premature activation during the infectious cycle is ensured by downregulation of receptor synthesis by some viruses or, in the case of influenza, by a virus-encoded proton channel that elevates the pH of the *trans* Golgi to prevent HA activation during biosynthesis.

Consequences for Mechanism. What role do these molecular complexes or the mechanisms of their formation have in membrane fusion? A number of possibilities have been suggested for the SNAREs, some of which incorporate other molecules known to be involved in vesicle fusion in vivo (reviewed in Weis and Scheller, 1998). For virus protein-mediated fusion, studies of singly expressed proteins in vivo and of reconstituted virosomes in vitro show that, provided with the appropriate activation signal, such as low pH in the case of influenza HA, the virus fusion protein alone can mediate cell and liposome fusion. The structural similarities between the virus proteins and the SNARE core complex suggest, therefore, that the SNAREs may also be a central component of the vesicle-fusion machinery and give support to evidence that they could represent the minimal fusion complex (Weber et al., 1998). If this is the case, then virus fusion peptides would simply represent a substitute for the membrane anchor of a SNARE protein. Alternatively, it may be that virus fusion peptides have additional features that allow them, on insertion into membranes, to influence membrane structure; short synthetic virus fusion peptide analogs can fuse liposomes and lyze cells. Perhaps for the SNARE core complex or its associated proteins, a response to Ca^{2+} during fusion activation may result in similar protein–lipid interactions at a site determined by SNARE complex formation.

Conclusion

SNARE complexes from synapses and intracellular vesicles and the membrane fusion subunits of a group of virus glycoproteins form rod-shaped α-helical bundles that, although very different in structure, all appear to have the membrane-anchoring sequences at one end of the rods (Figure 2), where they could draw the participating membranes into apposition and possibly distort their structure (Figure 1). Some parallels, including exchanges in the location of α helices and the possibility of random coil-to-helix transitions, may exist between the regulation of assembly of SNAREs by the N-terminal domain of the t-SNARE, syntaxin, and the conformational refolding found in influenza virus HA and suggested to occur in other viral glycoproteins. SNARE complexes are apparently both primed and disassembled for recycling by a dedicated ATPase (NSF/α-SNAP), while viral proteins analyzed to date appear to be used only once during viral entry and then discarded.

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