Alteration of Sendai Virus Morphogenesis and Nucleocapsid Incorporation due to Mutation of Cysteine Residues of the Matrix Protein

Takemasa Sakaguchi,1* Tsuneo Uchiyama,2 Cheng Huang,1 Noriko Fukuhara,1 Katsuhiro Kiyotani,1 Yoshiyuki Nagai,3† and Tetsuya Yoshida1

Department of Bacteriology, Hiroshima University School of Medicine, Hiroshima 734-8551,1 Department of Virology, Tokushima University School of Medicine, Tokushima 770-8503,2 and AIDS Research Center, National Institute of Infectious Disease, Tokyo 162-8640,3 Japan

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The matrix (M) protein of Sendai virus (SeV) has five cysteine residues, at positions 83, 106, 158, 251, and 295. To determine the roles of the cysteine residues in viral assembly, we generated mutant M cDNA possessing a substitution to serine at one of the cysteine residues or at all of the cysteine residues. Some mutant M proteins were unstable when expressed in cultured cells, suggesting that cysteine residues affect protein stability, probably by disrupting the proper conformation. In an attempt to generate virus from cDNA, SeV M-C83S, SeV M-C106S, and SeV M-C295S were successfully recovered from cDNA, while recombinant SeVs possessing other mutations were not. SeV M-C83S and SeV M-C106S had smaller virus particles than did the wild-type SeV, whereas SeV M-C295S had larger and heterogeneously sized particles. Furthermore, SeV M-C106S had a significant amount of empty particles lacking nucleocapsids. These results indicate that a single-point mutation at a cysteine residue of the M protein affects virus morphology and nucleocapsid incorporation, showing direct involvement of the M protein in SeV assembly. Cysteine-dependent conformation of the M protein was not due to disulfide bond formation, since the cysteines were shown to be free throughout the viral life cycle.

Sendai virus (SeV), an enveloped virus with a single-stranded negative-sense RNA genome of 15,384 bases, belongs to the genus Respirovirus of the family Paramyxoviridae. The virus particle displays spherical morphology of relatively uniform size with a diameter of about 200 nm. The envelope comprises a lipid bilayer derived from the host plasma membrane and two inserted viral glycoproteins, fusion (F) and hemagglutinin-neuraminidase (HN) proteins. Lining beneath the envelope are the matrix or membrane (M) proteins. The nucleocapsid represents the internal structure, which comprises the genome RNA complexed with the nucleocapsid (N) protein and polymerase consisting of the L (large) protein and the P (phospho) protein (11).

There is increasing evidence suggesting that the M protein plays a key role in the assembly of paramyxoviruses and related RNA viruses. The M protein has been suggested to be essential to cross-link the external envelope proteins and the internal nucleocapsid. It also promotes the condensation of viral glycoproteins into a patch in the plasma membrane, an immediate precursor of the envelope (3, 7, 15). Bending of membranes may also be facilitated from the outside by glycoproteins (17, 18). We previously showed that the SeV M protein expressed from plasmid was released into the culture supernatant, as was seen in the case of the M protein of vesicular stomatitis virus (12, 22). Recently, the F protein as well as the M protein was shown to cause the budding of vesicles from cells (25). The SeV M protein as well as the F protein therefore has an intrinsic nature to be a driving force of virus budding.

The SeV M protein is 348 amino acids in length and contains five cysteine residues. Cysteines can form intrachain and interchain disulfide bonds and thereby contribute to the folding of polypeptides as well as homologous and heterologous protein-protein covalent interactions. Cysteines in various enzymes function as an active center, and those in various proteins sometimes form zinc finger motifs to bind metabolically important zinc ions (5, 13). The last function has recently been exemplified for a nonstructural protein of SeV, the V protein (8). Little is known, however, about the cysteine residues of the SeV M protein. It is unlikely that the cysteines in the M protein are intracellularly oxidized to form disulfide bonds because the M protein is localized in the cytosol, the reducing milieu. However, it is not known whether the cysteines of the M protein form disulfide bonds in virus particles in an oxidizing extracellular environment. Nevertheless, the fact that cysteine residues are well conserved in a wide variety of paramyxovirus M proteins suggests that the functions of the M proteins are important. We therefore focused on cysteine residues at the targets of site-directed mutagenesis and investigated the actual contribution of the M protein to SeV assembly.

MATERIALS AND METHODS

Cells and viruses. LLC-MK2 cells were grown in Eagle’s minimal essential medium (MEM) supplemented with 10% fetal calf serum. The wild-type SeV
(SeV wt) derived from cDNA of the Z strain and its mutants were propagated in embryonated chicken eggs, and infectivity was measured by an immunofluorescent focus assay (9) and expressed as cell infectious units (CIU)/ml.

Plasmid construction. DNA manipulation was performed basically according to a manual (2). Mutations were introduced into the plasmid pGEM-M, possessing the cDNA of the SeV M protein (20), using unique site elimination mutagenesis (4). The recombinant clones were confirmed by sequence analysis using a 310 genetic analyzer (PE Biosystems, Foster City, Calif.). The M cDNA was further transferred to the pSeV (+) plasmid, possessing the entire genomic cDNA of SeV, through three steps of subcloning as described previously (8, 20).

Protein analysis by SDS-PAGE. For analysis of virus particles, proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using an 11% gel as described previously (21) and stained with Coomassie brilliant blue R 250. Protein bands were scanned and quantitated using an FX Molecular Imager (Bio-Rad Laboratories, Hercules, Calif.).

For analysis of intracellular M proteins, subconfluent monolayers of LLC-MK2 cells in a 3.5-cm-dish were infected with vTF7.3, a recombinant vaccinia virus expressing the T7 RNA polymerase (6; a kind gift from B. Moss), and transfected with pGEM-M or its mutant plasmid. After 5 h, the cells were labeled with [35S]cysteine-methionine ([35S]Pro-mix; 3.7 MBq/ml; Amersham Pharmacia Biotech, Piscataway, N.J.) for 15 min in cysteine- and methionine-free Dulbecco’s modified MEM and solubilized in a radioimmunoprecipitation assay (RIPA) buffer (10 mM Tris-HCl [pH 7.4], 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, and 150 mM NaCl). Polypeptides were immunoprecipitated with anti-SeV serum and analyzed by SDS-PAGE. An autoradiogram was analyzed using by a BAS2000 image analyzer (Fuji Film, Tokyo, Japan).

Recovery of SeV from cDNA. SeV was recovered from the pSeV(+) plasmids as described previously (10). Briefly, LLC-MK2 cells were infected with vTF7.3 and subsequently transfected with three plasmids, pGEM-N, pGEM-P, and pGEM-L, supporting the initiation of the SeV life cycle from cDNA. After a 48-h incubation, the cell lysates were injected into embryonated chicken eggs and incubated at 35°C for 3 days. The recovered allantoic fluid, containing roughly 10° CIU of SeV/ml and 10° PFU of vTF7.3/ml, was diluted to 10⁻⁷ and inoculated again into eggs to eliminate contaminating vTF7.3.

Electron microscopy. Negative staining and electron microscopy were performed as described previously (27) with some modifications. Briefly, the virus pellet after ultracentrifugation was placed on Formvar-carbon-coated nickel grids, stained with 4% uranyl acetate, and examined under a JEOL JEM-1200EX II transmission electron microscope.

The long diameter (2a) and short diameter (2b) of a virus particle were measured on photographs, and the tentative volume (V) was calculated (V = 4/3πr³) on the assumption that a virus particle was shaped like a rugby ball. The diameter (2r) was calculated using the following equation: V = 4/3πr³. For each mutant virus, 200 to 500 particles were measured and diameters were individually estimated, and the percent distribution of the diameters was shown in a histogram.

Sucrose density gradient ultracentrifugation. Allantoic fluid was collected from SeV-inoculated embryonated eggs, clarified by low-speed centrifugation, and subsequently centrifuged at 100,000 × g for 60 min at 4°C. One milligram of the pellet was layered on the top of a 20 to 50% (wt/wt) sucrose/phosphate-buffered saline (PBS) linear density gradient and centrifuged at 24,000 rpm for 18 h in an SW40 rotor (Beckman, Palo Alto, Calif.). The tubes were then photographed, and fractions were taken with an Auto-Densi-Flow fractionator (Labconco, Kansas City, Mo.). The densities of the fractions were measured by weighing each fraction.

Modification of thiol in free cysteines. Purified virions in PBS were treated with 2 mM N-ethylmaleimide (NEM; Sigma-Aldrich, St. Louis, Mo.) or 2 mM 4-acetamide-4’-maleimidystilbene-2’,2’-disulfonic acid (AMS; Molecular Probes, Eugene, Ore.) at room temperature for 20 min in the presence of 0.1% Triton X-100. Virus particles were concentrated by centrifugation at 180,000 × g in a Beckman TL-A100 rotor to remove unreacted reagents. Polypeptides were then suspended in 2% SDS sample buffer (4% SDS, 60 mM Tris-HCl [pH 6.8], 40% [wt/vol] glycerol, and a trace amount of bromophenol blue) and analyzed by SDS-PAGE and Coomassie brilliant blue R 250 staining. Alternatively, cell lysates in the RIPA buffer, after expression of the M protein with the Vac/T7 transient expression system and metabolic labeling with [35S]cysteine-methionine, were treated with 2 mM NEM or AMS and subsequently processed for immunoprecipitation with anti-SeV antiserum.

For fluorolabeling of free cysteine residues, purified virus particles were incubated in an 80-μl reaction mixture containing 0.5 mM Oregon Green 488-maleimide (OGM; Molecular Probes), 0.1% Triton X-100, 0.3 M sodium acetate, pH 5.2, and 0.9% NaCl at room temperature for 20 min. The reaction mixture was then diluted to 500 μl with 0.9% NaCl, and virus particles were collected by ultracentrifugation. The pellet was suspended in 25 μl of 1% SDS and then boiled for 2 min. After addition of an equal amount of 2× SDS sample buffer, the sample was resolved by SDS-10% PAGE. The gel was then fixed in 10% methanol and 10% acetic acid and scanned using an FX Molecular Imager to detect fluorescent signals. Proteins were further stained with Coomassie brilliant blue R 250 and also scanned using the FX imager in a densitometric mode.

RESULTS

M proteins with mutations at cysteine residues. We introduced mutations into the cDNA of the SeV M protein to replace cysteine with serine at one of the following positions: 83, 106, 158, 251, and 295. The mutants were designated M-C83S, M-C106S, M-C158S, M-C251S, and M-C295S, respectively (Fig. 1). Another mutant, in which all five of the cysteine residues had been changed to serine residues, was created and designated M-C(--). (Fig. 1).

Proteins were expressed from the plasmids by the Vac/T7 transient expression system using the bacteriophage T7 RNA polymerase-expressing vaccinia virus (6). They were metabolically labeled with [35S]cysteine-methionine for 15 min, immunoprecipitated with anti-SeV antibody, and analyzed by SDS-PAGE. Almost equivalent amounts of the M protein were observed, and a phosphorylated form of the M protein, B protein, was also observed (Fig. 2A). A 15-min pulse-labeling and subsequent chase demonstrated that the amount of the M(--)-protein detected after the pulse decreased by 80% after a 3-h chase, indicating an impairment of stability due to the mutation (Fig. 2B). M-C158S, M-C295S, and the wild-type M protein (M wt) were quite stable and, rather, acquired antigenicity during the chase period, probably due to matura-
M-C106S, and M-C295S mutations were successfully recovered in a Vac/T7 transient expression system and pulse labeled with \[^{35}\text{S} \text{cys}\]

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M-C83S, M-C106S, and M-C251S were relatively unstable, showing a decrease in the stability of the M protein as described before (24). In contrast, proteins were expressed from cDNA in LLC-MK2 cells by using the Vac/T7 transient expression system and pulse labeled with \[^{35}\text{S} \text{cys}\].

Recovery of SeV M-cysteine mutant viruses. We next constructed the entire genomic cDNA of SeV, possessing each of the cysteine mutations described above, and tried to recover virus from the plasmids. The viruses that retained the M-C83S, M-C106S, and M-C295S mutations were successfully recovered from the eggs and designated SeV M-C83S, SeV M-C106S, and SeV M-C295S, respectively. Efficiencies of the recovery of the mutant viruses were equivalent to that of the control wild-type virus (data not shown).

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To examine protein expression in virus-infected cells, LLC-MK2 cells infected with recombinant SeV at an input multiplicity of infection of 20 were labeled with \[^{35}\text{S} \text{cys}\]-methionine for 15 min and subsequently incubated in chase medium for 0, 1.5, and 3 h. Immunoprecipitation with anti-SeV antibody (Fig. 3A) demonstrated that protein expression at 0 h postinfection is equivalent among the viruses, showing no prominent difference in the protein composition in virus-infected cells. Chase experiments and quantification of the M protein showed that M mutant proteins continuously decreased during a 3-h chase in the context of SeV infection, while the wild-type M protein once increased at a 1.5-h chase and decreased at a 3-h chase (Fig. 3B). For M-C295S, protein stability of the M protein during the chase period was different between expression from plasmid and virus infection (Fig. 2B and Fig. 3B). This may be due to factors other than protein stability, such as loss of the M protein by virus budding.

When 1,000 CIU of each recovered virus was inoculated into an egg and incubated at 33°C for 3 days, virus infectivities reached 3.3 \times 10^9 CIU/ml (mean value from three eggs) for SeV M-C83S, 1.5 \times 10^9 CIU/ml for SeV M-C106S, and 3.5 \times 10^9 CIU/ml for SeV M-C295S. These values were only two- to fourfold smaller than that of the SeV wt (6.6 \times 10^9), which was prepared similarly in parallel, indicating equivalent growth of the mutant viruses. The ratio of infectivity to hemagglutinating unit (I/H ratio) of SeV wt was 2.1 \times 10^3 (mean value from three eggs), that of SeV M-C83S was 1.3 \times 10^3, that of SeV M-C106S was 5.7 \times 10^2, and that of SeV M-C295S was 2.2 \times 10^2. The I-to-H ratio of SeV M-C106S was significantly lower than those of the other viruses (Mann-Whitney U test; P = 0.05), suggesting an impairment of infectivity relative to the particle number in this mutant population.

Protein profile of recovered virus particles. To investigate the protein profiles of the viruses, virus particles were purified from the allantoic fluid of infected eggs and analyzed by SDS-PAGE and Coomassie blue staining (Fig. 4). The density of the M protein was quantified in a gel in a reducing condition, and ratios of M to N are shown in Fig. 4. No prominent difference was found in the incorporation of the M protein into the virus particles. Careful quantification with changes in the amount of loading on the gel confirmed that there was no remarkable difference in protein profiles among the mutants and SeV wt in a reducing condition (data not shown).

In a nonreducing condition, the HN protein migrated more slowly than it did in a reducing condition due to retention of the disulfide bond to form a homodimer (Fig. 4). A disulfide-linked F1 plus F2 band was also found in this condition. Besides, the M protein migrated faster as heterogeneous bands than it did in a reducing condition. In the case of mutants, the M protein bands sometimes showed even greater heterogeneity (Fig. 4). This was notable for SeV M-C295S and SeV M-C106S. The migration patterns of the M proteins were similar between SeV M-C83S and the wild type, except that the fastest migrated band was missing in the former. These results strongly suggest that the introduced mutations damaged the conformation of the M protein in virus particles.
Alteration of size and shape of SeV virions caused by cysteine mutations. To examine virus morphology, virus particles were pelleted from the infected supernatant by ultracentrifugation, negative stained with uranyl acetate, and observed under an electron microscope (Fig. 5). SeV M-C83S appeared to have particles of similar size to those of SeV wt (Fig. 5A and B). The long and short diameters of each virus particle were measured on photographs, and the particle diameter was estimated as described in Materials and Methods. A histogram of the size distribution after measuring about 500 particles showed that the diameters of the SeV wt virions had a bell-shaped distribution with the peak at 175 to 225 nm and that the distribution of particle diameters of SeV M-C83S was similar to that of SeV wt (Fig. 6).

Virus particles of SeV M-C106S appeared to be smaller than those of SeV wt (Fig. 5C), and this was confirmed by a histogram (Fig. 6). Figure 5C also shows that there were many small vesicles with no visible nucleocapsids that were less than 100 nm in diameter in the SeV M-C106S preparation. The small vesicles appeared to have spikes on their surface. The virus preparation was then purified by equilibrium ultracentrifugation in a linear sucrose gradient. Several fractions corresponding to the virus band, judged by protein staining, were pooled and observed under an electron microscope. The diameters of these purified virions were also estimated and included in the histogram (Fig. 6, after purification). Small particles (less than 75 nm in diameter) found before the sucrose gradient ultracentrifugation were lost thereafter, indicating that SeV M-C106S generated small particles that were not banded by sucrose gradient ultracentrifugation. The presence of such vesicles was consistent with the low I/H ratio of SeV M-C106S. Such particles were also found in SeV wt and the other mutants, although their number was smaller than that of SeV wt.

FIG. 3. Expression of M protein in virus-infected cells. (A) LLC-MK2 cells were infected with SeV M-cysteine mutants, metabolically labeled with [35S]cysteine-methionine for 15 min, and subsequently incubated in a chase medium for 0, 1.5, or 3 h. Proteins were immunoprecipitated with anti-SeV antiserum and analyzed by SDS-PAGE. F0, the uncleaved precursor of the F protein. (B) Amounts of the M protein from panel A were quantitated by an image analyzer, and ratios of radioactivity compared with that after zero hours of chase are plotted. The means from two gels are plotted.

FIG. 4. Virus particles of SeV M-cysteine mutants. Virus particles were purified from the allantoic fluid of infected embryonated hen’s eggs by ultracentrifugation and analyzed by SDS–11% PAGE under a reducing condition or a nonreducing condition, followed by Coomassie blue staining. Positions of the viral proteins are shown. HN*, a disulfide-linked dimer of the HN protein; F1 and F2, disulfide-linked subunits of the F protein.
M-C106S. Furthermore, the mean diameter of the SeV M-C106S particles after purification (157 ± 46 nm [mean ± standard deviation]) was about 75% of that of the SeV wt particles (200 ± 48 nm). Thus, M-C106S gave rise to smaller virions.

From the loss of small particles by sucrose density ultracentrifugation (Fig. 6), it was expected that the small particles would be found in the top fractions in SeV M-C106S. After increasing the loaded amounts, we again performed sucrose density gradient ultracentrifugation as described above and

FIG. 5. Electron microscopy of virus particles. The allantoic fluid of infected eggs was subjected to ultracentrifugation, and the pellet was then placed on grids, negative stained with 4% uranyl acetate, and examined under a transmission electron microscope. (A) SeV wt; (B) SeV M-C83S; (C) SeV M-C106S; (D, E, and F) SeV M-C295S. Bars, 500 nm.
found a visible band around the top of the gradient. The fraction had a density of 1.09 g/ml. Electron microscopic observation demonstrated that the fraction contained many small vesicles and also some large vesicles (Fig. 7A). Proteins in the fraction contained abundant M proteins and some N proteins; the N-to-M ratio in the fraction was about 100 times lower than that in virus particles (Fig. 7B). These findings suggest that the small vesicles did not contain the RNA genome.

Electron microscopy of SeV M-C295S demonstrated that there was a large range of particle sizes in the virus (Fig. 5D). Some large particles were spherical (Fig. 5E) while others were racket shaped (Fig. 5F). Figure 6 confirmed a wide distribution of estimated diameters of particles, ranging from less than 75 nm to more than 475 nm. The mean diameter of SeV M-C295S particles was apparently larger than that of SeV wt particles.

Densities of SeV M-cysteine mutant viruses. Equilibrium ultracentrifugation in a 20 to 50% linear sucrose gradient demonstrated reproducible differences (Fig. 8). The virus particles of SeV M-C83S, whose particle sizes were found to be similar to those of SeV wt, also had a similar density (1.18 g/ml), although they banded more sharply. Virus particles of SeV M-C106S had a larger density (1.20 g/ml) than those of SeV wt did. This was probably related to the fact that SeV M-C106S had smaller particles with less lipid envelope and an equivalent amount (one copy) of the nucleocapsid. The smaller amount of light lipid was thought to make the whole virus particle heavy. SeV M-C295S banded broadly with a density of 1.18 to 1.22 g/ml (Fig. 8). This could be related to the fact that SeV M-C295S particles are highly heterogeneous in size.

Viruses possessing a cysteine-to-alanine or cysteine-to-glycine mutation. Although cysteine and serine have similar structures, there was the possibility that the introduced serine residues underwent a modification such as phosphorylation, which in turn affected viral morphogenesis. To examine this possibility, we generated recombinant SeVs whose cysteine at position 295 of the M protein was converted to alanine (A) or glycine (G). The M protein in virus particles of SeV M-C295A...
and SeV M-C295G showed phenotypes similar to that of SeV M-C295S in SDS-PAGE in a nonreducing condition and in sucrose density gradient ultracentrifugation (data not shown). Thus, the phenotypes were thought to be due to a lack of a cysteine residue and not due to the presence of a novel serine residue, at least in the case of SeV M-C295S.

Cysteine residues of the M protein are free without forming a disulfide bond with a molecular mass of 536.4 Da, shifted the M protein upward under both reducing and nonreducing conditions. Thus, there is the possibility that the distinct conformation, while M-C(−), in which all the cysteine residues were individually converted to serine residues, migrated to the position where it did in a reducing condition, clearly showing that the migration difference between the M proteins is cysteine dependent. The relatively weak signal of M-C(−) is probably due to the absence of cysteine residues in the metabolic labeling with [35S]cysteine-methionine.

NEM and AMS treatments affected the migration of M wt as observed in virions (Fig. 9A), showing that the cysteines were modified with the reagents. A slight shift and blur of the M-C(−) band in the AMS treatment is probably due to additional modification of residues other than cysteines, such as histidines. In SeV wt-infected cells, a similar phenomenon was also observed (data not shown). These results indicate that cysteine residues of the M protein are free in the cell, a reducing milieu, and that the M protein forms a specific conformation via the free cysteines.

To confirm the above results, purified virions were treated at pH 5.2 with OGM, which modifies proteins specifically at thiols, especially at low pH. Oregon Green 488 possesses a stable fluorescent nature similar to that of fluorescein isothiocyanate. After modification, proteins were resolved by SDS-PAGE and both Oregon Green 488 signals and Coomassie blue staining were scanned using an image analyzer (Fig. 9C). OGM labeling was especially prominent in the M protein as well as in the P and N proteins compared with their respective Coomassie blue stainings. These findings indicate that internal proteins, especially the M protein, have free cysteines in the molecule. Viral glycoproteins, HN and F1, were not strongly labeled with OGM, showing that their abundant cysteines had already formed disulfide bonds in the ER during synthesis and that this labeling procedure did not disrupt disulfide bonds.

The OGM labels of mutant M proteins standardized with Coomassie blue staining were 0.85 for SeV M-C83S, 0.82 for SeV M-C106S, and 0.77 for SeV M-C295S when the label of the wild-type M protein was set at 1.00. The lower values of single-cysteine knockout mutant proteins (about 80% of that of the wild-type M protein) strongly suggest that all of the five cysteine residues are free in the M protein in virus particles.

DISCUSSION

In the present study, we found that the mutated M protein at cysteine residues affected viral morphology and, in some cases, incorporation of the viral genome. Mutations at cysteine residues altered migration of the M protein in SDS-PAGE under a nonreducing condition, generating multiple bands in some cases. These findings suggest that the mutations affected protein conformation of the M protein and could generate multiple populations of molecules. SeV M-C83S was shown to generate virus particles of sizes similar to those of SeV wt. The PAGE profiles of the M proteins in the two viruses were also similar. In contrast, both the size distribution and the PAGE profile of the M protein were different from those of the wild-type M protein for either SeV M-C106S or SeV M-C295S.
It thus appears that migration of the M protein in gel electrophoresis is related to viral morphology. We therefore assumed that a mutation at these cysteine residues of the M protein caused its conformational change and that this change was responsible for the morphological alteration. M-C106S, with a distinct conformation, could generate a stronger bend of the plasma membrane by its association, resulting in the formation of smaller virus particles in the budding process. On the other hand, the regulatory mechanism for formation of a viral patch (bud) of regular size could somehow have been impaired by M-C295S, so that the size distribution became enormously broad.

The M protein of the rhabdovirus was shown to condense the nucleocapsid and bind the nucleocapsid to the plasma membrane rich in the M protein and viral glycoprotein (19). Although nucleocapsid condensation by the M protein has not been found in SeV, it has been shown that interaction between the M protein and the nucleocapsid is essential for nucleocapsid incorporation into progeny virus particles and virus assembly in SeV infection (28, 29). The mutant M protein M-C106S generated abundant small vesicles. The vesicles had a higher ratio of the M protein and lower ratios of other proteins, particularly N and P proteins. These findings indicate that fewer nucleocapsids were incorporated into the vesicles. M-C106S may be deficient in an interaction with the nucleocapsid. In the concentrated fraction of the small vesicles, larger particles (usually larger than normal virus particles) were often found. These may have been generated by fusion of the small vesicles during the procedure of concentration, since no such large vesicles were found before sucrose gradient ultracentrifugation. Alternatively, these vesicles may have been pushed to bud by an intrinsic activity of the M protein in the absence of nucleocapsids available for assembly.

The presumed cysteine-dependent conformational changes in the M protein are not thought to be related to disulfide bond formation; cysteine residues were free in both infected cells and virions. It is possible that the M protein binds heavy metals like zinc ions through free cysteine residues. Such binding has been indicated to be important for protein conformation (13). We therefore purified the M protein from virions as described before (14), and zinc ions in the preparation were analyzed by atomic absorption spectroscopy. The finding that roughly 0.5 atoms of zinc ion was in a molecule of the M protein does not strongly suggest involvement of zinc ions in the M protein conformation (data not shown). The nature of the cysteine-dependent conformation remains to be clarified.

Cysteine residues of the M protein have been found to be...
relatively well conserved among the members of the subfamily Paramyxovirinae according to the alignment of the M protein of SeV with those of other paramyxoviruses by the CLUSTALW program (26). Particularly remarkable is the perfect conservation of the cysteine residue at position 295 in all of the members of the subfamily Paramyxovirinae. The cysteine residue at position 295 may be critically important for paramyxovirus assembly in general. Our study showed that a change in this residue greatly affected the virion size of SeV and that the cysteine at 295 may be related to the size determination of virus particles. M-C158S and M-C251S did not support virus recovery. In particular, the high degree of stability of M-C158S in cultured cells suggested proper folding and conformation of the M protein.

In conclusion, the present results indicate that a single-point mutation at a cysteine residue of the M protein affects virus morphology and nucleocapsid incorporation. This structure-function analysis demonstrated that the M protein is directly involved in SeV assembly. This study has raised an issue about the involvement of free cysteine residues in structure and function of the M protein.

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