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Conformational Changes in Newcastle Disease Virus Fusion Glycoprotein during Intracellular Transport

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The migration on polyacrylamide gels of nascent (pulse-labeled) and more processed (pulse-labeled and then chased) forms of nonreduced Newcastle disease virus fusion glycoprotein were compared. Results are presented which demonstrate that pulse-labeled fusion protein, which has an apparent molecular weight of 66,000 under reducing conditions (Collins et al., J. Virol. 28:324–336), migrated with an apparent molecular weight of 57,000 under nonreducing conditions. This form of the Newcastle disease virus fusion protein has not been previously detected. This result suggests that the nascent fusion protein has extensive intramolecular disulfide bonds which, if intact, significantly alter the migration of the protein on gels. Furthermore, upon a nonradioactive chase, the migration of the fusion protein in polyacrylamide gels changed from the 57,000-molecular-weight species to the previously characterized nonreduced form of the fusion protein (molecular weight, 64,000). Evidence is presented that this change in migration on polyacrylamide gels is due to a conformational change in the molecule which is likely due to the disruption of some intramolecular disulfide bonds: Cleveland peptide analysis of the pulse-labeled nonreduced fusion protein (molecular weight, 57,000) yielded a pattern of polypeptides quite different from that obtained from the more processed form of the fusion protein (molecular weight, 64,000). However, the pattern of polypeptides obtained from the nonreduced 64,000-molecular-weight species was quite similar to that obtained from the fully reduced nascent protein (molecular weight, 66,000). This conformational change occurred before cleavage of the molecule. To determine the cell compartment in which the conformational change occurs, use was made of inhibitors which block glycoprotein migration at specific points. Monensin allowed the appearance of the 64,000-molecular-weight form of the fusion protein, whereas carboxyl cyanide *m*-chlorophenylhydrazine blocked the appearance of the 64,000-molecular-weight form of the fusion protein. Thus, the fusion protein undergoes a conformational change as it moves between the rough endoplasmic reticulum and the medial Golgi membranes.

Newcastle disease virus (NDV), a prototype paramyxovirus, is an enveloped, negative-stranded RNA virus (2, 4). The virion is composed of a core and a surrounding membrane. The core contains the genomic RNA as well as three proteins, the nucleocapsid protein (NP), a phosphoprotein (P), and a presumed transcriptase (L) (2, 4, 11, 15, 22, 38). The membrane contains a nonglycosylated membrane protein (M) and two glycoproteins, the hemagglutinin-neuraminidase (HN) glycoprotein and the fusion (F) protein (2, 4, 22, 24).

During infection, the glycoproteins of simple, enveloped RNA viruses such as NDV are processed by the well-characterized host cell pathways involved in the transport of cellular plasma membrane glycoproteins to the cell surface (29). As glycoproteins are inserted into the membrane of the rough endoplasmic reticulum and transported through the Golgi membranes to the plasma membrane, they are subject to a variety of co- and posttranslational modifications such as glycosylation, proteolytic cleavage, and fatty acid acylation (29, 31, 33, 35, 36). In addition, most classes of extracellular proteins, including plasma membrane glycoproteins, are subjected to intramolecular or intermolecular disulfide bond formation or both (7).

The NDV fusion protein is subjected to all these posttranslational modifications; this protein is glycosylated (2, 4, 22, 31), proteolytically cleaved (23, 31, 33), and modified with the fatty acid palmitate (3), and it contains intramolecular disulfide bonds (34). The glycosylation, while not extensively characterized, is tunicamycin sensitive (19, 36) and, therefore, is likely to be of the *N*-linked variety. The polypeptide is synthesized as a precursor, termed F_0 (apparent molecular weight, 66,000) (6, 31). F_0 is cleaved into F_1 and F_2 polypeptides (apparent molecular weights, 54,000 and 10,000, respectively) (16), which remain associated by disulfide bonds. Thus, the uncleaved precursor must contain intramolecular disulfide bonds. The cleavage of the NDV fusion protein, mediated by a host cell enzyme, occurs intracellulary (30) in or near the *trans* Golgi membranes (21, 24, 37).

In the course of our studies of the intracellular processing of the fusion glycoprotein, we found that the nascent fusion protein (F_0) contains intramolecular disulfide bonds the reduction of which significantly alters its migration on polyacrylamide gels. Furthermore, we found that subsequent to its synthesis, F_0 undergoes a change in migration on polyacrylamide gels as well as an alteration in its susceptibility to partial proteolysis. These results suggest that F_0 undergoes a conformational change. This conformational change occurs soon after synthesis and well before the proteolytic cleavage of the molecule. We present evidence that this conformational change is due to disruption of some of the intramolecular disulfide bonds, resulting in a protein which is conformationally similar to the fully reduced nascent form of the protein. Thus, disulfide bond disruption as well as formation may be a possible posttranslational modification of viral glycoproteins.

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MATERIALS AND METHODS

Cells and viruses. Cells used were chicken embryo (CE) cells prepared as described previously (12). NDV (Australia-Victoria strain), was propagated and purified as described previously (12). This strain is a virulent NDV strain that undergoes a productive infection of CE cells to produce infectious virions (2, 12, 26).

Infection and radioactive labeling of cells. Monolayers of CE cells (2×10^6 cells) were infected at a multiplicity of 10 PFU per cell. Radioactive labeling was initiated at 5 h postinfection. Labeling with [^{35}S]methionine (100 μCi/ml, 1,450 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) was accomplished in methionine-free minimal essential medium (supplemented with nonessential amino acids and 7% dialyzed fetal calf serum) as described previously (20, 21). For pulse-chase experiments, labeling was for 5 min followed by the addition of chase medium (minimal essential medium containing 2.5% calf serum, 50 μg of cycloheximide per ml, 2.5% tryptone phosphate broth, penicillin, streptomycin, amphotericin B [Fungizone], and 2 mM cold methionine). The effectiveness of the chase was monitored by determining the trichloracetic acid-precipitable counts associated with cell extracts.

Cell fractionation. Total cytoplasmic extracts were prepared by lysing cells in RSB (10 mM NaCl, 1.5 mM $MgCl_2$, 10 mM Tris hydrochloride [pH 7.4]) containing 1% Tween 40 and 0.5% sodium deoxycholate. The plates were scraped with a rubber policeman, and material was removed, homogenized by passing through a syringe, and centrifuged to pellet the contaminating nuclei.

Cells were fractionated into Triton X-100-soluble and -insoluble fractions as previously described (17, 25). Briefly, cells were washed once with phosphate-buffered saline (0.15 M NaCl, 0.003 M KCl, 0.01 M Na_2HPO_4, 0.002 M KH_2PO_4) and once with E buffer (0.05 M NaCl, 0.01 M HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2- ethanesulfonic acid] [pH 7.4], 2.5 mM $MgCl_2$, 0.3 M sucrose). The Triton X-100-soluble fraction was prepared by adding E buffer containing 0.5% Triton X-100 to cell monolayers for 2 min on ice. The supernatant (Triton X-100-soluble fraction) was removed. The Triton X-100-insoluble fraction was solubilized by then adding RSB containing 1% Tween 40 and 0.5% sodium deoxycholate. The plates were then scraped with a rubber policeman, and the material was removed, passed several times through a syringe, and then centrifuged to remove nuclei.

CCCP treatment of cells. Infected CE monolayers were pulse-labeled for 5 min with [^{35}S]methionine as described above and then treated with carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) as previously described (9, 20, 21). A nonradioactive chase in the presence of CCCP was done as described previously (9, 20, 21).

Monensin treatment of cells. Monensin was added to infected CE monolayers (2×10^6 cells) at various concentrations at 2 h postinfection (10, 14, 21, 27). Radioactive labeling was done at 5 h postinfection in the presence of monensin as described above.

Polyacrylamide gel electrophoresis. Polypeptides were resolved in 10% polyacrylamide slab gels (14 by 22 by 0.15 cm) prepared and run as previously described (21). The gels were then fixed, dried, and subjected to autoradiography (X-ray film X-Omat AR; Eastman Kodak Co., Rochester, N.Y.). The resulting autoradiograms were scanned with a microdensitometer.

Cleveland gels. Protocols for Cleveland gels were essentially those described by Cleveland et al. (5). Radioactively labeled proteins present in fixed, dried gels were located by autoradiography. The region of the gel containing the polypeptide in question was excised, rehydrated in swelling buffer, and placed on top of a 12% polyacrylamide gel. The rehydration buffer contained β-mercaptoethanol where indicated. The gel slice was overlayered with swelling buffer containing 25% glycerol and then overlayered with swelling buffer containing 12% glycerol and various concentrations of *Staphylococcus aureus* V8 protease (Sigma Chemical Co., St. Louis, Mo.). Electrophoresis was as described previously (5).

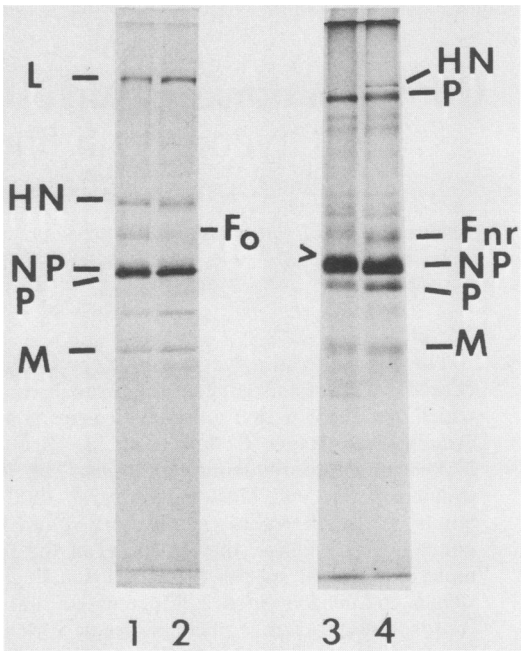

FIG. 1. Polyacrylamide gel electrophoresis of the uncleaved and cleaved forms of the fusion protein in the presence and absence of reducing agent. Monolayers of infected CE cells were pulse-labeled (5 min) with [^{35}S]methionine or pulse-labeled and then chased for 2 h as described in the text. Cells were lysed, and proteins present in total cell extracts were electrophoresed on 10% polyacrylamide gels in the presence (lanes 1 and 2) or absence (lanes 3 and 4) of β-mercaptoethanol (βME). Lanes: 1 and 3, pulse-labeled extracts; 2 and 4, 2-h chase extracts.

RESULTS

Polyacrylamide gel electrophoresis of uncleaved and cleaved forms of fusion protein in the presence and absence of reducing agent. The uncleaved fusion protein (F_0) present in infected cell extracts pulse-labeled with [^{35}S]methionine migrated on a polyacrylamide gel in the presence of reducing agents such as β-mercaptoethanol with an apparent molecular weight of 66,000 (6) (Fig. 1, lane 1). In extracts derived from cells subjected to a radioactive pulse followed by a 2.0-h chase, F_0 was completely cleaved, resulting in the disappearance of the 66,000-molecular-weight protein (Fig. 1, lane 2). F_1 comigrated with the P protein (6), and F_2 migrated near the bottom of the gel and was difficult to resolve in [^{35}S]methionine-labeled cell extracts (6). However, if the polypeptides shown in lane 2 were electrophoresed in the absence of reducing agents (Fig. 1, lane 4), the F_1 and F_2 polypeptides remained associated by disulfide bonds (34), and the F_1-F_2 complex (F_{nr}) migrated slightly

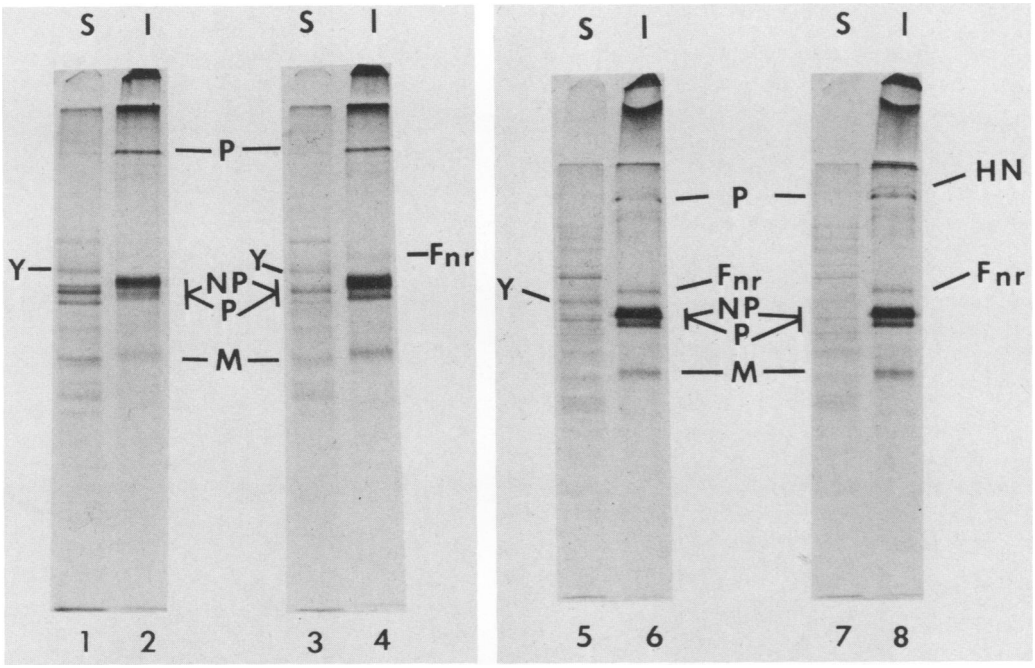

FIG. 2. Resolution of polypeptide Y on polyacrylamide gels. Monolayers of infected CE cells were pulse-labeled with [^{35}S]methionine (lanes 1 and 2) or pulse-labeled and then chased for 20 min (lanes 3 and 4), 40 min (lanes 5 and 6), or 2 h (lanes 7 and 8) as described in the text. Cells were fractionated into Triton X-100-soluble (S) or -insoluble (I) components, and proteins present in each fraction were electrophoresed on 10% polyacrylamide gels in the absence of reducing agent.

faster (64,000 molecular weight) than F_0 (66,000 molecular weight) on polyacrylamide gels (19, 36) (Fig. 1, compare lanes 1 and 4). A similar difference was noted between the reduced and nonreduced Sendai virus fusion protein and was attributed to interchain disulfide bonds (13).

Surprisingly, however, if the infected cell extracts which had been pulse-labeled for 5 min with [^{35}S]methionine were electrophoresed on polyacrylamide gels in the absence of reducing agent, no band at the position of the F_{nr} could be seen (Fig. 1, lane 3).

These results suggest that under nonreducing conditions, pulse-labeled fusion protein migrates in the polyacrylamide gel to a different position than the more processed fusion protein present in cells which had been pulse-labeled and then chased.

Altered migration of pulse-labeled fusion protein in the absence of reducing agents. Since pulse-labeled extracts contained no obvious polypeptide which might be the pulse-labeled, nonreduced fusion protein, it seemed likely that the pulse-labeled fusion protein comigrated with NP. Indeed, there appeared to be material migrating just above the NP band in the pulse-labeled samples (Fig. 1, lane 3, arrow) which disappeared upon chase. We therefore made use of a cell fractionation procedure which separated pulse-labeled NP from other pulse-labeled viral proteins. We observed that if pulse-labeled cells were fractionated into Triton X-100-soluble and -insoluble fractions, the NP and P proteins were found primarily in the Trition X-100-insoluble fraction (Fig. 2, lane 2), while the other viral proteins were present in the soluble fraction (Fig. 2, lane 1). Furthermore, after a nonradioactive chase, all viral proteins became associated with the Triton X-100-insoluble fraction (Fig. 2, lanes 7 and 8). The Triton X-100-soluble fraction of pulse-labeled cells contained a polypeptide marked Y which migrated just above NP (Fig. 2, lane 1). Importantly, this polypeptide gradually disappeared in chase samples concomitant with the appearance of F_{nr} in the Triton X-100-insoluble fraction. The polypeptide Y (molecular weight, 57,000) was, therefore, a candidate for the pulse-labeled fusion protein.

To determine if the Y polypeptide was the fusion protein, we used two approaches. First, if Y is the fusion protein, then upon the addition of a reducing agent it should comigrate with F_0 isolated from polyacrylamide gels containing β-mercaptoethanol (termed $F_{0_{red}}$). Indeed, if Y was excised from a gel and reduced with β-mercaptoethanol, its migration in a second polyacrylamide gel was retarded. The Y polypeptide comigrated with $F_{0_{red}}$ in the presence of reducing agent (Fig. 3, lane 3). However, in the absence of reducing agent, the Y polypeptide still migrated faster than F_0 at a position just above NP protein (Fig. 3, lane 6).

Second, to confirm the identity of Y, the pattern of polypeptides generated after proteolysis was compared with that generated from F_0 (Cleveland analysis). The patterns were nearly identical (Fig. 4). There was a minor difference near the bottom of the gel. The significance of this difference is under investigation. Importantly, digestion of Y and F_0 was done in the presence of β-mercaptoethanol to ensure that both polypeptides were reduced.

The results presented suggest that the pulse-labeled, nonreduced fusion protein (apparent molecular weight, 57,000) migrates faster in a polyacryamide gel than the reduced form of the fusion protein ($F_{0_{red}}$, 66,000 molecular weight) or the more processed, nonreduced fusion protein (apparent molecular weight, 64,000) found in extracts subjected to a pulse and then a nonradioactive chase. The addition of β-mercaptoethanol to the pulse-labeled fusion protein slowed the migration of the protein in a polyacrylamide gel to that characteristic of the reduced pulse-labeled F_0. This finding suggests that the nascent fusion protein contains intramolecular disulfide bonds which considerably speed its migration on polyacrylamide gels. In addition,

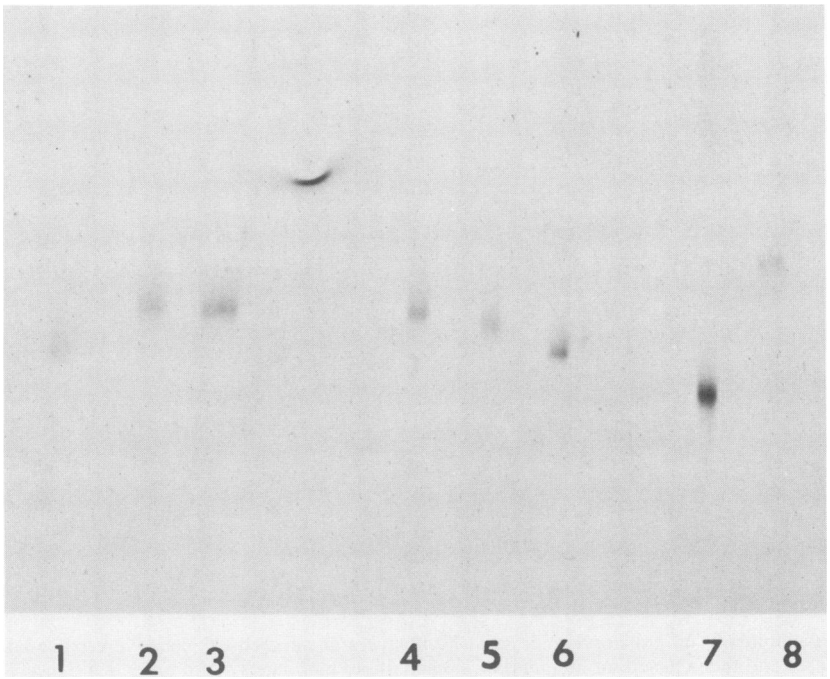

FIG. 3. Reelectrophoresis of Y polypeptide in the presence and absence of reducing agent. Cell extracts were prepared, and proteins present were electrophoresed on 10% polyacrylamide gels as described in the legends to Fig. 1 and 2. Polypeptides were excised from the fixed, dried gel, rehydrated in the presence (lanes 1, 2, and 3) or absence (lanes 4, 5, and 6) of β-mercaptoethanol, and reelectrophoresed on a 10% polyacrylamide gel. Lanes: 1 and 4, nonreduced F protein from extracts pulse-labeled and chased for 2 h; 2 and 5, F_0 protein from pulse-labeled extracts electrophoresed in the presence of β-mercaptoethanol; 3 and 6, Y protein from pulse-labeled extracts; 7, NP protein; 8, HN protein.

subsequent to its synthesis, the protein undergoes a change which, even in the absence of reducing agent, slows its migration in gels to that close to the fully reduced protein.

Alteration in migration is not due to glycosylation or cleavage. There are several obvious possibilities to account for the change in migration of the nonreduced fusion protein on polyacrylamide gels during a pulse-chase labeling protocol. Carbohydrate addition is well known to retard the migration of proteins in polyacrylamide gels. However, posttranslational carbohydrate addition is not a likely explanation. If carbohydrates were added to F_0 posttranslationally, then the reduced form of the fusion protein should increase in apparent size before cleavage or in the absence of cleavage. This phenomenon does not occur (21; see Fig. 6B). Such an argument also holds for other posttranslational modifications such as phosphorylation.

Such a change in migration on polyacrylamide gels could be attributed to a conformational change in the molecule. Indeed, it has been previously shown that the fusion protein of Sendai virus, another paramyxovirus, undergoes a conformational change concomitant with proteolytic cleavage (13). It therefore seemed possible that the alteration in migration of the nonreduced fusion protein in gels might reflect a conformational change related to the cleavage of F_0 to the F_1-F_2 complex. However, two different experiments suggest that this change occurs before proteolytic cleavage. First, the kinetics of cleavage was compared to the kinetics of appearance of the slower-migrating form (64,000 molecular weight) of the nonreduced fusion protein (Fig. 5). Clearly, the slower-migrating form of the fusion protein appears before significant cleavage occurs.

Second, an inhibitor known to block cleavage of F_0 was used. Monensin is a sodium ionophore which is reported to interfere with the transport of most membrane glycoproteins by preventing their exit from the medial Golgi membranes (10, 14, 27, 39, 40). We have previously shown that monensin blocks the cleavage of the fusion protein (21). However, the slower-migrating form of the nonreduced fusion protein appeared in monensin-treated cells (Fig. 6A). That cleavage was inhibited by monensin in these extracts is shown in Fig. 6B. These results clearly show that the change in the protein upon a chase reflected by altered gel migration is not related to proteolysis of the F_0 protein.

Conformational changes detected by Cleveland analysis. Alternatively, the change in migration of the nonreduced fusion protein could be due to a conformational change due to partial disruption of intramolecular disulfide bonds. If a protein is in a different conformation at different stages in its intracellular transport, this difference might be reflected in different patterns of peptides after partial proteolysis in the absence of reducing agent. Therefore, polypeptides generated by partial proteolysis of nonreduced, pulse-labeled protein Y, (57,000 molecular weight) were compared with those generated from the nonreduced fusion protein present in pulse-labeled extracts subjected to a 20-min nonradioactive chase (64,000 molecular weight). The polypeptide isolated from cells chased for 20 min contained the slower-migrating form of the fusion protein, but this protein had undergone minimal amounts of cleavage (Fig. 5). Figure 7 shows the results of partial proteolysis of these two forms of the fusion protein. The pattern obtained from the pulse-labeled protein (57,000 molecular weight) is clearly different from that obtained from the uncleaved but slower-migrating form (64,000 molecular weight) of the fusion protein. Iden-

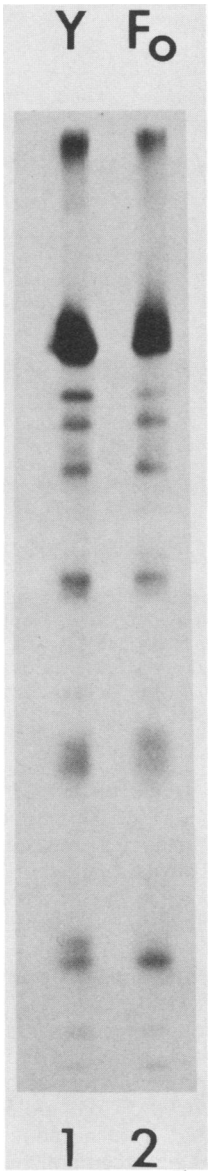

FIG. 4. Cleveland peptide analysis of Y polypeptide and F_0. Y polypeptide shown in Fig. 2, lane 1, and F_0 protein shown in Fig. 1, lane 1, were excised from the fixed, dried gel. The gel slices containing the proteins were rehydrated in the presence of β-mercaptoethanol and subjected to *S. aureus* V8 protease digestion as described in the text. The resulting polypeptides were electrophoresed on a 12% polyacrylamide gel.

tical results were obtained comparing the partial polypeptides obtained from Y protein and the totally uncleaved F_{nr} protein isolated from monensin-treated cells (not shown). Thus, the fusion protein contains different sites accessible to the V8 protease depending upon the age of the protein. This result argues for a conformational change in the fusion protein during intracellular transport.

Conformational change in the presence of CCCP. Monensin is reported to block the exit of glycoproteins from the medial or in some cases the *trans* Golgi membranes (10, 27, 39, 40). Since the conformational change in the fusion protein occurs in the presence of monensin, it is likely that the conformational change occurs before the entry of the protein into the distal parts of the Golgi membranes. To more precisely pinpoint the time of the conformation change, we used CCCP. CCCP, an inhibitor of oxidative phosphorylation, blocks the migration of pulse-labeled glycoproteins into Golgi membranes (9). We have previously shown that after CCCP treatment of NDV-infected cells, no radioactively labeled virus particles are released (21), no glycoprotein reaches the cell surface (20), and the F_0 protein remains uncleaved (21). CCCP treatment also blocked the appearance of the slower-migrating form (64,000 moleuclar weight) of the nonreduced fusion protein (Fig. 8). This result suggests that the conformational change in the fusion protein occurs concomitant with or after its exit from the rough endoplasmic reticulum.

DISCUSSION

Intracellular transport of glycoproteins is accompanied by numerous cotranslational and posttranslational modifications which may include glycosylation and modification of the oligosaccharide core, proteolytic cleavage, fatty acid acylation, sulfation, and phosphorylation (29). Most extracellular proteins, including plasma membrane glycoproteins, also contain intramolecular and sometimes intermolecular disulfide bonds (7). This form of modification may be a quite important determinant of the conformation of a protein.

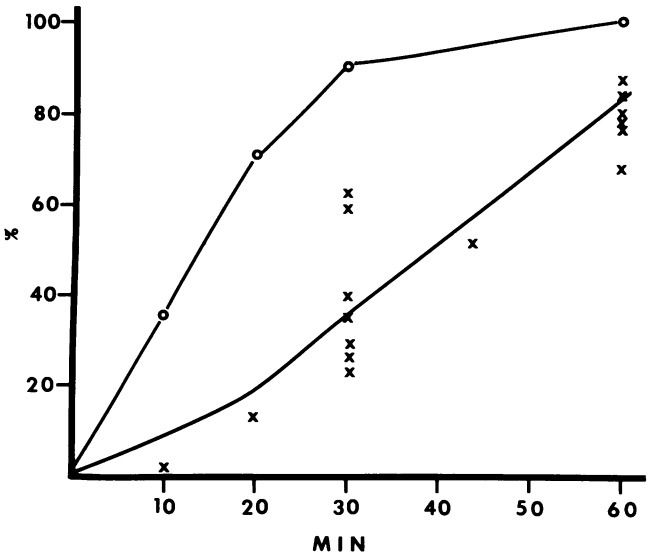

FIG. 5. Comparison of the kinetics of F_0 cleavage and the fusion protein conformational change. To determine the kinetics of cleavage of F_0, monolayers of infected CE cells were pulse-labeled with [^{35}S]methionine for 5 min and then chased as described in the text. Cells were lysed at various times after the onset of the chase, and proteins present in total cytoplasmic extracts were electrophoresed on 10% polyacrylamide gels. The amount of F_0 at the end of the pulse is taken as 100. The percentage of F_0 at each time point is subtracted from 100 to plot the percentage of cleavage at each time point. Thus, 100% cleavage represents no detectable F_0 in cell extracts. Multiple points at each time represent separate experiments. The kinetics of the conformational change was determined by scanning autoradiograms similar to those shown in Fig. 2 and quantitating the amount of Y polypeptide (57,000 moleuclar weight) and F_{nr} polypeptide (64,000 molecular weight) at each time point. The total amount of fusion protein (Y protein and F_{nr}) remained constant during the chase. The appearance of the F_{nr} is plotted. Symbols: ○, kinetics of the appearance of F_{nr}; ×, kinetics of cleavage of F_0.

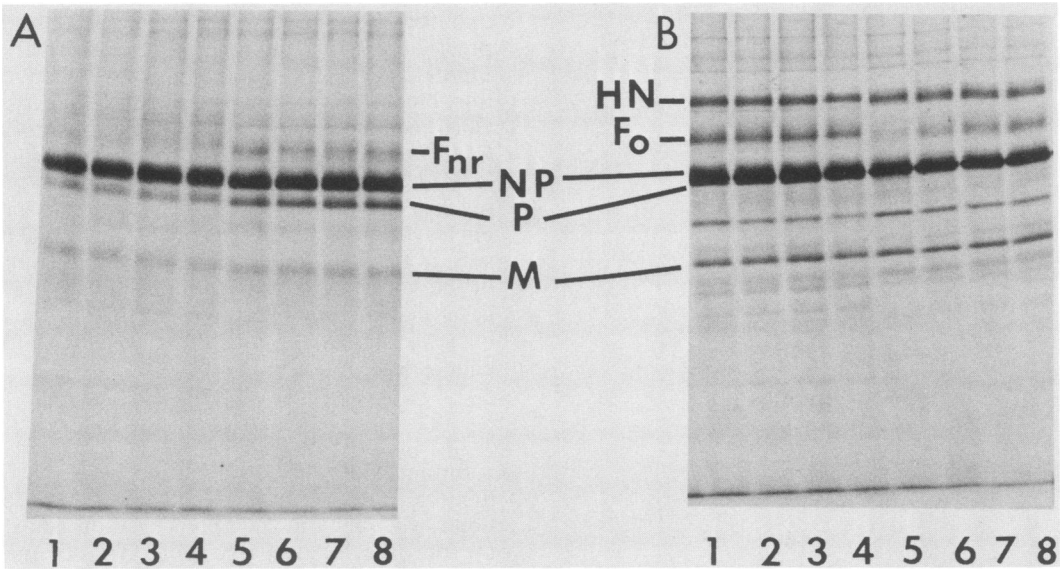

FIG. 6. Monensin does not block the conformational change. Monolayers of infected CE cells treated with various concentrations of monensin beginning at 2 h postinfection were pulse-labeled with [^{35}S]methionine for 5 min at 5 h postinfection. Duplicate monolayers were subjected to a 2-h chase in the presence of monensin. Cells were lysed, and proteins present in total cell extracts were resolved on 10% polyacrylamide gels in the absence of reducing agent (A). The same samples were electrophoresed in the presence of β-mercaptoethanol (B). Lanes: 1 to 4, pulse-labeled extracts; 5 to 8, 2-h chase extracts; 1 and 5, no monensin; 2 and 6, 10^{-7} M monensin; 3 and 7, 10^{-6} M monensin; 4 and 8, 10^{-5} M monensin.

Current evidence suggests that disulfide bond formation and disruption are mediated by an enzyme called protein disulfide-isomerase (7), which is located in the lumen of the rough endoplasmic reticulum and, to some extent, smooth membranes (7, 8). In systems studied, intramolecular disulfide bonds form on the nascent chain (1, 28, 32).

We have presented evidence that newly made (pulse-labeled) NDV fusion protein contains intramolecular disulfide bonds which significantly alter the migration of the protein on polyacrylamide gels. When the disulfide bonds are intact, the protein migrates with an apparent molecular weight of 57,000, while disruption of the bonds results in an apparent molecular weight of 66,000. There is a precedent for altered migration due to intramolecular disulfide bonds. Scheele and Jacoby (32) found that pancreatic secretory proteins migrate on sodium dodecyl sulfate-polyacrylamide gels much faster when the intramolecular disulfide bonds are intact. They proposed that intramolecular disulfide bonds result in a more compact structure which causes the protein to move faster in gels. Thus, it is likely that disulfide bonds form in the nascent fusion protein which results in a more compact structure than the fully reduced form of the protein. This compact form of the protein migrates much faster on a gel than the fully reduced form of the protein.

In addition, we found that soon after its synthesis, the fusion protein in the absence of reducing agent changes from an apparent molecular weight of 57,000 to 64,000. This result suggests that the fusion protein undergoes a posttranslational modification which significantly alters its migration on a polyacrylamide gel.

It may be argued that this change in migration of the fusion protein on a polyacrylamide gel is directly due to a posttranslational modification such as the addition of sugars. However, the faster-migrating pulse-labeled fusion protein can be converted to a slower-migrating form (66,000 molecular weight) of the fusion protein solely by the addition of β-mercaptoethanol. The addition of β-mercaptoethanol to the 64,000-molecular-weight uncleaved protein merely converted the molecule to a 66,000-molecular-weight protein (Fig. 6). These results are inconsistent with changes in migration due to posttranslational addition of carbohydrates. Rather, these results argue that the nascent polypeptide is subjected to a change in its conformation which slows its migration on gels. This change cannot be due to the cleavage of the molecule since the change occurs in the presence of monensin which blocks cleavage (21).

We detected a significant conformational change in the fusion protein by partial proteolysis or Cleveland peptide analysis (5). The partial polypeptides generated from the pulse-labeled, nonreduced, 57,000-molecular-weight protein and F_0 protein isolated from a reducing gel were nearly identical if proteolysis was accomplished in the presence of reducing agent. However, if β-mercaptoethanol was omitted during proteolysis, the pattern of polypeptides generated from the pulse-labeled 57,000-molecular-weight protein and the nonreduced but uncleaved 64,000-molecular-weight protein were quite different. This result argues for a significant difference in the conformation of the pulse-labeled F protein and the more processed form of the F protein, resulting in different sites accessible to the protease. It should also be noted that the conformation of the 64,000-molecular-weight form of the protein and the fully reduced form of the protein (66,000) are quite similar as determined by Cleveland peptide analysis (compare Fig. 4, F_0, and Fig. 7, lanes 3 plus 4).

These results are consistent with the idea that the nascent polypeptide contains extensive intramolecular disulfide bonds. Some of these bonds are disrupted as the protein is transported through the cell, resulting in a polypeptide which is less compact. The protein thus has different sites accessible to the V8 proteases than does the pulse-labeled protein. The more processed from of the protein (chase protein) migrates only slightly faster than the fully reduced protein. Cleveland analysis suggests these two forms of the protein have similar sites accessible to protease. Thus, the more

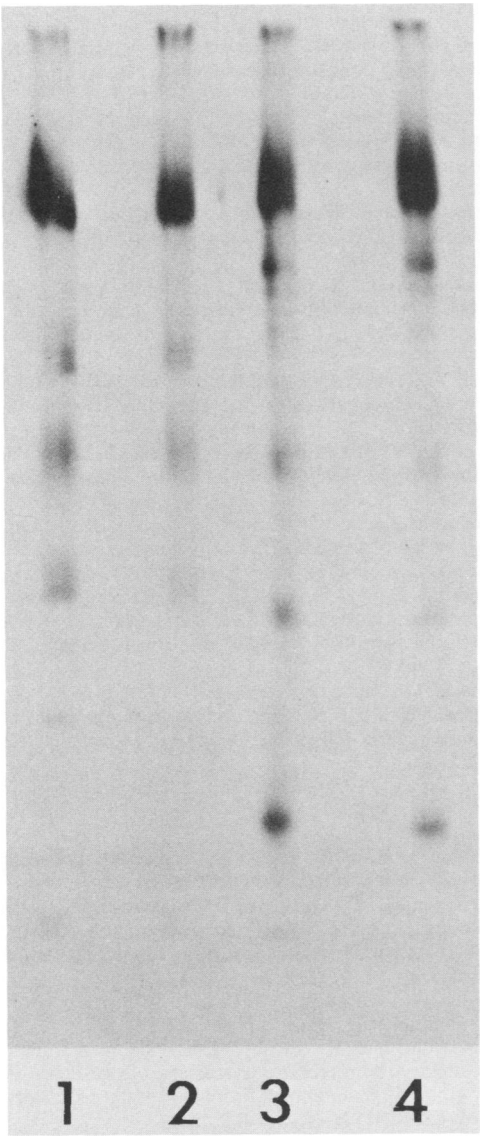

FIG. 7. Cleveland peptide analysis of Y polypeptide and F_0 in the absence of β-mercaptoethanol. Y polypeptide shown in Fig. 2, lane 1, and F_{nr} (primarily F_0) shown in Fig. 2, lane 4, were excised from the fixed, dried gel. The gel slices containing the polypeptides were rehydrated in the absence of β-mercaptoethanol and subjected to *S. aureus* V8 protease digestion (lanes 1 and 3, 10 μg/ml; lanes 2 and 4, 20 μg/ml) as described in the text. The resulting polypeptides were electrophoresed on a 12% polyacrylamide gel. Lanes: 1 and 2, Y polypeptide; 3 and 4, F_{nr}.

processed fusion protein is in conformation very similar to that of the fully reduced nascent fusion protein.

It is possible that another posttranslational modification is indirectly responsible for the loss of some disulfide bonds. For example, alterations in the core oligosaccharide might be indirectly responsible for changes in the intramolecular disulfide bonds. While we cannot eliminate this possibility, we feel it is unlikely since we have evidence (L. W. McGinnes and T. Morrison, manuscript in preparation) that the fusion glycoprotein contains only simple oligosaccharide side chains.

We attempted to determine the cell compartment in which this conformational change occurs by using inhibitors which block glycoprotein migration at specific points within the cell. Our results suggest that the fusion protein undergoes a conformational change as it moves between the rough endoplasmic reticulum and the medial or *trans* Golgi membranes.

The role of this conformational change in the transport or activity of the F glycoprotein is unclear at this time. It has been suggested that conformation of glycoproteins may play an important role in the efficient transport of a protein from the rough endoplasmic reticulum. For example, McQueen et al. (18) have constructed hybrid genes containing sequences from the vesicular stomatitis virus glycoprotein and the influenza HA glycoprotein. The expression of these genes results in the synthesis of hybrid glycoproteins which are inserted into the rough endoplasmic reticulum, but these proteins are not transported beyond the rough endoplasmic reticulum. On the basis of these results, the authors suggest a role of conformation in transport. Indeed, if a conformational change such as we have seen with the NDV fusion protein is required for the transport of the protein to the *cis* Golgi membranes, then such a hybrid protein would perhaps be unable to undergo such a change and would, therefore, fail to be transported.

In sum, we have presented evidence which suggests that the nascent NDV fusion glycoprotein contains intramolecular disulfide bonds, some of which are disrupted as the protein is transported into the *cis* or medial Golgi membranes. This alteration in disulfide bonds results in a change in conformation of the protein which has been detected by changes in migration on polyacrylamide gels and by Cleveland peptide mapping. Thus, disruption as well as formation of disulfide bonds may be a possible posttranslational modification.

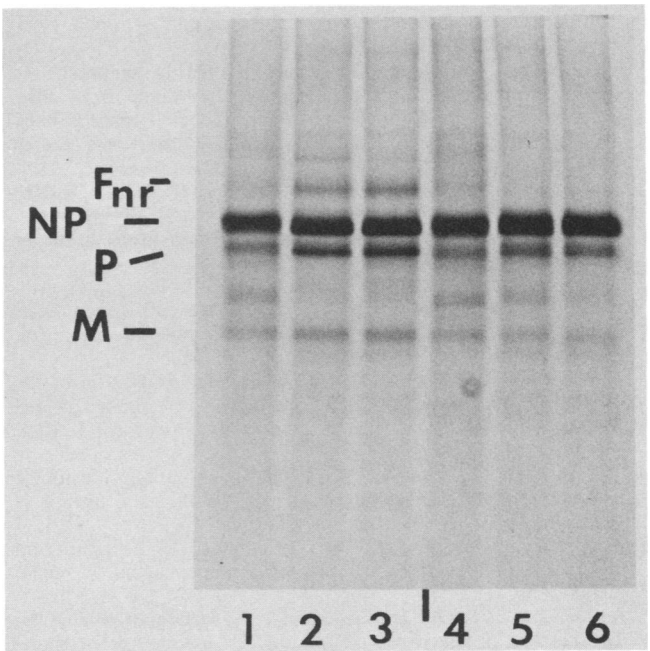

FIG. 8. CCCP blocks the fusion protein conformational change. Monolayers of infected CE cells were pulse-labeled with [^{35}S]methionine or pulse-labeled and then chased for 1 or 2 h as described in the text. Cells were lysed, and proteins present in total cytoplasmic extracts were electrophoresed on 10% polyacrylamide gels in the absence of β-mercaptoethanol. Lanes: 1 to 3, untreated cells; 4 to 6, CCCP-treated cells; 1 and 4, pulse-labeled proteins; 2 and 5, 1-h chase; 3 and 6, 2-h chase.

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