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Functional Interaction of Paramyxovirus Glycoproteins: Identification of a Domain in Sendai Virus HN Which Promotes Cell Fusion

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The cell fusion activity of most paramyxoviruses requires coexpression of a fusion protein (F) and a hemagglutinin-neuraminidase protein (HN) which are derived from the same virus type. To define the domain of the HN protein which interacts with the F protein in a type-specific manner, a series of chimeric HN proteins between two different paramyxoviruses, Sendai virus (SN) and human parainfluenza virus type 3 (PI3), was constructed and coexpressed with the SN-F protein by using the vaccinia virus T7 RNA polymerase transient-expression system. Quantitative assays were used to evaluate cell surface expression as well as fusion-promoting activities of the chimeric HN molecules. A chimeric HN protein [SN(140)] containing 140 N-terminal amino acids derived from SN-HN and the remainder (432 amino acids) derived from PI3-HN was found to promote cell fusion with the SN-F protein. In contrast, a second chimeric HN with 137 amino acids from SN-HN at the N terminus could not promote fusion with SN-F, even though the protein was expressed on the cell surface. A construct in which the PI3-HN cytoplasmic tail and transmembrane domain were substituted for those of SN in the SN(140) chimera still maintained the ability to promote cell fusion. These results indicate that a region including only 82 amino acids in the extracellular domain, adjacent to the transmembrane domain of the SN-HN protein, is important for interaction with the SN-F protein and promotion of cell fusion.

Paramyxoviruses contain two membrane glycoproteins, the fusion protein (F) and the hemagglutinin-neuraminidase protein (HN). The F protein is synthesized as an uncleaved, inactive form (F_0) and is cleaved into F_1 and F_2 subunits. Cleavage of the F protein by a trypsin-like protease is required for virus infectivity (9, 27). The exposure of a new hydrophobic domain at the N terminus of the F1 subunit appears to be essential for the membrane fusion activity of the F protein (14). The other glycoprotein, HN, binds to sialic acid-containing receptors and possesses neuraminidase activity (22).

A number of previous studies have demonstrated that the cell fusion induced by paramyxovirus glycoproteins requires expression of both glycoproteins (3, 5, 7, 13, 21, 26, 32). Furthermore, the requirement for the HN protein is generally restricted to HN proteins from the same species of virus (2, 3, 7, 11, 15, 34). It has been suggested that a type-specific interaction occurs between F and HN proteins and is involved in fusion activity (7, 12). Analysis of two deletion mutants of Newcastle disease virus (NDV) HN indicated that the attachment function could be separated from the fusion-promoting activity (29). On the basis of site-directed mutagenesis, it was also reported that mutations in the transmembrane domain of the HN protein of NDV affect the tetrameric structure, attachment activity, and fusion promotion activity (19).

In the present study, we have investigated the virus type-specific fusion promotion domain in the Sendai virus (SN) HN protein. A series of chimeric HN proteins between the SN-HN protein and the human parainfluenza virus type 3 (PI3) HN protein was constructed and coexpressed with the SN-F protein. Quantitative assays were used to evaluate the levels of expression of each protein on cell surfaces, the abilities of the proteins to bind to sialic acid-containing receptors, and their activities in promoting cell fusion. The results are compared with those obtained with other paramyxovirus HN proteins (2, 4, 33).

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MATERIALS AND METHODS

Virus and cells. HeLa-T4 cells and CV1 cells were maintained in Dulbecco's modified Eagle's minimal essential medium supplemented with 10 and 5% fetal calf serum, respectively. The recombinant vaccinia virus vTF7-3 (6) and vaccinia virus strain IHD-J were kindly provided by Bernard Moss (National Institutes of Health, Bethesda, Md.). Both vaccinia virus stocks were propagated and titrated on CV-1 cells.

Recombinant plasmids carrying F, HN, and β-galactosidase genes. Recombinant plasmids carrying the SN-F and SN-HN genes (pGEM4-F and pGEM4-HN, respectively) were generously provided by Laurent Roux (University of Geneva, Geneva, Switzerland). The PI3-F and PI3-HN genes were kindly provided by Mark Galinski (Cleveland Clinic Foundation, Cleveland, Ohio) and were subcloned into the pGEM plasmid vector (35). The recombinant plasmid pG1NT7β-gal was kindly provided by Edward Berger (National Institutes of Health). All of these plasmids were transformed into *Escherichia coli* DH5α cells, and the plasmids were purified with a Qiagen Plasmid Kit according to the manufacturer's instructions (Qiagen, Chatsworth, Calif.).

Construction of the chimeric HN plasmids. All of the chimeric HN plasmids encoding various part of the SN-HN and PI3-HN genes used in this study were generated by using the overlapping PCR technique (8, 10, 35). DNA fragments of the SN-HN gene were amplified by PCR with synthetic oligonucleotide primers and with the SN-HN plasmid as the template, and DNA fragments of the PI3-HN gene were similarly amplified with the PI3-HN plasmid as the template. The resulting DNA fragments were designed to overlap at one end of the SN-HN fragment and the other end of the PI3-HN fragment. In a subsequent PCR, the two DNA fragments were used in a 5-cycle reaction without primers and an additional 25-cycle reaction with 5′-SN and 3′-PI3 primers. The final PCR products were subcloned into the pGEM-3 vector. The schematic structures of the chimeric HN proteins are shown in Fig. 1 together with those of wild-type SN-HN and PI3-HN.

Transient expression of the genes. All of the cDNAs used in this study were expressed by the vaccinia virus T7 RNA polymerase transient-expression system (6). Semiconfluent HeLa-T4 cells were infected with recombinant vaccinia virus vTF7-3 containing the T7 RNA polymerase gene at a multiplicity of infection of 1 to 2 for 1 h, and 5 μg of recombinant DNA mixed with 10 μl of Lipofectace (GIBCO-BRL, Gaithersburg, Md.) was added to the cells. Expression of the proteins was examined after 14 to 16 h posttransfection.

Isotopic labeling, immunoprecipitation, and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. The transfected HeLa-T4 cells were labeled with

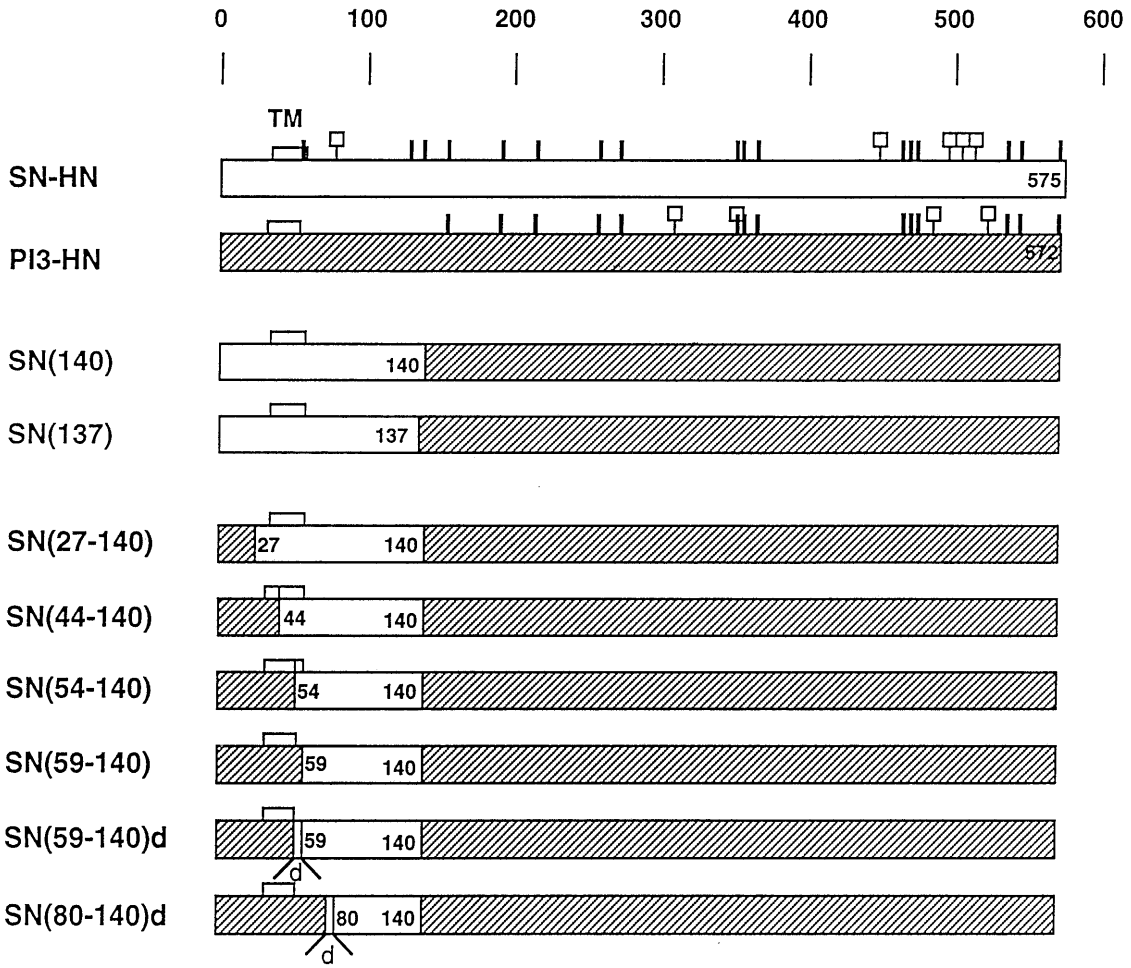


FIG. 1. Schematic diagram of SN- and PI3-HN proteins and chimeric HN proteins. The amino acid sequence numbers of the SN-HN are shown at the top. The transmembrane domain (TM), potential glycosylation sites (Ψ), and positions of cysteine residues (I) are shown for the wild-type HN proteins. Open and shaded bars indicate SN-HN and PI3-HN sequences, respectively.

methionine- and cysteine-deficient minimal essential medium containing 100 μCi of [^{35}S]methionine-cysteine ([^{35}S]Met-Cys) (Protein labeling mix; Amersham) per ml for 2 h. The radiolabeled cells were lysed with cell dissociation buffer (10 mM Tris-HCl [pH 8.0], 250 mM NaCl, 0.5% Triton X-100, and 0.5% sodium deoxycholate) (17) and clarified by centrifugation. The cell extract was mixed with SN-hyperimmune rabbit serum or PI3-hyperimmune guinea pig serum following precipitation with protein A-agarose (ImmunoPure; Pierce, Rockford, Ill.). The precipitates were analyzed on 8% acrylamide gels containing sodium dodecyl sulfate (SDS) and subjected to by fluorography.

Cell surface biotinylation analysis. To quantify the expression levels of the HN proteins on the cell surface, a biotinylation assay was performed as described previously (35). The transfected cells were biotinylated with sulfo-NHS-biotin (Pierce) and lysed with cell dissociation buffer. Immunoprecipitation and SDS-PAGE were performed as described above. The proteins were blotted onto nitrocellulose membranes and probed with horseradish peroxidase-conjugated streptavidin. After the membrane was washed, the specific proteins were detected by chemiluminescence (Amersham enhanced chemiluminescence Western blotting [immunoblotting] kit). The image on the exposed film was captured, and the bands of interest were quantitated with the National Institutes of Health image program (version 1.56).

Quantitative fusion assay. To quantitate the cell fusion activity, the fusion-dependent reporter gene activation method reported by Nussbaum et al. (24) was employed with modifications (35). Briefly, HeLa-T4 cells were infected with the recombinant vaccinia virus vTF7-3 and transfected with the SN-F plasmid together with the HN or chimeric HN recombinant plasmids. Another population of the HeLa-T4 cells was infected with wild-type vaccinia virus (IHD-J) and transfected with the plasmid pG1NT7β-gal, which contains the β-galactosidase gene under the control of the T7 promoter (24). After 14 h of incubation, the cells were mixed in the presence of trypsin (0.1 μg/ml) (type XIII; Sigma Chemical Co.) and further incubated for 5 h. Cell fusion provides transmission of T7 RNA polymerase from vTF7-3-infected cells to the pG1NT7β-gal-transfected cells, enabling expression of the β-galactosidase. The β-galactosidase produced was measured by A_{570} after addition of chlorophenyl red-β-D-galactopyranoside (CPRG) substrate. Fusion activity was expressed as a percentage of the amount of β-galactosidase production in the cells transfected with SN-F and wild-type SN-HN genes.

HAD activity. Transfected HeLa-T4 cells were incubated with 0.5% guinea pig erythrocytes for 30 min at 4°C, and unbound cells were removed by extensive washing. The cells were observed under the microscope. The bound erythrocytes were lysed with 50 mM ammonium chloride, and the eluted hemoglobin concentration (A_{459}) was determined as a measure of hemadsorption (HAD) activity (20).

RESULTS

Expression of the chimeric HN proteins. To characterize the chimeric HN proteins, the chimeric HN cDNAs were expressed in HeLa-T4 cells by using the vaccinia virus T7 RNA polymerase transient-expression system. At 15 h posttransfection, the cells were metabolically labeled with [^{35}S]Met-Cys for 2 h and lysed. The cell extracts were precipitated with anti-SN or anti-PI3 serum and subjected to SDS-PAGE and fluorography (Fig. 2). All of the chimeric HN proteins were found to be precipitated with anti-PI3 antibody (Fig. 2, lanes 4 to 11), indicating that the chimeric proteins maintain antigenic sites of the PI3-HN proteins. The electrophoretic mobilities of the

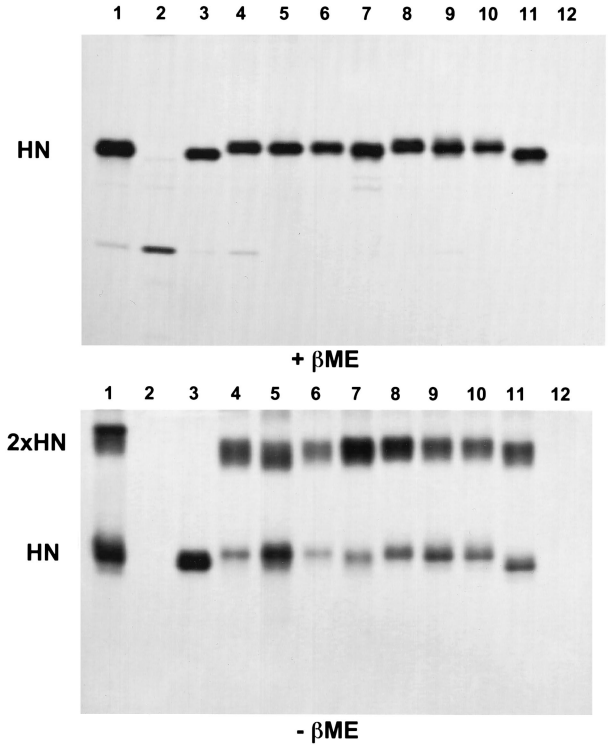


FIG. 2. Radioimmunoprecipitation analysis of the SN-HN, PI3-HN, and chimeric HN proteins expressed in HeLa-T4 cells by the vaccinia virus T7 transient-expression system. Lanes 1 and 3, precipitates from SN-HN- and PI3-HN-transfected cell extracts, respectively. Lanes 4 to 11, precipitates from cells transfected with chimeric HN SN(140), SN(137), SN(27-140), SN(44-140), SN(54-140), SN(59-140), SN(59-140)d, and SN(80-140)d, respectively. Lanes 2 and 12, cells transfected with pGEM vector DNA. Anti-SN hyperimmune serum was used in lanes 1 and 2. Lanes 3 to 12 were precipitated with anti-PI3 hyperimmune serum. The precipitated proteins were analyzed with 8% polyacrylamide gels containing SDS in the presence (+βME) or absence (−βME) of β-mercaptoethanol.

chimeric proteins were similar to that of the wild-type SN-HN (Fig. 2, top, lane 1), except for SN(80-140)d (lane 11), which lacked a potential glycosylation site (position 77 to 79) in the SN-HN sequence (Fig. 1). This observation suggests that the potential glycosylation site at this position of the SN-HN protein is actually utilized. When the chimeric proteins were analyzed by SDS-PAGE under nonreducing conditions (Fig. 2, bottom), the high-molecular-weight bands indicated as 2×HN were observed in all constructs except wild-type PI3-HN. This observation agrees with previous reports that disulfide-linked oligomerization occurs in the SN-HN proteins (16) but not in the PI3-HN proteins (13, 30). The results indicate that the ability to undergo homo-oligomerization as observed in SN-HN is maintained by the presence of the short SN-HN sequences present in the chimeric HN proteins. Comparison of the locations of cysteine residues in chimeric HNs and wild-type PI3-HN indicates that the cysteine at position 129 in the SN-HN protein (second cysteine in the SN-HN in Fig. 1) is responsible for intermolecular disulfide bond formation. This position is similar to that of a cysteine residue (position 123) identified in the formation of disulfide bonds between NDV-HN monomers (18).

Cell surface expression of the chimeric HN proteins. To examine the surface expression levels of the chimeric HNs, a surface biotinylation assay was performed. The cell surface proteins of HeLa-T4 cells infected with vTF7-3 and transfected with recombinant plasmid DNAs were biotinylated and immunoprecipitated with specific antibodies to the SN or PI3 viral proteins. After separation by SDS-PAGE, the proteins were blotted onto membranes and visualized by enhanced chemiluminescence. The membranes were exposed to X-ray films (Fig. 3), and each band of interest was quantified by densitometry analysis with the National Institutes of Health image program. The results were expressed as percentages of the value obtained for wild-type PI3-HN (Table 1). The SN(140) chimera was detected on the cell surface at a level of 68.1% of that of the PI3 wild-type HN proteins (Fig. 3, lane 5). The chimera SN(137), which differs in sequence from the SN(140) chimera by only three amino acids, was detected at a somewhat lower level (48.3%) on the cell surface (Fig. 3, lane 6). The second series of chimeric HNs with alterations in their cytoplasmic and/or transmembrane domains was also examined for cell surface expression, and it was found that they were expressed and transported efficiently to the cell surface and maintained the antigenicity of PI3-HN (Fig. 3, lanes 8 to 13) (62 to 132% of the wild-type PI3-HN level). Examination by indirect-immunofluorescence staining also showed that the all chimeric HN proteins were detected on the cell surface (data not shown).

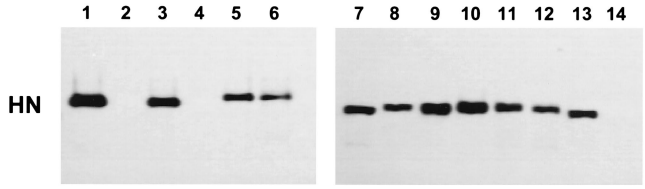


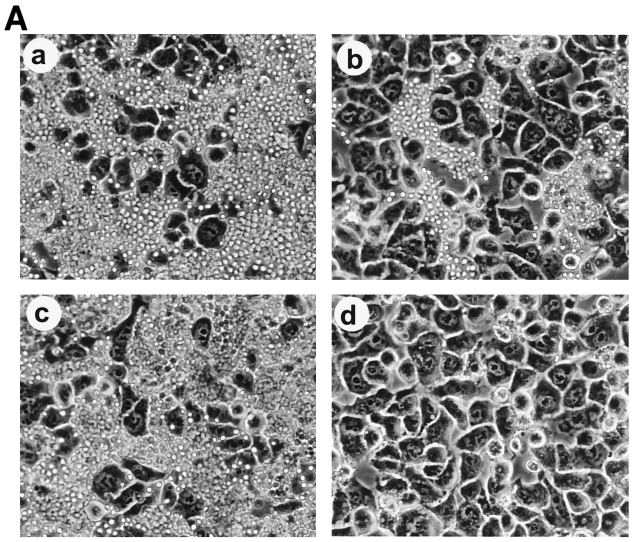
FIG. 3. Cell surface biotinylation assay of the wild-type and chimeric HN proteins. HeLa-T4 cells were transfected with various plasmids, as follows: lane 1, SN-HN; lanes 2, 4, and 14, pGEM vector; lanes 3 and 7, PI3-HN; lane 5, SN(140); lane 6, SN(137); lanes 8 to 13, SN(27-140), SN(44-140), SN(54-140), SN(59-140), SN(59-140)d, and SN(80-140)d, respectively. At 15 h posttransfection, cells were incubated with sulfo-NHS-biotin for 30 min at 4°C. Immunoprecipitation was done with anti-SN serum (lanes 1 and 2) and anti-PI3 serum (lanes 3 to 14). Membranes were probed with horseradish peroxidase-conjugated streptavidin and detected by using an enhanced chemiluminescence kit.

HAD activities of chimeric HN proteins. The HN proteins of paramyxoviruses have HAD activity as one of their biological activities, reflecting their ability to bind to sialic acid-containing receptors on erythrocytes. Representative results of the

TABLE 1. Cell surface expression and fusion-promoting activities of chimeric HN proteins

HN chimera	Cell surface expression (%)[a]	Fusion activity (%)[b]	Relative fusion-promoting activity[c]
SN-HN (wild type)	100.0	100.0	1.00
PI3-HN (wild type)	100.0	3.47	0.03
SN(140)	68.1	44.64	0.66
SN(137)	48.3	0	0
SN(27-140)	75.6	79.72	1.05
SN(44-140)	126.6	88.67	0.70
SN(54-140)	131.5	55.26	0.42
SN(59-140)	82.3	8.82	0.11
SN(59-140)d	61.6	31.33	0.51
SN(80-140)d	72.0	6.72	0.09

[a] The data were determined from Fig. 3.
[b] All HN proteins were coexpressed with SN-F. The data were determined from Fig. 5.
[c] Fusion activity divided by cell surface expression.



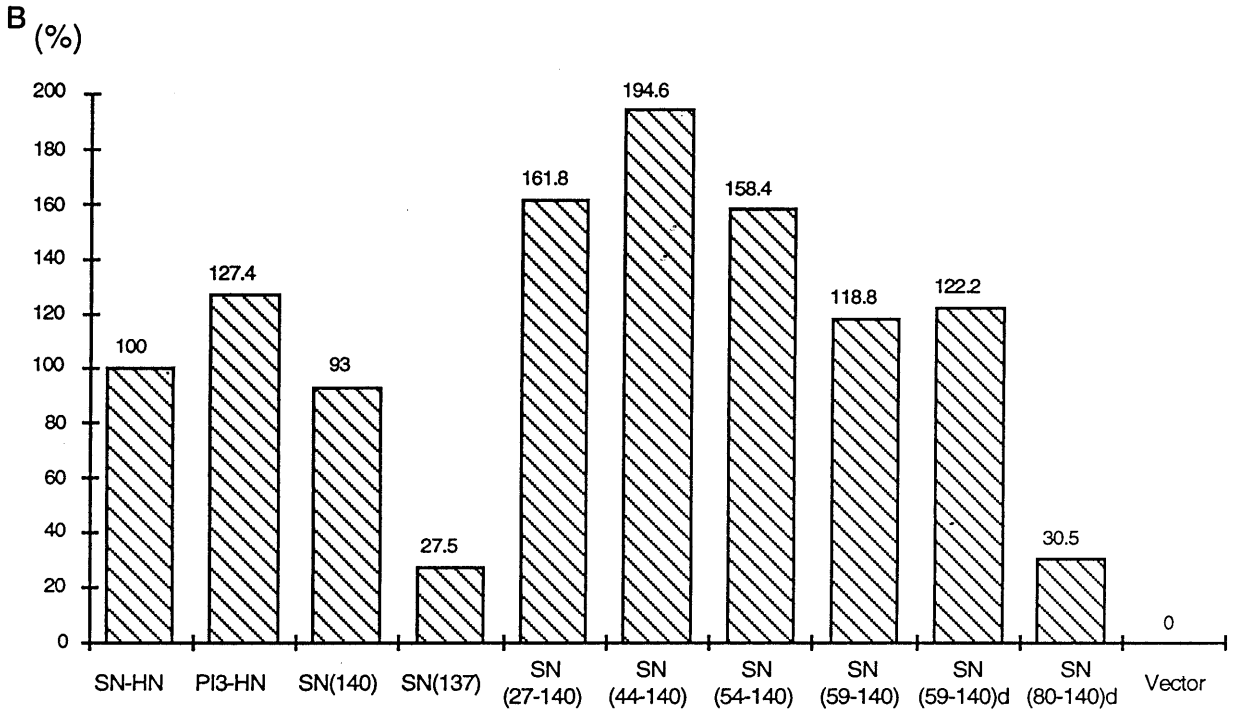


FIG. 4. HAD activities of chimeric HN proteins. The transfected cells were incubated with a 5% suspension of guinea pig erythrocytes for 30 min at 4°C. After removal of unbound cells by washing, the cells were observed with an inverted microscope. (A) Representative fields. Panels: a, SN-HN; b, SN(137); c, SN(27-140); d, vector DNA. (B) The bound erythrocytes were lysed with ammonium chloride, and the A_{549} was measured. The data are expressed as percentages of the concentration of released hemoglobin from SN-HN-expressing cells.

HAD assay are shown in Fig. 4A. The cells expressing chimeras SN(140), SN(44-140), SN(54-140), SN(59-140), and SN(59-140)d showed high levels of HAD, like wild-type SN-HN (Fig. 4A, panel a) or SN(27-140) (Fig. 4A, panel c). The chimeras SN(137) and SN(80-140)d showed lower activities (Fig. 4A, panel b). Vector DNA-transfected cells did not adsorb any guinea pig erythrocytes (Fig. 4A, panel d). To quantify the HAD activity, the bound erythrocytes were lysed with 50 mM ammonium chloride, and the A_{459} was measured. The results are shown in Fig. 4B as percentages of wild-type SN-HN HAD. The chimeras SN(140), SN(27-140), SN(44-140), SN(54-140), SN(59-140), and SN(50-140)d had HAD activity comparable to or higher than that of SN-HN. However, the chimeras SN(137) and SN(80-140)d showed reduced levels of HAD activity (27.5 and 30.5%, respectively, of that of wild-type SN-HN). These results demonstrate that all of the chimeric HN proteins were expressed on the cell surface and have detectable receptor binding activities and that the activity was not affected significantly in most of the chimeric proteins.

Fusion-promoting activities of the chimeric HN proteins. Extensive cell fusion was observed in the cell cultures which were transfected with a combination of SN-F and SN-HN and incubated with 0.1 μg of trypsin per ml. However, no cell fusion was observed with the heterologous combinations of the SN-F and PI3-HN proteins (data not shown). These results are in agreement with those reported previously for other paramyxoviruses, in which paramyxovirus-induced cell fusion was found to require coexpression of HN and F proteins de-

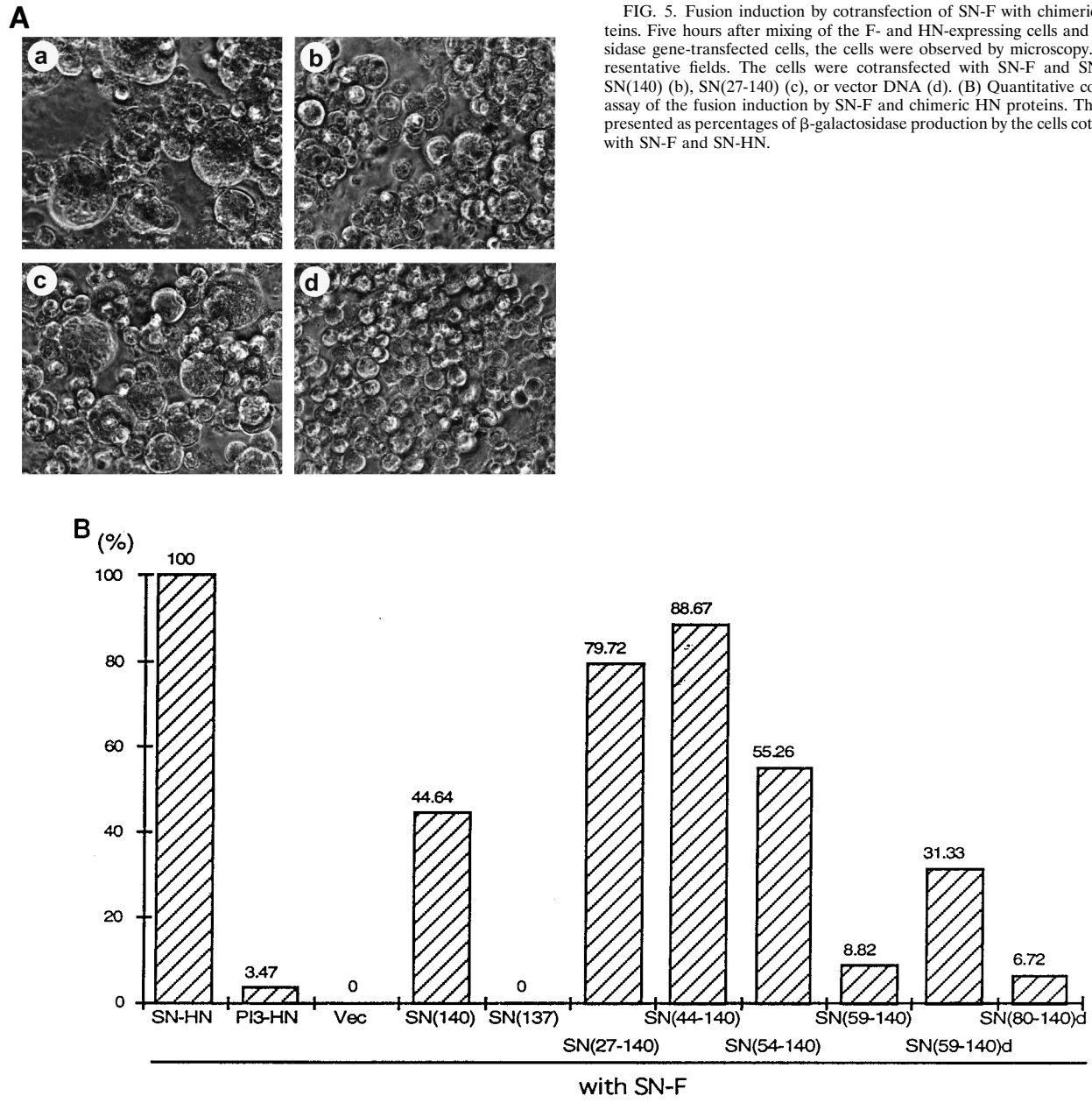


FIG. 5. Fusion induction by cotransfection of SN-F with chimeric HN proteins. Five hours after mixing of the F- and HN-expressing cells and β-galactosidase gene-transfected cells, the cells were observed by microscopy. (A) Representative fields. The cells were cotransfected with SN-F and SN-HN (a), SN(140) (b), SN(27-140) (c), or vector DNA (d). (B) Quantitative colorimetric assay of the fusion induction by SN-F and chimeric HN proteins. The data are presented as percentages of β-galactosidase production by the cells cotransfected with SN-F and SN-HN.

rived from the same virus type (7, 11, 12). To quantitatively analyze the fusion-promoting activities of the chimeric HN proteins, a fusion-dependent reporter gene activation assay was employed (24, 35). HeLa-T4 cells expressing SN-F and chimeric or wild-type HN were cocultivated with HeLa-T4 cells which were transfected with the pG1NT7β-gal plasmid. The mixed cells were incubated at 37°C for 5 h in the presence of trypsin (0.1 μg/ml) to allow cleavage of SN-F. Before extraction of β-galactosidase, the cells were observed by microscopy, and representative fields are shown in Fig. 5A. Syncytium formation was observed in the cells expressing SN(27-140)-HN and SN-F (Fig. 5A, panel c), as well as in those expressing wild-type SN-HN and SN-F (panel a). SN(140)-HN- and SN-F-expressing cells showed a lower level of syncytium formation (Fig. 5A, panel b), and the cells transfected with vector DNA and SN-F showed no syncytium formation (panel d).

The results of the colorimetric assay of β-galactosidase production showed that with a constant amount of SN-F DNA (2.5 μg), fusion activity increased progressively with the amount of SN-HN DNA added over a range of 0.1 to 5 μg (not shown). For comparison of the fusion promotion activities of chimeric HN molecules, we used 2.5 μg each of the F and HN plasmids. The data in Fig. 5B are presented as percentages of the β-galactosidase production in the cells transfected with wild-type SN-HN and SN-F. The first chimera we tested, SN(140), showed 44.6% of the wild-type level of fusion-promoting activity, but the SN(137) chimera did not show any detectable fusion-promoting activity. This result indicates that the three amino acid residues located between positions 140 and 137 are critical for the type-specific fusion promotion activity with the SN-F protein. The SN(27-140), SN(44-140), and SN(54-140) HN chimeras have segments replaced by PI3-HN sequences in

the cytoplasmic tail or cytoplasmic half of the transmembrane domain. All of these three chimeric HNs were found to promote cell fusion with SN-F, with activities of 79.7, 88.7, and 55.3% of that of the wild-type SN-HN, respectively. However, a chimera [SN(59-140)] in which the whole cytoplasmic tail and transmembrane domain of SN-HN were replaced by PI3-HN sequences showed very low activity (8.8% of that of wild-type HN). However, this construct, SN(59-140), contains a 5-amino-acid PI3 sequence in the ectodomain adjacent to the transmembrane domain (amino acid positions 54 to 58 of PI3-HN), which would alter the location of the SN-specific amino acids in relation to the membrane. To examine the possibility that this fragment may affect the structure of the chimeric HN required for promoting cell fusion of SN-F, another chimera, SN(59-140)d, was constructed, in which this 5-amino-acid PI3 sequence is deleted. The fusion promotion activity of this construct was found to be restored to 31.3% of the wild-type level. This observation indicates that the length of the external domain of HN as well as the precise amino acid sequence is important for the type-specific interaction with the F protein. The construct SN(80-140)d lost almost all fusion promotion activity (6.7%), although the protein was expressed on the cell surface and showed HAD activity (Fig. 3 and 4). The fusion activities were normalized for cell surface expression levels, and the relative fusion-promoting activities of the chimeric HN molecules are presented in Table 1. SN(140), SN(44-140), SN(54-140), and SN(59-140)d showed comparable activities, with scores of 0.42 to 0.7 (compared with SN-HN [1.00]). The activity of SN(27-140) was higher than that of the wild-type HN protein. In contrast, the SN(59-140) and SN(80-140)d constructs showed levels of only 0.11 and 0.09, respectively. All of these results indicate that the region from amino acid 59 to 140 (82 amino acids) of the HN molecule is the most important one for the promotion of cell fusion by the SN-F protein.

DISCUSSION

We have observed a requirement for coexpression of F and HN proteins for cell fusion induced by SN, which confirms previous reports (2, 31). The cell fusion activity also required that both glycoproteins be derived from the same virus type. The HN from a heterologous virus, PI3-, could not substitute for the SN-HN protein. This result agrees with previous observations with other paramyxoviruses (7, 11, 12). Recently, examples of cell fusion with some heterologous glycoprotein combinations have been reported, including SN-F with PI1-HN (2), simian virus 41 F or PI4A-F with human PI2-HN (33), and simian virus 5 F with NDV- or PI3-HN (1). These results are thought to reflect a high level of sequence similarity among the HN proteins or differences in requirements of the F proteins for induction of cell fusion.

We have constructed and expressed chimeric HN proteins between the SN-HN and PI3-HN proteins with the objective of defining the minimal segment of the SN-HN protein that will promote cell fusion in a virus type-specific manner. We observed that one chimera, SN(140), could promote cell fusion of SN-F but that a very similar chimera, SN(137), could not, even though both proteins were expressed on the cell surface and showed receptor binding activity. This result indicates that the region located between residues 140 and 137 in the SN-HN protein plays a critical role for the type-specific fusion promotion activity with the SN-F protein. Another chimeric HN, SN(59-140)d, in which the entire cytoplasmic and transmembrane domains were replaced by PI3-HN sequences, also maintained fusion promotion activity. However, SN(80-140)d, in which additional PI3-HN sequences were substituted, failed to induce cell fusion with SN-F. These results indicate that the cytoplasmic domain and transmembrane domain as well as most of the external domain can be replaced by a heterologous viral HN sequence and that a segment including 82 amino acids (position 59 to 140 of SN-HN) is the most important region for virus type-specific fusion-promoting activity of SN. This region includes a region previously identified in a deletion mutant of NDV-HN which lacked fusion promotion activity (amino acid positions 91 to 99) (28, 29) as well as the mutation site in antibody escape mutants of PI2-HN (positions 83 or 91) (36).

Recently, analysis of chimeric HN proteins for the combination of PI1 and SN was reported (2), and it was concluded that the middle 62%, which included much of the predicted globular domain, is responsible for the fusion promotion activity. In addition, the fusion promotion activities of HN mutants with mutations in a cysteine residue at position 55 located in the transmembrane region were examined (2). A Cys-to-Try mutation abolished fusion promotion activity, but a Cys-to-Gly mutation did not affect the activity. We found that a chimeric HN protein, SN(59-140)d, which lacks the cysteine residue at the corresponding position efficiently promoted cell fusion. This result demonstrates that this specific residue is not required for fusion promotion activity and that the entire transmembrane domain sequence can be functionally replaced by that of a heterologous HN.

In a recent study of chimeric HN proteins between NDV and PI3 (4), the transmembrane domain and 82 or 93 amino acids of the N-terminal ectodomain of HN were required to induce fusion with F derived from the same virus type. This portion of the ectodomain is similar to that found here for SN-HN (the first 82 amino acids in the extracellular domain), but the reported requirement for the transmembrane domain differs from our results. In another recent study, it was concluded that a region located at the membrane-proximal end of the ectodomain of PI2-HN (58 amino acids) and the N-terminal 94 amino acids of the simian virus 41 HN protein were important for type-specific fusion promotion (33). The observed differences in the requirement for the transmembrane domain and different lengths of the ectodomain may depend on the precise sequences present in the HN proteins. In addition, there may also be differences among HN proteins in the precise sequences required for fusion promotion activity.

In the first step in cell fusion, the receptor binding activity of the HN protein may bring the target cell membrane close to the effector cells (23). Since all HN proteins exhibit receptor binding activity, this step is unlikely to play a role in the type specificity of the F-HN interaction. It has been proposed that the HN protein may trigger a conformational change in the F protein which may allow the fusion peptide to insert into the target cell membrane (14). For this interaction between F and HN, the fusion-promoting domain of the HN protein may play a central role in a virus type-specific manner. Although the corresponding domain of the F protein which participates in the virus type-specific interaction has not yet been defined, it may be located in the region including the leucine zipper motif region adjacent to transmembrane domain (25).

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