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# Posttranslational Modification and Intracellular Transport of Mumps Virus Glycoproteins

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Analysis of the pronase-derived glycopeptides of isolated mumps virus glycoproteins revealed the presence of both complex and high-mannose-type oligosaccharides on the HN and $F_1$ glycoproteins, whereas only high-mannose-type glycopeptides were detected on $F_2$. Endoglycosidase F, a newly described glycosidase that cleaves N-linked high mannose as well as complex oligosaccharides, appeared to completely cleave the oligosaccharides linked to HN and $F_2$, whereas $F_1$ was resistant to the enzyme. Two distinct cleavage products of $F_2$ were observed, suggesting the presence of two oligosaccharide side chains. Tunicamycin was found to reduce the infectious virus yield and inhibit mumps virus particle formation. The two glycoproteins, HN and F, were not found in the presence of the glycosylation inhibitor. However, two new polypeptides were detected, with molecular weights of 63,000 ($HN_T$) and 53,000 ($F_T$), respectively, which may represent nonglycosylated forms of the glycoproteins. Synthesis of the nonglycosylated virus-coded proteins (L, NP, P, M, pI, and pII) was not affected by tunicamycin. The formation of HN oligomers and the proteolytic cleavage of the F protein were found to occur with the same kinetics. Analysis of the time course of appearance of mumps virus glycoproteins on the cell surface suggested that dimerization of HN and cleavage of F occur immediately after their exposure on the plasma membrane.

Mumps virus is a member of the family paramyxoviridae (9). In comparison with other paramyxoviruses, very little is known about the molecular biology of this virus. Mumps virus has only recently been adapted to cell culture, which has enabled more detailed studies of the viral components and their biosynthesis (4, 16, 20). It has been shown previously that mumps virions contain two glycoproteins, which are designated HN and F, like other members of the paramyxovirus genus. The former glycoprotein is thought to have hemagglutinating and neuraminidase activities, and the latter is thought to be involved in cell fusion and virus penetration (17), although this has not yet been directly demonstrated in the case of mumps virus. The F protein has been shown to be cleaved posttranslationally into two products, designated $F_1$ and $F_2$ (4, 16, 20), which are held together by disulfide bonds (16). Thus, the glycoproteins of mumps virus appear to resemble closely those of other paramyxoviruses (7, 13, 21, 22) in the properties that have been studied.

In the present study, we have investigated the glycosylation of the mumps viral glycoproteins as well as the posttranslational events involved in their biosynthesis. The time course of the posttranslational modification of the viral glycoproteins was determined and was correlated with the kinetics of their appearance on the cell surface.

## MATERIALS AND METHODS

**Cells.** Vero cells were obtained from the American Type Culture Collection and were grown in Dulbecco medium containing 5% newborn calf serum. LLC-$MK_2$ cells were purchased from Flow Laboratories and grown in the same medium.

**Virus.** The Enders strain of mumps virus was grown in Vero cells as described previously (4). Infectivity titers were determined by a plaque assay in Vero cells as described previously (4).

**Radiolabeling and purification of mumps virions.** Cells were infected as described (4). After adsorption, Eagle medium containing 5 μCi of [$^3$H]leucine, [$^3$H]glucosamine, or [$^3$H]mannose per ml was added. After incubation at 37°C for 40 h, virus was harvested and purified as described (4).

**Intracellular labeling.** For labeling of intracellular polypeptides, cells were grown in 35-mm dishes. At the time indicated, 100 μl of medium containing 100 μCi of [$^3$H]mannose or [$^3$H]leucine was added. After 15 min of labeling, cells either were processed for

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electrophoresis or immune precipitation or were further incubated with chase medium containing 20 mM unlabeled mannose before harvest.

**Radioimmune precipitation.** Radioimmune precipitation was carried out essentially as described by Kessler (8). In brief, labeled cells were lysed by adding 200 μl of a buffer containing 1% Nonidet P-40, 1% Triton X-100, 0.15 M NaCl, and 0.05 M Tris-hydrochloride, pH 7.3. Lysates were clarified by centrifugation in an Eppendorf microfuge, and 5 μl of a rabbit hyperimmune serum was added. The mumps virus-specific antiserum was raised by using purified virions grown in LLC-$MK_2$ cells. Antigen bound to antibodies was pelleted after the addition of Formalin-fixed Cowan I strain of *Staphylococcus aureus*. The immune complexes were dissociated by boiling in electrophoresis sample buffer containing no mercaptoethanol.

**SDS-polyacrylamide gel electrophoresis.** Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was performed as described previously (5).

**Isolation of glycoproteins for glycopeptide analysis.** Purified virions labeled with [$^3$H]glucosamine or [$^3$H]mannose were subjected to SDS-polyacrylamide gel electrophoresis. After electrophoresis, the gel strip was sliced into pieces 1 mm in length. Each slice was soaked in 0.5 ml of a buffer containing 1% SDS and 0.05 M $NH_4HCO_3$. After 48 h at 37°C, samples were counted in a liquid scintillation counter, and the peak fractions were pooled. The samples were lyophilized and twice ethanol precipitated, and the glycoproteins were dissolved in buffer for pronase digestion.

**Analysis of glycopeptides.** Pronase digestion and glycopeptide analysis on a Bio-Gel P6 column were carried out as described by Sefton (25).

**Treatment of glycopeptides with endo-H.** After chromatography on Bio-Gel P6, fractions containing glycopeptides were pooled and lyophilized. The samples were desalted by using a Bio-Gel P2 column, lyophilized, and dissolved in 100 μl of 0.05 M sodium acetate buffer, pH 5.5. Digestion was carried out by the addition of 10 μl of sodium acetate buffer containing 0.01 U of endo-β-*N*-acetylglucosaminidase H (endo-H) and incubation at 37°C. The reaction was terminated 16 h later by boiling for 2 min. Afterwards, the sample was analyzed on a Bio-Gel P6 column.

**Digestion of glycoproteins with endo-F.** Glycoproteins were separated from other viral proteins by Triton X-100 fractionation as described previously (6). Detergent was removed by precipitation of the proteins with *n*-butanol, and residual butanol was removed by lyophilization. The glycoproteins were dissolved in 1% Nonidet P-40–0.1% SDS–1% β-mercaptoethanol. After boiling for 2 min, 5 μl of 1 M phosphate buffer (pH 6.1) and 5 μl of 50 mM EDTA were added to a final sample volume of 50 μl. Digestion was carried out by the addition of 5 μl of a preparation of endo-β-*N*-glucosaminidase F (endo-F), which was kindly provided by John Elder. The preparation of the enzyme has been described by Elder and Alexander (2). At the time indicated, the reaction was terminated by boiling the sample and adding 50 μl of electrophoresis sample buffer.

**Electron microscopy.** Cells were harvested 32 h postinfection, fixed with glutaraldehyde and osmium tetroxide, and embedded in an epoxy resin mixture. Thin sections were stained with uranyl acetate and lead citrate and were examined in a Philips 301 electron microscope.

## RESULTS

**Glycopeptides of mumps virus glycoproteins.** The HN, $F_1$, and $F_2$ glycoproteins of mumps virus were separated by polyacrylamide gel electrophoresis of purified [$^3$H]mannose-labeled virions under reducing conditions. The isolated glycoproteins were digested extensively with pronase, and the resulting glycopeptides were analyzed by gel filtration on a Bio-Gel P6 column. Fig. 1a shows that for HN, three size classes of glycopeptides were resolved, which are designated I, II, and III. Their apparent molecular weights were determined by using the glycopeptides of Sindbis virus as size markers; molecular weight estimates of 2,600, 2,200, and 1,900 were obtained. To determine whether these three size classes represent complex or mannose-rich glycopeptides, samples of the peak fractions were pooled and digested with endo-H. This enzyme is known to cleave mannose-rich glycopeptides between the two glucosamine residues adjacent to asparagine; complex glycopeptides are resistant to the action of this endoglycosidase (1, 29). As shown in Fig. 1d, peak I was unaffected by endo-H treatment. Peaks II and III, however, disappeared after digestion with endo-H, and a new peak was found at a lower molecular weight position. This result indicates that the largest glycopeptide size class is complex, whereas the two smaller ones are composed of mannose-rich glycopeptides. This conclusion is also supported by the observation that peak I was more prominent, in comparison to peaks II and III, when the oligosaccharides were labeled with [$^3$H]glucosamine instead of [$^3$H]mannose (data not shown). Under the conditions used, peaks II and III of HN were completely digested only if the endo-H treatment was repeated a second time.

The $F_1$ glycoprotein also was found to contain three size classes of glycopeptides, designated I, II, and III (Fig. 1b). Their molecular weights were estimated to be 2,800, 2,000, and 1,600, respectively. Again, samples of the peak fractions were pooled, digested with endo-H, and rechromatographed. Fig. 1e shows that peak I was resistant to the enzyme treatment. Peaks II and III, however, were sensitive, as indicated by their change in position after endo-H digestion. Thus, peak I contains a complex type of glycopeptide, whereas the two smaller size classes of the $F_1$ glycopeptides are of the mannose-rich type. As in the case of the HN glycopeptides, the endo-H-resistant peak I was much more prominent than peaks II and III when the oligosaccharides were labeled with glucosamine instead of [$^3$H]mannose (not shown). Treatment of

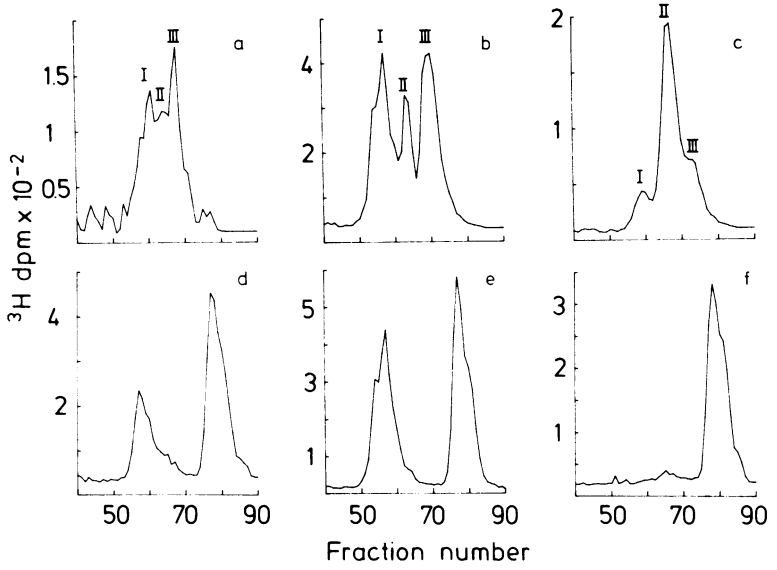


FIG. 1. Analysis of the glycopeptides of mumps viral glycoproteins. HN, $F_1$, and $F_2$ glycoproteins of purified [$^3$H]mannose-labeled mumps virions grown in Vero cells were separated by SDS-polyacrylamide gel electrophoresis, isolated, and digested extensively with pronase. The glycopeptides were analyzed by gel filtration on Bio-Gel P6 columns. One-third of the volume from each fraction was used for counting. The remaining two-thirds of the fractions containing the three peaks (56 through 72) was pooled, concentrated, and desalted. After digestion with endo-H, the glycopeptides were again chromatographed on Bio-Gel P6 columns. a and d, HN; b and e, $F_1$; c and f, $F_2$; a, b, and c, before endo-H treatment; d, e, and f, after endo-H treatment.

the $F_1$ glycopeptides with neuraminidase did not change the profile obtained after Bio-Gel P6 chromatography, whereas the glycopeptides of Sindbis virus, which were cochromatographed as a control, showed a change as expected (data not shown). Thus, like other paramyxoviruses that contain a neuraminidase, mumps virus apparently does not contain sialic acid in its glycoproteins.

Analysis of the glycopeptides of $F_2$ on Bio-Gel P6 is shown in Fig. 1c. The molecular weight of the major peak, designated II, was estimated as 1,700. Two minor peaks, designated I and III, with approximate molecular weights of 2,100 and 1,500, respectively, were also detected. All three peaks were sensitive to digestion with endo-H (Fig. 1f), suggesting that the $F_2$ cleavage product of the mumps fusion protein contains only oligosaccharides of the mannose-rich type.

**Treatment of mumps viral glycoproteins with endo-F.** To estimate the molecular weight of the protein portion of the mumps virion glycoproteins, attempts were made to remove the carbohydrate portion by digestion with endoglycosidases. The enzyme used was endo-F, which recently has been reported to cleave both mannose-rich and complex types of oligosaccharides via hydrolysis of the glycosidic bond of the di-*N*-acetylchitobiose core structure adjacent to asparagine (2). The glycoproteins were isolated by Triton X-100 fractionation of purified mumps virions labeled with [$^3$H]leucine, treated for various periods of time with endo-F, and analyzed by polyacrylamide gel electrophoresis (Fig. 2). Both HN and $F_2$ were found to be sensitive to the action of endo-F, whereas $F_1$ appeared to be more resistant to the enzyme. The HN band decreased in apparent molecular weight with the duration of enzyme treatment, and protein label which migrated ahead of the position of the untreated HN glycoprotein appeared. After 120 min, no original HN remained, and a diffuse band was found with an apparent molecular weight of 65,000. In the case of $F_2$, endo-F treatment resulted in the appearance of two new bands with molecular weights of about 14,000 and 12,000. The latter was the major species observed after prolonged digestion with enzyme. This suggests that $F_2$ probably contains two oligosaccharide side chains and that the 14,000-molecular-weight band may represent polypeptides from which only one oligosaccharide chain is removed.

**Effect of tunicamycin on the replication of mumps virus.** To obtain information about the importance of glycosylation for mumps virus replication, the effect of tunicamycin was analyzed. This drug has been shown to inhibit the formation of lipid-linked *N*-acetylglucosamine compounds, which are necessary for the synthesis of the common core structure of *N*-linked oligosaccharides (28, 30). It has been reported

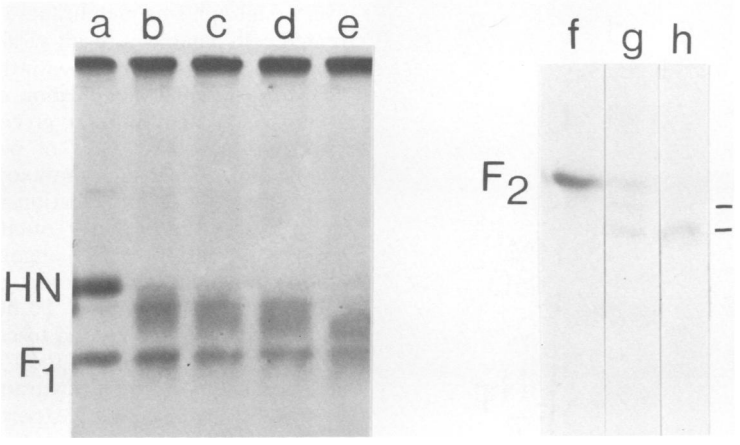


FIG. 2. Effect of endo-F on the glycoproteins of mumps virus. The glycoproteins of purified [$^3$H]leucine-labeled virions were isolated by Triton X-100 fractionation and digested with endo-F for various times. HN and $F_1$ were analyzed on a 10% gel (lanes a through e), and $F_2$ was analyzed on a 15% gel (lanes f through h). The incubation times with endo-F were as follows: a and f, 0 min; b and g, 60 min; c, 90 min; d and h, 120 min; e, 180 min. The positions of the $F_2$ cleavage products are indicated by markers.

that tunicamycin inhibits particle formation of a number of viruses (11, 15, 24, 26). With other viruses, particle formation occurs, but these virions may have reduced infectivity (12, 14, 24). As shown in Table 1, the amount of infectious mumps virus released into the medium was decreased drastically in the presence of tunicamycin. A concentration as low as 100 ng/ml caused the infectivity titer to drop by 99.9%. The hemagglutination titer was decreased proportionally. In the presence of tunicamycin, mumps virus-infected Vero cells released only low levels of radiolabeled material which could be pelleted by ultracentrifugation. After analysis by SDS-polyacrylamide gel electrophoresis, the material released from infected Vero cells in the presence of tunicamycin showed no similarity to the protein pattern obtained from untreated control samples. Not only were protein bands corresponding to the glycoproteins missing, but there were also no internal proteins L, NP, P, or M (data not shown). Thus, we conclude that tunicamycin inhibits the formation of mumps virions.

TABLE 1. Effect of tunicamycin on the growth of mumps virus in Vero cells[a]

| Tunicamycin concn (ng/ml) | Yield of infectious virus (PFU/ml) |
|---|---|
| 0 | $9.4 \times 10^7$ |
| 10 | $8.0 \times 10^7$ |
| 25 | $2.3 \times 10^7$ |
| 50 | $1.8 \times 10^6$ |
| 100 | $1.1 \times 10^5$ |

[a] Tunicamycin was added at the concentration indicated immediately after the adsorption period, and virus yields were determined by plaque assay at 40 h postinfection.

Analysis of the intracellular proteins synthesized in the presence of tunicamycin revealed no difference in the non-glycosylated viral proteins as compared with untreated cells. However, no protein band could be detected above the background of cellular proteins, which might correspond to unglycosylated counterparts of the glycoproteins. Therefore, these experiments were repeated under conditions which selectively reduced the synthesis of cellular proteins. An especially effective method proved to be an incubation of the infected cells with leucine-free medium for 4 h before labeling. Figure 3 shows that under these conditions all viral structural proteins could be detected easily; in addition, it was possible to see the two low-molecular-weight proteins pI and pII, which are not found in purified virions and which have been shown to be related in their amino acid sequence to the structural P protein (4). In the region of the gel where HN was expected, a doublet could be resolved. One of these two bands (p80) may represent a cellular protein since the synthesis of a protein with a molecular weight of 80,000 has been reported to be increased after infection with other paramyxoviruses (18). In the tunicamycin-treated cells, no $F_0$ could be detected, and only one protein was found in the doublet band mentioned above. Two new protein bands not present in the control sample were detected in tunicamycin-treated cells; their estimated molecular weights were 63,000 and 53,000. They were designated $HN_T$ and $F_T$ because they were believed to be unglycosylated forms of the mumps viral glycoproteins, as discussed below.

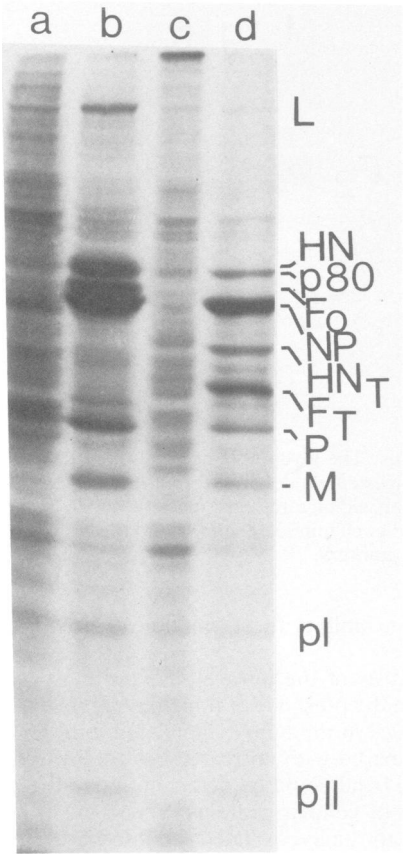


FIG. 3. Effect of tunicamycin on the synthesis of mumps viral proteins. Infected or mock-infected Vero cells were incubated with leucine-free medium at 18 h postinfection. Some of the dishes were treated with tunicamycin (1 μg/ml). After 4 h, cells were labeled with 100 μCi of [$^3$H]leucine per dish. After 20 min, cells were lysed and prepared for SDS-polyacrylamide gel electrophoresis. Lanes a and c, Mock-infected cells; lanes b and d, infected cells; lanes a and b, absence of tunicamycin; lanes c and d, presence of tunicamycin.

To investigate the effect of tunicamycin on viral morphogenesis, infected cells were examined by electron microscopy. In the absence of the drug (Fig. 4A and B), viral nucleocapsids were observed to be associated with regions of the plasma membrane exhibiting projections on the external surface; often, these projections formed an unusually dense layer (Fig. 4B). In the presence of tunicamycin, neither formation of virus nor association of nucleocapsids with the plasma membrane was observed, whereas assembly of nucleocapsids within the cytoplasm was apparently unaffected (Fig. 4C). These results suggest that viral envelope proteins are not inserted into plasma membranes of tunicamycin-treated cells, and as a result viral nucleocapsids do not become associated with the cell surface.

**Posttranslational modification of mumps viral glycoproteins.** In addition to glycosylation of the polypeptides, other types of posttranslational modifications occur in paramyxovirus glycoproteins. One type of modification involves cleavage of $F_0$ into the $F_1$ and $F_2$ subunits. A second involves formation of HN oligomers, probably dimers, in which the monomers are held together by disulfide bonds (16). To determine when the dimerization occurs and to correlate it with the proteolytic cleavage of the F protein, which has been shown to be a posttranslational event (4, 16, 20), a pulse-chase experiment was performed. Cells were labeled for 15 min with [$^3$H]mannose. After various chase periods, cells were lysed and were analyzed by SDS-polyacrylamide gel electrophoresis after immunoprecipitation (Fig. 5). For detection of HN oligomers, samples were analyzed under nonreducing conditions, i.e., in the absence of mercaptoethanol (Fig. 5, lanes a through d). Disulfide-linked HN glycoproteins were not detected in cells lysed 10 min after pulse-labeling (lane a) but were found after a 20-min chase period (lane b). The same samples were also analyzed under reducing conditions, i.e., in the presence of mercaptoethanol, to determine the kinetics of the proteolytic cleavage of $F_0$. As shown in Fig. 5, lane e, the cleavage products $F_1$ and $F_2$ were virtually absent in cells analyzed 10 min after pulse-labeling. After a chase period of 20 min (lane f), both proteins could be detected. Thus, both proteolytic cleavage of the $F_0$ protein and formation of disulfide bonds between HN monomers occur with similar kinetics. It is noteworthy that the glycoproteins were found as rather broad bands which actually were composed of multiple bands that could be seen after shorter exposure of the films. This indicates the existence of several forms of the glycoproteins, which are also found after longer chase periods. Some of these immature forms reach the cell surface, as shown in Fig. 6; they were not, however, detected in virions (data not shown).

**Appearance of mumps viral glycoproteins on the cell surface.** The results shown in Fig. 5 suggest that the proteolytic cleavage of the F protein as well as dimerization of the HN glycoprotein are late events in the pathway to the plasma membrane. Therefore, it was of interest to correlate these events with the rate of appearance of the mumps viral glycoproteins on the cell surface. In an experiment similar to that shown in Fig. 5, cells were labeled for 15 min with [$^3$H]mannose. After various chase periods, cells were analyzed for the appearance of newly synthesized glycoproteins on the cell surfaces.

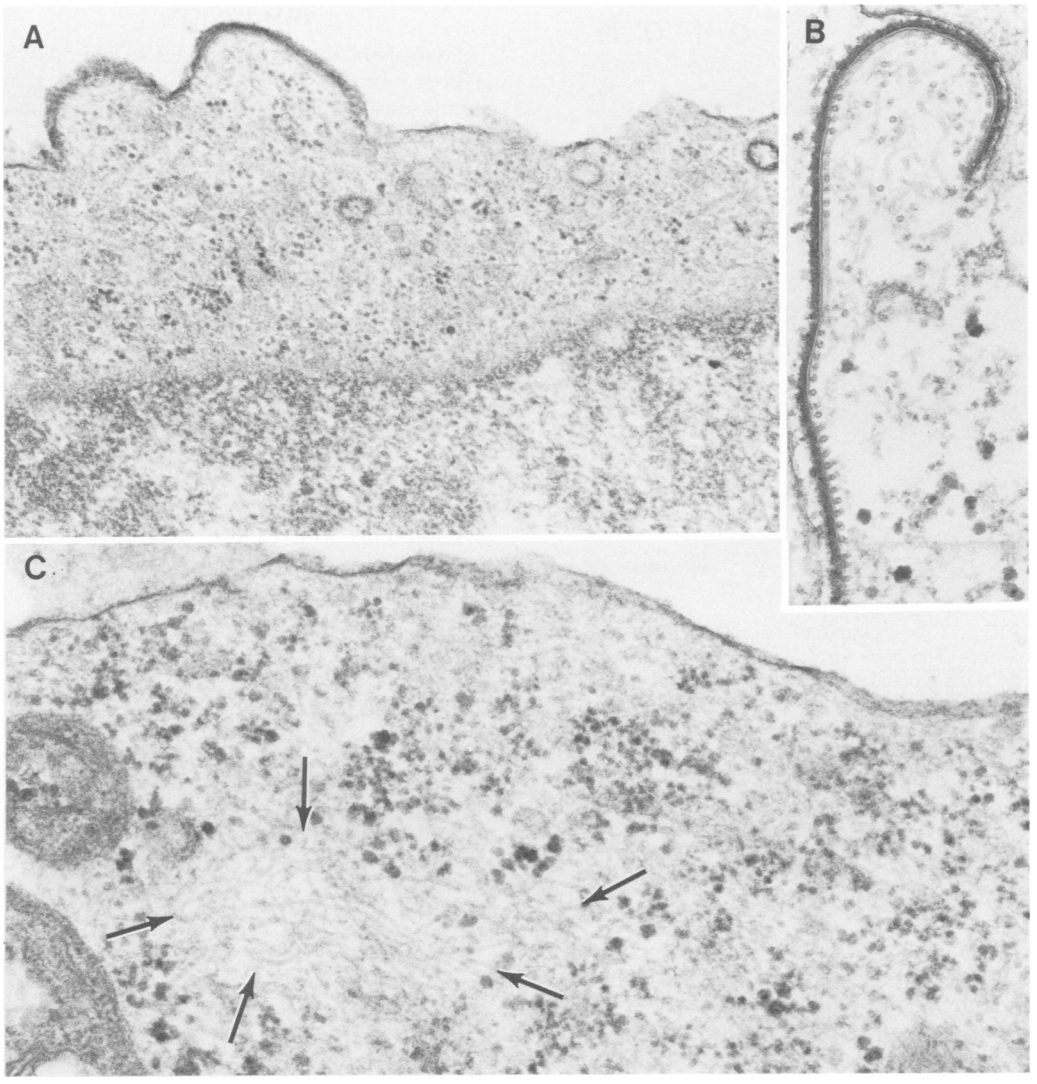


FIG. 4. Effect of tunicamycin on mumps virus morphogenesis. A and B, Thin sections of mumps virus-infected Vero cells in the absence of tunicamycin showing viral nucleocapsids aligned under regions of the plasma membrane. C, Region of the cytoplasm of an infected Vero cell from a culture incubated postinfection with tunicamycin at a concentration of 1 μg/ml. No virions were observed, nor were nucleocapsids found to be associated with the cell surface. The arrows outline an area of the cytoplasm filled with electron-dense strands with the morphology of viral nucleocapsids (see B); such structures were not observed in uninfected cells. Magnifications: A, ×39,000; B, ×68,000; C, ×60,000.

For this purpose, rabbit hyperimmune serum against mumps virus was added to intact cells at intervals after labeling. After the removal of unbound antibodies by thorough washing, cells were lysed and processed for immune precipitation. Upon analysis by SDS-polyacrylamide gel electrophoresis, only glycoproteins which were exposed on the cell surface should be detectable. The results (Fig. 6) show that some F protein could already be found on the cell surface after a 10-min chase period. At that time, the F protein was not yet in the mature form, as indicated by the absence of the cleavage products $F_1$ and $F_2$ (Fig. 6, lane e) and by the slightly increased electrophoretic mobility under nonreducing conditions (compare lane a with lanes b through d). The amount of this immature form of the mumps fusion protein decreased with longer chase periods. However, even after a 40-min chase, $F_1$ and $F_2$ could be seen as a doublet band, indicating the presence of some F protein in an immature form on the cell surface. As

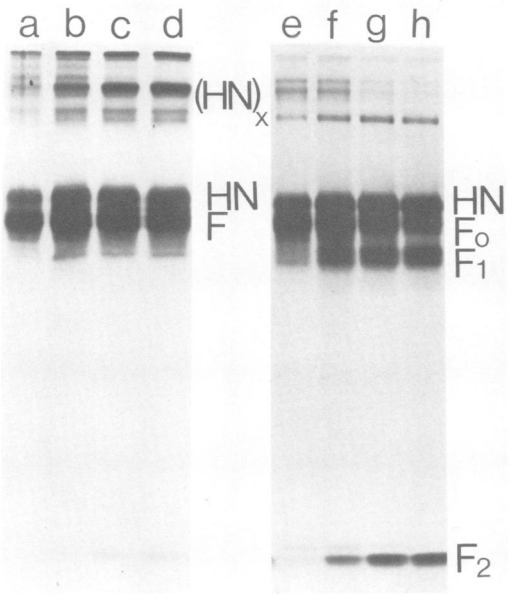


FIG. 5. Time course of the posttranslational modifications of mumps viral glycoproteins. Infected vero cells were labeled 18 h postinfection with 100 μCi of [$^3$H]mannose per dish. After a pulse of 15 min, cells were washed thoroughly and further incubated with medium containing 20 mM of cold mannose. After various times, cells were processed for immune precipitation. Analysis by SDS-polyacrylamide gel electrophoresis was performed under reducing (lanes e through h) or nonreducing (lanes a through d) conditions. Cells were chased for 10 (a and e), 20 (b and f), 30 (c and g), or 40 (d and h) min.

mentioned above, no band corresponding to the immature F protein was detected in purified virions. The cleaved F protein could be detected on the cell surface 20 min after the labeling period, and it increased in amount with longer chase intervals (Fig. 6, lanes f through h). Under reducing conditions, some HN could already be detected 10 min after labeling (lane e). This material could not be recovered quantitatively in the absence of mercaptoethanol, probably because of the formation of aggregates, which could be seen on top of the gel. HN oligomers could first be seen on the cell surface after a 20-min chase (Fig. 6, lane b). With longer chase periods, the amount of HN oligomers on the cell surface increased (lanes b through d). Most HN on the cell surface was in the form of oligomers; only a minor amount was present in the form of the monomer, which could be detected as a faint band just above the $F_1$-$F_2$ doublet band. Thus, the results shown in Fig. 5 and 6 indicate that the formation of HN oligomers and the cleavage of the F protein occur shortly after the glycoproteins appear on the cell surface.

## DISCUSSION

Analysis of the glycopeptides of mumps viral glycoproteins revealed that both complex and mannose-rich oligosaccharides are present on the F as well as the HN glycoprotein. These results differ from those obtained with other paramyxovirus glycoproteins, for which a more uniform glycopeptide pattern has been reported. The HN protein of egg-grown Sendai virus was reported to contain only complex carbohydrates (10), and the glycoproteins of simian virus 5 grown in MDBK cells were found to contain only complex oligosaccharides, all of which had the same composition (19). The finding that tunicamycin affects the synthesis of both the HN and the F proteins indicates that the carbohydrate chains of the mumps glycoproteins are attached via N-linkages to asparagine residues of the protein backbone. Digestion of mumps glycoproteins with endo-F suggested the presence of two oligosaccharides on the $F_2$ protein.

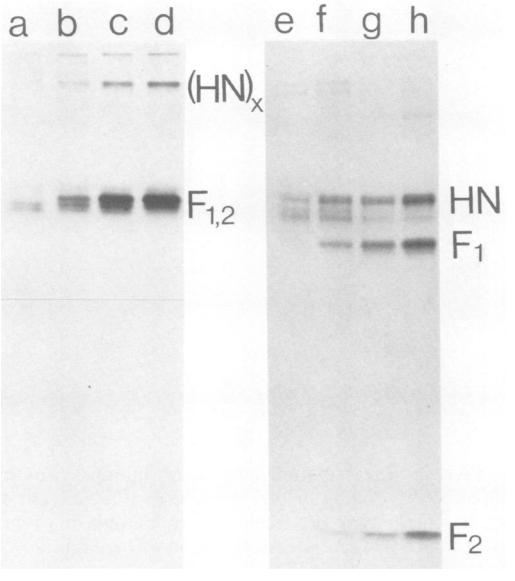


FIG. 6. Time course of the appearance of mumps viral glycoproteins on the cell surface. Infected Vero cells were labeled 18 h postinfection with 100 μCi of [$^3$H]mannose per dish. After a pulse of 15 min, cells were washed thoroughly and were further incubated with medium containing 20 mM of cold mannose. After various times, intact cells were treated in the cold with 20 μl of mumps antiserum in 100 μl of phosphate-buffered saline. After 30 min, cells were washed five times with cold phosphate-buffered saline. Cells were lysed and processed for immune precipitation using *S. aureus*. Analysis by SDS-polyacrylamide gel electrophoresis was performed under reducing (lanes e through h) or nonreducing (lanes a through d) conditions. Cells were chased for 10 (a and e), 20 (b and f), 30 (c and g), or 40 (d and h) min.

Although HN was also sensitive to the action of endo-F, the removal of oligosaccharides did not result in the appearance of distinct protein bands, which could have enabled estimation of the number of carbohydrate side chains. This is probably due to the larger number of oligosaccharides and to their variation in size. A rough estimate can be made, however, based on the molecular weight difference between fully glycosylated HN (79,000) and $HN_T$ (63,000). The conclusion that $HN_T$ represents the unglycosylated form of HN is supported by the finding that digestion with endo-F reduced the molecular weight of HN to 65,000. A somewhat larger molecular weight of HN is expected after endo-F treatment, as compared to $HN_T$, because the endoglycosidase does not remove the complete oligosaccharide but leaves one glucosamine residue bound to the protein backbone. The molecular weight difference of 16,000 between HN and $HN_T$, together with the size estimates of the HN glycopeptides, suggests the presence of about seven carbohydrate chains on the HN glycoprotein. Similarly, based on the molecular weight differences in $F_0$ (74,000) and $F_T$ (53,000), the molecular weight of the carbohydrate portion of $F_0$ can be estimated as 21,000. Taking into account the two oligosaccharides on $F_2$ and the size estimates of the $F_1$ glycopeptides, these results indicate that as many as seven or eight oligosaccharide chains may be present on the $F_1$ polypeptide.

Tunicamycin was found to inhibit the formation of mumps virions. Inhibition of particle formation by tunicamycin has been reported for a number of viruses, including two paramyxoviruses, Sendai virus and measles virus (11, 15, 24, 26). With other viruses, including Newcastle disease virus, another paramyxovirus, virions are produced in the presence of tunicamycin, but these virus particles are not infectious (12, 14, 24). So far, the prototype Indiana strain of vesicular stomatitis virus is the only enveloped RNA virus that has been reported to form infectious virions when glycosylation is inhibited (3). In the case of mumps virus, further studies are required to determine the mechanism by which particle formation is inhibited. However, since viral polypeptides are synthesized in the presence of the drug and assembly of nucleocapsids is observed, it seems likely that a block in surface expression of the glycoproteins occurs and results in inhibition of virus assembly.

Kinetic analysis of the appearance of mumps viral glycoproteins on the plasma membrane by surface immune precipitation revealed that both HN and F arrive at the cell surface with similar kinetics, indicating that both proteins have the same migration rate on their pathway from the rough ER to the plasma membrane. It has been reported that the secretory protein transferrin is externalized more slowly than the G protein of vesicular stomatitis virus (27). Also, the HN glycoprotein of Newcastle disease virus has been found to have a slower migration rate to the cell surface than the vesicular stomatitis virus G protein (23). As far as mumps virus is concerned, it is not known whether the similar migration rates of HN and F reflect an intrinsic property of the proteins or whether they are somehow associated and therefore reach the cell surface simultaneously. We have also compared the kinetics of appearance of mumps viral glycoproteins on cell surfaces with the time course of cleavage of the F glycoprotein and the formation of HN oligomers. Proteolytic cleavage of the fusion protein of Sendai virus is an extracellular event (7), whereas with Newcastle disease virus, cleavage is reported to occur on intracellular membranes (13, 23). With the latter virus, HN dimers have been found in the rough endoplasmic reticulum (23). Mumps virus apparently differs from Newcastle disease virus in that cleavage of F and formation of HN oligomers are posttranslational modifications which occur after the appearance of the glycoproteins on the surfaces of infected Vero cells. The factors which determine that both of these modifications are late events are unknown. One possibility is that after completion of glycosylation, conformational changes of the glycoproteins occur, which favor formation of HN oligomers and which make the F protein accessible to cleavage by protease. Alternatively, the conditions on the cell surface (pH, presence of suitable ions and proteases) may be necessary for the glycoprotein modifications.

## ACKNOWLEDGMENTS

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