A Histidine Switch in Hemagglutinin-Neuraminidase Triggers Paramyxovirus-Cell Membrane Fusion

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Most paramyxovirus fusion proteins require coexpression of and activation by a homotypic attachment protein, hemagglutinin-neuraminidase (HN), to promote membrane fusion. However, the molecular mechanism of the activation remains unknown. We previously showed that the incorporation of a monohistidylated lipid into F-virosome (Sendai viral envelope containing only fusion protein) enhanced its fusion to hepatocytes, suggesting that the histidine residue in the lipid accelerated membrane fusion. Therefore, we explored whether a histidine moiety in HN could similarly direct activation of the fusion protein. In membrane fusion assays, the histidine substitution mutants of HN (H247A of Sendai virus and H245A of human parainfluenza virus 3) had impaired membrane fusion promotion activity without significant changes in other biological activities. Synthetic 30-mer peptides corresponding to regions of the two HN proteins containing these histidine residues rescued the fusion promoting activity of the mutants, whereas peptides with histidine residues substituted by alanine did not. These histidine-containing peptides also activated F-virosome fusion with hepatocytes both in the presence and in the absence of mutant HN in the virosole. We provide evidence that the HN-mimicking peptides promote membrane fusion, revealing a specific histidine “switch” in HN that triggers fusion.

Sendai virus (SeV), Newcastle disease virus (NDV), human parainfluenza virus type 1 (hPIV1) to hPIV4, and several other enveloped animal and human viruses of the Paramyxoviridae family share a common receptor-ligand interaction and their mode of entry into host cells. The very first step for introgresion of their RNA genome into host cells is membrane fusion. The fusion requires a coordinated action of two envelope glycoproteins: a receptor-binding protein, hemagglutinin-neuraminidase (HN), and a fusion protein F (36). Most paramyxovirus F proteins require HN proteins from the same virus or closely related viruses for optimum membrane fusion. Although F proteins of some of the simian virus 5 strains and mutant F protein of NDV induce cell fusion independently (39), coexpression of homologous HN proteins (type specific) accentuates the fusion potential of the virus. Similarly, our previous studies established that Sendai viral envelope devoid of HN protein (F-virosomes [FV]) can fuse with liver cells in culture and in whole animals, but cografting of its HN protein in the same envelope significantly enhances the fusion activity (3, 4, 34). These findings indicate that a homotypic F-HN interaction is essential for efficient membrane fusion (41). In spite of recent biochemical, molecular, and structural investigations on HN and F proteins of paramyxovirus, the precise mechanism of HN-F interactions that leads to HN protein-mediated F protein activation and subsequent membrane fusion remains unknown (23). The detailed molecular structures of HN of NDV (13, 48) and hPIV3 (24) imply conformational changes or oligomerization of the HN protein subsequent to its interactions with host cell surface receptor. Earlier experiments using chimeric and mutant HN proteins support the involvement of both the stalk and the globular head regions of HN protein in the specific interaction with the F protein for fusion promotion at neutral pH (41, 43). Based on their experimental data, Yuan et al. proposed a model that indicated HN stalk region interacting with F for a complete membrane fusion (47). Using electron cryomicroscopy, Ludwig et al. have emphasized that the prefusion state of cleaved F protein of SeV requires stabilization by direct association with its HN protein (27). Furthermore, Lee et al. has demonstrated that some specific residues on F protein of canine distemper virus and attachment protein (H) of measles virus interact with each other for the formation of fusion active functional complexes (25). However, their assay did not pick up additional microdomains in mediating precise F-H interactions. Such studies suggest that HN appears to provide an activation signal to the F protein leading to fusion, but its exact molecular nature is still far from clear.

While developing the FV-based novel liver-specific drug/gene delivery vehicle (4, 30, 34) exploiting the high affinity of F-protein to asialoglycoprotein receptors (ASGPR) on the hepatocyte surface, we observed significant reduction in membrane fusion activity in the absence of its native attachment protein, HN. The fusion efficiency of FV increased on cografting a histidyl residue of a cationic amphiphile (L14) in the virosole membrane (L14-F-virosome) (45). It has been proposed that L14 probably activates F protein into a more fusion competent state by stabilizing the coiled-coil heptad repeats of F protein, leading to enhanced membrane fusion. Presumably, the “histidine” head group of L14 interacts with fusion primed F protein, analogous to HN-F interactions, leading to a significantly increased fusion activity of L14-F-virosome. To test this hypothesis, it is necessary to investigate the role of some hist-
tidine residue(s) of the HN protein, within its fusion promotion-domain, in their interaction with F-protein in transmitting the activation signal(s).

We attempted here to identify fusion-promoting histidine residue(s) of HN, if any, and tested whether the histidine-containing domain when present along with F-protein is able to enhance the fusion activity. To this end, a series of SeV HN mutants were prepared with histidine substituted by alanine. The fusion promotion activity was significantly decreased in H247A SeV HN mutant. A similar decrease in fusion activity was observed for the H245A mutant of hPIV3 HN. Furthermore, synthetic peptides corresponding to HN proteins containing histidine equivalent to H247 and H245 were found to rescue the fusion activity of respective HN mutants of SeV and hPIV3. The peptides also improved the fusion of FV with liver cells. Based on the results and in silico analyses, a model for HN-mimicking peptide-F interaction is proposed that demonstrates for the first time that a “histidine” residue of HN protein regulates the F protein in enhancing the membrane fusion.

MATERIALS AND METHODS

Reagents. Monoclonal anti-Sendai F and HN were available from CosmoBio Co., Ltd., Tokyo, Japan. Millipore supplied monoclonal anti-hPIV3 F and HN, RTIC (rhodamine isothiocyanate), NBD-taurine [N-(7-nitrobenzofurazan-4-yl)taurine], and R18 (octadecylrhodamine) were procured from Molecular Probes (Junction City, OR). NBD-PE (7-nitrobenzofurazan-4-yl)taurine] and R18 (octadecylrhodamine) were obtained from Molecular Probes (Junction City, OR). NBD-PE (N-4-nitrobenz-2-oxa-1,3-diazole phospha-thydylcholamine) was obtained from Avanti-Polar Lipids. All other reagents used were of analytical grade.

Cells and virus. CHO and HepG2 cells were obtained from the American Type Culture Collection and maintained in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum, 100 U of penicillin/ml, and 100 μg of streptomycin per ml at 37°C and 5% CO2. SeV, Z strain, was grown in the allantoic sac of the 10- to 11-day-old embryonated chicken eggs. The virus was harvested and purified according to standard procedures (3).

Cloning and mutagenesis of F and HN proteins. The full-length SeV HN and F genes in pGEMT were obtained as a gift from D. Kolakofsky, R. A. Lamb provided the hPIV3 HN and F cDNAs. All HN and F cDNAs were subcloned in eukaryotic expression vector pcDNA3(+) (Clontech) under cytomembranol virus promoter using BamHI/EcoRI restriction sites. Positive clones were screened by restriction mapping and confirmed by sequencing. All HN protein mutants were generated by using Stratagene’s QuikChange site-directed mutagenesis kit according to the manufacturer’s instructions. Synthetic oligonucleotid primers (from Microsynth) were used to introduce point mutation. Each mutation was confirmed by sequencing the DNA complementary to respective DNA strand.

Cell surface expression of HN and F proteins. In order to check surface HN (wild type and mutant) and F protein expression, immunofluorescence (32) and flow cytometry were used. CHO cells were plated in 35-mm tissue culture dishes at a density of 104 cells in 2 ml of Dulbecco modified Eagle medium. Subconfluent monolayers were transfected with 0.4 μg of desired DNA using Lipofectamine reagent according to the supplier’s protocol. CHO cells were transiently transfected with HN (wild type or mutant) and F cDNA. At 24 h posttransfection, cells were processed for immunofluorescence. Cells were washed with phosphate-buffered saline (PBS) twice and fixed in 2% paraformaldehyde in PBS at room temperature for 20 min. After fixing, cells were blocked with 1% bovine serum albumin in PBS for 1 h. This was followed by incubation with monoclonal mouse anti-HN or anti-F protein antibody for 1 h. After a washing step with PBS-Tween, the cells were incubated with secondary antibody—goat anti-mouse immunoglobulin G coupled to tetramethyl rhodamine isothio-cyanate (TRITC; Sigma) that could be visualized directly under fluorescence microscope. Fluorescence-activated cell sorting was performed to quantify cell surface expression of the HN wild type and mutants. Briefly, transfected cells were removed from plates with 5 mM EDTA and washed with PBS containing 2% fetal calf serum and 0.1% azide. Cells were further incubated with monoclonal anti-HN antibody for 30 min on ice. After being washed with PBS containing 0.1% azide, cells were incubated with goat anti-mouse immunoglobulin G coupled to TRITC. After three washes with PBS, the cells were subjected to flow cytometry (31). Cells transfected with vector alone and incubated with both primary and secondary antibody served as negative controls.

Design and synthesis of peptides. Two peptides, each 30 amino acids long, were designed from wild-type HN protein β1-sheet region (SeV and hPIV3 spanning H2347 and H245 residues) and named as SH and PH, respectively. Two more peptides with the histidines described above substituted by Ala were also synthesized and named SA and PA. All peptides were synthesized by the standard Fmoc (9-fluorenlymethoxy carbonyl) solid-phase method and purified to 95% purity using reversed-phase high-pressure liquid chromatography (UV, Ltd., Mumbai, India). The purity and identities of peptides were confirmed by mass spectrometry. Peptides were dissolved in diized distilled water and diluted in PBS or culture medium as required. Their overall conformation were probed by far-UV circular dichroism (CD) using a Jasco J-815 spectropolarimeter.

HAD and NA assay. Hemadsorption (HAD) activity was evaluated based on the ability of cell surface-expressed HN proteins to specifically bind erythrocytes (29). SeV and hPIV3 HN (both wild type and mutants)-transfected cells were incubated with 0.5% mouse red blood cells (RBCs) at room temperature for 30 min. After incubation, cells were washed extensively to remove unbound RBCs and viewed under an epifluorescence microscope (Nikon Eclipse TE 300) for cells with rosette of erythrocytes. The specificity of such binding was assured by detaching the RBCs in the presence of neuraminidase (NA) treatment. For quantitation of HAD activity, adsorbed erythrocytes were lysed in 50 mM NH4Cl, the lysates were clarified by centrifugation, and the absorbance was measured at 540 nm. Backgrounds obtained with cells expressing vector alone were subtracted.

NA activity was determined by the colorimetric method that detects N-acetyl-neuraminic acid released from fetuin (1). Cells were scraped 24 h after transfection, suspended in cold PBS, and lysed with 0.5% Triton X-100 for 100 min. The lysis was clarified by low-speed centrifugation, and fetuin, the substrate, was added to the supernatant. NA activity was determined by the colorimetric analysis of the released sialic acid at 549 nm. The background absorbance obtained with vector-expressing cells was subtracted.

Fusion assays. (i) Content mixing based on green and red fluorescent proteins. The abilities of the mutated HN proteins to complement the F protein in the fusion promotion were evaluated by using content mixing assay, and quantification was done by scoring the syncytia. Complete cell-cell fusion involves mixing of both the leaflet of the lipids and contents of donor and recipient cells (37). For the content mixing assay, two populations of CHO cells were used. For the first set, a cell population was cotransfected with the desired HN wild-type or mutant cDNA, along with F and enhanced green fluorescent protein (EGFP)-N1 plasmid DNA. In the second set, monolayers were transfected with Discosoma sp. red fluorescent protein (DsRed)-N1 plasmid DNA. After 24 h of transfection, EGFP-, HN-, and F cotransfected cells were treated with 5 μg of trypsin/ml (for activation of F, F, and F) and 0.22 mg of NA/ml before the addition of target cells. DsRed-expressing CHO or HepG2 cells (serving as target cell population) were lifted and overlaid on first set of cells. Cell-cell fusion was assayed in cells that showed both green fluorescence (450- to 490-nm-pore size excitation filter, 510-nm dichroic mirror filter, and low-pass 520-nm emission filter; Eclipse TE300 epifluorescence microscope [Nikon, Japan]) with a barrier filter of 510 nm and red fluorescence (BP546 excitation filter, 580-nm dichroic mirror filter, and low-pass 590-nm emission filter) with a barrier filter of 590 nm and a ×100/0.40 CF ACHRO LWD DL objective lens. Images were captured with a digital camera (Digital Sight DS-5 M [Nikon]) attached to a microscope that gave yellow color merging using the Image-Pro Plus version 5.1 (MediaCybernetics) software package as described by Sha et al. (40). No spectral overlap was observed under these conditions.

Quantification of syncytia was done by Giemsa staining (5). Cells were fixed with ice-cold methanol and stained with Giemsa solution (1:20 diluted in deionized water; Sigma) for 30 min. After incubation, the cells were washed with deionized water, and images were captured with a digital camera attached to an inverted phase-contrast microscope (Nikon, Japan) with an ×100/0.40 CF ACHRO LWD DL objective lens. The incidence of cell fusion was calculated from the ratio of the total number of nuclei in multinucleated cells to the total nuclei in 10 randomly chosen fields in which 1,000 nuclei or more were counted. Values obtained after transfection with the vector alone were subtracted.

(ii) Kinetics of lipid and content mixing during cell-cell fusion. Cell-cell fusion involves hemifusion, and a content mixing event followed sequentially. CHO cells (subconfluent monolayers) were cotransfected with HN wild type or mutant and F cDNA (SeV or hPIV3) and treated with NA and trypsin as described above. Monolayer cells were labeled with lipid (for content mixing)-loaded and NBD-taurine (for content mixing)-loaded RBCS were used to measure kinetics of membrane fusion as described previously (37). Transfected cells were incubated with labeled RBCS (R18 and NBD-taurine separately) at room temperature for 15 min to form RBC-CHO cell complexes. The unbound RBCS were removed by a wash with PBS solution.

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Attached RBC-decorated cells were then lifted from the flask with a solution of 0.5 mg of trypsin/ml and 0.2 mg of EDTA/ml, washed with cold PBS with 1.5 mM Ca\(^{2+}\), and stored on ice until use. In order to assess the initial rate of membrane fusion (both lipid and content mixing), online fusion measurements were made by using a spectrofluorimeter (FL3-22; Horiba Jobin) according to our published protocol (37). The time resolution for spectral measurements was 1 s, and the excitation and emission wavelengths were 473 and 515 nm for NBD-taurine and F, respectively. Briefly, 50 µl of the labeled RBC-CHO cell complex was placed in a cuvette containing 2 ml of PBS with 1.5 mM Ca\(^{2+}\) prewarmed to 37°C, and online data were recorded. To normalize the data, the percent fluorescence dequenching (% FDQ) at any time point was calculated according to the following equation: % FDQ = (F - F0/F = F0 × 100, where F0 and F are the fluorescence intensities at zero time and at a given time point, respectively, and F0 is the fluorescence intensity in the presence of 0.1% Triton X-100 and defined as fluorescence at “infinite” dilution of the probe (100%). The dye transfer was also examined separately by fluorescence microscopy (Nikon) with a ×40/0.55 CF ACHRO LWD DL objective lens after incubation of respective RBC-CHO cell complexes for 10 min at 37°C as described above.

(iii) Fusion kinetics of SeV FV with HepG2 cells. NBD-PE-labeled FV (NBD-PE-FV) were prepared, and its fusion in the presence of peptides with galactolipid liver cells in culture was carried out as described earlier (3, 45). Spectrofluorometric measurements of membrane fusion were performed as described above. To see the effect of SH and SA peptides on the FV-HepG2 cell fusion, NBD-PE-FV was coincubated with HepG2 cells in the presence of 10 µM SH or SA on ice for 60 min to allow binding, and then the fusion kinetics with HepG2 cells were evaluated as described above. The effect of peptides (SH/SA and PHEPA) on fusion activity was also tested by hemolysis assay and delivery of RETC-lysozyme into HepG2 (content-mixing assay) cells through NBD-PE-FV according to our published protocols (3, 45).

Preparation of mutant HN containing SeV FV (F/HNV) and effect on its fusion with HepG2 cells. NBD-PE-labeled F/HNV virosomes (NBD-PE-F/HNV) were prepared following our earlier protocol (3). The mutant HN (H274A) was expressed on the CHO cell surface as described above and purified to homogeneity (18). The recombinant mutant protein containing 15 µM SH or SA peptides was expressed in the NBD-PE-FV as described earlier (3), and its fusion in the presence of peptides (SH/SA) with HepG2 cells was studied as described above.

(iv) Effect of peptides on cell-cell fusion. To evaluate the role of SeV and hPIV3 peptides on cell-cell fusion, CHO cells were cotransfected with SeV or hP/IpV3 HN mutant, F, and EGFP-N1 cDNAs. After trypsin activation, cells were washed twice with serum-free medium containing 20 µg of soybean trypsin inhibitor/ml and then made to overlay with target cells (CHO and HepG2 cells) expressing DSRed along with different concentrations of peptide (0.001 to 15 µM). Fusion activity was monitored via content mixing and synectin assay as described earlier.

Intrinsic protein fluorescence. In order to assess any conformational changes in F protein induced by the peptides, the intrinsic protein fluorescence spectra of F V in the presence or absence of the relevant peptides were measured in a spectrofluorimeter (FL3-22). SeV FV (20 µg of F protein) were preincubated with 10 µM SH or SA on ice for 1 h. Unreacted peptides were removed by ultracentrifugation (50,000 rpm) for 1 h at 4°C. The resulting pellet was suspended in 20 µl of PBS, and emission spectra were recorded over 300 to 400 nm with excitation at 280 nm (16). The recorded spectra were subtracted from baseline spectra collected using the corresponding buffer and peptide without FV.

Limited proteolysis. Stability and conformational changes in F protein in the presence of peptides were also probed by using limited proteolysis. SeV FV (20 µg), preincubated with 10 µM SH or SA peptides, was treated with proteinase K (0.05 µg/ml) at 37°C for 30 min, and the extent of digestion was examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; reducing gel). It was further analyzed by densitometric scanning using ImageMaster total lab software (version 1.11; Amersham Pharmacia Biotech) (45). This was cross-checked by Western blot analysis using F-specific antibody.

In silico analysis. SeV HN shares ~70% sequence identity with hP/IV3 HN, and, as such, the three-dimensional structure of the latter determined by X-ray crystallography was used as a specified template (PDB ID 1V3B) for homology modeling of the former. Since SeV HN is known to be homodimeric and homotetrameric in nature (47, 48), attempt was made to model the SeV HN oligomer using the Swiss-PDB viewer (http://swissmodel.expasy.org/). For dimer modeling, the target protein sequence was submitted to SWISS-MODEL using “project move” (38), which returned the protein model and the corresponding template in PDB file format. Alternatively, homology models for each monomer of SeV HN were also obtained using chain A and chain B of hP/IV3 HN structures as templates with the help of servers such as ESPyPred3D (http://www.

RESULTS

Construction and expression of histidine mutants of HN proteins. The role of a “histidylated” lipid in promoting membrane fusion of reconstituted SeV envelope (FV) with liver cells in culture and in whole animal has been established (45). This kind of fusion enhancement is analogous to HN-mediated fusion promotion of F glycoprotein for paramyxovirus family, and so we decided to identify specific “histidine” residue(s) of the HN glycoprotein that might be involved in fusion promotion. The crystal structures of NDV HN and hP/IV3 HN (13, 24) have revealed that HN folds into a six-bladed β-propeller structure, with each blade consisting of a four-stranded β-sheet motif connected by loops. In the absence of an experimental three-dimensional structure for SeV HN, homology modeling with hP/IV3 V as a template (PDB ID 1V3B; ca. 70% identity) was used to obtain a representative dimer structure for SeV HN. The dimeric model, like other paramyxovirus HNs, is arranged in a six-bladed β-propeller structure with few histidines, either partially or completely exposed on the surface (Fig. 1A and 1A). Elaborate mutagenesis studies of surface-exposed residues in NDV HN indicate that fusion promotion domain of HN lies primarily in the first or sixth β-strand (41). Considering the importance of β1- and β6-sheets in fusion promotion and the fact that histidine residues that are amenable to solvent are more likely to influence fusion, five histidine substitution mutations of SeV HN and two histidine substitution mutations of hP/IV3 HN (Fig. 1A, B, and C) were introduced. The positions and locations of the histidines that have been mutated to alanine are shown on the model of SeV HN and crystal structure of hP/IV3 HN, as well as in the sequences for β-sheets of both SeV and hP/IV3 HN (Fig. 1A, B, and C).

Interestingly, sequence alignment of SeV and hP/IV3 HN in the β1 and β6 regions revealed conserved histidine residues at positions 247/245 and 539/538 as part of SHT and SCITH conserved sequence motifs, respectively, indicating that these residues could be significant targets for investigation (Fig. 1D).
The genes coding for HN and F proteins of SeV and hPIV3 were cloned in a cytomegalovirus promoter-driven expression vector (pcDNA) and used subsequently for construction of mutants outlined above. Protein expression was detected by indirect immunofluorescence at the cell surface at 24 h posttransfection in a majority of the cell population (>80%) (data not shown) and quantitated by flow cytometry (Table 1). All HN mutants efficiently expressed on the cell surface, and their expression levels were comparable to those of their wild-type counterparts.

The possibility also existed for residues other than histidine side chains in fusion promotion domain to influence fusion activity. Thus, a number of other substitutions of nonpolar and aromatic amino acids flanking the target histidine residue (Fig. 1C) were generated and tested for their biological activities. These included V245A, Y249A, and I251A of SeV and I243A and F247A of hPIV3. All such mutants were expressed at the cell surface with efficiencies comparable to those of their corresponding wild-type molecules (Table 2).

**Effect of mutations in HN protein on their biological activities and fusion promotion function.** (i) HAD and NA of mutant HN proteins. Before analyzing the ability of wild-type and mutant HN proteins in affecting cell-cell fusion, we checked...
their biological activities. The HN protein is known to exhibit both HAD and NA activity. In CHO cells expressing vector alone, virtually no RBC binding was observed; however, bound RBCs and HAD activities were seen in all cells expressing either SeV or hPIV3 wild-type HNs and their mutants except SeV H547A (Fig. 2). The NA activities of all of the HN proteins were critically assessed and are expressed as the percent relative activity compared to that of their wild-type counterparts (Fig. 2). In the case of SeV HN, H539A and H547A mutants exhibited much reduced NA activity (25 to 30% of the wild type) (Fig. 2A), whereas a reduction in NA activity was observed for the H538A mutant of hPIV3 HN (Fig. 2B). All other mutants showed NA activity at a par with the wild-type protein (Fig. 2). H247A, H304A, and H315A SeV HN showed HAD and NA activities equivalent to those of the wild-type proteins (Fig. 2A). The diminished activity in H539A and H547A SeV HN could be due to conformational changes introduced by mutation, which can affect catalytic residues involved in HAD or NA activities. Similarly, while H245A hPIV3 HN retained significant wild-type HAD and NA activities, H538A hPIV3 HN behaved like H539A SeV HN (Fig. 2B).

Thus, the corresponding mutants, with the positions of histidine conserved in SeV HN and hPIV3 HN (i.e., H245A/H247A and H539A/H538A), show similar results (Fig. 2) underlying a similar function for these residues.

(ii) Cell fusion activity of mutant proteins. Complete cell-cell fusion involves integration of the outer and the inner leaflet membrane lipids with concomitant mixing of aqueous contents of donor and recipient cells (37). We adopted a novel way of evaluating cell-cell fusion that involved monitoring the content mixing of EGFP-expressing CHO cells cotransfected with HN and F cDNAs overlaid with DsRed-expressing CHO cells. The fused cells appeared both red and green under fluorescence microscope and finally scored as yellow on superimposition (Fig. 3A and B). In addition, we quantified the fused cells by counting the syncytia after Giemsa staining (5). For both SeV and hPIV3, cells coexpressing wild-type HN and F formed extensive and big syncytia (Fig. 3A and B). In contrast, the SeV HN mutants H247A and H315A showed extensive fusion promoting abilities, which were indistinguishable from that of the wild type (Fig. 3A and C), and these neutral mutations are not suitable for further investigation. On the other hand, the SeV HN mutants H245A, H539A, and H547A showed significant reductions in fusion activity (5 to 10% relative activity com-

### TABLE 1. Surface expression of HN proteins

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<th>CSE</th>
<th>HAD</th>
<th>NA</th>
<th>Fusion</th>
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<td>86 ± 7</td>
<td>92 ± 5</td>
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<tr>
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<td>66 ± 7</td>
<td>79 ± 5</td>
<td>58 ± 3</td>
</tr>
<tr>
<td>I251A</td>
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<td>88 ± 6</td>
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hPIV3

<table>
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<th>Fusion</th>
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<td>87 ± 7</td>
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<td>83 ± 9</td>
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<td>68 ± 5</td>
<td>67 ± 7</td>
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<td>57 ± 9</td>
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### TABLE 2. Biological activity of other HN mutants expressed on the CHO cell surface

<table>
<thead>
<tr>
<th>Virus and mutant</th>
<th>% Activity relative to the wild type (mean ± SD)</th>
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<td></td>
<td>CSE</td>
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**a** CSE, cell surface expression.

**FIG. 2.** Biological activities of HN histidine mutants. (A) HAD and NA activity of SeV HN histidine mutants. HAD activity was determined by the ability of attachment protein to adsorb RBCs as described in Materials and Methods. The NA activity was assayed by determining the ability to cleave sialic acid from fetuin, which serves as a substrate. The data are represented as relative percentages of the HAD or NA activity of the mutant, with respect to wild-type HN treated in the same way. The results are expressed as mean ± the standard deviation for three independent experiments. (B) HAD and NA activities of hPIV3 HN histidine mutants. The data represent an average of three independent experiments; error bars represent the standard deviations (SD).
pared to that of the wild-type HN). However, H539A and H547A SeV HN mutants also showed reductions in HAD/NA activity, which in turn can affect fusion activity. Hence, these mutations are also not suitable for the present investigation. The other mutants, where nonpolar and aromatic amino acids flanking H247 (V245A, Y249A, and I251A) were substituted, had little effect on HAD and NA activities. Moreover, when coexpressed with respective wild-type F protein, these mutants had no inhibitory effects on syncytium formation or content mixing (Table 2).

In an attempt to investigate similar effects of HN mutant(s) on cell-cell fusion induced by other closely related member virus of the paramyxovirus family, hPIV3 was selected. As shown above, H245 and H538 of hPIV3 HN are conserved (Fig. 1D) and surface exposed in the crystal structure (Fig. 1B). The H245A hPIV3 HN mutant retained all biological activities except for fusion, which was reduced to 8% compared to that of the wild type, whereas the fusion activity of H538A was reduced to ca. 25% relative to wild-type fusion activity (Fig. 3B and D). This could be attributed to a significant reduction of NA activity (Fig. 2B). Note also that although H546 of hPIV3 HN aligns with that of SeV HN H547, the latter mutation leads to a reduction in both HAD and NA activity and resulted in diminished fusion activity. Furthermore, in order to confirm that the significant fusion defect observed for hPIV3 H245A was indeed due to histidine, other flanking substitution mutants in this region (i.e., I243A and F247A) were tested as well. These mutants failed to affect any of the biological activities of HN. Furthermore, in order to verify the importance of “histidine” per se in triggering F-mediated fusion, the residues H247 (of SeV HN) and H245 (of hPIV3 HN) were mutated to three other amino acids viz. I, W, and R (as shown in Table 2). All three HN mutants of SeV and hPVI3 expressed well on the CHO cell surface and retained their biological activities (HAD and NA). However, the mutants failed to activate fusion resembling their alanine counterparts. These findings together clearly indicated that fusion defects conferred by H247A mutant are due to histidine and not to any other residue(s) in the same domain.

(iii) Effect of synthetic peptides on fusion activity of HN mutants. Two 30-mer peptides, SH and PH, encompassing β1-sheet region corresponding to SeV and hPIV3 HN sequence and containing histidines equivalent to 247 and 245, respectively (Fig. 4A), along with their counterparts where histidines were replaced by alanines (SA and PA), were modeled in silico from first principles. The peptides show β-sheets similar to the ones present in their corresponding wild-type proteins, as shown for SH and SA in Fig. 4B (data not shown for PH and PA). This was further
confirmed by CD analysis of the peptides synthesized commercially (Fig. 4B). The CD spectra of SH and SA were typical of peptides containing high beta-sheet content. However, in both cases, the amount of beta-sheets were less than those in their wild-type counterparts viz. 30 to 40% according to the model and CD data compared to ca. 80% in the wild-type protein. Nonetheless, the target residues, histidine and alanine, still lie on the beta-sheets corresponding to beta-1 region in the respective proteins (Fig. 4B). The synthetic peptides were tested for their ability to rescue the fusion promotion activity of the mutated HN proteins. When these peptides were used in the cell-cell fusion assay described above, we observed dose dependence with significant (45 to 50%) enhancement of cell-cell fusion (Fig. 4C; data not shown for hPIV3), which appeared
saturated beyond 10 μM concentration (Fig. 4D and E). Similar fusion activity by SH and PH was observed in the other three mutants of SeV and hPIV3 HN (I, W, and R) as well (Fig. 5A). Thus, histidine at this location imparts unique characteristics to HN and substituting to other amino acids with diverse properties (A, I, W, and R) shows similar results. This finding further supports the importance and specificity of the histidine moiety in activating fusion by ruling out the effect of the charge, size, and hydrophobicity of the replaced amino acids. It is pertinent to state that considering the pH-independent nature of fusion exhibited by these viruses, the pK_a of histidine may not influence the observed fusion at neutral pH. It has been noticed that under certain conditions, mixing of outer leaflets of cells takes place without the formation of fusion pores, a process known as hemifusion (2). In order to determine the exact nature of the defect in HN mutant-mediated syncytium formation and to evaluate the effects of HN peptides in rescuing the fusion promotion kinetically, a dye redistribution assay (37) was performed (Fig. 7). R18 was used for membrane mixing, and NBD-taurine was used for cytosolic mixing assay. As shown in Fig. 7A and D, both SeV and hPIV3 HN mutants failed to induce lipid mixing in the presence of 10 μM SA and PA peptides. However, coinubcation of 10 μM SH/PH peptides could restore the initial rate, although not the extent of R18 redistribution (membrane mixing), to that of the wild-type HN proteins. A similar activity profile obtained in the NBD-taurine assay, except for a lag period of about 40 s at the start of the fusion event (Fig. 7B and E), confirmed these findings. The microscopic evidence presented (Fig. 7C and F) was in agreement with these kinetic events.

In light of the subtle difference in the mechanism of virus-cell fusion from that of virus-induced cell-cell fusion (3), the kinetics of FV fusion with HepG2 cells were evaluated. Data showing a fourfold increase in fusion efficiency in the presence of SH confirmed the ability of this peptide to enhance such fusion as well (Fig. 8A and B). Moreover, a content-mixing (RTIC-lysozyme transfer) profile of HepG2 cells induced by loaded FVs is in accord with the complete fusion and was activated by SH peptide (data not shown). It should be noted here that PH peptide fails to activate the fusion of FVs with HepG2 cells, as expected for a species-specific fusion. It is also important to note that SH peptide by itself could not induce membrane fusion, as evidenced by the fact that heat-treated FVs (inactive F) coinubcated with SH peptide failed to induce fusion (Fig. 8A). Also, mutant HN (H247A) reconstituted in FV completely failed to activate fusion with HepG2 cells. The SH peptide (but not the SA) could activate the fusion of F.HN(H247A)V with liver cell (Fig. 8C). The restoration of fusion in this case was ca. 50% of the fusion exhibited by F.HNV.
Peptide-induced conformational changes in F protein. The results presented thus far clearly indicate a specific physical interaction of HN protein (and its peptides) with its respective F partner, possibly through histidine residues. To investigate this further, we used fluorescence spectroscopy to probe the conformational changes in F protein in the presence of synthetic peptides (SH and SA). The alterations of intrinsic fluorescence are known to be sensitive indicators of any changes in the microenvironment of a protein due to physical interaction with partners (14). The fluorescence emission spectra of F protein showed an emission maximum at 337 nm (Fig. 9A). In the presence of SH peptide (10 μM) a considerable reduction in the intensity of the FV spectra and a hyperchromic shift in wavelength were noticed. As a control, with SA peptide the reduction in intensity was much less and, importantly, no concomitant hyperchromic shift was observed. The peptides (SH and SA) themselves showed negligible fluorescence intensity compared to the F protein. This may be an indication of a peptide-induced conformational change of F protein, resulting in its enhanced fusion activity. To further confirm this observation, limited proteolysis experiments were performed under similar conditions (Fig. 9B). This technique has been successfully used to probe changes in the structure, dynamics, and function of proteins (17). The FVs were subjected to proteinase K digestion (45) after incubation with 10 μM SH and SA peptides. SDS-PAGE analysis of proteinase K digestion of the F protein in the presence or absence of peptides, as shown in Fig. 9B, shows that F protein is significantly more resistant to proteinase K digestion in the presence of SH peptide, a finding suggestive of conformational changes in the F protein in the presence of SH peptide. As a control, it was also observed that the activity of proteinase K was unaltered in the presence of SH peptide. A corresponding Western blot analysis under similar experimental conditions provided further confirmation of conformational change leading to resistance to protease digestion (Fig. 9C).

DISCUSSION

Earlier studies with various paramyxoviruses have reported that both the stalk (15, 20, 42) and the head region (6, 44) of the HN protein are involved in fusion promotion. It has been also proposed that during the metastable prefusion state of F protein its hydrophobic fusion peptide resides within the radial...
channel and gets exposed after fusion activation (8). It is assumed that HN protein interacts with F protein through its hydrophobic surface and retains the fusion peptide in the radial channel. Upon receptor binding and subsequent conformational changes in the globular region of the HN protein, specific HN-F interaction is disrupted. This is known to be crucial in releasing the fusion peptide of F protein, leading to membrane fusion. The possible sites of HN protein involved in the functional activation of F protein were referred to as the fusion-promoting region (41). Such regions primarily consisted

FIG. 7. Kinetics of lipid mixing and aqueous content mixing in the presence of peptides. (A) Kinetics of fusion of R18-labeled RBCs (for lipid mixing) with cells coexpressing SeV HN (wild type) or H247A and homologous F protein in the presence of peptide SH or SA (10 μM). (B) Kinetics of fusion of NBD-taurine loaded RBC (for core mixing) with cells coexpressing SeV HN or H247A and F cell complex when coincubated with SH or SA. (C) Lipid mixing and core mixing monitored with dye distribution assay with R-18-labeled or NBD-taurine-loaded RBC-cell complex with cells coexpressing SeV-HN (wild type) or H247A and F protein in the presence of respective peptide SH or SA (10 μM). Bar, 50 μm. (D) Kinetics of R18-labeled RBCs with cells coexpressing hPIV3 HN (wild type) or H245A and F when coincubated in the presence of PH or PA (10 μM). (E) Kinetics of NBD-taurine-loaded RBC-cell complex with cells coexpressing hPIV3 HN (wild type) or H245A and F in the presence of PH or PA (10 μM). (F) Lipid mixing and core mixing monitored microscopically for cells coexpressing hPIV3 HN (wild type) or H245A and F when coincubated with PH and PA (10 μM). Bar, 50 μm. wt, wild type.
of HN encompassing the membrane proximal heptad repeats domain (β-sheet region). However, a specific molecular trigger on HN protein enabling the activation of F protein in catalyzing virus-cell and cell-cell fusion or the mechanistic details of HN-F interaction are yet to be elucidated. Deciphering a trigger in terms of specific amino acid residues or structural domains can be a mammoth task from first principles or rational mutagenesis or random alanine scanning, especially since the mechanism of HN-F interaction is not known. However, the suggestion that a "histidyl" moiety plays a significant role in activating F protein as evidenced from our earlier work (45) and the knowledge of predicted "fusion-promoting regions" in some HN proteins (41) could be combined together for an insight into the trigger. For such a purpose, β-sheet regions and exposed histidine residues in HN were primary targets for investigation.

In silico models of SeV HN protein, the crystal structure of hPIV3 HN, and analysis of amino acid sequences, their alignment, and homology have shown that five histidines of SeV HN and two histidines of hPIV3 remain fully or partially exposed and lie in the globular β-sheet region (Fig. 1). Among all of the target histidines that were mutated, only H247A showed a significant reduction in fusion activity while keeping HAD/NA activity unaltered, ensuring that the loss of fusion promotion activity was not due to defect in other biological activities. H245A hPIV3 HN behaved similarly, implying that His 247/245 play an important role in regulating fusion. None of the amino acid residues flanking these histidines impaired fusion when mutated, further confirming the unique role of these histidine residues.

Thus, a key histidine residue in HN (247 in SeV HN and 245 in hPIV3 HN) that regulates fusion has been identified, which lie in the first β-sheet (Fig. 1). A recent study of NDV HN has suggested that fusion promotion activity resides within the first or sixth β-sheet (41). This implies that the β1-sheet is a fusion-promoting domain, with histidine the prime site of action. Interestingly, the structures of both hPIV3 HN (24) and SeV HN reveal that the histidine residues as described above are located near the dimer interface and oriented on diagonally opposite sides relative to the interface, as represented in the model of SeV HN dimer (Fig. 10A). Various studies have shown the role of dimer interface in modulating the fusion promotion activity of HN (10, 12, 33). The X-ray crystallographic structures of NDV and hPIV3 HN suggest that upon...
binding sialic acid, the globular domain of HN undergoes minor conformational change that leads to an alteration in the association between monomers in each dimer (48). Interestingly, models of SeV HN dimer constructed in silico by two different methods (see Materials and Methods) and superimposed on each other (root-mean-square deviation = 0.7) indicated that the dimer interface allows a slight flip (Fig. 10B) such that one subunit aligns much better than the other. This suggests that there may be some sort of freedom associated with dimer interface that can support the required conformational change for fusion promotion by allowing two conformers of the dimer. Further analysis of hPIV3 HN crystal structures under different conditions (backbone alignment of PDB IDs 1V2I and 1V3B) also reveals that the monomers do have conformational flexibility enabling them to alter the dimer interface (data not shown). Thus, HN may be switched on to a fusion-promoting state through a series of conformational changes that are propagated from the sialic acid binding site to the dimer interface. Part of the dimer interface, using its conformational flexibility, may allow the fusion protein to relax and adopt its fusogenic state. Considering the role of the dimer interface in the fusion trigger process (33), the histidines at the dimer interface can very well be part of this design, since the flexibility of the monomer association results in two conformations.

FIG. 9. Peptide induced conformational change in FV. (A) Emission spectra of FV in the presence of peptide (SH or SA) and the spectra of peptides (SH and SA) alone. (B) Limited proteolysis of FV with (+) or without (−) proteinase K after pretreatment with 10 μM SH or SA as monitored by Coomassie blue-stained SDS-PAGE. Bovine serum albumin (BSA) served as a positive control. The arrow indicates the F1 fragment (45K) of F protein. (C) Western blot analysis reflecting a proteolysis pattern similar to that in panel B.

FIG. 10. Structural analysis of the importance of His247 in fusion promotion. (A) Surface representation of the dimeric model of SeV HN, color coded as in Fig. 1, showing the locations and orientations of His247 residues (green) in the two subunits. The residues are located at the dimeric interface (red) and oriented on diagonally on opposite sides relative to a plane demarking the interface. (B) Backbone alignment of the model of SeV HN obtained directly in the dimeric form (blue) with that obtained by homodimeric docking of the individual modeled monomers (gold). One subunit aligns well, while the other aligns poorly, showing that SeV HN can exist in two conformations. (C) Section of the alignment in panel B magnified to demonstrate the possible conformational changes in His247 (ball-and stick-representation). (D) Ribbon diagram of the tetrameric model of SeV HN, color coded as in Fig. 1, representing the location and orientation of histidine residues. All of the histidine residues are orientated facing the center of subunit interfaces.
ers of histidine as well (Fig. 10C), which can regulate fusion by appropriate changes in conformation.

Since biochemical studies have also shown evidence of the existence of SeV HN tetramers (47), the protein was modeled in tetrameric form as well (Fig. 10D). The location of the His 247 residues and the orientation of the dimers in the tetramer pose a striking difference from the dimer itself and represent an interesting situation. Although the two subunits in the dimer are antiparallel to each other, they are parallel in the tetramer such that all of the subunits are oriented similarly. All of the histidines are on the same side of the subunits and point in the same direction toward the center of the interfaces. They seem to be more accessible in this orientation than in the dimer and thus readily available for binding. The subunits in the dimer may simply twist to bring the histidines onto the same side in the tetramer. This feature enhances the possibilities of conformational changes required for fusion.

If His→Ala substitution mutants had impaired fusion due to a specific role of His 247/245, then it should be possible to rescue such a defect by using a “fusion promotion domain.” The β1-sheet region comprising of His 247/245 seems to be such a domain. Hence, synthetic peptides mimicking the amino acid sequences of β1-sheet of both SeV and hPIV3 HN (SH and PH, respectively) were used to test this concept. As controls, similar peptides with histidine substituted by alanine (SA and PA) were used. It is indeed interesting to observe that the small synthetic peptides (SH and PH) could significantly mimic HN protein function by restoring cell-cell fusion promotion ability of the mutants in a dose-dependent manner (Fig. 4). Moreover, these peptides seem not to compete with wild-type HN in their interaction with F protein in promoting fusion, thus indicating that full-length HN is much more potent and rapid to bring about the F protein activation than the peptides.

It is well accepted that to establish true membrane fusion per se, it is a must to check the content mixing defined by the lipid compartments comprising the membranes from two previously separated entities (50). It is evident from membrane and core mixing assays that SH and PH can achieve this objective for fusion-impaired mutants of SeV and hPIV3 HN (Fig. 7). These histidine-containing peptides restore the kinetics of lipid mixing and content mixing. The specificity and significance of the histidine residues in the peptides are reiterated by the fact that the alanine peptides cannot perform a similar rescue operation. Although the extent of recovery of fusion by the peptides is not 100%, the initial rate of fusion (till about 10 to 15 s from the onset) was almost the same. This may be taken as an indication of activation mechanisms of F proteins by the HN peptides that are similar to those of the intact HN proteins. The authenticity of these assays are also validated by a lag time (of ca. 40 s) in a core mixing assay (Fig. 7B and E) for both the virus species, as shown earlier in the case of influenza virus hemagglutinin-induced cell-cell fusion (37). More strikingly, the results in the case of the mutants support the total loss of fusion activity (both “hemifusion” and “core mixing”) despite having their normal binding and NA functions. This is in full agreement with an analogous previous report wherein a single histidine residue of the receptor binding subunit of murine leukemia virus was shown to be the key switchpoint between the receptor-induced conformation changes that expose fusion peptide and those that lead to a six-helix bundle formation (49). The importance of a single histidine residue in these two virus species (SeV and hPIV3) as a “switch” for triggering F-induced fusion may be considered novel.

To see whether fusion promotion ability of the peptides depend on the nature of interaction with target cells, the effect of peptide on SeV F-mediated cell-cell and virosome-cell fusion was evaluated using liver cells. It appeared from the results that, irrespective of initial attachment, either through F alone (ASGPR-mediated) or mutant HN/F together (dual attachment; sialic acid and ASGPR mediated) with the target cells, the peptides (SH and PH) could restore comparable magnitudes of F-mediated (of both SeV and hPIV3) core mixing process (Fig. 6B and C). From this, it is safe to conclude that the F protein can be triggered for activation by histidine moiety of HN protein leading to membrane fusion, once it binds tightly either directly through ASGPR on host cells or comes close to the target membrane with the help of its native HN attachment (45). The remarkable activation (~4-fold in both rate and extent; Fig. 8A) of the fusion of FV with HepG2 cells by the SH peptide provide further evidence. The kinetic data of fusion and results on dye redistribution (Fig. 8B) support our earlier hypothesis of histidine-induced F superfusion activity (45). Moreover, impairment of cell fusion of such mutant HN expressed in CHO cell membrane was further corroborated from recombinant virosomes containing SeV H247A HN and may be extrapolated to intact virus (Fig. 8C).

It is envisaged from Takimoto et al. (41) that NDV HN specifically interacts with its F protein in a virus type-specific manner to induce efficient membrane fusion with the identification of L224 and K536 (in the first or sixth β-sheet region) as the potential trigger residues by inducing structural change near the hydrophobic site of HN upon receptor binding. Although H247 and H245 of SeV and hPIV3 HN lie in this β1 region only, no histidine moiety was considered in the case of NDV HN that can affect such activation of F protein. It was thus worth investigating whether any exposed histidine residue(s) in this region can also function as potential trigger(s). Apart from this, there is also a report demonstrating a specific interaction of the NDV F-protein HR2 domain and the HN protein domain from amino acids 124 to 152 (the loop region preceding β550) with a histidine residue. However, the specific role of the histidine residue in fusion promotion was not examined (20). Similar HN-F interactions regulating membrane fusion involving a multiple domain of hemagglutinin protein (in the heptad repeat region) has been reported in measles virus (11). Considering these views on the direct HN-F contacts crucial for fine fusion regulation, we were encouraged to investigate by physicochemical techniques whether the SH peptide interacts with pure SeV F protein in its natural membrane environment. The specific and significant hyperchromic shift accompanying the reduction of FV fluorescence intensity (Fig. 9A) and resistance to protease digestion (Fig. 9B and C) are indicative of a significant conformational change in F protein. Such specific physical interaction(s) may be responsible for fusion promotion. It is interesting to note here that although both L14 (45) and the synthetic peptides cause similar conformational changes in F protein promoting fusion, while the larger peptide masks some protease cleavage sites resulting...
in enhanced protease resistance, L14 cannot do the same, thus reducing the resistance of F protein to protease degradation.

A proposed model (Fig. 11) attempts to summarize these overall findings, which may shed light on the possible role of histidine residue of HN protein (or the respective peptides) in triggering F protein. The mutant HN coexpressed with F could mediate binding but failed to trigger trypsin-activated F protein. On the other hand, such a mutant HN when coexpressed with respective histidine peptides along with target cells could rescue fusion activation. It is hypothesized that mutant HN, upon binding with target cells, undergoes conformational changes but is not capable of an sending appropriate trigger response, thus not allowing F protein to achieve the relaxed fusion-triggered state and membrane fusion, whereas in the presence of histidine peptides, F protein could attain the relaxed state and mediate complete membrane fusion (Fig. 11III). In a similar situation, when histidine peptides were added with FV, where F protein is in advanced fusion primed state (26), such peptides could trigger an appropriate activation signal, leading to a complete fusion process analogous to L14-mediated fusion activation (Fig. 11III). It is well known that in native virion or cells coexpressing HN (wild type) the F protein is restored in a metastable prefusion state. Upon binding to sialic receptor conformational changes within HN protein could expose the histidine residue. The potential of the specific histidine residue to attain various conformational states in the dimer or tetramer, as well as the dimer-tetramer equilibrium, can very well define the requisite conformational changes. Such an event is crucial to activate and propel the trigger response to F protein, thus permitting it to attain the relaxed fusion-triggered state followed by membrane fusion (Fig. 11I). It is well known that fusion-primed F protein exposes its N-terminal helical region, along with the DIII or DI region (19, 29, 35). Therefore, it may be concluded that the histidine-mediated activation signal is transmitted to F protein from HN or peptides to these regions.

Finally, testing recombinant SeV and hPIV3 containing H247A and H245A HN, respectively, may provide additional support for such peptide-mediated fusion activation of the F protein. Also, the efficiency of gene/drug-loaded FVs for enhanced cytosolic delivery to liver cells in vivo by SH peptide remains to be explored. Notwithstanding these, our present data establish a platform and hold promise for deciphering the detailed mechanism and specificity of HN-F interactions.

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REFERENCES

8. Chen, L., J. J. Gorman, J. McKimm-Breschkin, L. J. Lawrence, P. A. Tul-
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