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Analysis of residues near the fusion peptide in the influenza hemagglutinin structure for roles in triggering membrane fusion

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Abstract

Influenza virus entry occurs in endosomes, where acidification triggers irreversible conformational changes of the hemagglutinin glycoprotein (HA) that are required for membrane fusion. The acid-induced HA structural rearrangements have been well documented, and several models have been proposed to relate these to the process of membrane fusion. However, details regarding the role of specific residues in the initiation of structural rearrangements and membrane fusion are lacking. Here we report the results of studies on the HA of A/Aichi/2/68 virus (H3 subtype), in which mutants with changes at several ionizable residues in the vicinity of the "fusion peptide" were analyzed for their effects on the pH at which conformational changes and membrane fusion occur. A variety of phenotypes was obtained, including examples of substitutions that lead to an increase in HA stability at reduced pH. Of particular note was the observation that a histidine to tyrosine substitution at HA1 position 17 resulted in a decrease in pH at which HA structural changes and membrane fusion take place by 0.3 relative to WT. The results are discussed in relation to possible mechanisms by which HA structural rearrangements are initiated at low pH and clade-specific differences near the fusion peptide.

Keywords: Influenza; Hemagglutinin; Membrane fusion; Fusion peptide; HA structure; Conformational change

Introduction

Membrane fusion is a biological process required for a variety of fundamental viral and cellular functions. Developments in recent years have significantly expanded the understanding of the fusion mechanisms for both class I and class II viral fusion glycoproteins (Barnard et al., 2006; Colman and Lawrence, 2003; Doms, 2004; Dutch et al., 2000; Earp et al., 2005; Eckert and Kim, 2001; Harrison, 2005; Huang et al., 2003; Kielian, 2006; Lamb et al., 2006; Sieczkarski and Whittaker, 2005; Skehel and Wiley, 2000; Smith and Helenius, 2004). Among the class I viral fusion proteins, related mechanisms for fusion have evolved in which a metastable form of the molecule is converted into a highly thermostable conformation during the fusion process. These thermostable structures are rod-like in appearance and all contain a central trimeric α-helical coiled coil and antiparallel polypeptide chains that pack against it. As a consequence of the refolding events, the hydrophobic transmembrane and fusion peptide domains of the glycoproteins, which are postulated to associate with the viral and cellular membranes, respectively, as part of the fusion process, are brought into close proximity with one another. For the class I viral fusion proteins, there are a number of mechanisms by which such conformational changes can be triggered to initiate the fusion process. These include receptor binding with the involvement of coreceptors, receptor binding and interaction with separate viral fusion proteins, and activation of a viral fusion protein by acidification following endocytosis.

Influenza A is a well-characterized example of a virus that enters the host cell via the endocytic pathway, and structural studies spanning the past three decades have made the HA

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glycoprotein a valuable paradigm for studies on viral membrane fusion in general (Bullough et al., 1994; Chen et al., 1998; Wilson et al., 1981). Similar to other class I viral fusion proteins, the polypeptide chains of the HA precursor (HA0) associate as homotrimers in the endoplasmic reticulum during biosynthesis. Each monomer of HA0 is subsequently cleaved at a surface loop into the disulfide-linked polypeptides HA1 and HA2. This generates hydrophobic HA2 N-terminal fusion peptide domains and transforms the molecule into its metastable conformation to activate the membrane fusion potential of HA. As a consequence, HA0 cleavage is required for virus infectivity (Appleyard and Maber, 1974; Klenk et al., 1975; Lazarowitz and Choppin, 1975). After attachment to host cells and internalization, HA undergoes irreversible conformational changes due to the acidification of the endosomal environment, and membrane fusion is induced.

The current study focuses on the analysis of residues near the fusion peptide of cleaved HA that may be involved in the initiation of the acid-induced HA conformational changes required for fusion. While it is known that amino acid substitutions at various locations in the HA trimer are capable of destabilizing the native structure leading to an increase in the pH of fusion, the possibility that protonation of specific residues provide the initial trigger for conformational changes remains unresolved. The region surrounding the fusion peptide in the native HA structure is of interest regarding potential triggers for fusion for several reasons. Among the many mutants with elevated fusion pH identified in studies based on amantadine resistance, site-directed mutagenesis, and reverse genetics, those involving amino acid substitutions in and around the fusion peptide are particularly well represented (Cross et al., 2001; Daniels et al., 1985; Lin et al., 1997; Steinhauer et al., 1993). In fact, nearly all amino acid substitutions in the N-terminal region of the fusion peptide that have been analyzed to date lead to increased fusion pH, regardless of the HA position or the amino acid introduced (Cross et al., 2001; Gething et al., 1986; Steinhauer et al., 1995). Studies on double mutant HAs also indicate that amino acid substitutions in and around the fusion peptide are dominant in dictating the pH of fusion when expressed in combination with substitutions elsewhere in the molecule (Steinhauer et al., 1996). Other studies using anti-peptide antibodies to detect changes in HA structure also suggest that conformational changes involving the fusion peptide and proximal residues precede the detrimerization of the HA1 head domains (White and Wilson, 1987).

When HA0 is cleaved into HA1 and HA2 to prime membrane fusion potential, only six residues at the C-terminus of HA1 and 12 residues at the N-terminus of HA2 are relocated (Chen et al., 1998) (Fig. 1). As the conformational changes that accompany cleavage are restricted to this region, the accessibility to solvent is altered for only a selected number of ionizable residues in the trimer. A comparison of the structures of different subtype HAs shows that some of the ionizable residues that are buried by the fusion peptide after cleavage are completely conserved, while others vary strictly along clade-specific lines (Gamblin et al., 2004; Ha et al., 2002; Russell et al., 2004; Wilson et al., 1981).

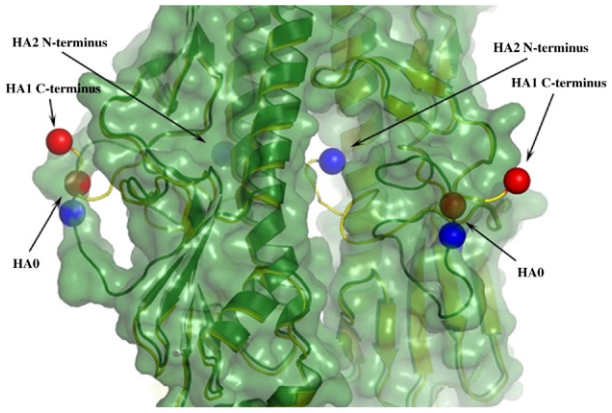

Fig. 1. Superimposed ribbon diagrams of uncleaved HA0 (green) and cleaved HA (yellow), which deviate only at positions 323–329 of HA1 and 1–12 of HA2. To highlight the structural relocations that occur upon cleavage, for two of the monomers, the positions of the C-termini of HA1 and the N-termini of HA2 are indicated by red and blue balls respectively. The structure of uncleaved HA0 is also shaded to indicate van der Waals radii. This highlights the loop structure of HA0 and the central cavity into which the N-terminal portion of the HA2 fusion peptide relocates following cleavage.

The recently determined H13 subtype HA structure (Russell et al., unpublished) now divides the HAs into five clades, and when structural characteristics of the fusion peptide region are compared the five clades can be separated into two groups as depicted in Fig. 2. The H1 group includes H1-, H9-, and H13-like viruses, and the H3 group includes the H3 and H7 clades. In all HAs HA2 residues K51, D109, and D112 are completely conserved, whereas the residues at positions HA1 17, and HA2 106, and 111 are group specific. Among H3 group HAs, such as the Aichi HA analyzed in this study, HA1 17 and HA2 106 are nearly always histidine, and HA2 111 is threonine. In the H1 group HAs, HA1 17 is tyrosine, HA2 111 is histidine, and HA2 106 is either a basic lysine or arginine residue (Fouchier et al., 2005; Kawaoka et al., 1990; Nobusawa et al., 1991; Rohm et al., 1996).

We postulate that these residues are involved in modulating the stability of cleaved HA, and that the introduction of substitution mutations at these positions will have a tendency to alter the pH at which conformational changes are induced. Changes to positions that result in increased stability (reduced pH of fusion) could possibly help identify residues that are involved in the triggering of conformational changes when protonated. To examine the possible role of specific residues in the initiation of fusion upon acidification, we generated a series of single, double, and triple amino acid substitution mutants in an H3 subtype HA. The mutant HAs were analyzed for folding, cell-surface transport, acid-induced structural changes, the pH at which they take place, and membrane fusion properties. A variety of phenotypes were detected, including examples that undergo conformational changes and mediate membrane fusion at reduced pH. A possible role for particular ionizable residues in the initiation of the fusion process is discussed. Furthermore, we compare the structures of this region among HA subtypes and discuss clade-specific differences with regard to acid-induced activation of membrane fusion.