Analyzing the fusion process of influenza hemagglutinin by mutagenesis and molecular modeling

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INTRODUCTION

Hemagglutinin (HA) is a protein on the surface of influenza virus that binds to sialic acid on cell membranes and leads to fusion of viral and cell membranes. Its crystal structure (Wilson *et al.,* 1981) shows that HA consists of two disulfide linked glycopolypeptide chains, HA1 and HA2, which extend as a trimeric spike 13.7 nm from the virus membrane (Booy *et al.,* 1985). The spike is surmounted by globular domains of three HA1's that contain the sialic acid binding sites. The fusion peptide, the apolar HA2 amino terminus, is buried near the base of the spike between three HA monomers. In an acidic environment, as found in endosomes, the protein undergoes an irreversible conformational change which exposes the fusion peptide. The fusion peptide is thought to interact with the target membrane and trigger membrane fusion. The pH-dependent conformational change of HA appears to be only part of a complicated fusion process (White, 1990).

To examine the fusion mechanism in more detail, we have studied effects of mutations on the fusion peptide and have used computer graphics and computational chemistry methods to develop plausible models for how HA may change conformations and associate with adjacent HA trimers during the fusion process.

METHODS

Recombinant SV40-HA virus was kindly provided by Mary-Jane Gething and Joe Sambrook (University of Texas Health Science Center, Dallas, TX). The vectors used were strain A/Japan/305/57. By oligonucleotide-directed mutagenesis, Gly in position 1 of HA2 was replaced by Glu (G1E), Gly in position 4 by Glu (G4E), and Glu in position 11 by Gly (E11G) (Gething *et al.,* 1986). Binding and fusion of R18-labeled red blood cells (RBC) to SV40-HA infected CV-1 cells were performed as described by Morris *et al.* (1989) and Sarkar *et al.* (1989). The fusion reactions were recorded in a spectrofluorimeter (model 800; SLM Instruments, Inc., Urbana, IL).

Plausible molecular models of the fusion mechanism were examined by using the crystal structure of the soluble portion of HA (Wilson *et al.,* 1981) and developing models of the viral transmembrane segments and the fusion peptides. The hydrophobic COOH-terminal portions of the three HA2's of the trimer were modeled as a triple-stranded coiled coil that spans the viral membrane. The fusion peptide (res 1–25) was modeled as an α-helix that is connected to the rest of the protein by a flexible chain (res 26–36). The theoretical methods used to develop the structural models are described elsewhere in this volume (Durell and Guy, 1992).

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RESULTS

Effects of mutations

In accordance with previous cell biological results (Gething *et al.,* 1986), G1E did not induce fusion, E11G showed the same general pattern as the wild type (WT), and G4E showed a shift of ~0.4 in pH threshold (Schoch and Blumenthal, 1991). The overall kinetics of G4E and WT were similar in lag times for the onset of fusion, rise times, and extent. The lag time could be dissected into two components: a commitment time of 5–10 s, and a maturation time of 20–30 s. Although the maturation times for WT and G4E were similar, the commitment time was more rapid for G4E. These results suggest that only the relatively rapid components in the overall fusion reaction depend on the structure of the fusion peptide, whereas the slower processes are governed by other factors.

Structural Models

Although the fusion peptide is thought to interact with the target membrane, its location near the base of the trimeric spike makes it unclear how this interaction could occur. The mechanisms that have been suggested are as follows: (*a*) *The HA trimer splits apart and collapses toward the viral membrane* (Doms and Helenius, 1986). Recent experiments indicate, however, that the trimers remain intact during the fusion process (Stegmann *et al.,* 1990). (*b*) *Lipids of the target membrane attach to the globular region and then creep down the spikes to the fusion peptide* (Bentz, 1991). (*c*) *The HA trimer bends near the base of the spike* (Stegmann *et al.,* 1990).

We propose the following alternative mechanism. The fusion peptides interact initially with the viral membrane to destabilize the perpendicular orientation of

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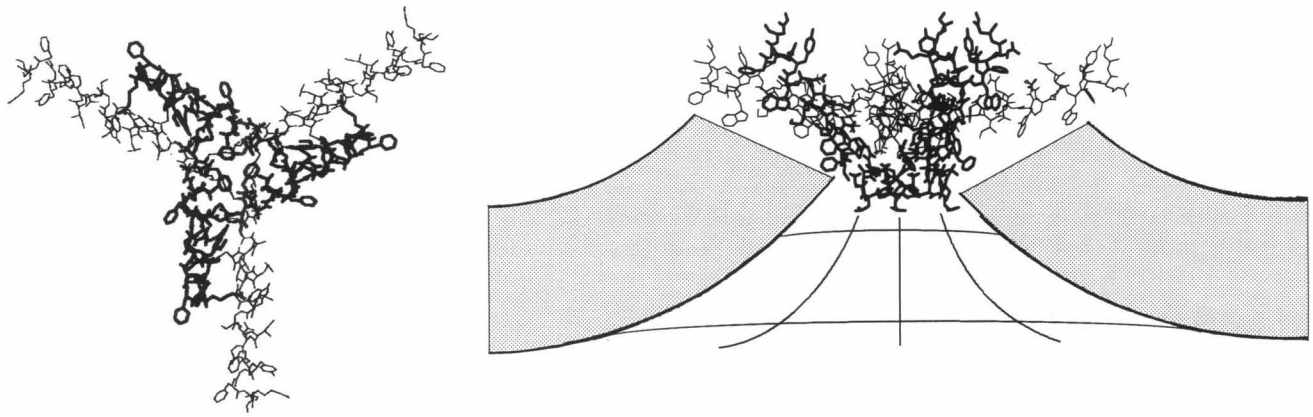

FIGURE 1 Model of fusion peptide assembly. (*Left*) View looking toward the membrane. Hydrophilic side chains are oriented upward and would be exposed to water; most hydrophobic side chains are oriented downward where they would be exposed to lipid chains or are buried between the helices. (*Right*) Side view. The shaded region represents the membrane.

HA relative to the membrane and/or to stabilize tilted orientations. The tilted orientations are also stabilized by interactions among adjacent HA trimers. Once the trimers have tilted, some of the fusion peptides may interact with the target membrane. This interaction is followed by creation of a "fusion pore" (Spruce *et al.*, 1989) in which HA trimers are tilted still more. Finally, the membranes fuse. To examine this mechanism, we have modeled ways in which fusion peptides and the viral transmembrane segments of adjacent trimers could interact with each other and the membrane when HA trimers are tilted.

First, fusion peptide assemblies were modeled. The first 25 HA2 residues were assumed to form an α-helix. The first 10 residues of this helix are hydrophobic and the remainder is amphipathic. If the peptide lies on the membrane surface, this pattern suggests that the NH_2-terminal will sink deeper into the membrane than the COOH-terminus. The hydrophobic NH_2-terminal portions of three helices can pack tightly together to form a trimer, as illustrated in Fig. 1. Small ambivalent residues are at the center of the helical bundle, the larger hydrophobic residues are on the surface postulated to be in contact with lipid, and hydrophilic residues are on the surface postulated to be exposed to water. Three additional fusion peptides (Fig. 1, thin lines) can lie on the

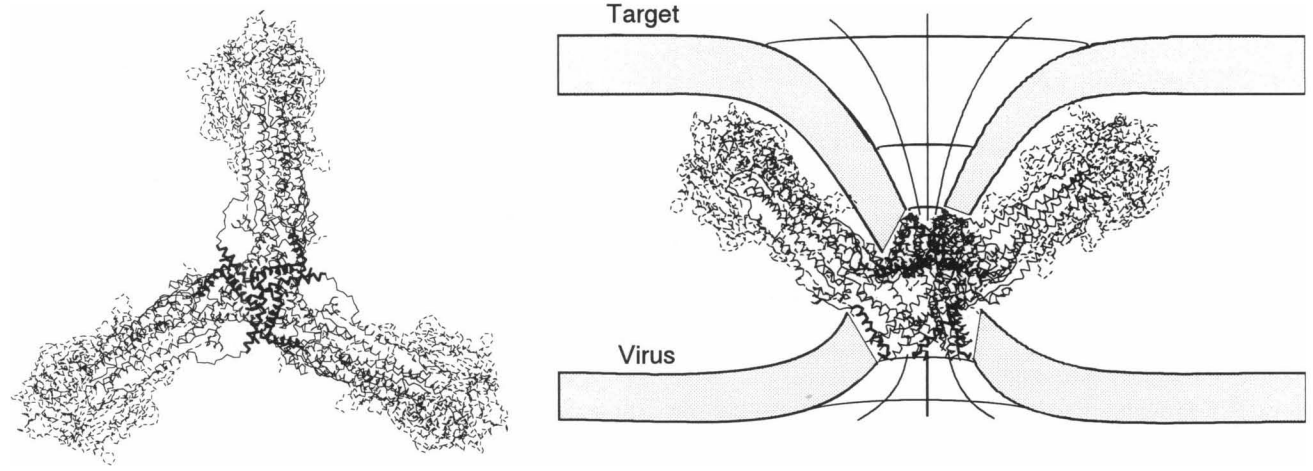

FIGURE 2 Alpha carbon drawing of aggregate of three tilted HA trimers. In this figure each trimer has been rotated 54° relative to the membrane normal. Note that the "fusion peptide" region (*thick lines*) of each monomer is in a different position: one extends toward the target membrane at the top, one extends through the viral membrane on the bottom and one fits between the trimers on the outer surface of the viral membrane. HA1 has dashed lines and HA2 solid lines. (*Left*) View looking toward the viral membrane. (*Right*) Side view. Shaded regions represent viral and target membranes.

membrane surface and fit between the first three. The interactions of the NH_2-terminal ends of these peptides with each other and with the first three helices are similar to interactions in the crystal structure. This model is consistent with the importance of residues one and four indicated by mutagenesis. Note that this assembly forms a cone-shaped structure that would distort a planar bilayer structure, but that could stabilize a highly curved bilayer.

A tilted orientation of HA could be stabilized by interactions among HA trimers involving other portions of the protein. Fig. 2 illustrates a model in which the transmembrane regions of three tilted trimers interact directly with each other to form a transmembrane bundle of nine α-helices. The complex is postulated to be stabilized by interactions of fusion peptides in the transmembrane region and possibly on the surface between the trimers. This still leaves three or six of the fusion peptides to interact with the target membrane. This assembly is postulated to represent only one of numerous metastable assemblies that occur before formation of the fusion pore. Additional HA trimers are likely to be involved in the complete fusion process.

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