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Effects of Deletions in the Cytoplasmic Domain on Biological Functions of Human Immunodeficiency Virus Type 1 Envelope Glycoproteins

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The role of the cytoplasmic domain of the human immunodeficiency virus type 1 (HIV-1) envelope glycoproteins in virus replication was investigated. Deletion of residues 840 to 856 at the carboxyl terminus of gp41 reduced the efficiency of virus entry during an early step in the virus life cycle between CD4 binding and formation of the DNA provirus without affecting envelope glycoprotein synthesis, processing, or syncytium-forming ability. Deletion of residues amino terminal to residue 846 was associated with decreased stability of envelope glycoproteins made in COS-1 cells, but this phenotype was cell type dependent. The cytoplasmic domain of gp41 was not required for the incorporation of the HIV-1 envelope glycoproteins into virions. These results suggest that the carboxyl terminus of the gp41 cytoplasmic domain plays a role in HIV-1 entry other than receptor binding or membrane fusion. The cytoplasmic domain of gp41 also affects the stability of the envelope glycoprotein in some cell types.

The presence of a long intracytoplasmic domain at the carboxyl terminus of the gp41 transmembrane protein of the human immunodeficiency virus type 1 (HIV-1) distinguishes the HIV-1 envelope glycoprotein from that of most retroviruses (4, 12, 34). The transmembrane proteins of the type C and type D retroviruses terminate within 50 amino acids carboxy terminal to the transmembrane domain (34). The presence of a cytoplasmic domain extending more than 100 amino acids beyond the transmembrane region in HIV-1, the simian immunodeficiency virus (SIV), visna virus, and the equine infectious anemia virus suggests a specific function for this region in the life cycle of the lentivirus subfamily of retroviruses (12, 34). HIV-2 and SIV are frequently truncated just after the membrane anchor domain, but these truncated forms appear to result from selection during tissue culture propagation in human cells (4, 19, 20, 22). The natural form of the SIV transmembrane protein is the full-length 41-kDa protein (20, 22).

The HIV-1 envelope glycoprotein is initially made as a 160-kDa glycosylated precursor that is cleaved to form the gp120 exterior and gp41 transmembrane subunits (7, 8, 40). The gp120 glycoprotein determines the tropism of HIV-1 for specific target cells by binding to the CD4 molecule (6, 21, 27). The gp41 transmembrane glycoprotein contains an extracellular domain that is required for membrane fusion and noncovalent association with the gp120 glycoprotein, a hydrophobic transmembrane region that anchors the gp120-gp41 complex in the cell or virion lipid bilayer, and an intracytoplasmic domain of unknown function (2, 11, 14, 24, 35). The cytoplasmic domain contains two highly conserved segments with the potential to form amphipathic α helices and may form a secondary association with the lipid bilayer (1, 14, 15, 39).

The cytoplasmic domain of gp41 is required for efficient HIV-1 replication, but the function of this domain in the virus life cycle is unknown (9, 26, 37). The cytoplasmic domain is not required for syncytium formation or binding to CD4 (7, 8, 24) but may modulate intracellular transport and processing of gp160 in some cell types (16). Whether the cytoplasmic domain is involved in virion assembly, for example, by facilitating the incorporation of envelope glycoproteins into virions, is unknown.

To investigate the role of the gp41 cytoplasmic domain in virus replication, the effect of deletions in this region on the synthesis, processing, cell surface expression, incorporation into virions, and biological function of the HIV-1 envelope glycoproteins was investigated.

MATERIALS AND METHODS

Plasmids. The pSVIIIenv plasmid expresses the HIV-1 *env* and *rev* genes of the HXB2 strain (31) under the control of the HIV-1 long terminal repeat (LTR) (17, 24). The pHXBΔenvCAT plasmid contains an HIV-1 provirus with an in-frame deletion from the *Bgl*II to *Bgl*II sites (nucleotides 6620 and 7200) of the sequence of Ratner et al. (32) in the *env* gene and a chloramphenicol acetyltransferase (CAT) gene replacing the *nef* gene (17, 36). The pHXBΔenvΔEcoCAT plasmid, derived from pHXBΔenvCAT, contains an additional deletion from *Eco*RI to *Eco*RI (nucleotides 4231 to 5325) overlapping the *pol* gene. These plasmids contain a simian virus 40 origin of replication. Envelope glycoprotein deletion mutants were made by *Bal* 31 exonuclease digestion at either the *Bam*HI site or the *Xho*I site (nucleotide 8053 or 8476, respectively) (24, 32, 37). The *env* open reading frame was restored when necessary either by creating blunt ends prior to religation or by inserting *Cla*I linkers. The Δ(767-856) mutant was made by inserting an *Eco*RI linker at an *Mnl*I site to create a frameshift at amino acid 767. Oligonucleotide-directed mutagenesis was used to create the Δ(796-803), Δ(796-804), Δ(846-856), Δ(851-856), and Δ(854-856) deletions in an *Eco*RI-to-*Xho*I (nucleotides 5325 to 8476) fragment of HXB2 subcloned into the pBluescript plasmid (Stratagene) (25). All plasmids were sequenced in the region

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of the introduced mutation. The pHXB2Δ(840-856) provirus was made by subcloning the deleted *Bam*HI-to-*Xho*I fragment into the pHXB2 plasmid (31). The *rev* expressor plasmid pSVIIIenvΔKS contains a *Kpn*I-to-*Stu*I (nucleotide 5926 to 6411) out-of-frame deletion in the envelope gene and expresses HIV-1 *rev* but not the envelope glycoproteins.

Virus replication studies in Jurkat lymphocytes. Jurkat T lymphocytes were transfected by the DEAE-dextran method (30) with 10 μg of pHXB2 or pHXB2Δ(840-856). Following transfection, cultures were maintained in RPMI 1640 plus 10% fetal calf serum with daily medium changes. Reverse transcriptase activity of pelleted virions was measured as previously described (33).

Radioimmunoprecipitation. For measurement of viral protein expression in infected Jurkat cells, 5×10^6 cells were metabolically labeled with 100 μCi (each) of [^{35}S]cysteine and [^{35}S]methionine per ml for 16 h. Virions were harvested for immunoprecipitation by centrifugation of supernatants for 10 min at 1,500 × *g* to remove cell debris and ultracentrifugation at 12,000 × *g* for 1 h. Cells or virions were lysed in RIPA lysis buffer, and HIV-1 proteins were immunoprecipitated with AIDS patient serum and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (23). The human monoclonal antibody (50-69) to gp41 was obtained from the National Institutes of Health AIDS Research and Reference Reagent Program and was a donation of Susan Zolla-Pazner. For measurement of envelope glycoprotein expression in transfected COS-1 cells, 3×10^6 cells were metabolically labeled with 100 μCi of [^{35}S]methionine per ml for 16 h at 48 h posttransfection with 5 μg each of an envelope expressor plasmid and the *rev* expressor plasmid by the DEAE-dextran method (5). The labeled cells were lysed in 10 mM Tris-HCl (pH 7.4)–150 mM NaCl–5% Triton X-114 at 0°C, and the detergent phase containing integral membrane proteins was isolated by phase separation at 30°C, dissolved in RIPA lysis buffer, clarified, and immunoprecipitated as previously described (3, 11). Virions produced in COS-1 cells were pelleted as described above following labeling of transfected COS-1 cells for 16 h and chasing for 3 h with medium containing excess unlabeled methionine and cysteine.

PCR assay for determining the efficiency of provirus formation following acute infection. Fresh virus stocks were prepared from supernatants of infected Jurkat cell cultures by centrifugation at 1,500 × *g* for 10 min and filtration (0.45-μm-pore-size filter) to remove cell debris. Virus stocks were treated with DNase (Worthington) (2 μg/ml) for 20 min at room temperature to eliminate plasmid DNA contamination (41). Heat-inactivated virus control supernatants were incubated for 90 min at 60°C. Jurkat cells (10^7) were incubated with HXB2 or HXB2Δ(840-856) for 7 h at 37°C. After 7 h, 2×10^6 cells were harvested by centrifugation, washed twice, lysed in 50 μl of 0.2% Nonidet P-40, and boiled for 15 min. The cell lysate was clarified by centrifugation at 12,000 × *g* for 2 min and stored at −20°C. For polymerase chain reactions (PCR), the DNA in 2.5 μl of the cell lysate (100,000 cell equivalents) was amplified by using the HIV-1 LTR R/U5 primers 5′ GGCTAACTAGGGAACCCACTG 3′ and 5′ CTGCTAGAGATTTTCCACACTGAC 3′, which are similar to the AA55 and M667 primer pair previously described (41). PCR reactions were performed according to the manufacturer's instructions (Perkin-Elmer Cetus Corp.) for 33 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min. The PCR products were analyzed by electrophoresis on 2% agarose gels.

Replication complementation assay for measuring the replicative potential of mutant envelope glycoproteins. A transient *trans*-complementation assay was used to assess the replicative potential of mutant envelope glycoproteins in a single round of virus replication (17). Cell-free virus transmission was assessed by cotransfecting COS-1 cells by the DEAE-dextran method (5) with 5 μg of an envelope expressor plasmid and 5 μg of pHXBΔenvCAT. At 48 to 72 h after transfection, the COS-1 cell supernatants were filtered (0.45-μm-pore-size filter) and the reverse transcriptase activity was measured (33). Equivalent reverse transcriptase units of cell-free supernatants were added to 5×10^6 Jurkat T lymphocytes. The Jurkat cells were incubated for 48 to 72 h and then assayed for CAT activity. In a similar *trans*-complementation assay, both cell-free and cell-to-cell virus transmission were measured by direct transfection of Jurkat T lymphocytes as previously described (17).

Syncytium formation assays. The ability of the mutant glycoproteins to mediate the formation of syncytia was assessed in COS-1 cells by cotransfection of 5 μg of the envelope expressor plasmid and 5 μg of the *rev* expressor plasmid followed by cocultivation with CD4-positive SupT1 lymphocytes for 6 h at 60 h posttransfection (38). The syncytium-forming abilities of the mutant envelope glycoproteins were assessed in Jurkat-*tat* cells by cotransfecting 8 μg of the envelope-expressing plasmid and 8 μg of the *rev* expressor plasmid into Jurkat *tat* cells and scoring syncytia at 60 h posttransfection (11).

Expression of envelope glycoproteins on the cell surface. COS-1 cells cotransfected with an envelope expressor plasmid and the *rev* expressor plasmid were metabolically labeled with [^{35}S]methionine for 16 h at 48 h posttransfection. The intact labeled cells were washed twice with ice-cold phosphate-buffered saline (PBS) containing 2% heat-inactivated fetal calf serum, incubated with a 1:100 dilution of AIDS patient serum reactive with the envelope glycoproteins at 4°C for 30 min, rinsed twice with PBS containing 2% fetal calf serum, and lysed in RIPA lysis buffer containing 2.5 μg of unlabeled gp120 (American BioTechnologies, Inc.) at 4°C. The cell lysates were clarified by ultracentrifugation, and bound envelope glycoproteins were immunoprecipitated by incubation with protein A-Sepharose as described above.

Soluble-CD4 inhibition of syncytium formation and virus replication. The effect of soluble CD4 on virus entry was measured by producing the recombinant HXBΔenvCAT proviruses in COS-1 cells as described above and preincubating equivalent amounts of virus as determined by measuring reverse transcriptase activity with different concentrations of full-length soluble CD4 (American BioTechnologies) for 1 h at 37°C prior to infection of Jurkat lymphocytes (38).

RESULTS

Effects of a deletion at the carboxyl terminus of gp41 on HIV-1 replication in Jurkat lymphocytes. A mutation which deletes residues 840 to 856 (28) at the carboxyl terminus of gp41 was introduced into an infectious HIV-1 provirus on plasmid pHXB2. To compare the replication rate of this mutant virus with that of the wild-type virus, the pHXB2 and pHXB2Δ(840-856) plasmids were transfected into Jurkat cells and virus replication was monitored by measuring reverse transcriptase activity in the culture supernatants. The Jurkat cultures transfected with the pHXB2Δ(840-856) plasmid demonstrated slowed virus replication, with a 5-day lag in the time required to reach peak reverse transcriptase activity compared with that of the wild-type virus (Fig. 1), which is consistent with previous studies (9, 26, 37). When

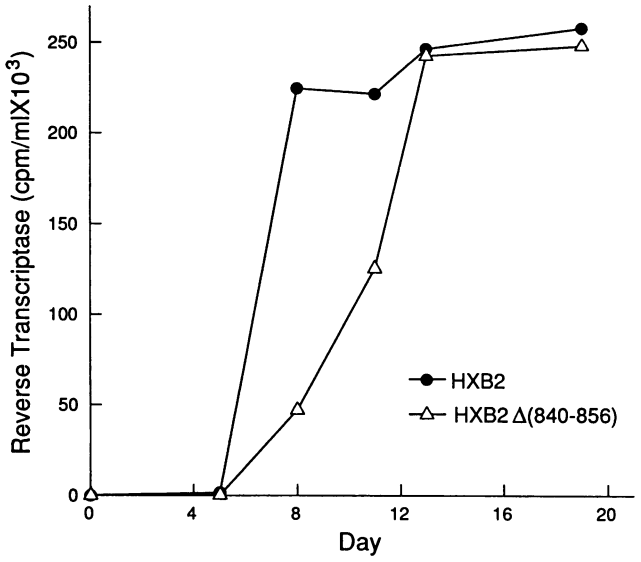

FIG. 1. Replication of HXB2 and HXB2Δ(840-856) viruses in Jurkat cells. Curves show reverse transcriptase activity in supernatants of Jurkat cell cultures transfected with 10 μg of pHXB2 or pHXB2Δ(840-856) DNA. The results are typical of those seen in at least three independent experiments.

similar levels of virus production were achieved, the number of syncytia formed in the pHXB2- and pHXB2Δ(840-856)-transfected cultures and the viability of these cultures were indistinguishable (data not shown).

To examine the effects of a deletion at the carboxyl terminus of gp41 on *gag* and *env* protein synthesis, processing, secretion, and incorporation into virions in Jurkat cells, steady-state and pulse-chase labeling experiments were performed at a time when virus production in HXB2- and HXB2Δ(840-856)-infected cultures was similar. At 14 to 21 days after transfection, equal numbers of viable cells were metabolically labeled with [^{35}S]cysteine and [^{35}S]methionine for 16 h and viral proteins were immunoprecipitated from cell lysates, supernatants, and pelleted virions. The steady-state levels of viral *gag* and *env* proteins were similar in the pHXB2- and pHXB2Δ(840-856)-transfected cell lysates, supernatants, and pelleted virions (Fig. 2A, B, and C and data not shown), although slightly less cell-associated gp160 was observed in the pHXB2Δ(840-856)-transfected culture (Fig. 2A). Pulse-chase analysis of infected Jurkat cell cultures demonstrated similar synthesis, processing, and release of envelope glycoproteins (Fig. 2D and E). However, the level of the mutant gp160 in cell lysates was approximately 50% of the wild-type level after a 2-h or longer chase (Fig. 2D). This decrease in the level of gp160 at the longer chase times was not accompanied by an increase in the level of cell-associated gp120 glycoprotein. The gp120 glycoprotein was released into the cell supernatants after a 1-h chase in both the wild-type and mutant cultures (Fig. 2E). These results suggest that deletion of residues 840 to 856 at the carboxyl terminus of gp41 results in decreased stability of the gp160 glycoprotein in infected Jurkat cells. Nonetheless, the synthesis, processing, secretion, and incorporation into virions of the mutant HXB2Δ(840-856) envelope glycoproteins appear to be comparable to those of the wild-type glycoproteins.

Deletion of the carboxyl terminus of gp41 reduces the efficiency of virus entry. To determine whether the cytoplasmic domain of gp41 has an effect on the efficiency of virus entry, HXB2 or HXB2Δ(840-856) virus stocks were used to infect Jurkat cells and the efficiency of HIV-1 provirus formation at 7 h after acute infection was measured by PCR amplification of the proviral DNA. Uninfected Jurkat cells and Jurkat cells incubated with heat-inactivated virus controls were used as controls (41). The HIV-1 LTR R/U5 primer pair used for PCR amplification detects the first region of the viral DNA made during reverse transcription and should detect virtually all HIV-1 DNA made, including partial DNA transcripts (41). Mapping of restriction sites within the amplified DNA product with *Hin*dIII, *Hin*fII, *Xho*II, and *Mbo*I restriction enzymes, which cut at internal sites, confirmed that the 140-bp fragment amplified by these primers was the correct HIV-1 LTR fragment (data not shown). Following acute infection of Jurkat cells, formation of the HXB2Δ(840-856) provirus as detected by PCR amplification of viral DNA was significantly reduced compared

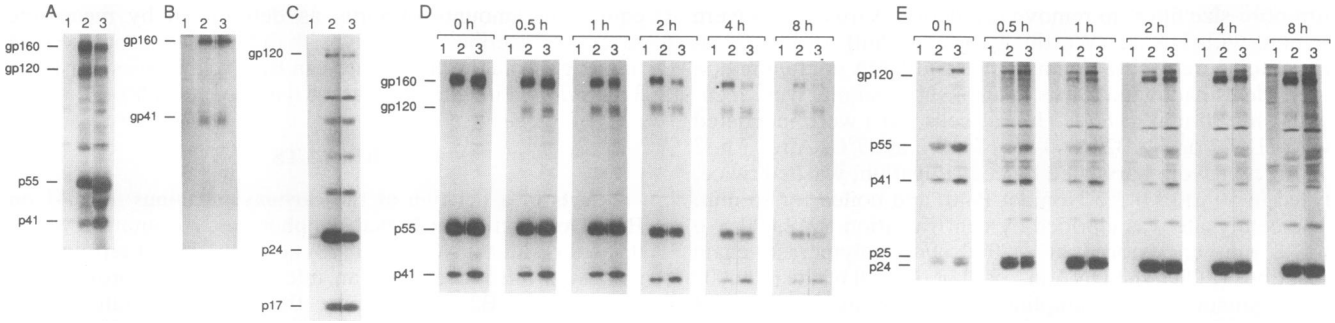

FIG. 2. HIV-1 *env* and *gag* proteins in HXB2- and HXB2Δ(840-856)-infected Jurkat cells and incorporated into virions. Immunoprecipitated cell lysates (A, B, D), virions (C), and supernatants (E) were assayed at days 14 to 21 following transfection of Jurkat cells with no DNA (lanes 1), 10 μg of pHXB2 (lanes 2), or 10 μg of pHXB2Δ(840-856) (lanes 3). Cells were metabolically labeled with [^{35}S]cysteine and [^{35}S]methionine for 16 h (A, B, C) or by a pulse-chase protocol (D, E). The gp160, gp120, and gp41 envelope glycoproteins and the p55, p24, and p17 *gag* products are marked. (A) Immunoprecipitated cell lysates with AIDS patient serum analyzed by 4 to 12% gradient SDS-PAGE; (B) immunoprecipitated cell lysates with a human monoclonal antibody (50-69) that recognizes gp41 analyzed by 12.5% SDS-PAGE; (C) immunoprecipitated pelleted virions with AIDS patient serum analyzed by 12.5% SDS-PAGE; (D and E) pulse-chase analysis of cell lysates (D) and supernatants (E) immunoprecipitated with AIDS patient serum and analyzed by 4 to 12% gradient SDS-PAGE. Cells were labeled for 10 min and chased for the indicated times (in hours) with medium containing excess unlabeled cysteine and methionine.

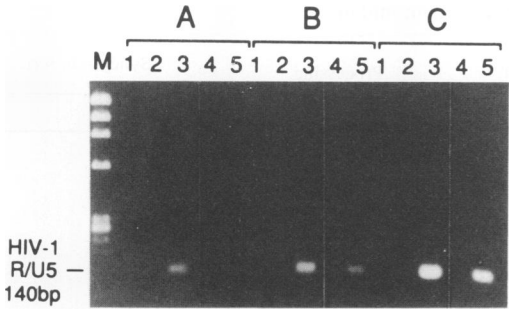

FIG. 3. Detection of HIV-1 provirus formation in Jurkat cells by PCR amplification of viral DNA following acute infection with HXB2 or HXB2Δ(840-856) viruses. PCR amplification was performed by using the HIV-1 LTR R/U5 primer pair M667-AA55 (41) for 33 cycles to amplify DNA from 100,000 Jurkat cell equivalents harvested 7 h after acute infection with 4,000 (A), 20,000 (B), or 100,000 (C) cpm of reverse transcriptase units of no virus (lane 1), heat-inactivated HXB2 (lane 2), HXB2 (lane 3), heat-inactivated HXB2Δ(840-856) (lane 4), or HXB2Δ(840-856) (lane 5) per ml. The 140-bp PCR product was analyzed by electrophoresis on 2% agarose gels and visualized with ethidium bromide. A DNA standard (*Phi*X-*Hae*III digest) is shown in lane M.

with that of wild type (Fig. 3). As shown in Fig. 3, the effect of the mutation on the efficiency of virus entry was most marked at the lower multiplicities of infection (4,000 and 20,000 cpm/ml). These results suggest that the carboxyl terminus of gp41 has an effect on the efficiency of virus entry during an early stage in the virus life cycle prior to formation of the DNA provirus.

Mutational analysis of sequences in the cytoplasmic domain of gp41 required for replication. The cytoplasmic domain of gp41 contains a strongly hydrophilic region (amino acids 724 to 745) followed by a region of alternating hydrophobicity and hydrophilicity (amino acids 746 to 856) (4, 25). The latter region also contains two segments with the potential to form amphipathic α helices (amino acids 770 to 794 and 824 to 856) (1, 39). A series of mutants containing deletions in the cytoplasmic domain was constructed in the pSVIIIenv envelope expressor plasmid (Fig. 4). Because many of these mutants overlap the *tat* or *rev* second open reading frames, *rev* or *rev* plus *tat* was supplied in *trans* by cotransfecting either the *rev* expressor plasmid or pHXB2ΔenvCAT, respectively. Of note, the truncation in the Δ(726-856) mutant is similar to the truncation found in some isolates of SIV and HIV-2 (4, 10). The effect of the Δ(814-856) mutation in the

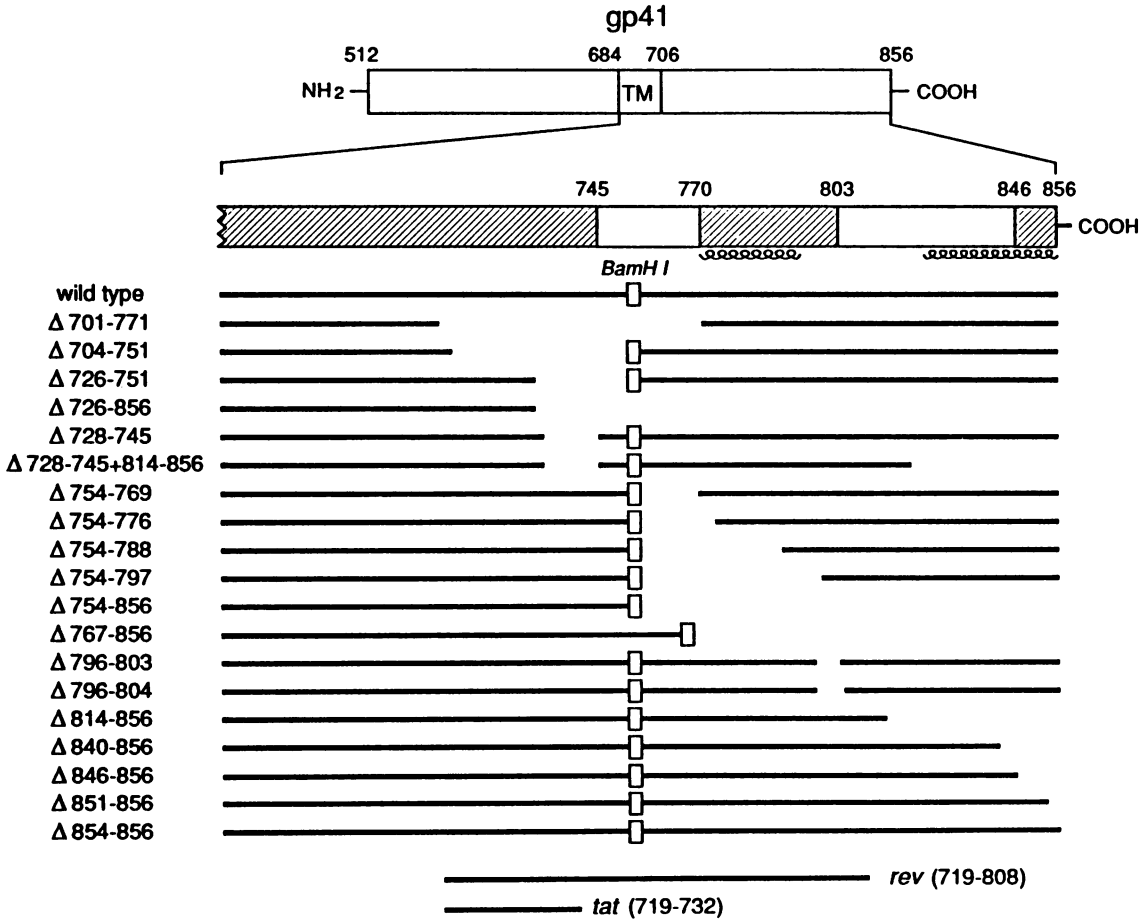

FIG. 4. Diagram of the mutant HIV-1 gp41 transmembrane glycoproteins. The wild-type HIV-1 gp41 transmembrane glycoprotein is depicted at the top, with the hydrophobic transmembrane region designated TM. The cross-hatched regions are highly conserved among HIV-1 isolates (28). Regions of potential amphipathic α helices at residues 770 to 794 and 824 to 856 (39) are indicated by coils. The positions of the second open reading frames of *tat* and *rev* are shown.

TABLE 1. Phenotypes of gp41 cytoplasmic domain mutants[a]

Mutant(s)[b]	Replication complementation[c]		Relative cell-associated gp120[c]	Processing index[d]	Syncytium formation[c]	
	COS-1	Jurkat			COS-1	Jurkat
None	2	5	0	ND	9	5
Wild type	100	100	100	1.0	100	100
Δ701-771	8	25	27	0.6	38	10
Δ704-751	38	50	28	0.7	68	96
Δ726-751	32	86	58	0.8	67	60
Δ726-856	28	7	32	1.0	71	83
Δ728-745	31	88	63	0.9	64	56
Δ728-745 and 814-856	9	10	ND	ND	ND	ND
Δ754-769	60	95	ND	ND	ND	ND
Δ754-776	63	37	ND	ND	ND	ND
Δ754-788	69	38	49	0.9	37	63
Δ754-797	31	10	29	0.9	20	33
Δ754-856	6	9	7	1.0	71	106
Δ767-856	6	12	32	1.5	30	92
Δ796-803	50	14	56	1.1	72	ND
Δ796-804	4	7	33	1.2	65	ND
Δ814-856	24	17	57	1.1	47	126
Δ840-856	34	24	48	1.1	63	135
Δ846-856	47	24	96	1.0	102	92
Δ851-856	50	28	121	0.9	80	122
Δ854-856	89	32	111	1.0	80	86

[a] Data reported represent the means of at least two or three independent experiments. ND, not done.
[b] The number of the mutant refers to the amino acid residues of the HXB2 strain of HIV-1, where 1 is the initial methionine (31).
[c] Percentage of the wild-type value. Values for replication complementation represent the CAT conversion in target Jurkat cells for the mutant envelope glycoprotein relative to the value for the wild type. Values shown for cell-associated gp120 were determined by gel densitometry of radioimmunoprecipitates analyzed by SDS-PAGE and autoradiography.
[d] A measure of the conversion of mutant gp160 to gp120 relative to that of the wild-type glycoprotein. The amounts of gp160 and gp120 were determined by gel densitometry of autoradiograms of SDS-PAGE gels. The processing index was calculated by the following formula: processing index = $[(\text{total gp120})_{\text{mutant}} \times (\text{gp160})_{\text{wild type}}]/[(\text{gp160})_{\text{mutant}} \times (\text{total gp120})_{\text{wild type}}]$.

context of an infectious HIV-1 provirus has already been described elsewhere (37).

To identify sequences in the cytoplasmic domain that are required for efficient viral replication, the ability of the mutant envelope glycoproteins to support cell-free virus transmission was determined by using a transient complementation assay (17). In this assay, COS-1 cells cotransfected with an envelope expressor plasmid and an envelope-defective provirus expressing the CAT gene produce recombinant virions that are used to infect Jurkat T lymphocytes (17). The efficiency of cell-free transmission of the recombinant viruses is determined by measuring CAT activity in the infected Jurkat culture. Most deletions within the carboxy-terminal 160 amino acids of gp41 reduced replication complementation of cell-free virus transmission to 4 to 69% of the wild-type value (Table 1). To determine whether similar regions are required for replication in Jurkat cells under conditions in which most of the virus transmission occurs by cell-to-cell spread, the abilities of the mutant glycoproteins to support a single round of virus replication were examined by using a similar transient complementation assay in transfected Jurkat cell cultures (17). The hydrophilic region extending from amino acids 726 to 751 and the immediately carboxyl region (amino acids 754 to 769) were relatively dispensable for replication in Jurkat cells (Table 1). Otherwise, the regions in the cytoplasmic domain required to support the transmission of viruses produced in COS-1 cells and Jurkat cells were similar. These results suggest that the function of some regions of the cytoplasmic domain depends on the cell type in which the envelope glycoproteins are expressed or on the mode of virus transmission.

Deletions in the cytoplasmic domain of gp41 are associated with decreased envelope glycoprotein stability in COS-1 cells. To determine whether mutations in the cytoplasmic domain of gp41 alter envelope glycoprotein synthesis or processing in COS-1 cells, mutant envelope glycoproteins expressed in COS-1 cells were immunoprecipitated with AIDS patient serum and analyzed on SDS-polyacrylamide gels. The steady-state levels of mutant glycoproteins with deletions within residues 846 to 856 at the carboxyl terminus were similar to those of the wild-type glycoproteins (Fig. 5A and Table 1). The steady-state levels of mutant glycoproteins containing deletions amino terminal to residue 846 were reduced (Fig. 5A and Table 1). Two mutants containing deletions overlapping the transmembrane domain (amino acids 701 to 777 and 704 to 751) exhibited a processing defect (Fig. 5A and Table 1). The levels of wild-type and mutant gp120 in the transfected COS-1 cell supernatants correlated with the levels of cell-associated gp120 (data not shown). When labeling COS-1 cells for 16 h was followed by a 3-h chase with excess cold methionine and cysteine, the levels of mutant envelope glycoproteins that were low in the steady state were further reduced (Fig. 5B and Table 2). This result suggests that deletion of residues amino terminal to amino acid 846 results in decreased envelope glycoprotein stability in COS-1 cells.

The cytoplasmic domain of gp41 is not required for incorporation of envelope glycoproteins into virions. To determine whether the cytoplasmic domain of gp41 is required for the

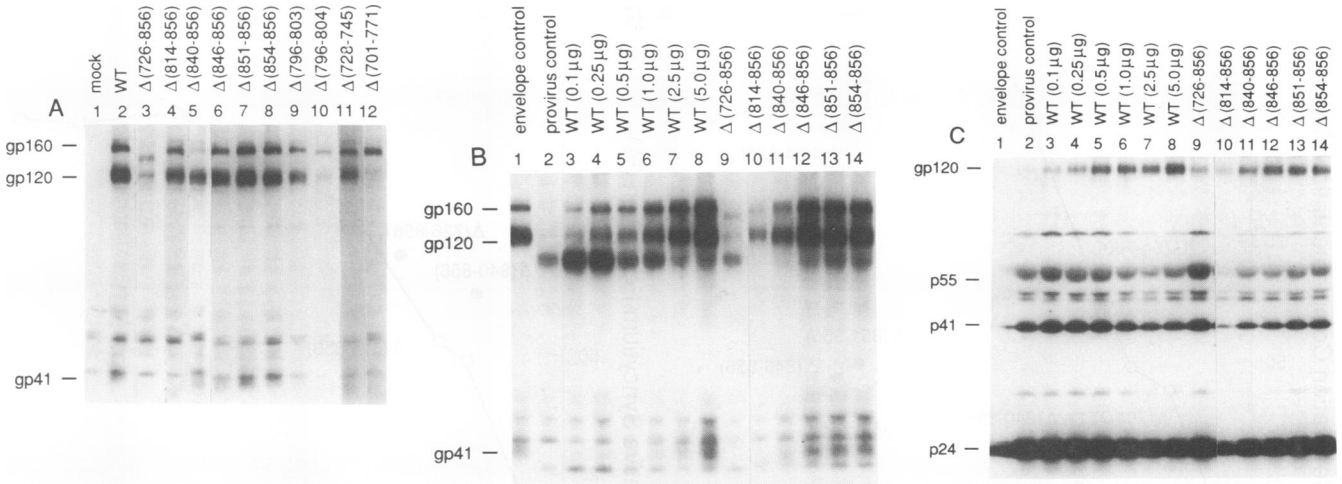

FIG. 5. Wild-type and mutant HIV-1 envelope glycoproteins in COS-1 cell lysates (A, B) and virion pellets (C). Cell lysates and virions were immunoprecipitated with AIDS patient serum and analyzed by 10% (A, B) or 12.5% (C) SDS-PAGE. (A) Steady-state levels of mutant envelope glycoproteins expressed in COS-1 cells transfected with 5 μg of the *rev* expressor plasmid plus no DNA (lane 1), 5 μg of wild-type envelope expressor plasmid (lane 2), or 5 μg of the mutant envelope expressor plasmids (lanes 3 to 12). (B) Expression of wild-type (lanes 1 and 3 to 8) and mutant (lanes 9 to 14) envelope glycoproteins in COS-1 cells after 16 h of labeling and 3 h of chase. COS-1 cells were transfected with 5 μg of the wild-type envelope expressor plasmid (lane 1), pHXBΔenvΔEcoCAT (lane 2), or pHXBΔenvΔEcoCAT plus 0.1, 0.25, 0.5, 1.0, 2.5, or 5.0 μg of the wild-type envelope expressor plasmid (lanes 3 to 8, respectively) or 5 μg of the mutant envelope expressor plasmids (lanes 9 to 14). (C) Virion-associated envelope glycoproteins. The lanes are the same as in panel B. The positions of the gp120 envelope glycoprotein and p55, p41, and p24 *gag* products are marked.

TABLE 2. Effect of mutations in the gp41 cytoplasmic domain on virion-associated envelope glycoproteins[a]

Mutant[b]	Relative cell-associated gp120 after 3-h chase[c]	Virion-associated gp120 (gp120/p24)[c]	Virion association index[d]
Wild type (0.1 μg)[a]	9	15	1.7
Wild type (0.25 μg)	16	22	1.4
Wild type (0.5 μg)	22	46	2.0
Wild type (1.0 μg)	39	35	0.9
Wild type (2.5 μg)	56	57	1.0
Wild type (5.0 μg)	100	100	1.0
Δ701-771	4	25	5.6
Δ704-751	18	25	2.6
Δ726-856	4	15	4.0
Δ728-745	58	70	1.4
Δ754-788	12	20	1.7
Δ754-856	4	4	ND
Δ767-856	15	32	2.6
Δ814-856	39	33	1.0
Δ840-856	62	42	0.8
Δ846-856	100	76	0.8
Δ851-856	84	72	0.9
Δ854-856	104	83	0.8

[a] Data reported represent the means of at least two independent experiments.

[b] The numbers in parentheses refer to the amount of wild-type envelope expressor plasmid DNA used for transfection. For all of the mutant glycoproteins, 5 μg of plasmid DNA was used.

[c] Percentage of the wild-type value at 5 μg of wild-type transfected plasmid DNA. The values shown were determined by gel densitometry of radioimmunoprecipitates analyzed by SDS-PAGE and autoradiography.

[d] Calculated to normalize the level of virion-associated gp120 for the level of cell-associated gp120. The virion-association index was calculated by using the formula $[(\text{virion-associated gp120})_{\text{mutant}} \times (\text{p24})_{\text{wild type}} \times (\text{cell-associated gp120})_{\text{wild type}}]/[(\text{p24})_{\text{mutant}} \times (\text{virion-associated gp120})_{\text{wild type}} \times (\text{cell-associated gp120})_{\text{mutant}}]$. ND, not done.

efficient incorporation of HIV-1 envelope glycoproteins into virions, the effect of deletions on the level of virion-associated envelope glycoproteins was examined. COS-1 cells cotransfected with an envelope expressor plasmid and pHXBΔenvΔEcoCAT were used to produce recombinant virions. Pelleted virions were immunoprecipitated with AIDS patient serum and analyzed on SDS-polyacrylamide gels. The amount of transfected wild-type envelope expressor plasmid DNA was incrementally increased from 0.1 to 5.0 μg to allow comparison of the level of wild-type and mutant virion-associated gp120 under conditions in which the levels of cell-associated envelope glycoproteins were similar (Table 2 and Fig. 5B and C). A virion association index was calculated to normalize the level of virion-associated gp120 for the level of cell-associated gp120 (Table 2). Deletions amino terminal to residue 846 caused reductions in the level of virion-associated gp120 to 70% or less of the wild-type level (Fig. 5C and Table 2). However, the virion association index of the mutant glycoproteins was similar to or greater than that of the wild-type envelope glycoproteins under conditions in which the levels of cell-associated gp120 were similar (Table 2). Moreover, the relative incorporation of gp120 into virions was generally more efficient when the levels of wild-type or mutant cell-associated gp120 were relatively low (Table 2). These results demonstrate that the cytoplasmic domain of gp41 is not required for the efficient incorporation of the gp120 envelope glycoprotein into virions. It can also be inferred that the incorporation of the gp41 glycoprotein is not defective, since the cell or virus association of the gp120 glycoprotein depends on an interaction with gp41 (24).

To determine whether the observed decrease in the absolute amount of virion-associated mutant envelope glycoprotein was the sole cause of the replication defect in virions made in COS-1 cells, replication complementation by the wild-type and mutant envelope glycoproteins was compared under conditions in which levels of virion-associated gp120

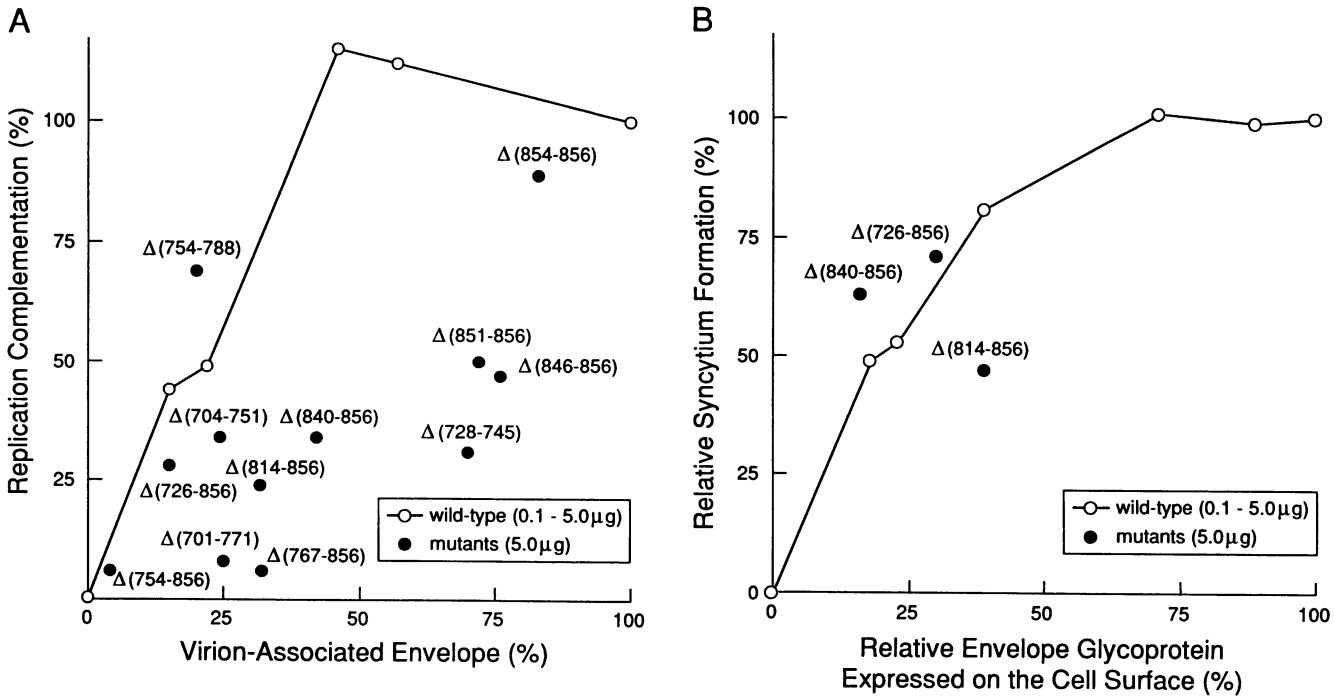

FIG. 6. (A) Levels of virion-associated envelope glycoproteins and replication complementation. Values for virion-associated envelope glycoproteins were derived from Table 2. Values for replication complementation were determined by using the replication complementation assay in COS-1 cells (Table 1). All values are expressed as a percentage of the value for the wild-type envelope glycoprotein at 5 μg of transfected plasmid DNA. Open circles represent the values, in consecutive order, for the wild-type envelope glycoproteins when 0.1, 0.25, 0.5, 2.5, or 5.0 μg of envelope expressor plasmid DNA was transfected. For the mutant envelope glycoproteins, 5 μg of envelope expressor plasmid DNA was transfected. (B) Levels of envelope glycoproteins expressed on the cell surface and syncytium-forming ability. The expression of envelope glycoproteins on the cell surface was determined by immunoprecipitation of cell surface-accessible envelope glycoproteins following incubation of intact COS-1 cells with AIDS patient serum, analysis by SDS-PAGE, and quantitation by gel densitometry. All values are expressed as a percentage of the value for the wild-type envelope glycoproteins at 5 μg of transfected plasmid DNA. Open circles represent values, in consecutive order, for the wild-type envelope glycoproteins when 0.1, 0.25, 0.5, 1.0, 2.0, or 5.0 μg of plasmid DNA was transfected. For the mutant envelope glycoproteins, 5.0 μg of plasmid DNA was transfected.

were similar. The relationship between the levels of virion-associated wild-type glycoprotein and degree of replication complementation was nonlinear, with saturation of the latter value when the level of virion-associated glycoprotein was only 35% of the maximum value (Fig. 6A). As shown in Fig. 6A, relatively low levels of wild-type virion-associated envelope glycoprotein can mediate virus entry efficiently. In contrast, most mutant envelope glycoproteins containing deletions within the cytoplasmic domain exhibited a replication defect that could not be solely attributed to the reduced level of virion-associated envelope glycoproteins (Fig. 6A).

The gp41 cytoplasmic domain is not required for syncytium formation. To determine whether a defect in fusion was the cause of the reduced replication complementation ability of the mutant envelope glycoproteins produced in COS-1 cells, COS-1 cells were cotransfected with a wild-type or mutant envelope expressor plasmid and cocultured with CD4-positive SupT1 cells, and syncytium-forming ability was scored. The syncytium-forming abilities of most of the mutant glycoproteins expressed in COS-1 cells were less than those of the wild-type glycoproteins (Table 1). Since previous studies had demonstrated that syncytium formation in other cell types did not require the cytoplasmic domain (7, 24), the syncytium-forming abilities of the mutant envelope glycoproteins were also examined in Jurkat-*tat* cells (11, 18). In Jurkat-*tat* cells, the syncytium-forming abilities of the mutant glycoproteins were usually equal to or greater than those of the wild-type glycoproteins (Table 1). These results suggest either that the syncytium-forming ability of the mutant glycoproteins is dependent on the cell type in which the envelope glycoproteins are expressed or that syncytium formation in COS-1 cells might be reduced as a consequence of decreased expression of the mutant envelope glycoproteins on the cell surface.

To determine whether the decrease in syncytium formation observed in COS-1 cells could be solely attributed to reduced expression of envelope glycoproteins on the cell surface, the expression of selected mutant envelope glycoproteins on the cell surface was determined by immunoprecipitation, analysis on SDS-polyacrylamide gels, and quantitation by gel densitometry. The syncytium-forming abilities of the wild-type and mutant envelope glycoproteins were compared under conditions in which similar levels were expressed on the cell surface. For the Δ(726-856), Δ(814-856), and Δ(840-856) mutant glycoproteins, the level of envelope glycoproteins expressed on the cell surface was not significantly different from the wild-type level when the levels of intracellular envelope glycoproteins were similar (Table 2 and Fig. 6B). Furthermore, the syncytium-forming abilities of the mutant envelope glycoproteins made in COS-1 cells were similar to that of wild type when similar levels of envelope glycoproteins were expressed on the cell surface (Fig. 6B). These results demonstrate that the cytoplasmic domain is not required for the cell surface expres-

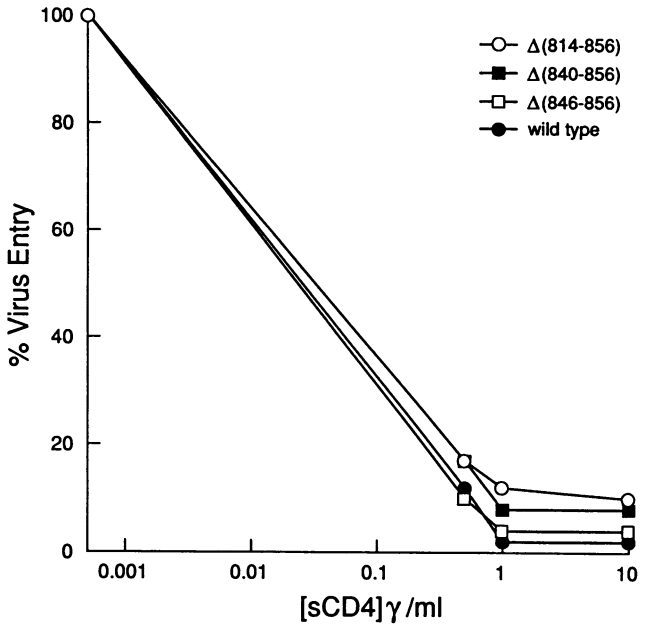

FIG. 7. Effect of mutations in the cytoplasmic domain on the sensitivity of replication complementation to soluble-CD4 (sCD4) inhibition. The effects of different soluble-CD4 concentrations on the CAT activity transferred to Jurkat lymphocytes by recombinant virions carrying the wild-type or mutant envelope glycoproteins are shown. Each value represents the percentage of CAT activity observed for a given mutant in the presence of soluble CD4 relative to the activity observed for the mutant in the absence of soluble CD4. γ, micrograms.

sion or the fusion function of envelope glycoproteins expressed in COS-1 cells.

Soluble-CD4 sensitivity of the mutant envelope glycoproteins. HIV-1 envelope glycoproteins that exhibit more than a twofold decrease in relative CD4-binding ability are less sensitive than the wild-type glycoprotein to soluble CD4 inhibition of virus entry (38). To investigate the possibility that deletions in the cytoplasmic domain of gp41 affect the CD4-binding ability of the functional envelope glycoprotein multimer, the effect of mutations in the cytoplasmic domain on soluble CD4 inhibition of virus entry was examined. Equivalent amounts of recombinant virions produced in COS-1 cells as determined by measuring reverse transcriptase activity were incubated with different concentrations of soluble CD4 for 1 h at 37°C prior to infection of Jurkat target cells. The Δ(814-856), Δ(840-856), and Δ(846-856) mutants exhibited the same sensitivity to soluble-CD4 inhibition of virus entry as the wild-type envelope glycoprotein (Fig. 7). From these results, it can be inferred that deletions in the cytoplasmic domain of gp41 do not significantly alter the CD4-binding ability of the native, multimeric envelope glycoproteins. This conclusion is consistent with previous observations that alterations in the gp41 cytoplasmic domain do not affect the CD4-binding ability of the monomeric soluble form of the gp120 glycoprotein (2, 7, 8, 24).

DISCUSSION

This study demonstrates that the cytoplasmic domain of the HIV-1 transmembrane glycoprotein has at least two effects on viral replication. A highly conserved region within residues 840 to 856 at the carboxyl terminus of gp41 is important for efficient virus entry during an early step in the virus life cycle between CD4 binding and formation of the DNA provirus. The entire cytoplasmic domain amino terminal to residue 846 affects the stability of envelope glycoproteins made in COS-1 cells. The entire cytoplasmic domain appears to affect the efficiency of virus entry during the cell-free transmission of viruses made in COS-1 cells, whereas residues 726 to 769 are dispensable for cell-to-cell transmission in Jurkat cells. Whether these differences are a consequence of the cell type in which the envelope glycoproteins are made or reflect differences in the requirement for this domain during cell-free versus cell-to-cell transmission remains to be determined.

The reduced efficiency of HIV-1 provirus formation following acute infection with HXB2Δ(840-856) demonstrates that the carboxyl terminus of the HIV-1 transmembrane glycoprotein has an effect on the efficiency of virus entry during an early stage in the virus life cycle prior to formation of the provirus. The steps in the virus life cycle required for virus entry include CD4 binding, fusion of the virus and host cell membranes, and penetration and uncoating of the viral core (13). Deletion of the carboxyl terminus of gp41 is unlikely to affect CD4 binding, since the CD4-binding domains are all contained within the gp120 subunit (24, 27). The lack of requirement for the cytoplasmic domain for syncytium formation and the lack of altered sensitivity to soluble CD4 provide further evidence that deletions in this region do not significantly affect the CD4-binding affinity of the multimeric glycoprotein complex. Deletions in the cytoplasmic domain of gp41 are also not likely to affect the fusion function, since this domain is not required for syncytium formation, as demonstrated here and in previous studies (7, 24). The possibility that the membrane fusion function of only the virion-associated envelope glycoproteins is defective has not been excluded, but reliable methods for measuring the fusion function of the small fraction of virions relevant to infection are not available. The competence of the mutant envelope glycoproteins for CD4 binding and membrane fusion in conjunction with inefficient formation of the DNA provirus raises the possibility that these truncated envelope glycoproteins are defective for a step involved in the uncoating or penetration of the viral core. The location of the gp41 carboxyl terminus within the virion close to the viral core is consistent with the possibility that this region plays a role in virus uncoating or penetration.

Independent of a role in virus replication, the integrity of the gp41 cytoplasmic domain amino terminal to residue 846 is important for the stability of envelope glycoproteins expressed in COS-1 cells. The observation that the stability of gp160 in HXB2Δ(840-856)-infected Jurkat cell cultures is decreased suggests that the cytoplasmic domain also has an effect on the stability of the envelope glycoproteins in other cell types. The effect of the cytoplasmic domain on envelope glycoprotein stability appears to be cell type dependent, judging by the observation that the effect is relatively minor in Jurkat cells and the finding that some mutant glycoproteins that showed decreased stability in COS-1 cells were fully functional, and thus presumably stable, in Jurkat cells. These results are consistent with previous studies demonstrating that the stability and processing of the HIV-1 envelope glycoproteins depend on the cell type in which the envelope glycoproteins are expressed (8, 18). In COS-1 cells, approximately 75% of gp160 is cleaved to produce the gp120 subunit, whereas in Jurkat cells, only a small fraction (10 to 20%) of gp160 is cleaved. This dependence of the efficiency

of gp160 cleavage on cell type might, at least in part, account for some of the cell type-specific effects observed in this study, since a decrease in the steady-state level of gp160 would be less deleterious in cells which process gp160 inefficiently. In a previous study, Willey et al. demonstrated that intracellular sorting results in the transport of most uncleaved gp160 to lysosomes, where it is degraded (40). The mechanism by which the cytoplasmic domain affects envelope glycoprotein stability is unknown. One possibility is that deletions in the cytoplasmic domain result in altered transport of gp160, as observed in one study (16), which might affect localization to subcellular compartments where degradation occurs.

The cytoplasmic domain of the transmembrane protein does not appear to be required for the efficient incorporation of the HIV-1 envelope glycoproteins into virions. These findings are consistent with those of previous studies demonstrating that SIV and equine infectious anemia virus containing truncated envelope glycoproteins are still infectious (4, 34). Similar studies of deletions of the shorter cytoplasmic domain of the Rous sarcoma virus envelope glycoprotein also demonstrated that this region was dispensable for virion incorporation, although in this case, infectivity was not affected (29). The studies reported herein, combined with systematic mutagenic analysis of the HIV-1 gp41 transmembrane region (11), essentially eliminate the possibility that interaction of specific gp41 sequences with *gag* proteins is a necessary step for incorporation of envelope glycoproteins into virions. The incorporation of the HIV-1 envelope glycoproteins into virions occurred more efficiently when levels of wild-type or mutant cell-associated envelope glycoproteins were low. This result suggests that a mechanism for concentrating the envelope glycoproteins into virions is involved in HIV-1 virion assembly. Whether the cytoplasmic domain is required for another step in virus assembly is not known, but the examination of HXB2(840-856) virions by electron microscopy and determination of the rate of *gag* precursor processing (Fig. 2D and E) did not reveal any gross defects in virus assembly or maturation (11a).

An HIV-1 provirus containing a 4-amino-acid deletion and an additional 15 random residues in the gp41 cytoplasmic domain, X10-1, was reported to result in a replication-competent HIV-1 exhibiting the ability to form syncytia but no ability to efficiently lyse single cells (9). A subsequent study also proposed a role for the cytoplasmic domain of gp41 in viral cytopathic effects (26). Contrary to these earlier studies, Kowalski et al. demonstrated that the replication and cytopathogenicity of the X10-1 mutant in Jurkat T lymphocytes were indistinguishable from those of the wild-type virus (23). The results of the present study provide further evidence that the cytoplasmic domain of gp41 does not play a major role in viral cytopathic effect independent of the effects of changes in this region on viral replication rate.

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REFERENCES

1. **Andreassen, A., H. Bohr, J. Bohr, S. Brunak, T. Bugge, R. M. J. Cotterill, C. Jacobsen, P. Kusik, B. Lautrup, S. B. Petersen, T. Saermark, and K. Ulrich.** 1990. Analysis of the secondary structure of the human immunodeficiency virus (HIV) proteins p17, gp120, and gp41 by computer modeling based on neural network methods. J. Acquired Immune Defic. Syndr. **3**:615–622.
2. **Berman, P. W., W. M. Nunes, and O. K. Haffar.** 1988. Expression of membrane-associated and secreted variants of gp160 of human immunodeficiency virus type 1 in vitro and in continuous cell lines. J. Virol. **62**:3135–3142.
3. **Bordier, C.** 1981. Phase separation of integral membrane proteins in Triton X-114 solution. J. Biol. Chem. **256**:1604–1607.
4. **Chakrabarti, L., M. Emerman, P. Tiollais, and P. Sonigo.** 1989. The cytoplasmic domain of simian immunodeficiency virus transmembrane protein modulates infectivity. J. Virol. **63**:4395–4403.
5. **Cullen, B. R.** 1987. Use of eukaryotic expression technology in the functional analysis of cloned genes. Methods Enzymol. **152**:684–703.
6. **Dalgleish, A. G., P. C. L. Beverly, P. R. Clapham, D. H. Crawford, M. F. Greaves, and R. A. Weiss.** 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature (London) **312**:763–766.
7. **Earl, P. L., S. Koenig, and B. Moss.** 1991. Biological and immunological properties of human immunodeficiency virus type 1 envelope glycoprotein: analysis of proteins with truncations and deletions expressed by recombinant vaccinia viruses. J. Virol. **65**:31–41.
8. **Earl, P. L., B. Moss, and R. W. Doms.** 1991. Folding, interaction with GRP78-BiP, assembly, and transport of the human immunodeficiency virus type 1 envelope protein. J. Virol. **65**:2047–2055.
9. **Fisher, A. G., L. Ratner, H. Mitsuya, L. Marselle, M. E. Harper, S. Broder, R. C. Gallo, and F. Wong-Staal.** 1986. Infectious mutants of HTLV-III with changes in the 3′ region and markedly reduced cytopathic effects. Science **233**:655–659.
10. **Franchini, G., C. Gurgo, H. G. Guo, R. C. Gallo, E. Collati, K. A. Gargnoli, L. F. Hall, F. Wong-Staal, and M. S. Reitz.** 1987. Sequence of simian immunodeficiency virus and its relationship to the human immunodeficiency viruses. Nature (London) **328**:539–543.
11. **Gabuzda, D., U. Olshevsky, P. Bertani, W. A. Haseltine, and J. Sodroski.** 1991. Identification of membrane anchorage domains of the HIV-1 gp160 envelope glycoprotein precursor. J. Acquired Immune Defic. Syndr. **4**:34–40.

11a. **Gabuzda, D., and J. Sodroski.** Unpublished data.

12. **Gallaher, W. R., J. M. Ball, R. F. Garry, M. C. Griffin, and R. C. Montelaro.** 1989. A general model for the transmembrane proteins of HIV and other retroviruses. AIDS Res. Hum. Retroviruses **5**:431–440.
13. **Grewe, C., A. Beck, and H. R. Gelderblom.** 1990. HIV: early virus-cell interactions. J. Acquired Immune Defic. Syndr. **3**:965–974.
14. **Haffar, O. K., D. J. Dowbenko, and P. W. Berman.** 1988. Topogenic analysis of the human immunodeficiency virus type 1 envelope glycoprotein, gp160, in microsomal membranes. J. Cell Biol. **107**:1677–1687.
15. **Haffar, O. K., D. J. Dowbenko, and P. W. Berman.** 1991. The cytoplasmic tail of HIV-1 gp160 contains regions that associate with cellular membranes. Virology **180**:439–441.
16. **Haffar, O. K., G. R. Nakamura, and P. W. Berman.** 1990. The carboxy terminus of human immunodeficiency virus type 1 gp160 limits its proteolytic processing and transport in transfected cell lines. J. Virol. **64**:3100–3103.
17. **Helseth, E., M. Kowalski, D. Gabuzda, U. Olshevsky, W. Haseltine, and J. Sodroski.** 1990. Rapid complementation assays measuring replicative potential of human immunodeficiency virus type 1 envelope glycoprotein mutants. J. Virol. **64**:2416–2420.
18. **Helseth, E., U. Olshevsky, D. Gabuzda, B. Ardman, W. Haseltine, and J. Sodroski.** 1990. Changes in the transmembrane region of the human immunodeficiency virus type 1 gp41 enve-

lope glycoprotein affect membrane fusion. J. Virol. **64**:6314–6318.

19. **Hirsch, V., N. Riedel, and J. L. Mullins.** 1987. Genome organization of STLV-3 is similar to that of the AIDS virus except for a truncated transmembrane protein. Cell **49**:307–319.
20. **Hirsch, V. A., P. Edmondson, J. Murphey-Corb, B. Arbeille, P. R. Johnson, and J. L. Mullins.** 1989. SIV adaptation to human cells. Nature (London) **341**:573–574.
21. **Klatzmann, D., E. Champagne, S. Chamaret, J. Gruest, D. Guetard, T. Hercent, J. C. Gluckman, and L. Montagnier.** 1984. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature (London) **312**:767–768.
22. **Kodama, T., D. Wooley, Y. Naidu, H. W. Kestler, M. D. Daniel, Y. Li, and R. Desrosiers.** 1989. Significance of premature stop codons in *env* of simian immunodeficiency virus. J. Virol. **63**:4709–4714.
23. **Kowalski, M., L. Bergeron, T. Dorfman, W. Haseltine, and J. Sodroski.** 1991. Attenuation of human immunodeficiency virus type 1 cytopathic effect by a mutation affecting the transmembrane envelope glycoprotein. J. Virol. **65**:281–291.
24. **Kowalski, M., J. Potz, L. Basiripour, T. Dorfman, W. C. Goh, E. Terwilliger, A. Dayton, C. Rosen, W. Haseltine, and J. Sodroski.** 1987. Functional regions of the envelope glycoprotein of human immunodeficiency virus type 1. Science **237**:1351–1355.
25. **Kunkel, T. A., J. D. Roberts, and R. A. Zakour.** 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. Methods Enzymol. **154**:367–382.
26. **Lee, S. J., W. Hu, A. Fisher, D. Looney, V. Kan, H. Mitsuya, L. Ratner, and F. Wong-Staal.** 1989. Role of the carboxyl-terminal portion of the HIV-1 transmembrane protein in viral transmission and cytopathogenicity. AIDS Res. Hum. Retroviruses **5**:441–449.
27. **McDougal, J. S., M. Kennedy, J. Sligh, S. Cort, A. Mowie, and J. Nicholson.** 1986. Binding of the HTLV-III/LAV to T4+ T cells by a complex of the 110 K viral protein and the T4 molecule. Science **231**:382–385.
28. **Myers, G., S. F. Josephs, A. B. Rabson, T. I. Smith, and F. Wong-Staal (ed.).** 1988. Human retroviruses and AIDS. Los Alamos National Laboratory, Los Alamos, N.Mex.
29. **Perez, L. G., G. L. Davis, and E. Hunter.** 1987. Mutants of the Rous sarcoma virus envelope glycoprotein that lack the transmembrane anchor and cytoplasmic domains: analysis of intracellular transport and assembly into virions. J. Virol. **61**:2981–2988.
30. **Queen, C., and D. Baltimore.** 1983. Immunoglobulin gene transcription is activated by downstream sequence elements. Cell **33**:741–748.
31. **Ratner, L., A. Fisher, L. L. Jagodzinski, H. Mitsuya, R. S. Liou, R. C. Gallo, and F. Wong-Staal.** 1987. Complete nucleotide sequence of functional clones of the AIDS virus. AIDS Res. Hum. Retroviruses **3**:57–69.
32. **Ratner, L., W. Haseltine, R. Patarca, K. J. Livak, B. Starcich, S. F. Josephs, E. R. Doran, J. A. Rafalski, E. A. Whitehorn, K. Baumeister, L. Ivanoff, S. R. Petteway, M. L. Pearson, J. A. Lautenberger, T. S. Papas, J. Ghrayeh, N. T. Chang, R. C. Gallo, and F. Wong-Staal.** 1985. Complete nucleotide sequence of the AIDS virus, HTLV-III. Nature (London) **313**:277–284.
33. **Rho, H. M., B. Poiesz, W. Ruscetti, and R. C. Gallo.** 1981. Characterization of the reverse transcriptase from a new retrovirus (HTLV) produced by a human cutaneous T-cell lymphoma cell line. Virology **112**:355–360.
34. **Rice, N. R., L. E. Henderson, R. C. Sowder, T. D. Copeland, S. Oroszlan, and J. F. Edwards.** 1990. Synthesis and processing of the transmembrane envelope protein of equine infectious anemia virus. J. Virol. **64**:3770–3778.
35. **Stein, B. S., S. D. Gouda, J. D. Lifson, R. C. Penhallow, K. G. Bensch, and E. G. Engleman.** 1987. pH-independent HIV entry into CD4-positive T cells via virus envelope fusion to the plasma membrane. Cell **49**:659–668.
36. **Terwilliger, E., B. Godin, J. Sodroski, and W. Haseltine.** 1989. Construction and use of replication-competent human immunodeficiency virus that expresses the CAT enzyme. Proc. Natl. Acad. Sci. USA **86**:3857–3861.
37. **Terwilliger, E., J. Sodroski, C. Rosen, and W. Haseltine.** 1986. Effects of mutations within the 3′ *orf* open reading frame region of human T-cell lymphotropic virus type III (HTLV-III/LAV) on replication and cytopathogenicity. J. Virol. **60**:754–760.
38. **Thali, M., U. Olshevsky, C. Furman, D. Gabuzda, J. Li, and J. Sodroski.** 1991. Effects of changes in gp120-CD4 binding affinity on human immunodeficiency virus type 1 envelope glycoprotein function and soluble CD4 sensitivity. J. Virol. **65**:5007–5012.
39. **Venable, R. M., R. W. Pastor, B. R. Brooks, and F. W. Carson.** 1989. Theoretically determined three-dimensional structures for amphipathic segments of the HIV-1 gp41 envelope protein. AIDS Res. Hum. Retroviruses **5**:7–22.
40. **Willey, R. L., J. S. Bonafacino, B. J. Potts, M. A. Martin, and R. D. Klausner.** 1988. Biosynthesis, cleavage, and degradation of the human immunodeficiency virus 1 envelope glycoprotein gp160. Proc. Natl. Acad. Sci. USA **85**:9580–9584.
41. **Zack, J. A., S. J. Arrigo, S. R. Weitsman, A. S. Go, A. Haislip, and I. S. Y. Chen.** 1990. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. Cell **61**:213–222.