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Characterization of Human Immunodeficiency Virus Type 2 Envelope Glycoproteins: Dimerization of the Glycoprotein Precursor during Processing

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Four glycoproteins with apparent molecular weights of 300,000, 140,000, 125,000, and 36,000 (gp300, gp140, gp125, and gp36) were detectable in human immunodeficiency virus type 2 (HIV-2)-infected cells. gp125 and gp36 are the external and transmembrane components, respectively, of the envelope glycoproteins of HIV-2 mature virions. gp300 and gp140 are only detectable in virus-infected cells. They have identical isoelectric points, suggesting that gp300 might be a dimeric form of the immature precursor, gp140. The purified gp300 can be dissociated in a slightly acidic buffer to give rise to monomers of 140,000 molecular weight. Such dissociated monomers and the purified gp140 showed identical patterns of polypeptides after partial proteolysis with *Staphylococcus aureus* V8 protease. Pulse-chase experiments indicated that gp300 is formed after synthesis of gp140 and before the detection of the mature external envelope glycoprotein, gp125. These results were confirmed by using various inhibitors of glycosylation and inhibitors of trimming enzymes. Dimer formation of the envelope glycoprotein precursor was also observed in cells infected with simian immunodeficiency virus (SIV), a virus closely related to HIV-2. On the other hand, the envelope glycoprotein precursor of HIV-1 did not form a dimer during its processing. Therefore, dimer formation seems to be a specific property of HIV-2 and SIV envelope gene expression. Such transient dimerization of the glycoprotein precursor might be required for its efficient transport to the Golgi apparatus and for its processing.

The etiological agent of acquired immunodeficiency syndrome (AIDS) is the retrovirus referred to as human immunodeficiency virus (HIV) (39). To date, two related but distinct types, HIV type 1 (HIV-1) and HIV-2, have been identified (2, 4, 6, 7, 20, 30, 46, 61). HIV-2 is closely related to simian immunodeficiency virus (SIV), which causes an AIDS-like disease in macaques (5, 10, 55). HIV-1, HIV-2, and SIV show all the features of retrovirus family members (5, 20, 38, 60). Their proviral genomes contain the two long terminal repeats and the three essential genes required for virus replication, which encode the viral internal structural proteins (*gag*), the reverse transcriptase (*pol*), and the envelope glycoproteins (*env*) of the virus. In addition to these genes, both HIVs and SIV contain additional genes encoding the proteins which regulate viral expression (*tat* and *rev*) and three other genes encoding proteins of unknown function (*vif*, *nef*, and *vpr*). The only notable difference in the genetic organizations of HIV-1, HIV-2, and SIV resides in the open reading frame referred to as vpx, which is absent in HIV-1. Alignments of the nucleotide sequences of HIV-1, HIV-2, and SIV reveal considerable homology between HIV-2 and SIV. These two viruses share about 75% overall nucleotide sequence homology, but they are only distantly related to HIV-1, with about 40% overall homology (5, 20). At the protein level, the *gag* and *pol* proteins of HIV-1, HIV-2, and SIV are antigenically cross-reactive, whereas the *env* proteins are cross-reactive only between HIV-2 and SIV (7). HIV-1, HIV-2, and SIV are both tropic and cytopathic for CD4-positive T lymphocytes (7, 9, 15, 24, 25, 37). A great number of studies have indicated that CD4 functions as the cellular receptor for HIV-1 (62).

The HIV-1 *env* gene codes for a 160-kilodalton (kDa) glycoprotein that is proteolytically cleaved to yield the extracellular and transmembrane proteins, gp120 and gp41, respectively (40). It has been demonstrated that HIV-1 recognition of CD4 is mediated by gp120. This complex, gp120-CD4, can be identified by coimmunoprecipitation, using antibodies specific for the CD4 antigen (36). After gp120 is bound to CD4, the entry of HIV-1 into the cell might occur by viral envelope cell membrane fusion (32, 35, 54, 56). A putative fusogenic domain in gp41 (27), which has a sequence homologous to other fusion peptides (Phe-Leu-Gly; 16), might provide at least one HIV fusion site necessary for this process (34).

In this report, we have characterized the processing of HIV-2 envelope glycoproteins. Four glycoproteins, referred to as gp300, gp140, gp125, and gp36, were synthesized in HIV-2-infected cells. gp125 and gp36 correspond to the external and transmembrane glycoproteins of the HIV-2 virion, whereas gp300 and gp140 are only detectable in infected cells. gp300 is a dimeric form of gp140, which is the immature precursor of the HIV-2 envelope glycoprotein. This dimer is very stable since it resists ionic and nonionic detergents, high levels of salt, 4 M urea, and reducing agents. However, it can be dissociated in low pH to yield gp140. Dimerization occurs in the endoplasmic reticulum after the action of trimming enzymes. We speculate that conformational modifications brought about by the formation of this dimer are necessary for transport of the glycoprotein precursor to the Golgi apparatus.

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Transient dimer formation of the glycoprotein precursor seems to be an intrinsic property of the polypeptide moiety of the HIV-2 envelope. This is a novelty in the mechanism of glycoprotein processing with N-linked oligosaccharide chains.

MATERIALS AND METHODS

Materials. L-[^{35}S]methionine (specific activity, >1,000 μCi/mmol), L-[6-^{3}H]fucose (specific activity, 45 to 70 μCi/mmol), D-[6-^{3}H]glucosamine (specific activity, 20 to 40 μCi/mmol), and D-[U-^{14}C]mannose (specific activity, 200 to 300 mCi/mmol) were purchased from Amersham (London, United Kingdom). Bromoconduritol, castanospermine, 1-deoxymannojirimycin (dMM), 1-deoxynojirimycyn (dNM), swainsonine, and tunicamycin were obtained from Boehringer GmbH (Mannheim, Federal Republic of Germany). Endo-β-*N*-acetylglucosaminidase H (endo H) and *Staphylococcus aureus* V8 protease were from Calbiochem-Behring (San Diego, Calif.). Ampholines were purchased from Pharmacia (Uppsala, Sweden).

Virus and cells. The HIV-1_{BRU} isolate of HIV-1 (39), the HIV-2_{ROD} isolate of HIV-2 (6), and the SIV isolate SIV_{mac142} (10) were used in this study.

The different cell lines and human lymphocytes were cultured in the suspension medium RPMI 1640 (GIBCO Laboratories, Cergy-Pontoise, France) containing 10% (vol/vol) fetal calf serum; 2 μg of Polybrene (Sigma Chemical Co., St. Louis, Mo.) per ml was added for HIV-infected cell cultures. CEM clone 13 cells were derived from the human lymphoid cell line CEM (ATCC CCL119) and express T4 antigen to a high level. At 5 days after infection with HIV-1_{BRU} or HIV-2_{ROD} isolates, about 80 to 90% of the cells produced viral particles which could be identified by a cytopathic effect corresponding to vacuolization of cells and the appearance of small syncytia. The HUT-78 cell line is another human T4-positive lymphoid cell line (17) that is highly permissive for the replication of SIV_{mac142} (10). Peripheral blood lymphocytes from healthy blood donors were stimulated for 3 days with 0.2% (wt/vol) phytohemagglutinin fraction P (Difco Laboratories, Detroit, Mich.) in RPMI 1640 medium supplemented with 10% fetal calf serum. Cells were then cultured in RPMI 1640 medium containing 10% (vol/vol) T-cell growth factor (Biotest). After infection with HIV-2, lymphocytes were cultured in the presence of 10% (vol/vol) T-cell growth factor and 2 μg of Polybrene per ml.

Metabolic labeling of cells. For metabolic labeling of proteins, infected cells were incubated for 16 h at 37°C in minimum essential culture medium without L-methionine and serum but supplemented with 200 μCi of [^{35}S]methionine per ml. For metabolic labeling of glycoproteins, infected cells were incubated for 16 h at 37°C in minimum essential culture medium lacking serum and glucose but supplemented with 200 μCi [^{3}H]fucose per ml, 200 μCi of [^{3}H]glucosamine per ml, or 25 μCi of [^{14}C]mannose per ml.

Cell and viral extracts. Cell pellets corresponding to 10^7 cells were suspended in 100 μl of buffer (10 mM Tris hydrochloride [pH 7.6], 150 mM NaCl, 1 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride [PMSF], 100 U of aprotinin [Iniprol; Choay, Paris, France] per ml) before the addition of 100 μl of the same buffer containing 2% (vol/vol) Triton X-100. Cell extracts were centrifuged at 12,000 × *g* for 10 min, and the supernatant was stored at −80°C until used. For viral extract preparations, 100 μl of 10× lysis buffer (100 mM Tris hydrochloride [pH 7.6], 1.5 M NaCl, 10 mM EDTA, 10% [vol/vol] Triton X-100, 100 U of aprotinin per ml) was added per ml of clarified supernatant from infected CEM cells and processed as described above.

Preparation of an immunoadsorbent with antibodies from HIV-2-seropositive patient serum. Immunoglobulins from the serum of an HIV-2-seropositive patient were precipitated with 50% $(NH_4)_2SO_4$, dissolved in 20 mM sodium phosphate (pH 8.0), and further purified on a DEAE-cellulose column (DE 52; Whatman, Inc., Clifton, N.J.) by elution with 20 mM sodium phosphate (pH 8.0). Immunoglobulins purified in this manner were judged to be 90% pure. The antibodies were subsequently coupled to CNBr-activated Sepharose CL 4B according to a technique described by Berg (3). Then, 2 mg of immunoglobulin G was coupled per ml of Sepharose CL 4B. This immunoadsorbent is referred to as HIV-2 serum-Sepharose.

Binding of HIV-2 proteins on the immunoaffinity column. Cell extracts from HIV-2-producing CEM cells were first diluted in 2 volumes of binding buffer (20 mM Tris hydrochloride [pH 7.6], 50 mM KCl, 150 mM NaCl, 1 mM EDTA, 1% [vol/vol] Triton X-100, 20% [vol/vol] glycerol, 7 mM β-mercaptoethanol, 0.2 mM PMSF, 100 U of aprotinin per ml) before incubation with 1 volume of HIV-2 serum-Sepharose. Supernatants from HIV-2-producing cells were processed as cell extracts, except that only 1/10 of binding buffer concentrated 10 times was added per volume of supernatant. The binding was carried out overnight, and then the column was washed batchwise in binding buffer. Proteins that bound to the column were eluted by being boiled in electrophoresis sample buffer (125 mM Tris hydrochloride [pH 6.8], 1% [wt/vol] sodium dodecyl sulfate [SDS], 2 M urea, 20% glycerol, 1% β-mercaptoethanol). Eluted proteins were resolved by electrophoresis on 7.5% polyacrylamide-SDS gels containing 6 M urea and 0.1% bisacrylamide instead of 0.2% (wt/vol).

Preparative electrophoresis. HIV-2 glycoproteins that were eluted from the affinity column were resolved by polyacrylamide gel electrophoresis as previously described (28), and the regions of the gel containing the viral glycoproteins were cut out by reference to the positions of prestained molecular weight protein markers (Bethesda Research Laboratories, Inc., Gaithersburg, Md.).

Glycoproteins were eluted by incubation for 16 h at 4°C in elution buffer (0.1 M $NaHCO_3$, 0.5 mM EDTA, 0.05% [wt/vol] SDS, 0.2 mM PMSF). The glycoprotein fractions thus obtained were lyophilized and kept refrigerated until used.

Two-dimensional gel isoelectric focusing. Two-dimensional gel isoelectric focusing was performed as described by O'Farrel (42), with the following modification. L-[^{35}S]methionine-labeled proteins bound on the HIV-2 serum-Sepharose column were eluted by being boiled in electrophoresis sample buffer as described above before dilution in a volume of buffer containing 9.5 M urea, 8% (vol/vol) Nonidet P-40, 1.6% (wt/vol) ampholines (pH ranges 6.5 to 9), 0.4% (wt/vol) ampholines (pH ranges 3 to 10), 100 U of aprotinin per ml, and 0.5% (vol/vol) β-mercaptoethanol.

RESULTS

Identification of HIV-2 envelope glycoproteins. Recently, it has been reported that the envelope gene of HIV-2 (ROD isolate) encodes a precursor glycoprotein that is then cleaved proteolytically to yield a 120-kDa extracellular glycoprotein and a 36-kDa transmembrane glycoprotein (6, 7).

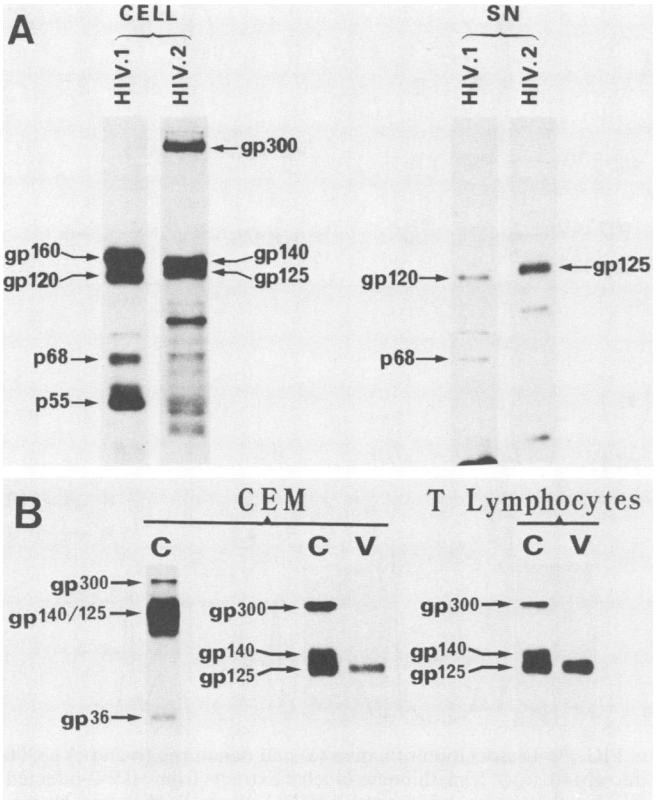

FIG. 1. HIV-2 envelope glycoproteins. (A) Comparison of high-molecular-weight proteins of HIV-1 and HIV-2. CEM cells infected with HIV-1 or HIV-2 were labeled with [^{35}S]methionine (200 μCi/ml; 4×10^6 cells per ml) for 18 h. Extracts from these infected cells (CELL) and their corresponding culture medium (SN) were purified on specific immunoaffinity columns: HIV-1, serum-Sepharose specific for HIV-1 proteins (28); HIV-2, serum-Sepharose specific for HIV-2 proteins (see Materials and Methods). These purified proteins were analyzed by electrophoresis in a 7.5% polyacrylamide-SDS gel containing 6 M urea. A fluorograph of the gel is presented. The sizes of the HIV-1 and HIV-2 proteins are indicated at the left and right of the lanes. p68 and p55, Reverse transcriptase and *gag* precursor, respectively; gp160 and gp120, glycoprotein precursor of the HIV-1 envelope and its cleaved product; gp300, gp140, and gp125, glycoprotein precursors and the cleaved product of the HIV-2 envelope. (B) Identification of HIV-2 glycoproteins. HIV-2-infected CEM cells and T lymphocytes were labeled with [^{3}H]glucosamine (200 μCi/ml; 4×10^6 cells per ml) for 18 h. Extracts from infected cells (lanes C) and culture medium containing virus (lanes V) were purified on the HIV-2 serum-Sepharose column, and the labeled proteins were analyzed by electrophoresis in a 7.5% gel. In the lane on the far left, electrophoresis of HIV-2 glycoproteins synthesized in CEM cells was analyzed in a 12.5% acrylamide gel to show the presence of gp36. This part of the figure had to be overexposed to see gp36, and for this reason gp140 and gp125 are resolved as a thick band.

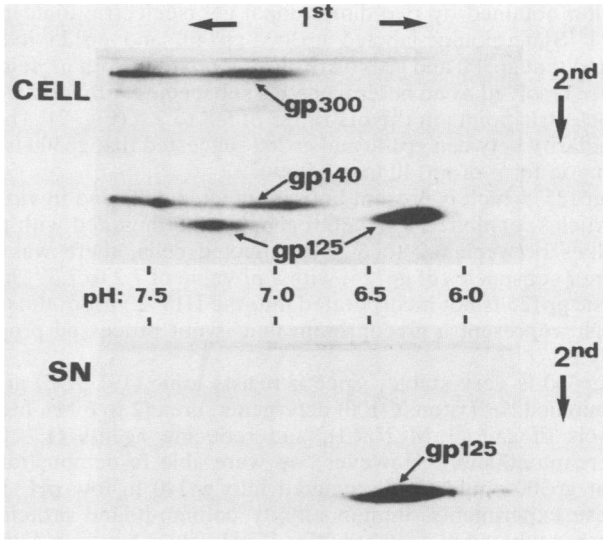

FIG. 2. Two-dimensional gel isoelectric focusing of gp140, gp300, and gp125. [^{35}S]methionine-labeled gp300, gp140, and gp125 purified from HIV-2-infected CEM cells (CELL) and culture medium containing virus (SN; prepared as described in the legend to Fig. 1) were analyzed by two-dimensional gel electrophoresis (see Materials and Methods). The pH gradient obtained by the isoelectric focusing (first dimension) was as shown. In the second dimension, proteins were resolved on a 7.5% polyacrylamide-SDS gel containing 6 M urea. Fluorographs of the gels are presented.

To identify the precursors of the HIV-2 glycoproteins, we studied viral proteins in infected cells as well as in virus particles. For comparison, we also studied the synthesis of HIV-1 proteins in cells infected with HIV-1 (BRU isolate).

Three major high-molecular-mass proteins of 300, 140, and 125 kDa were detectable in HIV-2-infected cells (Fig. 1A). The proteins are specific to HIV-2 because they are absent in noninfected cells and because they could be consistently identified by all HIV-2- but not by HIV-1-seropositive sera in an immunoprecipitation assay (data not shown). In side-by-side comparisons, the electrophoretic mobilities of these three HIV-2 proteins are clearly different from those of the 160-kDa HIV-1 precursor glycoprotein (gp160) and one of its cleaved products, the 120-kDa external envelope glycoprotein (gp120; Fig. 1A). It should be noted that the resolution of the 140- and 125-kDa proteins of HIV-2 from one another can be clearly observed in polyacrylamide-SDS gels containing a high concentration of urea. In the absence of urea, these proteins migrated as a thick band. The 300- and 140-kDa proteins were only detectable in infected cells, whereas the 125-kDa protein was detectable both in infected cells and in the virus (Fig. 1A).

The glycosylation of the 300-, 140-, and 125-kDa proteins was demonstrated by metabolic labeling with [^{3}H]glucosamine (Fig. 1B). Moreover, in a 12.5% polyacrylamide gel, the fourth glycoprotein of 36 kDa (gp36) was identified. gp36 is only slightly glycosylated, and its detection required longer exposure times. These results indicated that among the glycoproteins detectable in HIV-2-infected cells, gp125 and gp36 correspond to the virion envelope, whereas gp300 and gp140 might be precursors of the envelope glycoproteins. The presence of gp300 and gp140 was not restricted to infected CEM cells, since they were also detectable in HIV-2-infected T4 lymphocytes (Fig. 2B). As in CEM cell cultures, gp300 and gp140 were detectable only in infected cells, whereas gp125 was present both in cells and in HIV-2 particles.

In order to increase the resolution of the high-molecular-weight proteins, we used 0.1% bisacrylamide instead of 0.2% (wt/vol) in all polyacrylamide gels (see Fig. 1 to 10). In a 5% polyacrylamide gel containing 0.05 or 0.1% bisacrylamide, gp300 almost comigrated with the 280,000-molecular-weight protein marker obtained from BDH Biochemical Reagents, Dorset, England (data not shown).

Characterization of gp300 and gp140. The pattern of reso-

lution obtained by two-dimensional gel isoelectric focusing of [^{35}S]methionine-labeled gp300, gp140, and gp125 indicated that gp300 and gp140 are closely related. Both proteins were resolved as an heterogeneous subspecies with identical isoelectric points in the pH range of 6.8 to 7.8 (Fig. 2). This similarity between gp140 and gp300 suggested that gp300 is a dimeric form of gp140 (see below).

gp125, which is present both in infected cells and in virus particles, exhibited less heterogeneity and migrated with pI values between 6.2 to 6.5. In infected cells, there was a minor subspecies of gp125, with a pI value of 7.2 to 7.3. This basic gp125 is not incorporated into the HIV-2 virion; thus it might represent a glycoprotein that is not processed properly.

gp300 is very stable, since it resists ionic (1% SDS) and nonionic (2% Triton X-100) detergents, urea (2 to 6 M), high levels of salt (1 M NaCl), and reducing agents (1% β-mercaptoethanol). However, we were able to demonstrate that gp300 could be dissociated into gp140 in low pH. In these experiments, immunoaffinity column-bound proteins were incubated in acetate buffer at pH values between 4 and 7. These samples were then analyzed by polyacrylamide gel electrophoresis. Figure 3a shows that the band of gp300 shifted to the position of gp140 when the sample was incubated at pH 4. Further experiments were carried out by using purified preparations of gp300 obtained by preparative gel electrophoresis. Such denatured samples of gp300 were dissociated completely in acetate buffer at pH 6.0 (Fig. 3b). In Tris hydrochloride buffer, the dissociation was less efficient. At pH 7.5, there was only a slight dissociation of gp300 to gp140, but it increased with decreasing pH values. In Tris buffer at pH 6.0, the dissociation was about 80% (Fig. 3c). During dissociation of the pure gp300 in either acetate or Tris hydrochloride buffer, no proteins other than gp140 were detectable (experiments carried out in 15% polyacrylamide gels; data not shown). These results indicated that gp300 is a dimeric form of gp140, the precursor of HIV-2 envelope glycoprotein. It seems most likely that during processing of the envelope glycoprotein, two molecules of gp140 become fused by a pH-dependent mechanism.

Partial proteolysis of gp300 and gp140 by *S. aureus* V8 protease. In order to compare the polypeptide pattern of gp300 and gp140, we carried out partial proteolysis experiments with *S. aureus* V8 protease, which manifests specificity for glutamoyl bonds (22). For this purpose, gp300, gp140-gp125 (from infected cells), and gp125 (from virus extracts) were purified by immunoaffinity chromatography and by preparative gel electrophoresis. Each sample was then subjected to partial proteolysis by using different concentrations of V8 protease (Fig. 4a, lanes 2 to 4). The 140-kDa protein arising from the dissociation of gp300 (section gp300) was partially or totally converted to a 125-kDa protein when incubated with increasing concentrations of V8 protease. A similar electrophoretic pattern was observed for the sample containing both gp140 and gp125; with increasing concentrations of the V8 protease, the gp140 band disappeared completely and the final product was a 125-kDa protein (Fig. 4a, section 140/125, lanes 2 and 4). gp125 was digested with increasing concentrations of V8 protease, but no specific polypeptides of smaller molecular size became apparent (Fig. 4a). Analysis of samples digested with V8 protease (samples as in lanes 3) by electrophoresis in a 15% polyacrylamide gel revealed the presence of a 30- to 36-kDa protein in addition to the 125-kDa protein (Fig. 4b). These data suggested that in gp140 there is a preferential cleavage site for the action of V8 protease and that when gp140 is cleaved, other sites become accessible due to a change in the conformation of the cleaved product.

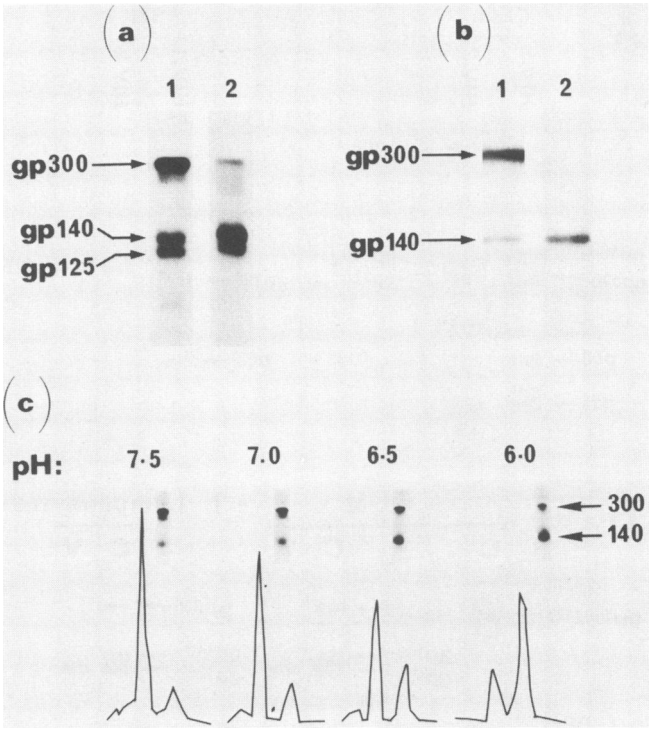

FIG. 3. Dissociation of native (a) and denatured (b and c) gp300 into gp140. (a) [^{35}S]methionine-labeled extracts from HIV-2-infected CEM cells were purified on the HIV-2 serum-Sepharose column. This sample was then divided into two aliquots. One was incubated in the binding buffer (lane 1), whereas the other was incubated in buffer containing 30 mM sodium acetate (pH 4.0), 0.2 mM PMSF, 100 U of aprotinin per ml, and 5 mM β-mercaptoethanol (lane 2). After 1 h at 37°C, the acidic medium was neutralized and both samples were analyzed by electrophoresis. (b) Purified and lyophilized [^{35}S]methionine-labeled gp300 was suspended in 100 μl of the sodium acetate buffer (pH 4.0) as described in panel a (lane 2). Incubation reactions were carried out for 30 min at 37°C before the addition of twofold electrophoresis sample buffer containing 2 M urea. In lane 1, the lyophilized gp300 was directly suspended in the electrophoresis sample buffer. (c) The purified and lyophilized [^{35}S]methionine-labeled gp300 was suspended in solution containing 30 mM Tris hydrochloride, 0.2 mM PMSF, and 100 U of aprotinin per ml; it was buffered with HCl at pH 7.5, 7.0, 6.5, and 6.0 (as indicated). After 60 min at 37°C, twofold electrophoresis sample buffer was added and the samples were analyzed by electrophoresis. Fluorographs of the gels are shown in panels a, b, and c. In panel c, gp300 and the dissociated gp140 were quantified by densitometric scanning of the fluorograph.

A potential cleavage site for the cellular protease between gp125 and gp36 has been described at amino acid 505, immediately after the Lys-Glu-Lys-Arg sequence (20). It is most likely, therefore, that the V8 protease preferentially cleaves this Gly-Lys peptide to give rise to 125- and 30- to 36-kDa products. In the envelope precursor (gp140), there are more than 30 glutamoyl bonds which represent a great number of putative cleavage sites for the V8 protease. Four of these glutamoyl bonds are composed of Gly-Lys peptides, among which one is localized in the potential cleavage site between gp125 and gp36 whereas the other three are found at different positions in gp36. At low concentrations of V8 protease, there might be a preferential cleavage of Glu-Lys

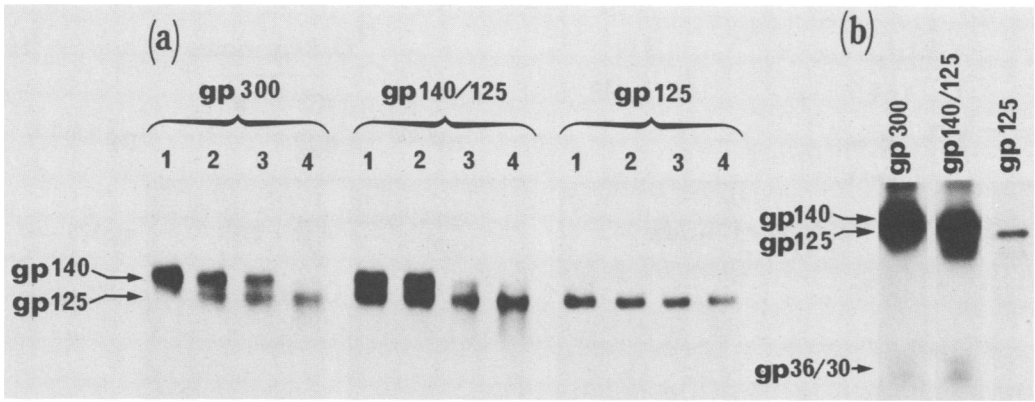

FIG. 4. Partial digestion of gp300, gp140, and gp125 with V8 protease. Purified and lyophilized gp300, gp140-gp125, and gp125 (see Materials and Methods) were investigated for susceptibility to digestion with V8 protease. The gp300 preparation was first dissociated to gp140 by being suspended in 50 μl of sodium acetate buffer at pH 6.0 (as described in the legend to Fig. 3) before the addition of 50 μl of buffer containing 125 mM Tris hydrochloride (pH 6.8) and 0.2% SDS (wt/vol). gp140-gp125 and gp125 were suspended directly in 100 μl of buffer containing 62.5 mM Tris hydrochloride (pH 6.8) and 0.1% SDS (wt/vol). The different samples were then incubated at 37°C for 30 min without (lanes 1) or with 0.1 (lanes 2), 0.5 (lanes 3) and 1 (lanes 4) g of *S. aureus* V8 protease. Reaction experiments were stopped by the addition of twofold electrophoresis sample buffer containing 2 M urea and boiling at 95°C for 5 min. All samples were analyzed by electrophoresis in a 7.5% polyacrylamide-SDS gel containing 6 M urea. A fluorograph of the gel is shown (panel a). Analysis of identical samples digested with 0.5 μg of V8 protease on a 15% polyacrylamide gel indicated the presence of a 30- to 36-kDa digestion product in addition of the 125-kDa protein in sample gp300 and gp140-gp125 (panel b). Panel b was overexposed in order to show the 30- to 36-kDa protein. No such protein was observed in the absence of V8 digestion (data not shown).

bonds, allowing the recovery of a major 125-kDa and a minor 30- to 36-kDa product. The level of the 30- to 36-kDa protein is low, probably due to the presence of three Glu-Lys bonds in its sequence, making it more susceptible to further digestion with V8 protease. At higher concentrations of V8 protease, gp125 becomes digested into small polypeptides. For this reason, the level of purified gp125 becomes decreased significantly with increasing concentrations of V8 protease (Fig. 4a, section gp125).

Effect of the glycosylation inhibitor tunicamycin on processing of HIV-2 glycoproteins. Tunicamycin blocks N-linked glycosylation of proteins (21, 31). In the presence of 2 μg of tunicamycin per ml, the overall N-linked glycosylation of HIV-2 envelope glycoproteins was completely blocked in infected CEM cells. This was demonstrated by the lack of [^{3}H]glucosamine incorporation in the viral glycoproteins, gp300, gp140, and gp125 (Fig. 5). Under these experimental conditions, protein synthesis was not affected in infected cells treated with tunicamycin (data not shown). Such cultures, isotopically labeled with [^{35}S]methionine, accumulated two major proteins with apparent sizes of 200 and 80 to 90 kDa, which migrated as wide bands (Fig. 5). These proteins were immunoprecipitated only with patient serum that was positive for HIV-2 envelope glycoproteins (data not shown). The molecular size of the 80- to 90-kDa protein corresponds to the expected molecular size of the unglycosylated HIV-2 envelope precursor estimated from its nucleic acid sequence (20). The 200-kDa protein is probably the dimeric form of the unglycosylated envelope precursor. Besides inhibition of glycosylation, tunicamycin treatment inhibits the processing and export of the envelope glycoprotein since the 80- to 90-kDa protein was not found in the extracellular medium (Fig. 5, lanes SN). The absence of unglycosylated forms of the envelope protein in the extracellular medium of tunicamycin-treated cells might be due to its rapid degradation. Several reports have suggested that the unglycosylated form of a protein is more sensitive to proteases than its glycosylated form (43, 53). Accordingly, the small-molecular-size proteins in [^{35}S]methionine-labeled cells cultured with tunicamycin might represent partially degraded products of the unglycosylated envelope protein (Fig. 5).

Digestion with endo H confirmed the presence of N-linked oligosaccharides (58) of the high-mannose type on HIV-2 glycoproteins. Upon endo H digestion of purified gp300, gp140, and gp125, the electrophoretic mobility of gp300 was reduced to a protein of 200 kDa, whereas gp140 and gp125 were converted into an 80- to 90-kDa protein (data not shown; experiments as in reference 28). Thus, the molecular sizes of these unglycosylated proteins coincided well with proteins detectable in HIV-2-infected cells in the presence of tunicamycin (Fig. 5).

Incorporation of mannose and fucose in gp300, gp140, and gp125. Metabolic labeling of HIV-2-infected cells with [^{14}C]mannose and [^{3}H]fucose resulted in the incorporation of mannose into gp300, gp140, and gp125, whereas only gp300 and gp125 were able to incorporate fucose (Fig. 6). Fucose residues are normally transferred on oligosaccharide chains late in the glycosylation pathway after the action of trimming enzymes of the endoplasmic reticulum and Golgi apparatus (14, 26). The fact that gp140 does not contain fucose residues suggested that it might be the precursor of gp300 and gp125 (discussed below).

Effect of oligosaccharide trimming inhibitors on synthesis and process of HIV-2 glycoproteins. Asparagine-linked oligosaccharides of glycoproteins undergo extensive modifications or processing after their attachment to nascent proteins (26). The trimming reactions occur in the lumen of the rough endoplasmic reticulum (RER) and in the Golgi apparatus by specific glucosidases and mannosidases.

Processing of oligosaccharide chains of glycoproteins can be manipulated with the aid of specific inhibitors of the trimming glucosidases and mannosidases (14, 52). In these experiments, we used different trimming inhibitors to investigate the localization of HIV-2 glycoprotein precursors and also to study the role of glycosylation in the processing of the envelope precursor. The inhibitors used were castanospermine, a plant alkaloid that inhibits glucosidase I (49); dNM, a glucose analog that inhibits trimming of glucosidase I and II (29, 45); dMM, a mannose analog that inhibits mannosi-

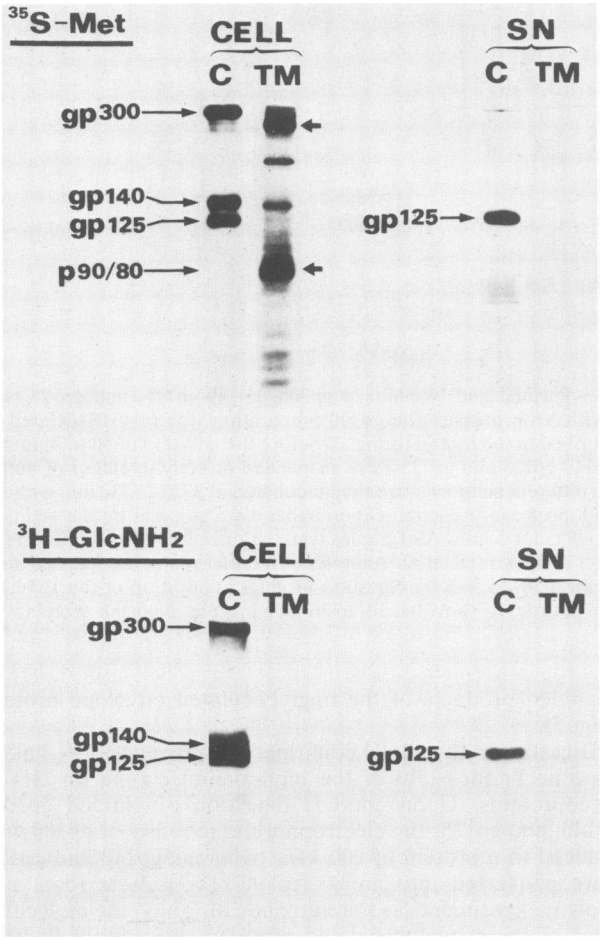

FIG. 5. Inhibition of N-linked glycosylation by tunicamycin. HIV-2-infected cells in the absence (lanes C) or presence of tunicamycin (TM; 2 μg/ml) were labeled with [^{35}S]methionine (panel ^{35}S-Met; 200 μCi/ml; 4 × 10^6 cells per ml) or with [^{3}H]glucosamine (panel ^{3}H-GlcNH$_2$; 200 μCi/ml; 4 × 10^6 cells per ml) for 16 h. Cells treated with tunicamycin were first incubated at 37°C with the antibiotic (2 μg/ml) for 2 h before being labeled with [^{35}S]methionine or [^{3}H]glucosamine. Extracts from infected cells (CELL) and from the culture medium containing virus (SN) were purified by HIV-2 serum-Sepharose and analyzed by 7.5% polyacrylamide gel electrophoresis. Fluorographs of the gels are presented. The positions of the unglycosylated envelope precursor (p90/80) and the unglycosylated dimer (200 kDa) are indicated by the arrows on the right. These 90- to 80- and 200-kDa proteins did not incorporate [^{3}H]glucosamine (panel ^{3}H-GlcNH$_2$, lanes TM).

dase-catalyzed reaction experiments (13); bromoconduritol (6-bromo-3,4,5-trihydroxycyclohex-1-ene), which inhibits glucosidase II (11); and swainsonine, an indolizidine alkaloid that inhibits Golgi mannosidase II (47). HIV-2-infected CEM cells were isotopically labeled with [^{35}S]methionine in the absence or presence of different trimming inhibitors, and intracellular and extracellular proteins were then purified by immunoaffinity chromatography (Fig. 7). As expected, control infected cells contained gp300, gp140, and gp125, whereas only gp125 was observed in the extracellular medium (Fig. 7, lanes T). In cells treated with castanospermine or dNM, there was a normal level of gp300, no gp125, and a small amount of a 150-kDa protein that probably corresponds to the glucosylated form of gp140. In such cells therefore, the processing of the envelope glycoprotein was

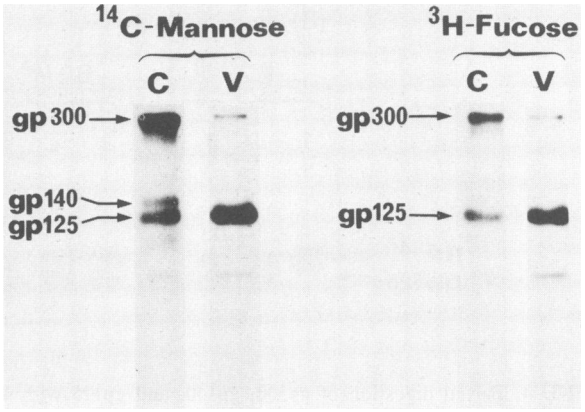

FIG. 6. Isotopic labeling of gp140, gp300, and gp125 with [^{14}C]mannose or [^{3}H]fucose. HIV-2-infected CEM cells were labeled for 18 h with [^{14}C]mannose (25 μCi/ml; 4 × 10^6 cells/ml) or [^{3}H]fucose (200 μCi/ml; 4 × 10^6 cells per ml). Extracts from infected cells (lanes C) and culture medium containing virus (lanes V) were purified on HIV-2 serum-Sepharose. Labeled glycoproteins were then analyzed by polyacrylamide gel electrophoresis. Fluorographs are shown.

blocked, since no gp125 was detectable in the extracellular medium despite the production of p26, the core protein of HIV-2 (Fig. 7, lanes Cast. and dNM). These results indicate that removal of the terminal glucose residues from the oligosaccharide chains of the envelope glycoprotein precursor is necessary for its processing and cleavage by the cellular protease. Bromoconduritol, which acts on glucosidase II, also inhibited the normal production of gp125 by 70 to 90%, but the levels of gp140 and gp300 remained normal (Fig. 7, lanes Bro.). In contrast to results with castanospermine and dNM (which inhibit removal of terminal glucose residue), bromoconduritol treatment (which inhibits removal of two inner glucose residues) did not completely block the processing of HIV-2 envelope glycoprotein. In fact, low amounts of gp125 were detectable intracellularly and extracellularly. This latter result suggests that a low level of mannose trimming can occur without removal of the two inner glucose residues. Such a phenomenon has been observed previously for the processing of other viral glycoproteins during bromoconduritol treatment (11). The mannosidase inhibitors, swainsonine and dMM, did not cause an apparent modification in the level of intracellular gp300, gp140, and gp125, but the level of extracellular gp125 was 50% less than that from the corresponding control cells (Fig. 7, lanes Sw and dMM). Thus, although the oligosaccharide chain was only deglucosylated, the glycoprotein precursor was proteolytically cleaved to yield a protein similar to gp125 but with a higher content of mannose, which probably affected the cellular transport of gp125. The molecular size of the extracellular glycoprotein produced in the presence of dMM was slightly higher than that produced in the absence of the inhibitor. This is probably due to the higher content of mannose residues in the extracellular protein synthesized by dMM-treated cells (Fig. 7, panel SN).

It should be emphasized that the effects of trimming enzyme inhibitors on the processing of HIV-2 envelope glycoprotein were specific, since the synthesis (data not shown) and production of HIV-2 p26 were not affected at all (Fig. 7, panel SN).

Effect of castanospermine and monensin on processing of HIV-2 glycoproteins. To study the intracellular processing of

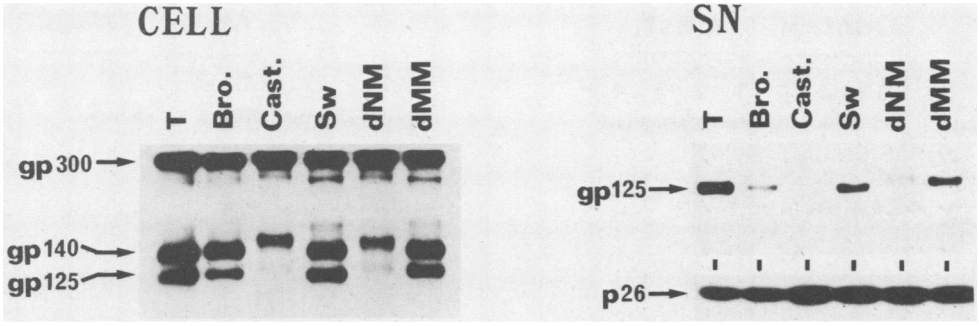

FIG. 7. Effect of oligosaccharide trimming inhibitors on the processing of HIV-2 envelope glycoprotein. HIV-2-infected CEM cells were labeled for 16 h at 37°C with [^{35}S]methionine (200 μCi/ml; 4 × 10^6 cells per ml) in the absence (lanes T) or presence of the oligosaccharide trimming inhibitor bromoconduritol (1 mM; lanes Bro.), castanospermine (1 mM; lanes Cast.), swainsonine (10 μg/ml; lanes Sw), dNM (3 mM; lanes dNM), or dMM (1 mM; lanes dMM). Extracts from infected cells (panel CELL) and from culture medium containing virus particles (panel SN) were purified on HIV-2 serum-Sepharose to identify the viral glycoproteins gp125, gp140, and gp300 in infected cells and gp125 in culture medium. All samples were analyzed by 7.5% polyacrylamide gel electrophoresis. In order to show that inhibition of gp125 production by cells treated with different inhibitors is specific to the viral glycoprotein, culture media were assayed for viral core protein p26 by an immunoprecipitation assay, using an HIV-2-seropositive serum (6, 8). p26 was analyzed by 12.5% polyacrylamide gel electrophoresis. The figure represents fluorographs, which show only one part of each gel.

HIV-2 glycoproteins, we performed pulse-chase experiments. HIV-2-infected cells were pulse-labeled for 15 min with [^{35}S]methionine before a chase for 0.5, 1.5, and 3 h (Fig. 8A, Control). gp140 was the first protein detectable, at 15 min after pulse-labeling. During the chase, gp300 became detectable at 0.5 h, whereas gp125 became detectable at 1.5 to 3 h. The facts that gp300 was observed after synthesis of gp140 and that gp125 was detectable only after formation of gp300 (Fig. 8A, lanes 1 to 4), suggest that dimerization is an intermediate step necessary for oligosaccharide processing toward the mature glycoprotein, gp125. This suggestion was confirmed by the use of castanospermine, which inhibits the trimming of the external glucose residue of polysaccharide chains. After 30 min of pulse-labeling in the presence of castanospermine, a 150-kDa protein was detectable along with gp300 (Fig. 8A, Cast., lane 1). The 150-kDa protein should correspond to gp140; the slight increase in the molecular size of the first precursor is ascribed to the presence of glucose residues in its oligosaccharide chains. Thus, gp140 synthesized in HIV-2-infected cells represents the precursor glycoprotein without its glucose residues. Accordingly, the 150-kDa protein (gp150) represents the first immature glycoprotein of the HIV-2 envelope. The removal of glucose residues in control cells has been reported to be a rapid process occurring during or briefly after cotranslational translocation of precursor glycoproteins into endoplasmic reticulum (29). After 30 min of pulse and 3 h of chase in the presence of castanospermine, the level of gp150 was gradually reduced, while gp300 accumulated (Fig. 8A, Cast., lanes 1 to 4). Under these conditions, the precursor was not cleaved to yield gp125.

Further characterization of the HIV-2 envelope glycoprotein was studied in pulse-chase experiments with monensin, a cationic ionophore that inhibits the transport of proteins from the Golgi apparatus to the plasma membrane or in some cases it might even block the transport of proteins at the level of the medial Golgi cisternae (18, 23, 52, 59). HIV-2-infected cells in the absence or presence of monensin were pulse-labeled for 30 min before a chase for 0.5, 1.5, and 3 h (Fig. 8B). In the presence of monensin, HIV-2-infected cells synthesized normal levels of gp140 and its dimeric form. However, no gp125 was detectable in monensin-treated cells. After 1.5 to 3 h of chase, monensin-treated cells accumulated a 135-kDa protein (gp135) that is probably the dissociated product of the dimer precursor. The slightly smaller molecular size of gp135 might be accounted for by the removal of some mannose residues by the action of RER and Golgi mannosidases. In view of these results, it is tempting to speculate that after deglucosylation, gp300 becomes trimmed by mannosidases of RER and Golgi before its dissociation into the mature precursor gp135 of the HIV-2 envelope. This gp135 could then be transported to the plasma membrane and also be cleaved by the cellular protease. Inhibition of protein transport by monensin blocks the mature glycoprotein gp135 in *trans* Golgi. No mature envelope glycoprotein gp125 was detectable in monensin-treated cells, intracellularly or extracellularly, although p26 was synthesized and excreted (data not shown).

Dimerization of the glycoprotein precursor also occurred in SIV_{mac}-infected cells. The nucleotide sequence of the HIV-2 envelope shows a considerable homology (75% amino acid identity) to that of SIV (5, 12, 20). For this reason, it was important to investigate whether dimerization of envelope glycoproteins was detectable in SIV-infected cells. SIV proteins were purified by the immunoaffinity column containing antibodies specific for HIV-2 proteins, since the *gag*, *pol*, and *env* proteins of HIV-2 and SIV are antigenically cross-reactive. Figure 9 shows that SIV-infected cells synthesize three high-molecular-size proteins analogous to those synthesized in HIV-2-infected cells: gp300, gp140, and gp130. Evidence that these proteins in SIV-infected cells are glycoproteins was provided by isotypic labeling with [^{14}C]mannose and [^{3}H]fucose. All three proteins incorporated mannose, but only SIV gp300 and SIV gp130 incorporated fucose. SIV gp300 and SIV gp140 are intracellular proteins, whereas SIV gp130 is the extracellular glycoprotein. The fact that SIV gp300 and SIV gp130 can incorporate fucose suggested that they are processed products of SIV gp140.

These results indicated that doublet formation of the envelope glycoprotein precursor is a specific property of HIV-2 and SIV envelope gene expression. It should be emphasized that HIV-1 envelope glycoprotein does not undergo dimerization during its processing. HIV-1-infected cells in the presence of castanospermine or dNM did not accumulate envelope dimers (data not shown) as was the case for HIV-2 or SIV.

Dimerization of gp140 in HIV-2-infected cells was inhibited

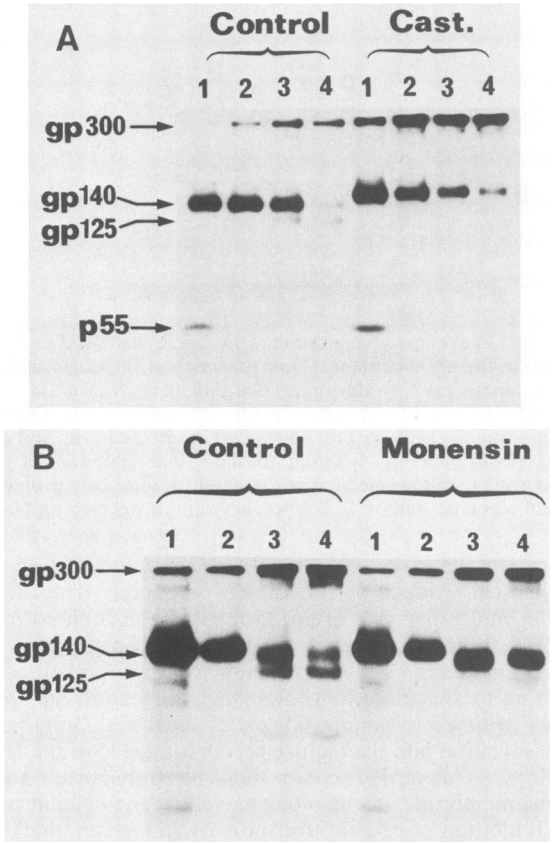

FIG. 8. Effect of castanospermine and monensin on the processing of gp300. (A) Pulse-chase experiments in HIV-2-infected CEM cells in the absence (Control) or presence of 1 mM castanospermine (Cast.). Control, Infected cells were incubated for 1 h at 37°C in methionine-free medium before 15 min of pulse-labeling with [^{35}S]methionine (200 μCi/ml; 4 × 10^5 cells per ml; lane 1). The radioactive label was then chased in culture medium containing 5 mM cold methionine for 0.5, 1.5, and 3 h (in lanes 2, 3, and 4, respectively). Cast., HIV-2-infected CEM cells were incubated for 1 h at 37°C in methionine-free medium containing castanospermine before 30 min of pulse-labeling with [^{35}S]methionine (lane 1). These cells were then chased as described above but in the presence of castanospermine for 0.5, 1.5, and 3 h (lanes 2, 3, and 4, respectively). (B) Pulse-chase experiments in HIV-2-infected cells in the absence (Control) or presence of 1 μM monensin. Infected cells with or without monensin were incubated for 1 h at 37°C in methionine-free medium before 30 min of pulse-labeling with [^{35}S]methionine (lanes 1). Labeled cells were then chased in culture medium containing 5 mM cold methionine for 0.5, 1.5, and 3 h (lanes 2, 3, and 4, respectively). Extracts were purified on HIV-2 serum-Sepharose, and labeled proteins were analyzed by polyacrylamide (7.5%) gel electrophoresis. Fluorographs are shown. p55, *gag* precursor.

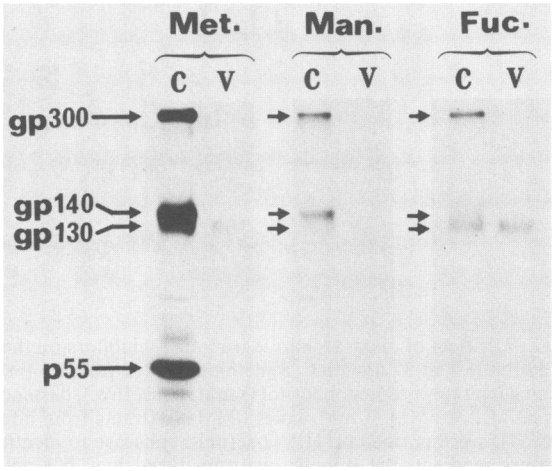

FIG. 9. Precursors of SIV envelope glycoproteins. SIV-infected HUT-78 cells were labeled for 16 h at 37°C with [^{35}S]methionine (200 μCi/ml; 4 × 10^6 cells per ml), [^{3}H]fucose (200 μCi/ml; 4 × 10^6 cells per ml), and [^{14}C]mannose (25 μCi/ml; 4 × 10^6 cells per ml). Extracts from infected cells (lanes C) and from the culture medium containing SIV (lanes V) were purified on HIV-2 serum-Sepharose. Because of cross-reactivity between HIV-2 and SIV proteins, the HIV-2-positive serum could be used to immunoprecipitate SIV proteins. All samples were analyzed by 7.5% polyacrylamide gel electrophoresis (see Materials and Methods). Fluorographs of the different gels are shown. The electrophoretic mobilities of SIV gp300 and SIV gp140 correspond to those of HIV-2 glycoproteins gp300 and gp140 (data not shown). SIV gp130 has a slightly higher mobility than HIV-2 gp125. The p55 labeled with [^{35}S]methionine is probably the *gag* precursor of SIV.

at reduced temperatures. It has been shown that protein transport in the endoplasmic reticulum and in the Golgi apparatus is blocked at reduced temperatures (48). For example, the transport of Semliki Forest virus membrane glycoproteins is blocked in the endoplasmic reticulum at 15°C, whereas at 20°C it is blocked in the Golgi apparatus (48). In order to investigate the intracellular site in which dimerization of gp140 occurs, we carried out a pulse-chase experiment at reduced temperatures, using HIV-2-infected cells. At 37°C, cells were pulse-labeled with [^{35}S]methionine for 15 min before a chase for 0.5, 1, 2, and 3 h each at 15, 20, 28, and 37°C (Fig. 10). After 15 min of pulse, the only protein detectable in HIV-2-infected cells was gp140. In cultures incubated at 37°C, the dimer was formed between the 0.5- and 1-h chases and then its level decreased with increasing time. In contrast to the results observed at 37°C, dimer formation was delayed or was not at all detectable at reduced temperatures. In fact, in cultures incubated at 15°C, the level of gp140 remained stable and dimerization was completely blocked (Fig. 10). These results indicated that formation of the dimer requires transport of gp140 in a compartment with an environment favoring the process of dimerization. This latter might occur at a stage between the endoplasmic reticulum and the Golgi apparatus, for example, in a vesicle involved in the process of transport to the Golgi. Whatever the case, these results emphasized that dimerization is a natural step in the processing pathway of gp140, i.e., dimerization is not due to accumulation of unprocessed gp140 nor is it an artifact of the experimental procedure. Dimerization observed at late chase points in cultures incubated at 20 or 28°C might reflect the slow leakage of gp140 in a compartment which favors dimerization.

Production of the mature glycoprotein gp125 was not detectable in cultures incubated at 20°C, even though the dimer gp300 was observed after 3 h of chase. This was probably due to a block in the Golgi transport of gp300. The data shown in Fig. 10 emphasize that efficient processing of the envelope glycoprotein required incubation at 37°C. Low levels of gp125 accumulated in infected cells were caused by the incorporation of gp125 in the budding virus.

It should be noted that the pulse-chase experiments at 37°C shown in Fig. 8 and 10 had slightly different kinetics of gp140 processing. This was probably due to small differences in the kinetics of virus infection, although similar experimental conditions were used in all studies. Consistently, gp140

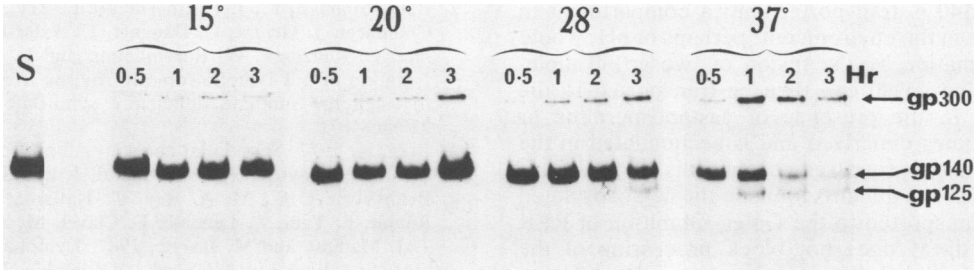

FIG. 10. Effect of temperature on dimerization of the HIV-2 envelope precursor. HIV-2-infected cells were incubated for 1 h at 37°C in methionine-free medium before 15 min of pulse-labeling with [^{35}S]methionine (200 Ci/ml; 4×10^5 cells per ml; lane S). The radioactive label was then chased in culture medium containing 5 mM cold methionine for 0.5, 1, 2, and 3 h each at 15, 20, 28, and 37°C. Extracts were then purified on HIV-2 serum-Sepharose, and labeled proteins were analyzed by 7.5% polyacrylamide gel electrophoresis. Fluorographs are shown.

was the first envelope glycoprotein synthesized in HIV-2-infected cells. gp300 was detected at 20 to 30 min after the start of metabolic labeling, whereas gp125 was detectable only after 60 to 90 min (data not shown).

DISCUSSION

Here we have provided evidence to describe for the first time processing of the HIV-2 envelope precursor glycoprotein and to point out a novelty in the mechanism of glycoprotein processing with N-linked oligosaccharide chains. The envelope glycoproteins of HIV-2, the extracellular gp125 and transmembrane gp36, arise from a common precursor glycoprotein (20). The unusual feature of this glycoprotein precursor is that it requires the formation of a homologous dimer in order to become transported and processed through the Golgi apparatus. The mechanism of dimerization of the envelope glycoprotein is not yet clear. The fact that the purified dimer can be dissociated at low pH (pH 6.0) suggests that dimerization might be pH dependent. Oligosaccharide chains on the precursor glycoprotein are not essential for dimer formation. Evidence for this was obtained by two different experiments. (i) Digestion with endo H resulted in a shift in the electrophoretic mobility of the dimer without dissociating it. (ii) In the presence of tunicamycin, HIV-2-infected cells synthesized an unglycosylated envelope precursor (80 to 90 kDa) that can form a dimer (200 kDa). These results emphasize that dimer formation is an intrinsic property of the polypeptide moiety of the envelope precursor.

Pulse-chase experiments in the absence or presence of castanospermine (Fig. 8) suggested that dimerization of the glycoprotein precursor normally occurs after removal of glucose residues in the RER. In the presence of castanospermine, the dimer becomes accumulated in the RER and it is not processed. However, once the glucose residues are removed, inhibition of the RER mannosidase did not prevent formation of a dimer and its processing through the Golgi apparatus (Fig. 7). Accordingly, the glucose residues in the oligosaccharide chains of the dimer precursor prevented its exit from the RER. In accord with this, it has been postulated that glucose trimming is necessary for efficient transport from the RER to the Golgi, possibly because the deglucosylated oligosaccharide forms part of a recognition site for a transport receptor (29, 33). It might also be possible that glucose removal is crucial for the precursor dimer to achieve a correct functional configuration (51) that favors the action of trimming mannosidases.

In view of our results, we propose the following pathway for the processing of HIV-2 envelope glycoproteins (Fig. 11). The first envelope precursor synthesized in HIV-2-infected cells is gp150, which becomes rapidly trimmed by RER glucosidases I and II and mannosidase to give rise to gp140.

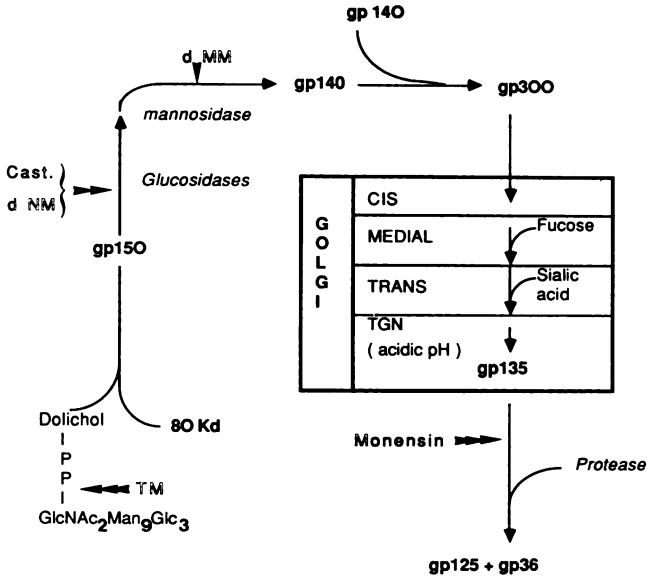

FIG. 11. Schematic pathway of HIV-2 envelope glycoprotein processing. During synthesis, the envelope polypeptide becomes glycosylated to give rise to the immature glycoprotein precursor gp150, which becomes rapidly trimmed by RER glucosidases I and II and mannosidase to give rise to gp140. At this stage, gp140 is transported into a compartment in which a difference in the environment, perhaps of pH, would trigger dimer formation by the fusion of two gp140 molecules. The resulting gp300 dimer intermediary precursor can then be transported to the Golgi apparatus. Further trimming of gp300 is carried out by the Golgi mannosidases before the transfer of fucose and sialic acid residues in the medial and *trans* Golgi. Finally, the dimer becomes dissociated into the mature precursor gp135, due to the low pH of the TGN compartment. gp135 is transported to the plasma membrane and cleaved by the cellular protease to yield the mature HIV-2 envelope glycoproteins gp125 and gp36. Tunicamycin (TM) inhibits assembly of dolichol-*P*-*P*-glycan; castanospermine (Cast.) and dNM inhibit RER glucosidases; dMM inhibits trimming of mannosidases in the RER and in *cis* and medial Golgi; and monensin inhibits the transport of membrane glycoproteins and secretory proteins from the Golgi apparatus. The 80-kDa protein represents the unglycosylated envelope protein which could be detected only in the presence of tunicamycin.

At this stage, gp140 is transported into a compartment in which a difference in the environment, perhaps of pH, would trigger dimer formation by the fusion of two gp140 molecules. The resulting gp300 can then be transported to the Golgi apparatus. In the presence of castanospermine or dNM, gp150 becomes dimerized and is accumulated in the RER. This dimer is not processed because it is glucosylated. However, as long as the dimer is found in the deglucosylated form, it can be transported to the Golgi; inhibition of RER mannosidase by dMM does not block processing of the dimer precursor. In the Golgi, gp300 traverses the different compartments probably by vesicular transport (19, 57) during which the oligosaccharide chain is further trimmed by Golgi mannosidases before the addition of other sugars such as fucose and sialic acid. Evidence for fucose incorporation has been obtained by isotypic labeling of gp300 with [^{3}H]fucose. Evidence for sialic acid incorporation was obtained indirectly by digesting gp300 with neuraminidase, an enzyme that hydrolyzes terminal *N*-acetylneuraminic acid in various glycoproteins (50). The gp300 of HIV-2 is susceptible to digestion with neuraminidase, as evidenced by a significant decrease in the electrophoretic mobility of the dimer (data not shown). The results are consistent with the precursor keeping its dimeric form all through its processing in the Golgi apparatus before its transport to the *trans*-Golgi network (TGN; 19) in which it dissociates due to a drop in the pH of this compartment. The dissociated dimer yields glycoproteins (gp135) of slightly lower molecular weight than the first detectable glycoprotein precursor (gp150 and/or gp140). gp135 could then be transported to the plasma membrane and could also be cleaved by the cellular protease to yield the mature glycoproteins of the HIV-2 envelope, gp125 and gp36. Monensin most probably inhibits transport from the Golgi to the plasma membrane; for this reason gp135 accumulates in the Golgi.

It is well accepted that the Golgi apparatus is implicated in the mechanism of sorting secretory and plasma membrane proteins, which seems to take place in the last Golgi compartment referred to as the TGN (19). This compartment, on the *trans* side of the Golgi stack, previously has been referred to as Golgi endoplasmic reticulum lysosomes and recently as post-Golgi vacuoles or the *trans*-most cisternae of the Golgi stack (41, 44, 48). Interestingly, the pH of the TGN has been considered to be mildly acidic, i.e., about pH 6 (1, 19). The low pH in the TGN could then account for the dissociation of the processed dimer.

The results discussed here illustrate that the processing of the envelope glycoproteins of HIV-2 is a multistep process involving synthesis of an immature precursor gp150 and/or gp140, the intermediary dimer precursor gp300, and finally the mature precursor gp135. Despite their evolutionary relationship, HIV-1 and HIV-2 have found different mechanisms for the processing of their envelope glycoproteins. Whether these differences are involved in their pathogenesis is under investigation.

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