Membrane Interactions of Synthetic Peptides Corresponding to Amphilathic Helical Segments of the Human Immunodeficiency Virus Type-1 Envelope Glycoprotein*

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Received for publication, September 19, 1991

Vol. 267, No. 10, issue of April 5, pp. 7121-7127, 1992
Printed in U.S.A.

The human and simian immunodeficiency virus envelope glycoproteins, which mediate virus-induced cell fusion, contain two putative amphophilic helical segments with large helical hydrophobic moments near their carboxyl-terminal ends. In an attempt to elucidate the biological role of these amphophilic helical segments, we have synthesized peptides corresponding to residues 768-788 and 826-864 of HIV-1/WMJ-22 gp160. Circular dichroism studies of the peptides showed that the a helicity of the peptides increased with the addition of dimyristoyl phosphatidylcholine (DMPC) indicating that the peptides form lipid-associating amphiphilic helices. The peptides solubilized turbid suspensions of DMPC vesicles, and electron microscopy of peptide-DMPC mixtures revealed the formation of discoidal complexes, suggesting that the peptides bind to and perturb lipid bilayers. The peptides were found to lyse lipid vesicles and caused carboxy-fluorescin leakage from dye-entrapped egg phosphatidylcholine liposomes. The peptides also lysed human erythrocytes and were found to be toxic to cell cultures. At subtoxic concentrations, the peptides effectively inhibited the fusion of CD4+ cells infected with recombinant vaccinia virus expressing human immunodeficiency virus (HIV)-1 envelope proteins. Based on these results, and reported studies on the mutational analysis of HIV envelope proteins, we suggest that the amphophilic helical segments near the carboxyl terminus of HIV envelope glycoproteins may play a role in lysis of HIV-infected cells and also may modulate the extent of cell fusion observed during HIV infection of CD4+ cells.

The human immunodeficiency virus (HIV) is the etiologic agent responsible for acquired immunodeficiency syndrome (AIDS), a chronic disease primarily affecting the immune and nervous systems. HIV primarily infects CD4+ T lymphocytes and macrophages. The viral envelope glycoproteins play an important role both in early and late events in viral infection (1, 2). The HIV envelope glycoprotein is first synthesized as a precursor polyprotein gp160; it is then cleaved intracellularly to give rise to a surface (SU) glycoprotein designated as gp120, and a transmembrane (TM) glycoprotein designated as gp41 (3, 4). The SU and TM proteins remain associated as a complex held together by noncovalent interactions (5). The SU protein is located on the outer surfaces of the viral envelope and infected cells, and functions as the viral attachment protein which binds to CD4 receptor on cell surfaces, whereas the TM protein serves as the membrane anchor. The amino terminus of the TM domain, generated upon cleavage of gp160, contains a stretch of hydrophobic amino acids termed the "fusion domain." This domain is necessary for fusion of the viral envelope with cellular membrane (6, 7). When cell-free virus interacts with a potential target cell, the envelope glycoproteins specifically bind to the CD4 antigen, the primary cellular receptor for HIV. Subsequently, virus-cell membrane fusion allows entry of the HIV nucleocapsid (8-10). Later in the viral life cycle, the HIV envelope glycoprotein is transported to the plasma membrane where it is available for virus assembly and budding. The surface-expressed envelope protein is also capable of inducing syncytium formation with neighboring uninfected CD4+ cells (2). Virus-induced cell fusion is a hallmark of HIV infection both in vivo and in cell culture and represents a major mechanism of virus-induced cell killing. Lysis of single virus-infected cells has also been observed with HIV infection (11).

Eisenberg and co-workers (12, 13) have identified two segments with relatively large hydrophobic moments, which can fold into amphophilic a helical structures, near the carboxyl terminus of HIV gp160. By computer modelling, Venable et al. (14) concluded that these amphophilic helices are structurally similar to regions of various proteins which form transmembrane selective channels. Some isolates of HIV-2 and simian immunodeficiency virus (SIV) carry mutations in their envelope genes which result in truncated TM proteins that lack the amphophilic helical segments (15, 16). These viruses, as well as certain mutants of HIV-1 with truncated TM proteins, are infectious and induce cell fusion but do not cause extensive single cell killing (17, 18). In this paper we show that synthetic peptides corresponding to the amphophilic helical segments near the carboxyl terminus of HIV-1 gp160 form a helical structures in nonpolar solvents and in the presence of lipids. In an attempt to elucidate the biological role of these amphophilic helical segments, we have investigated the interaction of these peptides with lipid bilayers and determined their effects on HIV-induced cell fusion.
EXPERIMENTAL PROCEDURES

Hydrophobic Moment Plots, Helical Wheel Plots, and Analysis of Amphipathic Helices—The nucleotide sequences of various HIV-1, HIV-2, SIV, and other lentiviruses were obtained from GenBank. Helical hydrophobic moments were calculated according to Eisenberg and co-workers (12, 13) and plots were generated using Macvector gene analysis software (International Biotechnologies, Inc., New Haven, CT). The Schiff-Edundson helical wheels (19) were plotted using a program developed for vaxstations that orients the hydrophobic moment (approximately representing center of nonpolar face) toward the top of the wheel (29). The values for the hydrophobic moment (GES scale) are expressed as mean hydrophobic moment per residue. The values for the hydrophobicity of the amphipathic peptides are expressed as the mean hydrophobicity per residue on the nonpolar face and were calculated by averaging the hydrophobicity of the residues that map to the nonpolar face of the amphipathic helix.

Synthesis, Purification, and Characterization of Peptides—Peptides were synthesized by the solid-phase procedure using an automated peptide synthesizer (Advanced Chemtech Inc., Louisville, KY) and Boc chemistry, as described previously (20). The amino acids, as their Boc derivatives, were attached to the benzhydylamine resin (Bachem, Torrence, CA) through a 4-oxymethyl-p-hexazoyl group using dicyclohexyl carbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) (Sigma). Benzyl-based side chain protecting groups were used for Asp, Glu, and Lys residues; Arg residues were protected by the Tns group, whereas the His residues were protected by Boc group. Boc groups at each stage of the synthesis were removed by treatment with 50% trifluoroacetic acid in methylene chloride and the Boc-amino acids were aminoethylylated with 15% HOBt. The peptides were cleaved from the resin by anhydrous hydrogen fluoride yielding a free carboxylic acid group at the carboxyl-terminal residue. To obtain the protected peptide, the Boc derivative of the first amino acid was directly attached to the benzhydylamine resin, and the resin-bound peptide was first treated with trifluoroacetic acid to remove the Boc group and then treated with acetic acid in the presence of DCC and HOBt. Upon cleavage with hydroxides fluoride, the released peptide was protected by an acetyl group at the amino terminus and an amide group at the carboxyl terminus. The crude peptides were first analyzed by HPLC under various solvent systems to identify optimum separation conditions. These conditions were translated to preparative systems and the peptides were purified by preparative HPLC using a Vydac C-4 column (25 × 2.2 cm). The purity of the peptides was ascertained by analytical HPLC and amino acid analysis. The concentrations of peptide solutions were determined by amino acid analysis and Pierce protein assay reagent.

Peptide-Lipid Interactions—Peptide-DMPC (dimyristoylphosphatidylcholine, Avanti Polar Lipids, Birmingham, AL) complexes were prepared at molar ratios of 1:5 and 1:10 by previously described procedures (21). Briefly, an ethanolic solution of DMPC was evaporated under nitrogen and resuspended in phosphate-buffered saline (PBS, pH 7.4) to remove lipid. An appropriate amount of the peptide in PBS was added to the DMPC suspension. The absorbance at 400 nm was recorded after 20 h incubation at 25 °C. The peptide-DMPC mixtures were also examined by electron microscopy. Samples after 20-h incubation were placed on formvar-coated copper grids, stained with uranyl acetate, and examined with a Philips EM 301 electron microscope.

Circular Dichroism (CD)—The peptides were dissolved in PBS or 80% trifluoroethanol at a concentration of 15 μM; peptide-DMPC complexes were prepared as described above. The samples were placed in a 10-mm sample cell, and CD spectra were obtained using a Jasco J-810 spectropolarimeter connected to a DPN500 data processor unit. The instrument was calibrated with d-10-camphorsulfonic acid. Approximately 8–16 scans were averaged and corrected for the base line. The corrected data was used to estimate the α-helical contents according to the calculations described by Greenfield and Fasman (22).

Effect of Peptides on Dye-entrapped Egg PC Vesicles—Carboxyfluorescein-entrapped egg PC liposomes were prepared according to previously described procedures (23). Briefly, 15 ng of egg PC (Sigma) was dried in vacuo and resuspended with 100 μM carboxyfluorescein (pH 7.4) by repeated vortexing for 30 min at room temperature. The suspension was sonicated with a Branson Sonifier Cell Disruptor (model 200) at room temperature. The resulting mixture was subjected to gel filtration on a Sepharose 4B column (40 cm × 8 cm) in 0.01 M Tris buffer containing 0.15 M NaCl, 0.01% EDTA, and 0.1% sodium azide (pH 7.4). Fractions containing carboxyfluorescein-entrapped unilamellar vesicles were collected and used in dye leakage assays. An increase in the fluorescence intensity of the dye-entrapped liposomes after incubation with peptides was used as a measure of peptide-induced liposomal leakage. Briefly, 20-μl aliquots of an appropriately diluted liposomal solution was mixed with varying concentrations of peptides in a total volume of 500 μl. The carboxyfluorescein was excited at 498 nm and emission at 525 nm was measured with an Aminco SPP 500 spectrophotometer. Release of carboxyfluorescein with Triton X-100 (0.1%) was considered as 100%.

Ehrlichocyte Lysis—Freshly drawn heparinized human blood from healthy donors was centrifuged and washed three times in PBS (pH 7.4) to remove plasma and the buffy coat. After the last wash, the
cells were suspended in an equal volume of PBS and the hematocrit was determined. A series of doubling dilutions of the peptides was prepared in PBS. Appropriate amounts of erythrocyte suspension were added to the diluted solutions of peptides to provide a final erythrocyte concentration of 1%. After a 30-min incubation at 37 °C, the samples were centrifuged for 5 min at 750 g, and the absorbance of the supernatant at 540 nm was measured. Complete lysis was obtained by the addition of Triton X-100 (final concentration, 1%) to the erythrocyte suspension.

Cells, Viruses, and Cell Fusion Assays—Vero cells (Clone 76, American Type Culture Collection) and CD4+ HeLa cells were maintained in Dulbecco's modified minimal essential medium supplemented with 5% newborn calf serum. CD4+ T lymphocytic cell line Molt4 and CD4- HSB cells were maintained in RPMI supplemented with 5% newborn calf serum. CD4+ T lymphocytic cell line Molt4 and CD4+ HSB cells were infected at a multiplicity of 0.1 and treated with peptide solutions infected HSB cells and Molt4 cells, according to previously described procedures (27).

Envelope Protein Synthesis and Processing—Monolayers of CD4+ HeLa cells grown on 12-mm glass coverslips in 24-well cluster plates were infected at a multiplicity of 0.1 and treated with peptide solutions as described above. At 10 h post-infection, intact unfixed monolayers were examined by indirect immunofluorescence assay using HIV immunoglobulin and fluorescein isothiocyanate-conjugated goat anti-human Ig (Southern Biotechnology, Inc., Birmingham, AL) as described previously (28).

RESULTS

Amphipathic Helical Segments in HIV and SIV Envelope Glycoproteins—Helical hydrophobic moment plots of the HIV envelope glycoprotein, gp160, reveal the presence of two highly amphipathic segments. One of these segments is located at the carboxyl-terminal end of the molecule (Segment I; residues 826–854), whereas the other (Segment II) corresponds to residues 768–788, near the carboxyl terminus of the molecule (Fig. 1). Similar segments with large hydrophobic moments are also observed near the carboxyl termini of various strains of HIV-2 and SIV, and representative examples are shown in Fig. 1. Interestingly, envelope glycoproteins of bovine (BIV) and feline (FIV) immunodeficiency viruses and other animal lentiviruses (not shown) did not display such amphipathic helical segments near their carboxyl termini.

Genetic variation in HIV is extreme, particularly in its envelope gene. We therefore analyzed the effect of such variation on the conservation of the amphipathic helical segments. A comparison of the amino acid sequences (deduced from nucleotide sequences of HIV envelope genes available from the GenBank data base) of HIV envelope proteins in the region that corresponds to the putative carboxyl-terminal amphipathic helical regions is shown in Figs. 2 and 3. Extensive sequence variation was observed with Segment I, which usually involved a substitution of a polar residue with another polar residue or a nonpolar residue with another nonpolar residue. However, several changes were nonconservative and involved a substitution of a charged residue with a nonpolar residue or vice versa. In certain isolates, an Arg' residue at position 19 was replaced by a Val or Thr residue. In several isolates the sequence changes occurred in pairs, wherein a change of hydrophobic residue to a charged residue was accompanied by the change of a charged residue to a hydrophobic residue (Fig. 3). Importantly, all these changes occurred at the polar face of the helix without disrupting its amphipathic nature. In Segment II, all but two changes were conservative in nature. In one instance, a His residue at position 2 which maps to the polar face of the helix (Fig. 3) was replaced by an Arg residue. In other instances, a Lys' residue located at the polar-nonpolar interface was replaced with a hydrophobic residue (Thr, Val, or Ala). None of these sequence variations altered the amphipathic helical motif. These results suggest the presence of a strong selective pressure to conserve the amphipathic helical segments, despite the extensive sequence variation in HIV.

Recently, Segrest et al. (29) have analyzed amphipathic
helix sequences from a large number of published protein sequences and classified them into seven classes. We therefore compared the structural features of the two COOH-terminal amphipathic HIV gp160 peptides with the above mentioned classes of amphipathic helical segments. The HIV gp160 amphipathic peptides appear to be distinct from the seven previously described groups of amphipathic helices, although they share several common features with amphipathic helices found in the apolipoproteins, as well as the amphipathic helices found in calmodulin-binding proteins. Like the apolipoprotein class of amphipathic helices, the gp160 amphipathic helices contain a cluster of positively charged residues at the polar-nonpolar interface. However, they are distinct from the apolipoprotein class of amphipathic helices in that: (i) the nonpolar face of the helices are more hydrophobic than the apolipoprotein class of amphipathic helices. (ii) The helices contain arginine residues in the center of the polar face and at the polar-nonpolar interface. (iii) The center of the most amphipathic of the gp160 helices (Segment I) contains a proline residue. It is therefore possible that these three unique features of the gp160 helices of gp160 produce a bent helix that can penetrate deeply into the interior of the cell (or viral) membrane, perhaps inducing instability and leakage.

The gp160 peptides also share several common features with the amphipathic peptides found in calmodulin-binding proteins, in that both helices have a very broad polar face which is highly charged, and contain several positively charged residues on their polar face. However, the gp160 amphipathic helices exhibit a much a greater hydrophobic moment. Interestingly, the amphipathic helices from lytic polypeptides and polypeptide hormones also contain a predominance of positively charged residues at the center of their polar face, although they have a much narrower polar face. The precise function of the gp160 amphipathic helical segments is presently not clear. We have therefore investigated the properties of synthetic peptide analogs corresponding to these amphipathic helical segments, in an attempt to elucidate their possible role in HIV envelope protein function.

**Lipid Affinity of gp160 Amphipathic Helical Peptides**—Two peptides corresponding to residues 826–854 (Peptide I) and 768–788 (Peptide II) of gp160 from HIV-1 (W32-22) (Fig. 2 and 3) were synthesized by the solid-phase method. Acetylated Peptide II, Ac(768–788)NH₂, was also synthesized to investigate the effects, if any, of end group modifications. An analytical HPLC profile of the purified peptides is shown in Fig. 4. The CD spectra of the peptides in aqueous and nonpolar solvents revealed minima at 222 and 208 nm, characteristic of α helical structures (Fig. 5). Peptide II displayed a greater α helical content both in buffer and in trifluoroethanol, compared with Peptide I. The acetylated Peptide II had a greater α helical content compared with its nonacetylated counterpart, consistent with the idea that end group modifications influence helix stability. In the presence of a lipid such as DMPC, the peptides showed an increase in the α helical content which is consistent with the surface-active amphipathic helical nature of the peptides. The peptides also clarified turbid DMPC suspension as evidenced by a decrease in the absorbance at 400 nm. Electron microscopy of the peptide-DMPC solution suggested that the peptides bind to DMPC and form discoidal complexes (Fig. 6). These results are consistent with the idea that the peptides form lipid-associating amphipathic α helices.

The gp160 Amphipathic Peptides Disrupt Phospholipid Bilayers—The interaction of the amphipathic peptides with phospholipid vesicles was further investigated by dye leakage experiments. Incubation of carboxyfluorescein entrapped egg PC vesicles with the amphipathic peptides resulted in the leakage of liposomal contents in a time-dependent and dose-dependent manner (Fig. 7). Peptide II was significantly more potent in inducing liposomal dye leakage in that 100% leakage was accomplished by 0.2 μM peptide, whereas only a 25% leakage was observed with Peptide I at this concentration. The minimum concentration of Peptide I required to induce 100% dye leakage was 3 μM. Although similar amounts of acetylated and nonacetylated Peptide II were required to induce 100% leakage, the leakage was found to be complete within a minute by acetylated Peptide II as opposed to 8 min with the nonacetylated Peptide II.

**Effects of gp160 Amphipathic Peptides on Eucaryotic Cells**—Biological membranes are far more complex than synthetic phospholipid bilayers and are likely to respond differently to membrane perturbing agents. We therefore investigated the effects of the gp160 amphipathic peptides on human erythrocytes. All of the gp160 amphipathic peptides were found to lyse human erythrocytes (Fig. 8). The percent of erythrocytes lysed increased gradually with increasing amounts of the peptide, and 100% lysis was observed at a peptide concentration of 6 μM acetylated Peptide II and 25 μM Peptide II; at the highest concentration tested (25 μM), Peptide I induced only a 25% lysis. These results suggest that the gp160 amphipathic peptides interact with and disrupt biological membranes, although the concentrations required to disrupt the biological membranes are much greater than the concentrations required to disrupt synthetic phospholipid bilayers. We have also investigated the effects of gp160 amphipathic peptides on cultured, nucleated cells (e.g., Vero cells, CD4+ HeLa cells, and Molt-4 cells) and found them to be cytotoxic at 100 μM or higher concentrations (data not shown).

**Effects of gp160 Amphipathic Peptides on Virus-induced Cell Fusion**—The effects of the peptides on HIV-induced cell fusion was investigated using rVVenv1, a recombinant vaccine virus expressing the HIV-1 envelope glycoproteins and Molt4 or CD4+ HeLa cells. Molt4 or CD4+ HeLa cells show extensive cell fusion upon infection with rVVenv1; incubation of rVVenv1-infection Molt4 or CD4+ HeLa T4 with the gp160 amphipathic peptides resulted in a significant reduction in cell fusion. The extent of fusion inhibition was found to be dose-dependent, and all the three peptides showed similar results and inhibited fusion at ~50 μM concentrations. A representative example of rVVenv1-infected Molt4 cells maintained in the absence or presence of the peptides is shown.
FIG. 5. CD spectra of Peptide I (A), Peptide II (B), and the acetylated Peptide II (C). CD spectra of the peptides were determined in buffer (top curves), in presence of DMPC at a peptide:lipid molar ratio of 1:10 (middle curves), or in 80% trifluoroacetic acid (bottom curves). The α-helical content (% helicity) in buffer, in presence of DMPC, and in TFE were 15, 17, and 23 for Peptide I, 20, 32, and 41 for Peptide II, and 27, 45, and 62 for acetylated Peptide II, respectively.

FIG. 6. Electron micrographs of peptide-DMC complexes. The peptide and DMPC were mixed together at a molar ratio of 1:10, and after 20-h incubation at room temperature, the complexes were stained with uranyl acetate and examined under an electron microscope. A shows the presence of multilamellar DMPC vesicles in samples that did not contain any peptide, and B shows the disruption of DMPC vesicles in the presence of Peptide II (magnification: ×65,000). Essentially similar results were observed with Peptide I and acetylated Peptide II.

FIG. 7. Liposomal dye leakage induced by gp160 peptides. Carboxyfluorescein-entrapped egg PC liposomes were incubated in the presence of 0.2 μM concentration of Peptide I (triangles), Peptide II (circles), or acetylated Peptide II (squares), and the fluorescence changes observed in the presence of varying concentrations of Peptide II after a 10-min incubation and shows the concentration dependence of dye leakage.

in Fig. 9. Approximately a 60–80% inhibition of fusion was observed at ~25 μM, and a nearly complete inhibition was observed at ~50 μM with Peptide II. The gp160 amphipathic peptides did not inhibit the synthesis, processing, or surface expression of HIV envelope glycoproteins in rVVenv1-infected HeLa or Molt-4 cells (data not shown), suggesting that the inhibition of fusion was not due to toxic effects of the peptides.

Virus-induced cell fusion was also studied in a two-cell coculture assay where a CD4+ cell was co-cultivated with a vaccinia-env recombinant-infected non-CD4 expressing cell.

We used a two-cell assay involving rVVenv-1 infected CD4+ HSB cells and CD4 expressing Molt-4 cells to dissect the components of the fusion process affected by the amphipathic peptides. Uninfected Molt-4 cells or rVVenv1-infected HSB cells were incubated overnight with gp160 amphipathic peptides, prior to cocultivation. Extensive cell fusion was observed when neither cell type was pretreated with the peptides (Fig. 10A). A significant reduction in fusion was observed when rVVenv1-infected HSB cells were pretreated with the gp160 amphipathic peptides (Fig. 10C), whereas pretreatment of CD4+ Molt-4 cells with the peptides did not result in fusion inhibition (Fig. 10B). These results indicate that the amphipathic peptides exert their inhibitory effect on the cell membranes expressing the HIV envelope glycoproteins and not on uninfected cells.

In order to determine whether the peptide-mediated effects are virus-specific, we also studied the effects of the gp160 amphipathic peptides on HSV-infected cell fusion (data not shown). Vero cells infected with HSV-1 (MP) at multiplicity of 10 plaque-forming units/cell showed extensive cell fusion involving nearly the entire monolayer within 16-18 h after infection. However, incubation of HSV-infected Vero cells in the presence of Peptide I or Peptide II resulted in complete inhibition of virus-induced cell fusion, and 100% inhibition of cell-fusion was achieved with peptide concentrations as low as ~1 μM. These results suggest that the fusion inhibitory
Properties of Amphipathic Helical Peptides from HIV-1 gp160

Fig. 9. Effect of gp160 peptides on HIV-induced cell fusion in Molt4 cells. Molt4 cells were infected at a multiplicity of 1 with rVVenv1, a recombinant vaccinia virus that expresses HIV-1 envelope glycoproteins, and maintained in the absence (A) or in the presence of 25 μM (B), 50 μM (C), or 100 μM (D) Peptide II for 18-20 h. The cells were examined under a light microscope for syncytium formation. In absence of the peptide, rVVenv1-infected Molt4 cells showed extensive syncytium formation (indicated by the arrows), whereas syncytium formation was significantly reduced in cultures incubated with 25 μM (B) and inhibited completely in presence of 50 μM peptide (C). Cultures maintained in 100 μM peptide (D) primarily show dead cells and suggest that the peptide is cytotoxic at these concentrations. Essentially similar results were observed in rVVenv1-infected Molt4 cells maintained in the presence of acetylated Peptide II or Peptide I.

Fig. 10. Effect of gp160 peptides on HIV-induced cell fusion in the two-cell fusion assay. CD4-negative HSB cells were infected with rVVenv1, a recombinant vaccinia virus that expresses HIV-1 envelope glycoproteins at a multiplicity of 1, and maintained in the absence or presence of 50 μM Peptide-1. Molt-4 cells were also maintained in the absence or presence of 50 μM Peptide-1. After 24 h, approximately 1.5 x 10^5 rVVenv1-infected HSB cells were mixed with an equal number of Molt4 cells and cocultivated for 12-16 h and examined microscopically for syncytium formation. A shows cocultures of rVVenv1-infected HSB cells and Molt4 cells, B shows cocultures of rVVenv1-infected HSB cells and peptide-treated Molt4 cells, whereas C shows cocultures of peptide-treated rVVenv1-infected HSB cells and Molt4 cells. Cell fusion is readily observed in cocultures of rVVenv1-infected HSB cells and Molt4 cells, as well as rVVenv1-infected HSB cells and peptide-treated Molt4 cells. However, cell fusion was inhibited in cocultures of peptide-treated rVVenv1-infected HSB cells and Molt4 cells.

effects of gp160 amphipathic peptides are not virus-specific. The precise mechanisms involved in peptide-mediated inhibition of cell fusion induced by different viruses remain to be elucidated.

**DISCUSSION**

The carboxyl termini of the HIV and SIV envelope glycoproteins contain two amphipathic helical segments. Available evidence indicates that these carboxyl-terminal amphipathic segments of gp160 may fold into α helical structures and associate with membranes when present as part of the viral glycoprotein complex (30, 31). It is possible that the membrane association of the gp160 amphipathic helical segments may modulate membrane-related events (e.g., virus budding, cell fusion, or single-cell lysis) in the viral life cycle.

Our studies on the synthetic amphipathic peptide analogs of HIV-1/WMJ-22 gp160 demonstrate that they lyse synthetic phospholipid vesicles and human erythrocytes, and are cytotoxic to cultured cells. Miller et al. (32) have recently synthesized a peptide corresponding to Segment I of HTLV-III/LAV and also observed that this peptide is lytic to prokaryotic and eukaryotic cells. Unlike these in vitro studies, where the amphipathic peptides were exogenously added to the cells, the gp160 amphipathic segments are located intracellularly in HIV-infected cells. Studies with other cytolytic amphipathic peptides suggest a requirement for the formation of peptide oligomers for their lytic effects (34, 35). The HIV envelope glycoproteins exist in the membranes as oligomers (either trimers or tetramers) and tend to concentrate in localized areas of the plasma membrane such as the sites of virus budding (36-39). Therefore, the gp160 amphipathic helical segments may interact with the cytoplasmic leaflets of the plasma membranes and play a role in the lysis of virus-infected cells.

Studies on the naturally occurring variants and mutants of HIV and SIV also support the idea that the amphipathic helical segments in the viral envelope proteins may play a role in lysis of virus-infected cells (15-18). Analysis of HIV-1 mutants with deletions in the carboxyl terminus of gp160 shows that mutations which disrupt the putative amphipathic helical segments result in viruses with reduced cytopathicity (18). For example, a variant of HIV-1, designated as X10-1, codes for a mutant envelope protein in which the 14 carboxyl-terminal residues have been replaced with 15 exogenous residues (K-R-R-R-R-W-V-F-Q-S-H-L-R-Y-L) and is fully capable of virus expression and syncytium formation but is no longer able to kill human T cells (40). Likewise, another mutant X9-3, in which the last five amino acids of gp160 were substituted with 153 amino acids of the 3′ Orf also had a diminished cytopathic potential (40). These changes disrupt the amphipathic nature of one of the two putative carboxyl-terminal amphipathic helical segments in gp160 (Segment I). Sakai et al. (41) have molecularly cloned multiple virus genotypes from a cytopathic HIV isolate, which differ markedly in their infection kinetics and cytopathogenic properties. Evidence was obtained that the weakly cytopathogenic isolates contained changes in the 3′ end of the envelope gene and 3′ Orf regions. In light of earlier studies which show that the 3′ Orf does not play a role in viral cytopathology, these results support the notion that the carboxyl-terminal regions of gp160 may contribute to viral cytopathology. Nevertheless, the amphipathic segments of gp160 are not likely to be the sole determinants of cell lysis in HIV-infected cultures (42, 43). Studies on mutant envelope glycoproteins, wherein the amphipathic helical segments have been disrupted by site-specific mutagenesis, without modifying other viral components, should provide a better understanding of their role in the viral life cycle.

Several naturally occurring variants of HIV-2, SIV, and mutants of HIV-1 which show TM protein truncations and fail to express the amphipathic helical segments in gp160, replicate well in culture, and release infectious virus particles containing truncated transmembrane proteins (15-18, 40, 41). These variants also induce syncytium formation in CD4+ cells. These reports, therefore, suggest that the amphipathic helical segments in gp160 are not required for virus budding.
or syncytia formation. We have shown that the gp190 amphipathic peptides exert a significant inhibitory effect on cell fusion induced by HIV-1 and an unrelated herpes simplex virus as well. It is therefore possible that the amphipathic helical segments may modulate the extent of cell fusion observed during HIV infections. Certain mutant HIV-1, HIV-2, and SIV glycoproteins with truncated cytoplasmic sequences lack the amphipathic helical segments and are fusion-incompetent or render them fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incompetent or fusion-incom...