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Assignment of Disulfide Bridges in the Fusion Glycoprotein of Sendai Virus

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The mature fusion (F) glycoprotein of the paramyxovirus family consists of two disulfide-linked subunits, the N-terminal F_2 and the C-terminal F_1 subunits, and contains 10 cysteine residues which are highly conserved at specific positions. The high level of conservation strongly suggests that they are indeed disulfide linked and play important roles in the folding and functioning of the molecule. However, it has not even been clarified which cysteine residues link the F_2 and F_1 subunits. This report describes our assignment of the disulfide bridges in purified Sendai virus F glycoprotein by fragmentation of the polypeptide and isolation of cystine-containing peptides and determination of their N-terminal sequences. The data demonstrate that all of the 10 cysteine residues participate in disulfide bridges and that Cys-70, the only cysteine in F_2, and Cys-199, the most upstream cysteine in F_1, form the interchain bond. Of the remaining eight cysteine residues clustered near the transmembrane domain of F_1, the specific bridges identified are Cys-338 to Cys-347 and Cys-362 to Cys-370. Although no exact pairings between the subsequent four residues were defined, it seems likely that the most downstream, Cys-424, is linked to Cys-394, Cys-399, or Cys-401. Thus, we conclude that the cysteine-rich domain indeed contributes to the formation of a bunched structure containing at least two tandem cystine loops.

The fusion (F) glycoprotein of members of the family *Paramyxoviridae* mediates fusion between the viral envelope and the target cell membrane to initiate infection and between an infected cell and an adjacent cell to cause syncytium formation. The F glycoprotein is a type I transmembrane glycoprotein which possesses the N-terminal signal peptide and the C-terminal membrane anchoring domain. It is synthesized as an inactive precursor, F_0. The biologically active form is produced through posttranslational cleavage of F_0 into two disulfide-linked subunits, the N-terminal (extracellular) F_2 and C-terminal (transmembrane) F_1 subunits (10, 22, 23, 29, 30). The hydrophobic stretch of some 25 amino acids at the F_1 N terminus is thought to act as the direct mediator of membrane fusion (5, 11). The cleavage that occurs at the carboxyl side of a particular arginine by the action of host cell proteases is classified into two categorically different types on the basis of the cleavage site sequence motifs and the proteases utilized (6, 20, 21, 38). When the site contains multiple basic residues with an Arg-X-Lys/Arg-Arg consensus sequence, the precursors are cleaved within the constitutive secretory pathway by ubiquitous Golgi-localized proteases such as furin (8). The precursors which contain a single arginine at the site are resistant to these and are cleaved by proteases produced by and secreted from special types of epithelial cells, including factor Xa in chicken embryo cells (7, 9, 20, 26, 34) and tryptase Clara in rat lung cells (36). Thus, the proper match between the cleavage site sequence motif and the protease is of prime importance in determining whether the virus spreads efficiently throughout the body or is localized to particular tissues and organs (20, 22, 24). Also, the F glycoprotein, together with another glycoprotein, hemagglutinin-neuraminidase (HN), is important as a critical target for the host immune response.

A comparison of published F-glycoprotein sequences revealed prominent conservation of certain amino acids (18, 21). In addition to the conservation of the amino acid sequence at the cleavage site and the adjacent fusion-inducing domain, the conservation of cysteine residues at specific positions is particularly striking. One of the conserved cysteine residues is present in the F_2 subunit (positioned at Sendai virus residue 70), another is located near the fusion-inducing domain of the F_1 subunit (residue 199), and the other eight are clustered in a narrow region near the F_1 transmembrane domain, residues 338, 347, 362, 370, 394, 399, 401, and 424. In protein families, cysteine residues forming disulfide bridges are crucial for stabilization of the characteristic three-dimensional structures and are therefore generally well conserved. The high level of conservation of cysteine residues in paramyxovirus F glycoproteins suggests that they are indeed covalently linked to each other and play important structural and functional roles. In fact, correct folding of the F glycoprotein appears to be a dynamic and complex process which involves intracellular disruption and rearrangement of disulfide bonds (16, 19). Also, specific monoclonal antibodies capable of inhibiting fusion activity and neutralizing infectivity often select escape mutants with amino acid changes in the cysteine-rich domains (4, 25, 27, 37).

Despite their potential importance, however, the disulfide bonds in the paramyxovirus F glycoprotein have remained totally undefined. It has not even been clarified whether all or only some of the cysteine residues actually participate in disulfide bridge formation nor which cysteine residues function to hold the F_2 and F_1 subunits. This report describes our assignment of the disulfide bridges in the Sendai virus F glycoprotein. Our results show that all 10 cysteine residues in the mature F glycoprotein are involved in disulfide bonds.

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Also, we identified the two residues responsible for bridging of the F_2 and F_1 subunits, as well as at least two cysteine pairs in the cysteine-rich domain of the F_1 subunit.

(Part of this work was performed in fulfillment of the requirements for the doctoral thesis of A.C.S.)

MATERIALS AND METHODS

Preparation of F glycoprotein. The Sendai virus Z strain was grown in 10-day-old chicken embryos, and the virions harvested from allantoic fluid at 48 h postinfection were purified by sucrose gradient centrifugation (22). Purified virions were treated with a disruption buffer (1% Triton X-100, 1 M KCl, 20 mM phosphate buffer, pH 7.2), and the nucleocapsids were removed by centrifugation at 190,000 × *g* for 1 h. The supernatant was dialyzed against 20 mM phosphate buffer, pH 7.2, to precipitate membrane protein, which was then removed by centrifugation at 50,000 × *g* for 30 min (28). The resulting supernatant, containing mainly F and HN proteins, was subjected to anion-exchange fast protein liquid chromatography (Pharmacia Biotechnology, Uppsala, Sweden) with a Mono Q HR5/5 column (5 by 50 mm; Pharmacia Biotechnology) in a linear gradient of 160 mM to 1.6 M NaCl in 20 mM phosphate buffer, pH 7.2, containing 0.1% Triton X-100 (39). All of the fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the fractions containing F protein in pure form were collected, dialyzed against water, and lyophilized.

Detection of free cysteine and cystine residues. Disulfide bonds in F glycoprotein were detected with thiol-specific fluorogenic reagent SBD-F (ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate; Dojindo, Kumamoto, Japan) (33) as follows. An aliquot of purified F glycoprotein in 350 μl of 2.5 M borate buffer, pH 9.5, containing 4 mM EDTA, 25 μl of a 0.4-mg/ml SBD-F solution, and 500 μl of a 0.2-mg/ml dimethylacetamide solution was mixed vigorously. For one assay, a set of two tubes were used and one of them was further treated with 5 μl of tributylphosphine (Wako Pure Chemicals, Osaka, Japan) to cleave disulfide bonds completely, while another was left untreated for detection of native free cysteine residues. After each reaction mixture was heated at 60°C for 1 h and then cooled to room temperature, its fluorescence intensity was measured by excitation at 385 nm and emission at 515 nm with an RF-5000 spectrofluorophotometer (Shimadzu, Tokyo, Japan). As a reference, oxidized glutathione (Sigma Chemical Co., St. Louis, Mo.) was used.

Digestion of F glycoprotein. The F glycoprotein was cleaved by digestion with a combination of lysyl endopeptidase (Wako Pure Chemicals) and trypsin (type XIII; TPCK [*N*-tosyl-L-phenylalanyl chloromethyl ketone] treated; Sigma Chemical Co.) or with cyanogen bromide (CNBr). Prior to digestion, the samples were treated with 5 mM iodoacetamide to block any free cysteine residues. For digestion with endopeptidases, 2 mg of the protein was dissolved in 400 μl of 50 mM Tris-HCl buffer, pH 7.0, containing 8 M urea and incubated at 37°C for 1 h. The solution was diluted with an equal volume of 50 mM Tris-HCl, pH 7.0, and 0.1 mg of lysyl endopeptidase was added. After 4 h of incubation at 37°C, 800 μl of 50 mM Tris-HCl buffer, pH 7.0, and 0.1 mg of trypsin were added. After further incubation for 10 h at 37°C, the sample solution was adjusted to pH 3.0 with 1 N HCl. For CNBr digestion, 0.5 mg of the protein was treated with an excess amount of CNBr in 70% formic acid at room temperature for 24 h in the dark. Digestion was stopped by adding 9 volumes of water, and the sample was lyophilized.

Peptide separation. Peptides were separated by high-performance liquid chromatography (HPLC) with a 600 Multisolvent Delivery System (Waters, Milford, Conn.) on a Synchropak RP-4 column (4.6 by 250 mm; pore size, 300 Å [30 nm]; Synchrom, Lafayette, Ind.) with a linear gradient of 0 to 60% acetonitrile in 0.1% trifluoroacetic acid at a 1.5-ml/min flow rate. Peptides were monitored with a Waters Programmed Multiwavelength Detector at 215 and 280 nm. Further purification of peptides was performed on a μBondasphere C-18 column (3.9 by 150 mm; pore size, 300 Å; Waters, Tokyo, Japan) with the same linear gradient of acetonitrile in dilute aqueous trifluoroacetic acid at a 0.5-ml/min flow rate.

The CNBr digest was separated by SDS-PAGE under nonreducing and reducing conditions, principally with a buffer system described by Laemmli (14). The digest was first run on a 13% acrylamide gel under nonreducing conditions. The gel was fixed and stained with 0.1% Coomassie blue in 50% methanol and 10% acetic acid for 20 min and destained with 10% acetic acid and 5% methanol. Individual protein bands were excised from the gel and soaked in water for 30 min and then in the sample buffer containing 4% 2-mercaptoethanol for 30 min. Each gel piece was then placed in the well bottom of a 13% gel and subjected to a second electrophoresis under reducing conditions. For the first electrophoresis, a buffer system at neutral pH was also used to minimize disulfide exchange or rearrangement reactions (13, 17). Fragments separated by the second electrophoresis were electroblotted onto a polyvinylidene difluoride membrane (Immobilon-P; Millipore Corp., Bedford, Mass.) by the semidry blotting method with 10 mM CAPS (3-cyclohexylamino-1-propanesulfonic acid; Dojindo) buffer, pH 11, containing 10% methanol (15). The membrane was stained with 0.1% Coomassie blue in 40% methanol and 1% acetic acid and destained with 50% methanol. Each protein band was subjected to amino acid sequence analysis.

Determination of amino acid composition and amino acid sequence. An aliquot of each peptide obtained by HPLC was hydrolyzed in 6 N HCl at 110°C for 15 h and analyzed with an L-8500 Amino Acid Analyzer (Hitachi, Tokyo, Japan). Cystine- or cysteine-containing peptides thus identified were subjected to sequence determination with an ABI 470A or 473A Protein Sequencer connected to a 120A PTH Analyzer (Applied Biosystems, Foster City, Calif.). CNBr fragments on polyvinylidene difluoride membrane were also analyzed with an ABI 473A Protein Sequencer.

Western immunoblotting. To detect the F_2 subunit on polyvinylidene difluoride membrane, Western blotting was performed with a rabbit antibody (F2C12) directed to the C terminus of the F_2 subunit (35). Peroxidase-conjugated anti-rabbit immunoglobulin G (Tago, Burlingame, Calif.) was used as the second antibody to visualize the F_2 band.

RESULTS

Isolation of Sendai virus F glycoproteins from virions. Purified virions were solubilized with Triton X-100 detergent, and the fraction containing the F and HN glycoproteins was subjected to fast protein liquid chromatography to separate these two glycoproteins. The F glycoprotein was eluted and recovered in several fractions around 0.4 M NaCl. These fractions were pooled, dialyzed against water, dried, and used as the starting material for further analysis. The F glycoprotein thus obtained was satisfactorily pure, giving a single band of 68 kDa in SDS-PAGE under nonreducing conditions (Fig. 1) and 16-kDa F_2 and 56-kDa F_1 bands under reducing conditions (data not shown).

Detection of disulfide bonds in F glycoprotein. Although

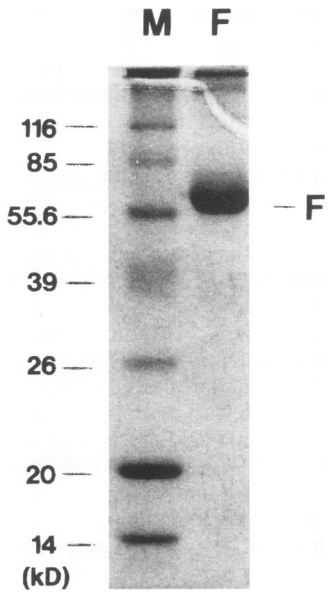

FIG. 1. SDS-PAGE, under nonreducing conditions, of the Sendai virus F glycoprotein isolated from virions. Lane M contained molecular mass markers.

there are 12 cysteine residues in the amino acid sequence deduced from the Sendai virus F gene nucleotide sequence, two of them are within the N-terminal signal peptide that is cotranslationally cleaved off (1). No other processing to remove cysteine residues takes place. Thus, 10 cysteine residues should be present in the mature F glycoprotein, and their positions are highly conserved in the paramyxovirus family. The cysteine and cystine contents of the F glycoprotein were assayed by using thiol-specific fluorogenic reagent SBD-F. As shown in Fig. 2, there was a remarkable linear elevation of the fluorescence intensity with increasing amounts of the protein reduced with tributylphosphine. In contrast, no such elevation of fluorescence was observed with increasing amounts of the

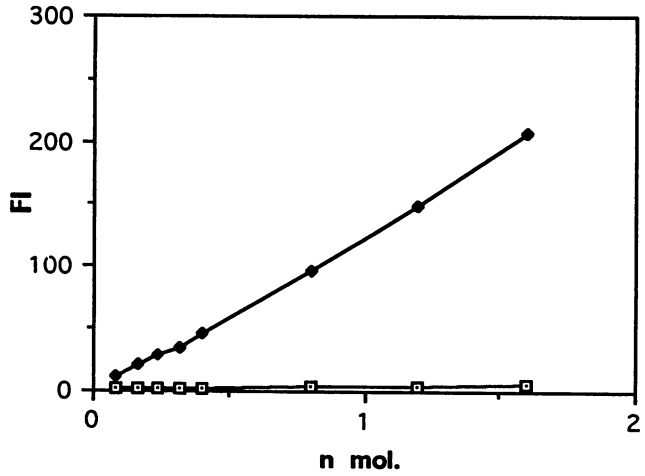

FIG. 2. Detection of free cysteine and cystine residues in F glycoprotein by using fluorescent probe SBD-F. F glycoprotein was treated with tributylphosphine (◆) to cleave disulfide bonds or left untreated (⊡) for detection of native free cysteine residues. FI, fluorescence intensity.

sample without tributylphosphine treatment. Thus, all 10 cysteine residues appeared to form disulfide bridges, without free cysteine residues, in the native F molecule.

Identification of intramolecular disulfide bonds in the F_1 subunit. Although there appeared to be no free cysteine residue, the sample was treated with iodoacetamide to minimize disulfide exchange reactions with free cysteine residues which might be present in contaminating proteins. In a preliminary experiment, we examined a number of different endopeptidases to generate fragments adequate in size and number for separation by HPLC. Acidic or neutral pH was used, because basic conditions might cause disulfide exchange reactions. The results indicated that the native F glycoprotein was resistant to most of the endopeptidases used, yielding highly insoluble products. However, pretreatment of the protein with 8 M urea and combination of lysyl endopeptidase and trypsin at a high enzyme-to-substrate weight ratio (1:20) yielded a satisfactory result. A digest with these endopeptidases was separated by HPLC. The elution profile was monitored by A_{215}. The huge peaks at the beginning and around 70 min (Fig. 3) represented urea and the detergent Triton X-100 in the sample solution, respectively. Satisfactorily high peaks, whose total number reached nearly 70, were all collected, and an aliquot of each peak was subjected to amino acid analysis. The results revealed that peaks 29, 55, and 67 contained cystine residues. These peaks were then subjected to N-terminal sequence analysis.

Peak 55 gave a sequence of Ala/Leu-Ser/Thr-Phe/Tyr-Leu/Ile-Gly/X-Gly/Pro-Ala/Arg (where X was undetermined), giving rise to two different residues in each cycle. By referring to the amino acid sequence deduced from the Sendai virus F gene nucleotide sequence (1, 32), this peak was determined to consist of two disulfide-linked peptides, Ala-327–Ser–Phe–Leu–Gly–Gly–Ala and Leu-343–Thr–Tyr–Ile–Cys–Pro–Arg (Fig. 4). In these peptides, Arg-342 and Arg-349 must be the C termini, as indicated by the substrate specificity of lysyl endopeptidase and trypsin. This assumption was supported by the amino acid composition of peak 55 (data not shown). Thus, it was concluded that Cys-338 and Cys-347 are covalently linked. Similarly, the linkage between Cys-362 and Cys-370 was identified by analysis of peak 29 (Fig. 4). In this case, however, the peak had to be further purified by HPLC on a μBondasphere C-18 column prior to sequence analysis. The sequencing protocol used was unable to distinguish phenylthiohydantoin-cystine from phenylthiohydantoin-tyrosine. Nevertheless, there were no ambiguities in the interpretation of the sequence data. The predicted C termini of the peptides beginning with Cys-362 and Cys-370 were Arg-369 and Lys-374, respectively (Fig. 4). Peak 67 yielded the sequence Phe/Gly-Ala/Val-Phe/Val-Val/Phe-Asn/Leu, indicating that the peak contained two disulfide-linked peptides, beginning with Phe-383 and Gly-415 and ending with Arg-405 and Arg-438, respectively (Fig. 4). There are three cysteine residues in the former peptide, Cys-394, Cys-399, and Cys-401, and one in the latter, Cys-424, which must participate in two-disulfide bridge formation as described above. Thus, Cys-424 appeared to be linked to one of the three cysteine residues in the former peptide (Fig. 4). To define which is the case, we needed to cleave between cysteine residues in the former peptide but were unsuccessful, even by using treatment with 12 N HCl at room temperature overnight. Thus, pairing of these four cysteine residues remains to be determined.

Identification of the intermolecular disulfide bond between the F_2 and F_1 subunits. We were able to identify eight cysteine residues participating in disulfide bridge formation in the F_1 subunit, but none of them was linked to Cys-70 in the F_2

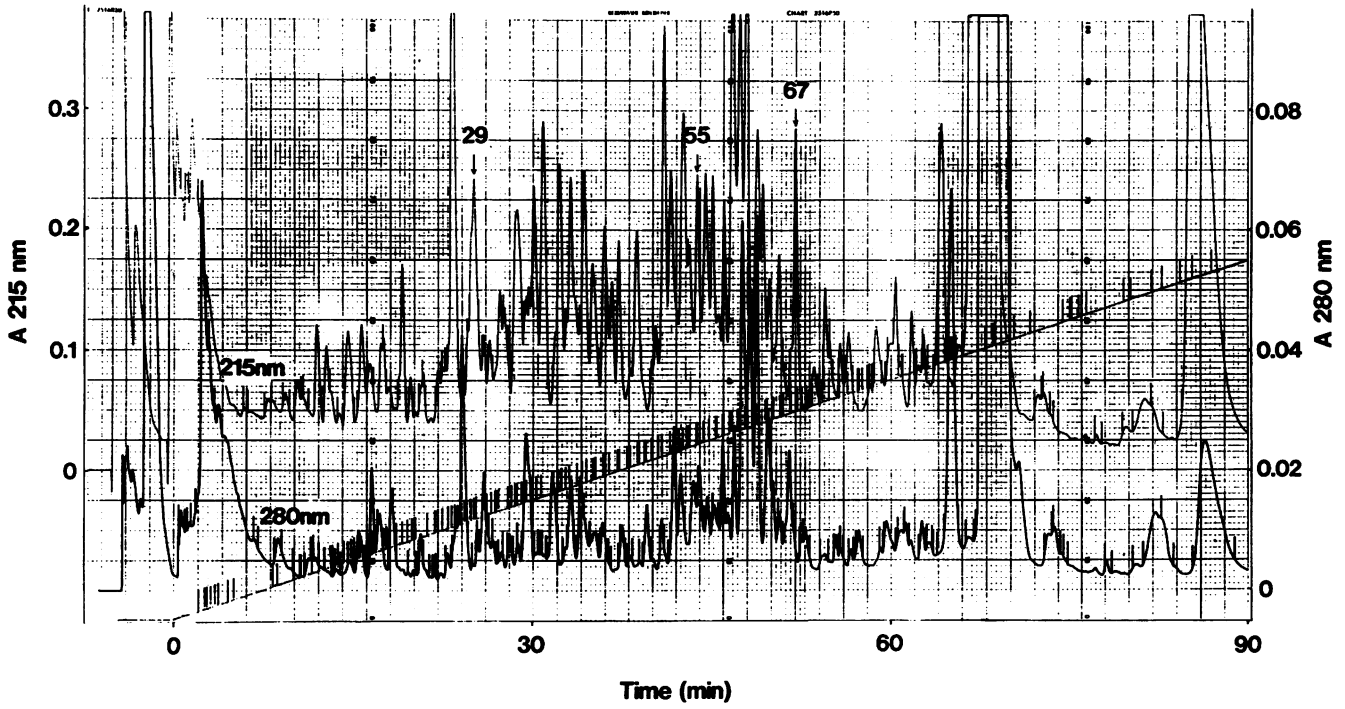

FIG. 3. HPLC elution profile of peptides obtained by digestion of F glycoprotein with lysyl endopeptidase and trypsin. Peptides were applied to a Syncropak RP-4 column and eluted with a linear gradient of 0 to 60% acetonitrile in 0.1% trifluoroacetic acid as shown by the straight, angled line climbing from the left to the right with the vertical ticks representing sampling into test tubes. The peptides were detected by A_{215}. Monitoring was also done at 280 nm to identify peptides containing aromatic amino acids. Peaks 29, 55, and 67 contained cystine residues.

subunit. It was therefore most likely that the intermolecular disulfide bond was formed between Cys-70 and Cys-199. We made several attempts to prove this and found that CNBr treatment of the intact F glycoprotein could yield an appropriate fragment. When the F glycoprotein was treated with CNBr, the digest showed very low solubility. However, by SDS-PAGE under nonreducing conditions, the digest was separated into three major protein bands, A1, A2, and A3 (Fig. 5). After blotting onto a polyvinylidene difluoride membrane, A1 gave the sequence Phe-Phe-Gly-Ala-Val, which was identical to the N terminus of the F_1 subunit (Fig. 4). The N-terminal sequence of both A2 and A3 was found to be Val-283–Thr–Leu–Ser–Val. Taking into account their apparent molecular masses and the positions of methionine residues in the primary sequence, A3 was regarded as a nonspecific degradation product of A2. The three major bands were treated with reducing agent and applied to the second gel under reducing conditions. Compared with the nonreducing gel, the mobilities of A2 and A3 did not change; they migrated as single bands B2 and B3, respectively (data not shown). On the other hand, A1 was separated into two major bands, B1-2 and B1-3, and a faint minor band, B1-1 (Fig. 5). The N-terminal sequences and electrophoretic mobilities of B1-1 and A1 were found to be identical and perhaps B1-1 is the remnant due to incomplete cleavage of the disulfide bond under reducing electrophoresis conditions. The apparent molecular masses of B1-2 and B1-3 were 21 and 16 kDa, respectively, much lower than the 37 kDa of A1 or B1-1, indicating that B1-2 and B1-3 are disulfide bonded to form A1. B1-2 was again shown to begin with the sequence Phe-Phe-Gly-Ala-Val and thus was assumed to be the N-terminal fragment of F_1. The apparent molecular mass of B1-2 (21 kDa) suggested that this fragment terminates at Met-282. There are two more methionine residues between

Fraction 29	^{362}Cys-Ile-Leu-Gly-Asp- - -^{369}Arg ^{370}Cys-Pro-Val-Thr-^{374}Lys
Fraction 55	^{327}Ala-Ser-Phe-Leu-Gly-Gly-Ala- - -^{338}Cys- - -^{342}Arg ^{343}Leu-Thr-Tyr-Ile-^{347}Cys-^{349}Pro-Arg
Fraction 67	^{383}Phe-Ala-Phe-Val-Asn- - -^{394}Cys- - -^{399}Cys- -^{401}Cys- - -^{405}Arg ^{415}Gly-Val-Val-Phe-Leu- - -^{424}Cys- - -^{438}Arg
Band A1	^{26}Gln-Ile-Pro-Arg-Asp- - - - - - - -^{70}Cys- - - - - - - -^{116}Arg ^{117}Phe-Phe-Gly-Ala-Val- - - - - - - -^{199}Cys- - - - - - - -^{282}Met

FIG. 4. Assignment of amino acid sequences of peptides contained in peaks 29, 55, and 67 of Fig. 3 and in band A1 of Fig. 5A. Residue numbers correspond to those of the Sendai virus F glycoprotein amino acid sequence deduced from the nucleotide sequence. Sequences underlined were chemically determined in the present study. Cysteine residues of which the pairing was unequivocally defined are linked by solid lines. All of the four cysteine residues in peak 67 also participate in disulfide bonds, but the exact pairing was not defined. For details, see the text.

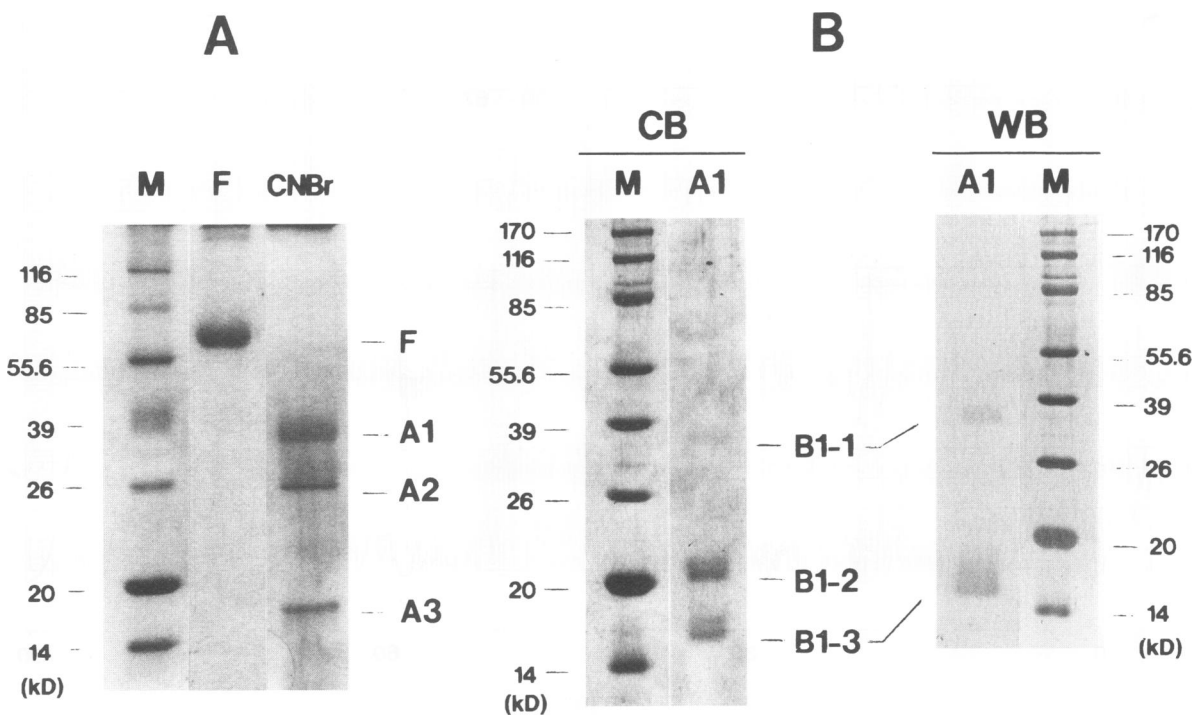

FIG. 5. (A) SDS-PAGE, under nonreducing conditions, of the intact F glycoprotein and its CNBr digest. Gels were stained by Coomassie blue. (B) Coomassie blue (CB) staining and Western blotting (WB) of SDS-PAGE of A1 under reducing conditions. Western blotting was performed with antibody F2C12, which is highly specific for the C terminus of F_2 (35). Lanes M contained molecular mass markers.

Phe-117 and Met-282, but they were not cleaved because both of them form a Met-Thr bond which is hardly cleavable by CNBr. No significant signal was observed for B1-3 by Edman degradation, suggesting that B1-3 is identical to residues 26 to 116 of the F_2 subunit with a blocked N terminus (Gln-26) (1, 5), because no methionine residues are present in the region from Gln-26 through Arg-116 of F_2 and because the apparent molecular mass of B1-3 (16 kDa) is very close to that of F_2. This was unequivocally demonstrated by Western blotting with an antibody highly specific for the C terminus of F_2 (Fig. 5). This immunoblotting further confirmed the presence of F_2 in B1-1 (Fig. 5), as well as in A1 (data not shown). Taken together, these results indicated that the two peptides Gln-26–Ile to Ser–Arg-116 and Phe-117–Phe to Tyr–Met-282 are linked by Cys-70 and Cys-199.

DISCUSSION

The F glycoprotein of paramyxovirus not only plays a crucial role in initiation of infection, spread of the virus in the host, and the immune response but also is important as a mediator of membrane fusion, a widely occurring biological event. To understand these pathological and biological processes further, we need to learn more about the structural features characteristic of the glycoprotein and their relevance to biological function. Since most cysteine residues are highly conserved in the F glycoproteins and could be structurally and functionally important (18, 21), assignment of disulfide bridges has been emphasized as one of the most important, if laborious, tasks to be performed. Here, we have been able to assign the cysteine residues participating in the covalent linking between the F_2 and F_1 subunits and two disulfide bridges in the cysteine-rich domain in the F_1 subunit. Although the remaining four cysteine residues in the C-terminal region were also found to participate in disulfide bridge formation, it has been difficult to assign their pairing. This appears to be due partly to the fact that they occur too close to be separated into different fragments. Figure 6 schematically shows the locations of assigned disulfide bridges relative to other structural and functional domains in the Sendai virus F glycoprotein. Because of the high level of conservation of cysteine residues in most other members of the paramyxovirus family, they all seem to have the above pattern of disulfide bridging.

There is only one cysteine residue in the F_2 subunit. This is linked to the most upstream cysteine in the F_1 subunit, forming the interchain bond. The remaining eight cysteine residues in the F_1 subunit are predicted to give rise to a bunched structure (1, 18, 21). Our data support this hypothesis. Two loops are formed in tandem by bridging between Cys-338 and Cys-347 and between Cys-362 and Cys-370, respectively. The most downstream cysteine, Cys-424, appeared to be linked to Cys-394, Cys-399, or Cys-401, and the remaining two must also be covalently linked. Thus, these four cysteine residues would also

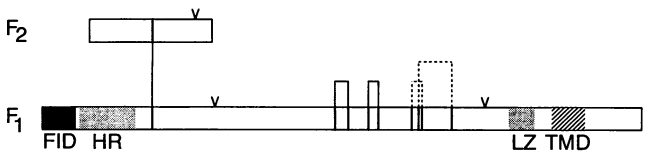

FIG. 6. Schematic diagram of disulfide bridges (———) in the F glycoprotein of Sendai virus. – – – –, not determined. The F_2 and F_1 subunits are diagrammed with the N terminus on the left and the C terminus on the right. FID, fusion-inducing domain; HR, heptad repeat; LZ, leucine zipper motif; TMD, transmembrane domain; V, potential glycosylation sites. Vertical bars represent cysteine residues. For details, see the text.

help to form loops in a bunched structure. The region between Cys-338 and Cys-424 is hydrophilic, and so the loops or the whole bunched structure could be exposed to the molecular surface. This is compatible with the hypothesis that the cysteine-rich domain of paramyxovirus F glycoproteins can be recognized by monoclonal antibodies (4, 25, 27, 37). Also, since these antibodies are all able to inhibit fusion activity and neutralize infectivity, it seems that the cysteine-rich domain has some functional relevance to the primary fusion domain at the F_1 N terminus, even though they are fairly distant from each other in the primary amino acid sequence (Fig. 6). In agreement with this concept, the variants escaping from the action of fusion-inhibiting peptides, which mimic the F_1 amino terminus, also undergo amino acid substitutions in the cysteine-rich domain (12). The residues surrounding Cys-70 in the F_2 subunit are mostly hydrophilic and are targeted by the host immune response (37).

For paramyxovirus F glycoprotein, and perhaps glycoproteins in general, formation of the correct intramolecular disulfide bonds does not appear to be a simple process but seems to involve disruption and rearrangement during intracellular transport (16, 19). In the mature virions, an F-glycoprotein spike appears to be present in a tetrameric form consisting of two identical dimers (31). The noncovalent interactions leading to this dimerization may be caused by a region of heptad repeat units that make up an α-helical coiled-coil conformation located adjacent to the fusion-inducing hydrophobic domain (3), as well as by the leucine zipper motif adjacent to the transmembrane domain (2) (Fig. 6). Interestingly, the cysteine residue participating in the interchain bond (Cys-199) immediately follows the heptad repeat and could play a role in adequate termination of the potentially long α-helices.

In view of these considerations, a series of events that cross-link the two subunits and produce intramolecular loop structures would be crucial for proper folding of the F-glycoprotein molecule and its intracellular transport, biological activity, and antigenicity. The mutation of each cysteine could be a reasonable approach to elucidation of the functional significance of each disulfide bond. Our present biochemical assignment of the disulfide bonds will help in the design of such site-directed mutagenesis studies and interpretation of the data obtained.

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