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Posttranslational Processing of Uukuniemi Virus Glycoproteins G1 and G2

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Uukuniemi virus, which matures specifically in the Golgi complex, contains two species of envelope glycoproteins, G1 (M_r, 70,000) and G2 (M_r, 65,000). These are translated as a polyprotein, p110, from an mRNA which is complementary to the medium-sized segment of the virion RNAs. By synchronized initiation of protein synthesis and pulse-labeling, it was shown that glycoprotein G1 is amino terminal in precursor protein p110. Apparently, the nonglycosylated forms of these proteins (M_r, 54,000 to 57,000), synthesized in the presence of tunicamycin, comigrate in sodium dodecyl sulfate-polyacrylamide gel electrophoresis, because a similar-sized protein could be isolated by immunoprecipitation with monoclonal antibodies directed against either G1 or G2. The G1 protein, which in the virion contains exclusively endoglycosidase H (endo H)-resistant glycans, was converted to the endo H-resistant form in a half time of about 45 min. The G2 protein, which in the virion has a heterogeneous glycosylation pattern as revealed by endo H digestion, attained this partial endo H resistance only after 90 to 150 min of chase. The transport time of Uukuniemi virus glycoproteins from the endoplasmic reticulum to the Golgi complex was considerably longer than that for alpha and rhabdovirus glycoproteins. Determination of the transport time of G1 and G2 to extracellular virions revealed that G1 is incorporated into mature virions about 10 min faster than G2, suggesting that G1 and G2 are transported with different kinetics to the site of virus maturation.

Studies of the biosynthesis of several viral and cellular membrane glycoproteins have revealed a common theme: the proteins are translated from their mRNAs in the rough endoplasmic reticulum (ER), translocated across the ER membrane, and transported through the Golgi apparatus to their final destinations (7, 9, 16). During this process, several maturation events occur, including proteolytic processing (8, 9), attachment and subsequent processing of oligosaccharides (6), fatty acid acylation (26), sulfation (33), and phosphorylation (11). Many viruses that mature at the plasma membrane have proved to be very useful in elucidating these steps.

Viruses which mature intracellularly, e.g., herpesviruses (4), coronaviruses (19), and bunyaviruses (2), are useful for studying the processing, transport, and addressing of membrane proteins within animal cells. Uukuniemi virus, which has a segmented, negative-stranded RNA (23, 24), provides an interesting model because it matures (12, 13), as do the other bunyaviruses (2, 20), by budding at the membranes of the Golgi complex. The virus contains two species of envelope glycoproteins, G1 (M_r, 70,000) and G2 (M_r, 65,000), and an internal nucleocapsid (N) protein (M_r, 25,000) (21, 22). Glycoproteins G1 and G2 are synthesized from the same mRNA, which is derived from the medium-sized RNA segment (M_r, 1.1×10^6), as precursor polypeptide p110 (M_r, 110,000). The precursor cannot be found in infected cells, but can be detected by in vitro translation of the glycoprotein mRNA (32). Thus, the precursor is probably cleaved cotranslationally to yield G1 and G2. In mature extracellular virions three types of protein-bound glycans can be found, the complex glycans and high-mannose glycans and, in addition, small endoglycosidase H (endo H)-resistant glycans (ratio, 2:2.8:1) (21). Glycoprotein G1 contains only endo H-resistant chains, whereas glycoprotein G2 contains mainly endo H-sensitive chains. However, after endo H treatment and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) analysis of G2 under nonreducing conditions, several bands were observed, suggesting that G2 contains variable amounts of endo H-resistant chains in addition to high-mannose chains.

I examined the size of the apoproteins of G1 and G2 as well as the order of the glycoproteins in precursor polypeptide p110. The kinetics of maturation of both G1 and G2 with respect to endo H resistance was slower than that of many other virus glycoproteins, suggesting slower transport from the ER to the Golgi complex for Golgi-specific Uukuniemi virus glycoproteins.

MATERIALS AND METHODS

Cells and virus. The origin and cultivation of the BHK-21 and chicken embryo cells and the preparation of stock virus from the prototype strain S-23 of Uukuniemi virus after several successive plaque purifications have been described previously (24). The titer of the stock virus was 10^8 PFU/ml. A multiplicity of about 10 PFU per cell was used in all experiments.

Pulse-chase experiments. Confluent monolayers of chicken embryo cells or BHK-21 cells, grown on 32-mm petri dishes, were infected with Uukuniemi virus. At 7 h after infection, the culture medium was replaced by methionine-free Eagle minimal essential medium supplemented with 0.2% bovine serum albumin and 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, pH 7.2). The cells were pulse-labeled at 8 h after infection with 100 to 500 μCi of [^{35}S]methionine (1.160 to 1.500 Ci/mmol; Amersham International) per ml, chased by a 50-fold excess of the normal amount of methionine. After the chase (or the pulse) the cells were washed with ice-cold phosphate-buffered saline and lysed with NET (1% Nonidet P-40, 0.005 M EDTA, 0.05 M Tris-hydrochloride [pH 8.0], 0.4 M NaCl, 100 IU of apoprotin per ml [Trasylol; Bayer, Leverkusen, Federal Republic of Germany]) buffer. To remove insoluble debris, the lysates were centrifuged at 10,000 × *g* for 15 min, and the supernatant was used for immunoprecipitation.

Synchronization of initiation of protein synthesis by hypertonic salt treatment. The order of glycoproteins in the glycoprotein precursor was determined by using synchronous initiation of protein synthesis (14, 25) and short pulses (2 to 6 min) of [^{35}S]methionine. Initiation of translation was inhibited by incubating the cells in minimal essential medium containing 335 mM NaCl (final concentration) for 40 min. To achieve synchronous initiation, the hypertonic medium was then replaced with 1 ml of pulse-labeling medium: methionine-free isotonic minimal essential medium supplemented with 20 mM HEPES and 100 to 500 μCi of [^{35}S]methionine per ml. Chase after the pulse and preparation of cell lysates for immunoprecipitation were carried out as described above.

Antibodies. Immunoblotting experiments, described in a previous report (13), have shown that the rabbit polyclonal G1-G2 antiserum used here in immunoprecipitations recognizes only G1 and G2 glycoproteins but not the N protein. However, when immunoprecipitations were carried out, as described below, the N protein coprecipitated. Because the coprecipitation of N could be prevented by using small concentrations of SDS, I concluded that, under the conditions used, the N protein precipitated by being in association with the glycoproteins. Routinely, SDS was not used because the G1-G2 antiserum, raised against Triton X-100-solubilized and purified glycoproteins, precipitated the undenatured forms of G1 and G2 better.

The specificities of the anti-glycoprotein monoclonal antibodies have been characterized by immunoblotting and immunoprecipitation (12). Immunoprecipitations here were carried out by using a mixture of three G1-specific or three G2-specific monoclonal antibodies (UG1-8, UG1-19, and UG1-22 for G1 and UG2-5, UG2-9, and UG2-16 for G2).

Immunoprecipitation and SDS-PAGE. Immunoprecipitation was carried out in 1.5-ml tubes by mixing 10 to 50 μl of lysate, 1.0 ml of NET buffer, and 5 to 10 μl of monoclonal antibody mixture (three G1-specific or three G2-specific antibodies, see above) or polyclonal G1-G2 antiserum. After incubation for 60 min at 25°C, 100 to 200 μl of 10% (vol/vol) protein A-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) was added, and the mixture was incubated overnight at 4°C in an end-over mixer. The precipitate was washed three times with NET buffer and once with 10 mM Tris-hydrochloride (pH 6.8). Finally, 50 μl of electrophoresis sample buffer was added, and the samples were boiled for 3 min and analyzed on 10% linear or 7.5 to 15% gradient polyacrylamide gels by the method of Laemmli (15). Fluorography was carried out as described by Bonner and Laskey (3).

Enzyme treatments. Purified virus or immunoprecipitated G1 and G2 were digested with 20 mU of endo H (Miles Laboratories, Inc., Elkhart, Ind.) per ml (30) for 20 h at 37°C in 0.125 M citrate buffer (pH 5.8), supplemented with 100 U of aprotinin per ml. It was found convenient to carry out the enzyme digestion of immunoprecipitated G1 and G2 on protein A-Sepharose beads, because the buffer for the enzyme treatment and the running buffer for electrophoresis could be changed easily by washing. To be sure that by the procedure used complete digestion was achieved, immunoprecipitated glycoproteins from mature virions and from cell lysates (pulse-labeled for 10 min and chased for 15 or 90 min) were boiled in 1% SDS for 2 min, diluted 1:10 with 0.125 M citrate buffer (pH 5.8) (to final SDS concentration of 0.1%), and digested with endo H exhaustively. Both procedures gave identical results.

To digest the terminal sialic acids of the protein-bound glycans, purified [^{35}S]methionine-labeled virus of immunoprecipitated glycoproteins were incubated in 0.1 M acetate buffer (pH 5.75) containing 0.5 U of neuramididase (from *Vibrio comma*, 1 IU/ml; Behringwerke AG, Marburg, Federal Republic of Germany) per ml for 1 h at 37°C. As a control, 5 μg of purified vesicular stomatitis virus was incubated under the same conditions. A clear and apparently quantitative shift in the mobility of the vesicular stomatitis virus G protein in SDS-gel (10) was observed (data not shown).

Labeling of G1 and G2 in the presence of tunicamycin. Chicken embryo or BHK-21 cells infected with Uukuniemi virus as described above were incubated in the presence of 2 μg of tunicamycin (Eli Lilly & Co., Indianapolis, Ind.) per ml at 6 to 8 h postinfection in methionine-free minimal essential medium supplemented with 0.2% bovine serum albumin and 20 mM HEPES. The cells were labeled with 100 μCi of [^{35}S]methionine per ml at 8 to 9 h postinfection in the presence of tunicamycin (29, 31), and a cell lysate was prepared and used in immunoprecipitation as described above.

Two-step procedure for virus purification. For kinetic analysis of secretion of labeled virus and for quantitation of G1 and G2 incorporated into virions, a two-step procedure for virus purification was used. The sample, 1 to 2 ml of culture medium from a culture dish, was centrifuged at 10,000 × *g* for 15 min to remove cells and cell debris, and the virus was concentrated by centrifugation through a 30% sucrose cushion in an SW41 rotor for 60 min at 30,000 rpm. The pelleted virus was dissolved in a small amount of electrophoresis sample buffer and loaded on a gel. The procedure gave pure preparations suitable for quantitation of the glycoproteins.

RESULTS

Nonglycosylated G1 and G2 are the same size. The fully glycosylated forms of Uukuniemi virus glycoproteins G1 and G2 have apparent M_rs of about 70,000 and 65,000, respectively (22), and are clearly resolved by SDS-PAGE, but only under nonreducing conditions (13, 21). To determine the size of the nonglycosylated forms (apoproteins) of G1 and G2, infected cells were labeled with [^{35}S]methionine at 8 to 9 h after infection in the presence of tunicamycin to inhibit the addition of asparagine-linked oligosaccharides (29, 31). When the nonglycosylated G1 and G2 were immunoprecipitated with polyclonal G1-G2 antiserum and analyzed by SDS-PAGE, only one band migrating with an apparent M_r of ca. 54,000 was seen (Fig. 1, lane c). The N protein seen at the bottom of the lane coprecipitated under the conditions used because of its strong affinity to one or both of the membrane proteins (see above). A protein with an apparent M_r of 54,000 could be immunoprecipitated with both G1-specific (lane d) and G2-specific (lane e) monoclonal antibodies (see above). In this experiment, the gel analysis was carried out under reducing conditions. Under nonreducing conditions, both apoproteins migrated slightly slower, with M_rs of about 57,000 (data not shown). Thus, the nonglycosylated forms of G1 and G2 are of about the same size.

G1 is amino terminal and G2 is carboxy terminal in the p110 precursor. The G1 and G2 proteins are synthesized in vitro as a nonglycosylated 110,000-dalton precursor (p110) (32). To determine the order of G1 and G2 in p110, the proteins were labeled in vivo by using short [^{35}S]methionine pulses after protein synthesis was synchronized with hypertonic salt (14, 25). After the cells were released from the hypertonic block by using isotonic medium, [^{35}S]methionine was added for 2, 4, or 6 min. The cells were then subjected to a

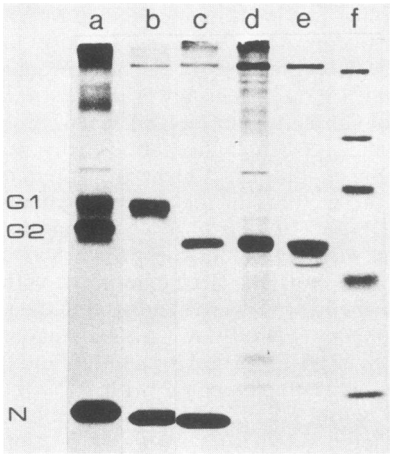

FIG. 1. Sizes of the nonglycosylated forms of G1 and G2. In lanes a and b, intracellular forms of viral proteins are shown, separated under nonreducing (lane a) and reducing (lane b) conditions. Nonglycosylated G1 and G2, synthesized in the presence of tunicamycin (2 μg/ml), comigrated in SDS-PAGE as a single band (M_r, 54,000), as shown in lane c. In lanes d and e, nonglycosylated G1 and G2, respectively, have been immunoprecipitated by G1- and G2-specific monoclonal antibodies. The molecular weight markers (lane f, top to bottom) are (kilodaltons): myosin (200), phosphorylase b (92.5), albumin (69), ovalbumin (46), and carbonic anhydrase (30).

chase period of 15 min in a medium containing an excess of unlabeled methionine. As a control, a 2-min pulse followed by a 15-min chase was given to a nonsynchronized virus-infected culture. After the chase, the glycoproteins were immunoprecipitated from cell lysates and analyzed by SDS-PAGE under nonreducing conditions (Fig. 2). When initiation of protein synthesis was not synchronized, roughly equal labeling of the two glycoproteins was observed after a 2-min pulse (lane a), whereas after the cells were released from the salt block, a 2-min pulse labeled only G1 (lane b) and after a 6-min pulse G1 and G2 were both clearly labeled (lane d). This indicates that G1 is located amino-terminally and G2 is located carboxy-terminally in the p110 precursor.

To confirm this, the viral proteins were synthesized in the presence of puromycin, which causes a premature release of nascent polypeptides from the ribosomes as puromycin peptides (1). Under limiting concentrations of puromycin, the probability of successful synthesis of the amino-terminal part of a polyprotein is greater than that for the carboxy-terminal region (27). Figure 3B shows the ratio of G1 to G2 labeled with [^{35}S]methionine at 8 to 11 h postinfection and synthesized in the presence of different concentrations of puromycin. The proteins were analyzed by SDS-PAGE from purified extracellular virus. Scanning of the different lanes of the fluorogram (Fig. 3A) showed that the labeled G2 glycoprotein disappeared before G1 and that the G1/G2 ratio increased with increasing puromycin concentrations (Fig. 3B). This confirmed that G1 is amino terminal to G2 in the p110 precursor.

Maturation of G1- and G2-linked glycans. G1 in mature virions apparently contains only endo H-resistant chains,

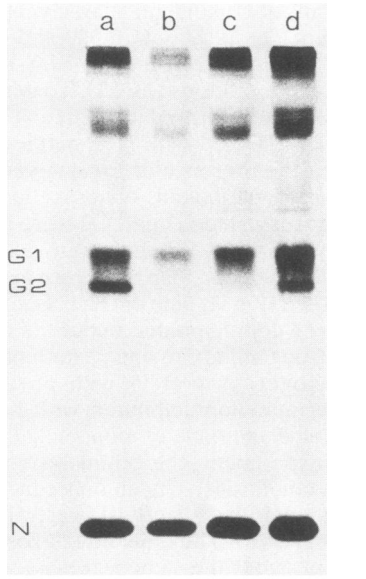

FIG. 2. Order of G1 and G2 in the glycoprotein precursor p110. Initiation of protein synthesis was synchronized by hypertonic salt treatment, and the cells were pulsed with [^{35}S]methionine. Lane a, Labeling (without synchronization) with a 2-min pulse gives roughly equal labeling of G1 and G2. Lanes b, c, and d, Labeling of G1 and G2 after 2-, 4-, and 6-min pulses, respectively. G1 was seen to appear before G2, indicating that G1 is located amino-terminally and G2 is located carboxy-terminally in the glycoprotein precursor p110.

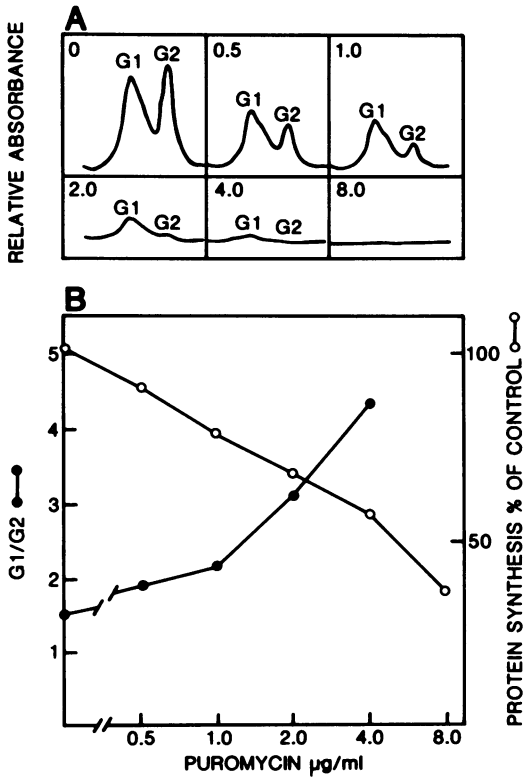

FIG. 3. Uukuniemi virus glycoproteins in extracellular virions, secreted from cells labeled with [^{35}S]methionine in the presence of various concentrations of puromycin were analyzed in a polyacrylamide gel under nonreducing conditions. In (A), the scanning profiles of the glycoproteins in separate lanes (puromycin concentration, 0 to 8.0 μg/ml) of the gel are shown. Note the disappearance of G2 before G1. The G1/G2 ratio, shown in (B), increases with increasing puromycin concentration.

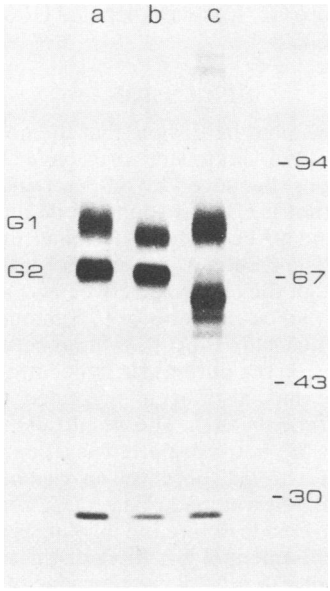

FIG. 4. Sialidase and endo H treatment of mature G1 and G2. In lane a, mature forms of G1 and G2, isolated from purified virus, were separated in a 10% Laemmli gel. Sialidase treatment of G1 and G2 (lane b) resulted in an apparent shift in the mobility of G1; in addition the G1 band has become sharper. A small shift in the mobility of G2 can also be seen. Endo H treatment, as shown in lane c, does not affect the mobility of G1, but the digestion results in a mobility shift of G2: a set of three bands, migrating faster than the untreated G2-band, is generated.

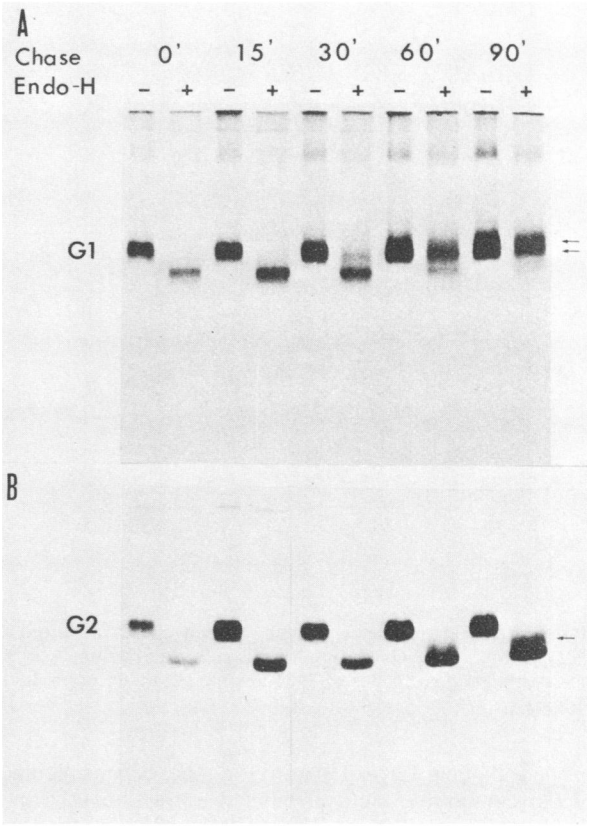

FIG. 5. Kinetics of processing of glycans associated with G1 and G2. (A) Kinetics of maturation of G1 to endo H resistance. After a 10-min pulse of [^{35}S]methionine, followed by chases of different lengths, the G1 protein was immunoprecipitated by using a mixture of G1-specific monoclonal antibodies. Endo H-treated and untreated samples were analyzed in a 10% Laemmli gel under reducing conditions. The glycans of G1 acquired endo H resistance in a half time of about 45 min. After a 90-min chase, the endo H-resistant form of G1 showed an additional migration shift, possibly due to sialylation. The two forms can be seen as a doublet, indicated by arrows. (B) Similar analysis of G2 as studied under reducing conditions. The glycans of G2 appear to remain endo H sensitive throughout the chase period. However, a fuzzy region, indicated by an arrow, is detected after a 60-min chase (compare, Fig. 7).

whereas G2 contains mainly endo H-sensitive glycans (21). These conclusions were based on mobility shifts of the proteins in SDS-gels after endo H treatment.

When the virion proteins were treated with sialidase (Fig. 4, lane b), a slight increase in mobility of both glycoproteins occurred, as compared with untreated controls (lane a). The mobility shift of G1 was clear and the band became sharper. The shift of G2 was smaller but still detectable. Endo H treatment had no effect on the mobility of G1, whereas G2 was resolved into three bands, all of which migrated faster than the untreated G2 (Fig. 4, lane c). The G2 triplet could be observed only in nonreducing SDS-gels. G2 therefore probably also contains some endo H-resistant glycans in addition to the high-mannose glycan chains.

The kinetics of acquisition of endo H resistance of G1 was studied by giving a 10-min pulse with [^{35}S]methionine at 8 h postinfection, followed by different chase periods. After the chase, cell lysates were prepared, and G1 was immunoprecipitated with a mixture of three G1-specific monoclonal antibodies (see above), treated with endo H, and analyzed by SDS-PAGE under nonreducing conditions. G1 became endo H resistant in a half time of roughly 45 min (Fig. 5A). The endo H-resistant G1 migrated faster than the G1 found in the virion. An additional mobility shift could be seen after longer chase periods (Fig. 6). A band with the same mobility as the virion G1 started to appear after a 60-min chase, and the bulk of the G1 had fully matured only after a 150- to 210-min chase.

The corresponding analysis of the maturation of G2 is shown in Fig. 5B (reducing conditions) and Fig. 7 (nonreducing conditions). When analyzed by SDS-PAGE under reducing conditions, G2 appeared to remain endo H sensitive up to a 90-min chase. A fuzzy region above the major band could, however, be seen at this time in the endo H-treated sample (Fig. 5B, arrow). Analysis under nonreducing conditions (Fig. 7) revealed the appearance of a faint triplet of bands after a 60-min chase. These bands which became more prominent only after longer chase periods had the same mobilities as those observed in virion G2 after endo H treatment. These results suggest that the maturation kinetics of G1 and G2 differ, G1 becoming endo H resistant more quickly than G2.

Kinetics of appearance of G1 and G2 in extracellular virions. Uukuniemi virus-infected cells were pulse-labeled for 5 min with [^{35}S]methionine at 8 h postinfection. After the pulse, chase medium was added, and the secreted virus was harvested at 30-min intervals from the same culture dish. The virus was purified and analyzed on a 10% polyacrylamide gel under nonreducing conditions. After fluorography, the G1 and G2 bands were quantitated by scanning from a moderately exposed film (Fig. 8A and B). Labeled virus was first detected in the medium at about 30 min, and a peak in the secretion of the pulse-labeled virus was reached after a

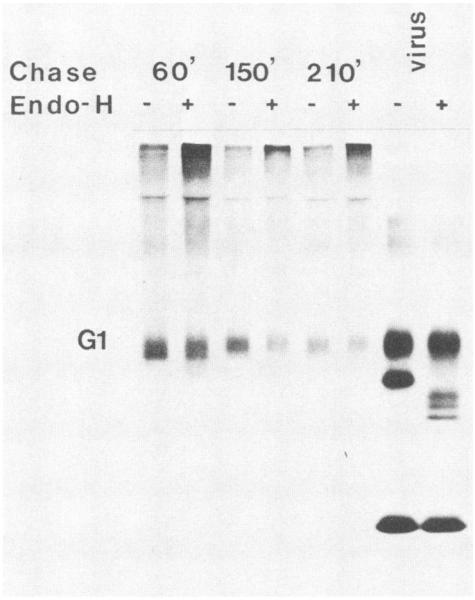

FIG. 6. The G1 doublet (Fig. 5A, 90-min chase) acquired the mobility of the virion G1 during a longer chase. The analysis was carried out in a 7.5 to 15% gradient Laemmli gel under nonreducing conditions.

chase period of ca. 120 to 150 min (Fig. 8B). When calculated from the cumulative yield of labeled extracellular virus, a half time of secretion of about 120 min was observed. Interestingly, labeled G1 was consistently detected in virions at ca. 10 min before G2. Likewise, labeled G1 also leveled off somewhat before G2.

DISCUSSION

The results presented here show that the nonglycosylated forms of G1 and G2 of Uukuniemi virus have about the same size (54,000 daltons) as judged by SDS-gel analysis. By two independent methods, G1 was found to be amino terminal, and G2 was found to be carboxy terminal in glycoprotein precursor p110. The results of the experiment with puromycin also suggest that the cleavage between G1 and G2 occurs cotranslationally, since the ratio of [^{35}S]methionine-labeled G1 to G2, which normally is near 1, varied between 2 and 4.5 (Fig. 3) depending on the puromycin concentration. Cotranslational cleavage has also been suggested from in vitro translation experiments (32). The result also suggests that the glycoproteins are not transported as a complex since the ratio of labeled G1 to G2, observed in virions, is different. The possibility that newly made G1 and G2 form complexes with glycoproteins made before the labeling is not excluded.

After endo H treatment of G2, three distinct bands migrating faster than untreated G2 were reproducibly observed in SDS-PAGE under nonreducing conditions (e.g., Fig. 6 and 7). The faster-migrating band comigrated with the nonglycosylated G2 made in the presence of tunicamycin. The triplet observed after endo H treatment was shown to appear kinetically in pulse-chase experiments (Fig. 7) (see below). Thus, it appears that the bulk of G2 is heterogeneously glycosylated, containing in addition to high-mannose-type chains also different numbers of endo H-resistant ones. The presence of terminally glycosylated endo H-resistant chains in G2 was further supported by the observation that sialidase

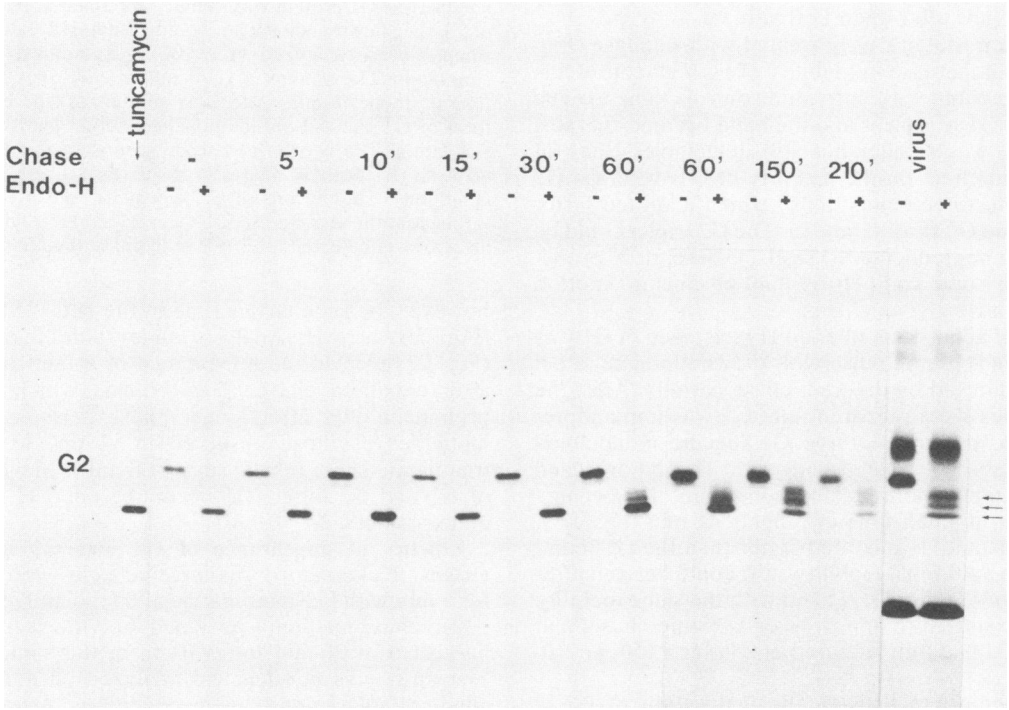

FIG. 7. Maturation of G2-associated glycans, as analyzed in a 7.5 to 15% gradient Laemmli gel under nonreducing conditions. The three faster-migrating bands, generated by endo H treatment of mature virion G2 (lane far right, arrows), appear in intracellular G2 after a 30- to 60-min chase, indicating that the bulk of G2 is heterogeneously glycosylated. The early phase (0 to 60 min) and the late phase (60 to 210 min) were analyzed in separate gels.

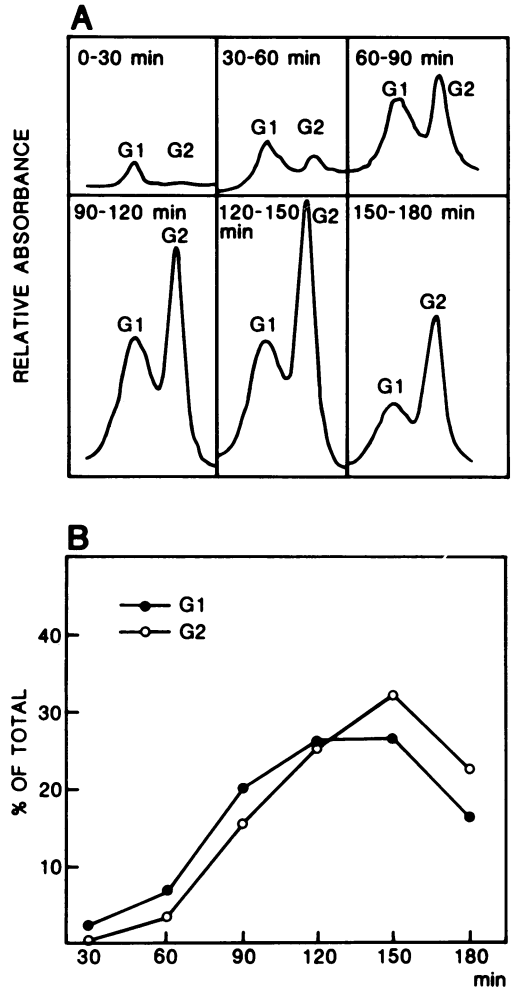

FIG. 8. Kinetics of transport of G1 and G2 to extracellular virions. After a 5-min pulse of [^{35}S]methionine, the virus secreted to the medium was harvested at 30-min intervals. After purification, the secreted virus was analyzed in a 10% Laemmli gel under nonreducing conditions, and the intensities of the G1 and G2 bands were scanned (A). The quantitation of the glycoprotein bands (B) shows that the half time for virus secretion was about 120 min. G1 appeared to be incorporated into virions faster than G2; the difference in the kinetics was about 10 min.

treatment resulted in a slight increase in the mobility of G2 in SDS-PAGE (Fig. 4).

One interesting feature of the bunyaviruses is that progeny virus particles bud into the Golgi complex (2, 12). During infection, the G1, G2, and N proteins accumulate in the Golgi region, and a relatively small fraction of the glycoproteins are found at the cell surface (13, 18). It has been suggested that inefficient intracellular transport of the glycoproteins results in their accumulation in the Golgi (13, 18), thereby increasing the probability of budding of virions into the Golgi or Golgi-derived vesicles (13).

It is generally considered that the time required for the acquisition of endo H resistance corresponds to the transport time from the ER to some location within the Golgi complex (28). Maturation of G1 to an endo H-resistant form occurred in a half time of roughly 45 min, and the resistant form was first detected at about 30 min. The final step of terminal glycosylation (sialylation), as judged by a mobility shift in SDS-PAGE, was first observed at 60 to 90 min and was completed by about 120 to 150 min. Maturation of G2 seemed to occur somewhat more slowly. The presence of all three bands was clearly evident at 60 min (Fig. 7). An endo H-protein pattern similar to that from virion G2 was obtained only after 90 to 150 min of chase.

Thus, the maturation of the glycans to endo H resistance and probably also the final sialylation of the Uukuniemi virus glycoproteins is unusually slow as compared with, for example, vesicular stomatitis virus G protein, which acquires endo H resistance in a half time of about 8 min (28). Recently it has been shown for several secretory proteins (17) and for some membrane proteins (5) that long half times of terminal glycosylation are a result of slow transport kinetics from the ER to the Golgi complex. At present we do not know whether, in the case of these Uukuniemi virus proteins, the transport from the ER to the *cis*-side of the Golgi is slow or whether transport within the Golgi is retarded.

[^{35}S]methionine pulse-label was first detected in extracellular virions after about 30 min, and the half time of secretion was about 120 min. Thus, although there is a very broad distribution of secretion time, the process itself can be relatively fast. Comparable kinetics of secretion has been reported for La Crosse virus (18), another bunyavirus. The similar half times for terminal glycosylation and virus secretion suggest that the virus budding may occur in distal parts of the functional Golgi complex. The other possibility is that the terminal glycosylation of the glycoproteins takes place on the virions. Interestingly, G1 appeared kinetically about 10 min before G2 in extracellular virions. This suggests that G1 and G2 may be transported independently of each other and with different kinetics to the site of virus maturation in the Golgi complex.

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