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Membrane Permeabilization by Different Regions of the Human Immunodeficiency Virus Type 1 Transmembrane Glycoprotein gp41

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The transmembrane glycoprotein (gp41) of human immunodeficiency virus type 1 (HIV-1) has been implicated in the cytopathology observed during HIV infection. The first amino acids located at the amino terminus are involved in membrane fusion and syncytium formation, while sequences located at the carboxy terminus have been predicted to interact with membranes and modify membrane permeability. The HIV-1 gp41 gene has been cloned and expressed in *Escherichia coli* cells by using pET vectors to analyze changes in membrane permeability produced by this protein. This system is well suited for expressing toxic genes in an inducible manner and for analyzing the function of proteins that modify membrane permeability. gp41 enhances the permeability of the bacterial membrane to hygromycin B despite the low level of expression of this protein. To localize the regions of gp41 responsible for these effects, a number of fragments spanning different portions of gp41 were inducibly expressed in *E. coli*. Two regions of gp41 were shown to increase membrane permeability: one located at the carboxy terminus, where two highly amphipathic helices have been predicted, and another one corresponding to the membrane-spanning domain. Expression of the central region of gp41 comprising this domain was highly lytic for *E. coli* cells and increased membrane permeability to a number of compounds. These findings are discussed in the light of HIV-induced cytopathology and gp41 structure.

The progression of AIDS in chronically infected patients is a complex process that depends on the host immune system and the selection of more pathogenic human immunodeficiency virus type 1 (HIV-1) variants (2, 23, 38, 55). There is a correlation between overt disease and killing of $CD4^+$ T lymphocytes. The emergence of more pathogenic viruses increases syncytium formation and killing of $CD4^+$ T lymphocytes (23, 38, 55). The mechanisms underlying both phenomena are clearly related to alterations of the plasma membrane (16, 39). Thus, syncytium formation is the consequence of increased cell fusion, an activity related to the virus glycoprotein gp41 (7, 25, 42, 44). The mechanisms of individual cell killing are more complex; several HIV-1 gene products, such as gp41 and Vpu, could participate in this process by inducing plasma membrane modifications (16, 28). Thus, permeability of membranes to a number of compounds, including ions, increases in HIV-1-infected T cells, suggesting the appearance of hydrophilic pores in the membrane (40) as occurs in other virus-cell systems (10). Therefore, perturbation of membrane permeability, not only in HIV-infected cells but also in other animal virus-infected cells, is thought to be involved in the cytopathic effects (10, 11). Understanding the cytopathology of HIV infection is of paramount importance to better understand the disease and its progression. Cytopathicity has been mapped to the *env* gene of HIV (7, 24, 33), which encodes a glycoprotein designated gp160. Proteolytic cleavage of gp160 produces gp120, which is the glycoprotein responsible for interaction of virions with the cellular receptor, and gp41, the transmembrane (TM) glycoprotein that anchors the complex gp120/gp41 to the membrane. Noncovalent binding between gp120 and gp41 produces the complex gp120/gp41 (31) as well as the formation of oligomers, most probably tetramers, on the cell surface and the viral membrane (45, 53).

Different functions have been ascribed to gp41: (i) fusion activity, (ii) interaction with gp120, (iii) anchoring of gp120/gp41 to the membrane, (iv) involvement in cytopathogenicity, and (v) participation in virion assembly. Upon binding of HIV-1 to its receptor (the CD4 molecule), fusion of the viral and the cellular plasma membrane takes place. This fusion activity is located at the amino terminus of gp41 (25) and can be modulated by the cytoplasmic domain (43, 47) and gp120 (3, 15). Similarities between a hydrophobic region located at the beginning of gp41 and other regions of viral proteins involved in membrane fusion have been noted (27). A synthetic peptide comprising 23 residues of the amino terminus of gp41 shows fusion activity (42). In addition, extensive genetic evidence supports the idea that the fusion activity of immunodeficiency viruses maps at the amino terminus of gp41 (3, 5, 6, 25, 33). Close to this fusion peptide (amino acids [aa] 512 to 534), there is a region (aa 559 to 587) with a leucine zipper-like motif that has been implicated in the interaction of gp41 with gp120 (14). Further toward the carboxy terminus, there is a cysteine loop that is highly conserved in retrovirus TM proteins, required for HIV-1 viability (18, 52). The TM region of HIV-1 gp41 is rich in hydrophobic residues and encompasses aa 684 to 706. This region contains a conserved Arg residue, which is very unusual in TM domains (54). Mutations in this TM domain also affect fusion activity (43). Toward the carboxy end of the gp41 molecule, there are two regions (aa 771 to 790 and 827 to 851) of predicted amphipathic helices (54). In fact, the carboxy-terminal portion is among the most amphipathic helices present in proteins (22). It is theoretically possible that these two amphipathic peptides associate with each other in spanning the membrane (54). Alternatively, these two regions of gp41 could interact with the cytoplasmic face of the membrane, distorting

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its structure. In accord with these predictions, synthetic peptides comprising the last amino acid residues of gp41 are toxic for both prokaryotic and eukaryotic cells (41) and directly interact with membranes (29). This toxicity is due to membrane permeabilization and cytolysis induced by the synthetic peptide (41). Perhaps the insertion of multimeric complexes into bacterial or mammalian cell membranes forms hydrophilic pores inducing cell lysis. This peptide has been designated the lentivirus lytic peptide (40, 41). Deletions of different portions of the cytoplasmic domain of gp41 profoundly influence cell fusion activity, virus assembly, and infectivity (13, 21, 26, 37, 47).

The involvement of different gp41 peptides in altering membrane function prompted us to test the permeability effects of the whole gp41 protein or fragments of it, in order to determine whether regions of gp41 other than the carboxy terminus are also able to permeabilize cells. Since the lentivirus lytic peptide, as well as membrane-active proteins, is equally active on both prokaryotic and eukaryotic cells, we made use of the inducible synthesis of these proteins in *Escherichia coli* cells to assay permeability changes. This system has been successfully used to analyze the permeabilizing capacity of several membrane-active proteins from other animal viruses (30, 34, 35, 48).

MATERIALS AND METHODS

Construction of expression plasmids. DNA manipulations for construction of the recombinant plasmids encoding the HIV-1 (BH10 isolate; kindly provided by R. Gallo, National Institute of Allergy and Infectious Diseases, Bethesda, Md.) gp41 and the different regions of this protein were performed by using standard cloning procedures. The different clones were obtained from recombinant plasmid pSP64, containing the *Sst*I insert of the BH10 clone from HIV-1 (46), by PCR. The oligonucleotide primers used for the amplification were designed to include codons to initiate and stop translation and to create unique *Nde*I and *Bcl*I restriction sites. The PCR products were purified, digested with the above-mentioned enzymes, and ligated with dephosphorylated pET-3a vector (kindly provided by B. A. Moffat and F. W. Studier, Brookhaven National Laboratory, Upton, N.Y.), previously digested with *Nde*I and *Bam*HI. After transformation of *E. coli* DH5 cells and restriction analysis of plasmids from positive clones, DNAs from DH5 cells were used to transform *E. coli* BL21(DE3)pLysS. The constructions were sequenced by the dideoxy method (Sequenase; U.S. Biochemical Corp.) to ensure that the PCR had not introduced any mutation.

Growth and induction of recombinant cultures. Single clones of BL21 (DE3)pLysS cells containing the indicated plasmid, were grown overnight at 37°C in LB medium in the presence of 100 μg of ampicillin and 34 μg of chloramphenicol per ml. Then, the cells were diluted 100-fold in M9 medium supplemented with 0.2% glucose and antibiotics and grown at 37°C and 250 rpm. Once the cultures reached an A_{600} of 0.5 to 0.6, they were induced by the addition of 1 mM of isopropylthiogalactopyranoside (IPTG). When indicated, 150 μg of rifampin (Boehringer) per ml was added 30 min after induction to inhibit transcription by *E. coli* RNA polymerase.

Labeling and electrophoretic analysis of proteins. For labeling the proteins synthesized by the transformed cells, 1-ml aliquots of cultures were collected and incubated with 1 μCi of [^{35}S]methionine (1.45 Ci/mmol; Amersham Corp.) per ml for 10 min at 37°C. The labeled bacteria were pelleted for 1 min at 12,000 rpm in an Eppendorf Microfuge and resuspended in electrophoresis sample buffer (10% glycerol, 0.1 M dithiothreitol, 160 mM Tris-HCl [pH 6.8], 1% sodium dodecyl sulfate [SDS], 0.024% bromophenol blue). The samples were sonicated and electrophoresed in 0.1% SDS–20% polyacrylamide gels. Fluorography was carried out by incubating the gel with 1 M salicylic acid. After drying, the gels were exposed to X-ray films (Agfa) at −70°C.

Uridine loading of *E. coli* cells. Cells were grown as described above and 90 min before induction were incubated with 2 μCi of [^{3}H]uridine (27.3 μCi/mmol; Amersham) per ml for 1 h. The cells were sedimented and washed twice with uridine-free, prewarmed growth medium. The cells were resuspended in the initial volume of growth medium and incubated at 37°C. Fifteen minutes later, the cells were induced with IPTG. At given times, 0.2-ml culture aliquots were removed and pelleted. The supernatants were mixed with L-929 scintillation cocktail (Dupont) to quantify the radioactivity released to the medium.

Flow cytometry analysis. *E. coli* BL21(DE3)pLysS cells were induced to express the particular protein as described above. Eighty-microliter aliquots of cells were collected at different times after induction with IPTG and resuspended in 500 μl of phosphate-buffered saline (PBS) with 0.005% propidium iodide (PI) for 10 min at 37°C. Cells were finally washed twice with PBS before flow cytometry analysis. The flow cytometry analysis was carried out in a FACScan flow cytometer (Becton Dickinson, San José, Calif.) with an argon laser tuned to 480 nm and power emission of 25 mW. To obtain resolvable signals from bacteria, the side scatter detector was set in log mode and forward scatter was used as the trigger parameter. PI fluorescence was collected with a BP 630/22 filter and directed to the photomultiplier (PMT, with a voltage of 600 V). The fluorescence signals were collected in log mode. All measurements were gated by the forward scatter channel. Signals from between 5,000 and 10,000 cells were collected per sample. Monodisperse latex particles (Sigma) were run before bacteria to check the machine.

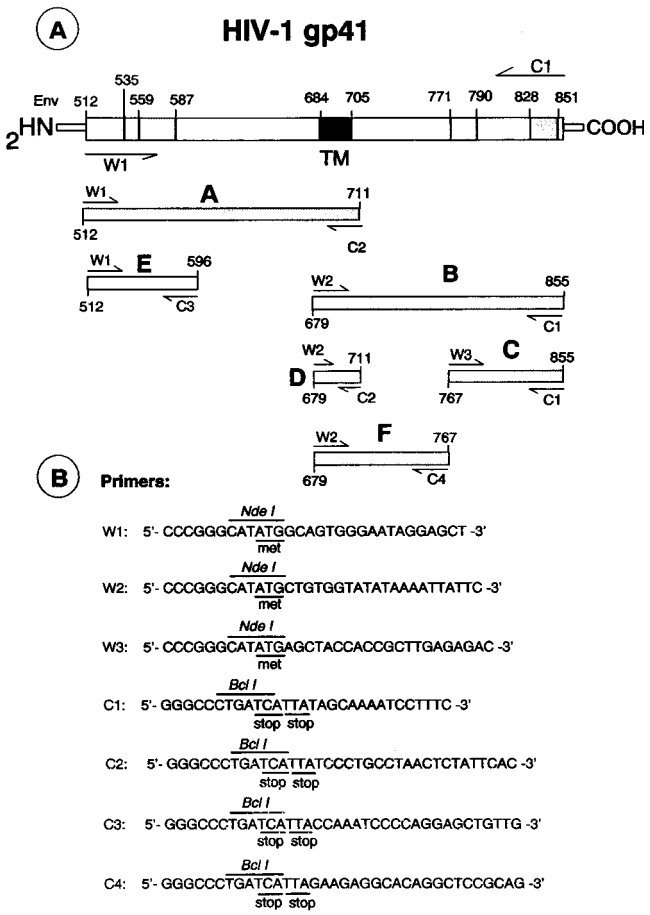

FIG. 1. Schematic representation of the HIV-1 transmembrane protein gp41 and the fragments studied in this work. The hydrophobic regions located at the N terminus, the TM domain, and the potential amphipathic helices located at the C terminus (shaded regions) are shown in panel A. The gp41 fragments studied are depicted. Panel B shows the sequences of the synthetic oligonucleotides used to obtain the different constructs.

RESULTS

Cloning and expression of HIV-1 gp41 and its fragments in *E. coli* cells. The sequences corresponding to HIV-1 gp41 were obtained from recombinant plasmid pSP64, containing 8.9 kb of the *Sst*I insert of the λ BH10 clone from HIV-1, by using standard PCR procedures and were cloned in pET vectors. To clarify the role of gp41 in the modification of cell permeability induced by the virus, the entire gp41 molecule or fragments of it were expressed in *E. coli* by means of the inducible system described by Studier et al. (51). Synthesis of phage T7 RNA polymerase is achieved in this system by addition of IPTG, which derepresses the *lacUV5* promoter, which governs transcription of the phage polymerase (50, 51). These cells bear plasmid pLys, encoding the T7 lysozyme, a natural inhibitor of the T7 RNA polymerase that counteracts its basal expression

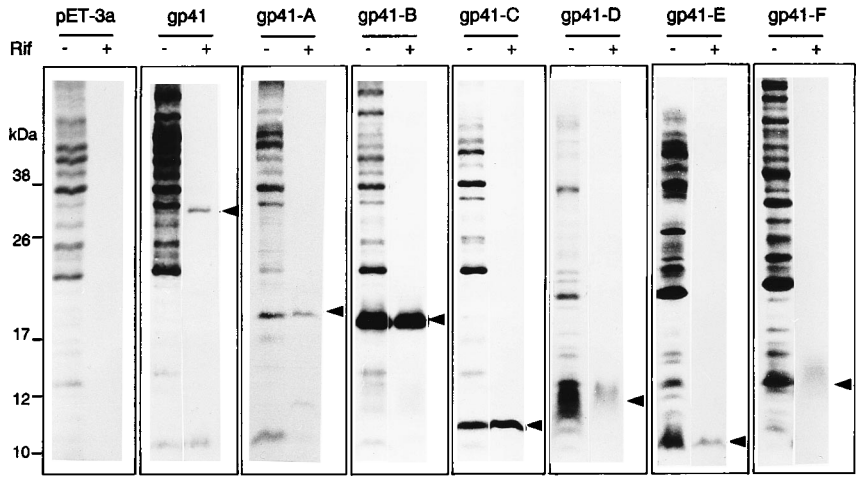

FIG. 2. Expression of the gp41 protein and its fragments in *E. coli* BL21(DE3)pLysS cells. Bacteria containing plasmid pET-3a without insert, pET3a-gp41 (gp41), and plasmids pET3a-gp41A, pET3a-gp41B, pET3a-gp41C, pET3a-gp41D, pET3a-gp41E, and pET3a-gp41F (fragments A to F from gp41) were induced with 1 mM IPTG, and after 1 h, cells were labeled with [^{35}S]methionine as described in Materials and Methods; 150 μg of rifampin (RF) was (+) or was not (−) added after 30 min of IPTG induction. Electrophoresis of cell extracts was carried out in SDS–20% polyacrylamide gels. The proteins induced are indicated by arrowheads.

(49). The presence of the T7 lysozyme also makes the cells more susceptible to lysis when the bacterial membrane is altered (34). Addition of rifampin together with IPTG blocks the expression of the *E. coli* genome and only synthesis of the plasmid genes is detected, greatly facilitating the analysis of the cloned products. Different regions of gp41 were expressed in *E. coli* cells to test their effects on cell permeability. The locations of these fragments and the oligonucleotides used for cloning are depicted in Fig. 1. Fragment A comprises the region between the amino terminus and residue 705, including the TM domain; fragment B spans the region from the TM domain and the carboxy terminus; fragment C includes the two potential amphipathic helices located toward the carboxy end; fragment D contains the TM domain; fragment E includes the amino terminus but not the TM domain; fragment F is as fragment B but lacking the amphipathic helices. Figure 2 shows the expression of the cloned products after induction with IPTG in the presence of rifampin. Some fragments, e.g., fragments B and C, were synthesized at rather high levels, while others, such as the whole gp41 and fragments E and F, were poorly expressed. The remaining proteins analyzed, fragments A and D, were made at intermediate levels. The explanation for this behavior in the case of the lytic fragments (see below) may reside in their cytotoxicity, but at present we have no explanation for the reduced synthesis of nonlytic products. The migration of unglycosylated gp41 and the fragments correlates well with their expected sizes. Fragments D and F give rise to a diffuse band, as observed with hydrophobic fragments that oligomerize; however, direct proof of such oligomerization has not yet been obtained. To our knowledge, the effect of gp41 expression in bacterial cells has not been described previously. Three different behaviors with respect to the kinetics of protein synthesis after induction of the different products were observed (Fig. 3). Some fragments (B and C) were synthesized at high levels for hours upon induction with IPTG alone or with IPTG in the presence of rifampin. These results indicate that expression of fragments B and C is tolerated by the cells for a period of several hours. Other fragments (A, D, E, and F) were clearly synthesized only during the first 1 or 2 h after induction. Moreover, in the presence of IPTG alone, the induction of fragments D and F ceased the synthesis of bacterial proteins (results not shown), most probably because of the membrane-active effects of these fragments. Finally, gp41 itself and fragments A and E were clearly made during the initial 2 h of induction with IPTG in the presence of rifampin, but these fragments did not inhibit the synthesis of cellular proteins in the presence of IPTG alone. The low level of expression of the different fragments correlated with a shorter period of synthesis, suggesting that for unknown reasons the synthesis of gp41 itself or some of its fragments is not well tolerated by *E. coli* cells, at least with the system and under the conditions used in this work.

Lytic properties of fragments D and F for *E. coli* cells. Several animal virus proteins that theoretically can form pores in membranes are lytic when inducibly expressed in *E. coli* (30, 35, 48). The lytic effects of gp41 and the fragments generated were tested by determining cell density at various times after induction with IPTG. Figure 4 shows that induction of the entire gp41, fragment A, or fragment B did not reduce cell growth as compared with *E. coli* BL21(DE3)pLysS cells containing plasmid pET-3a used as control, while expression of fragments D or F was highly lytic for bacteria. In the case of fragment D, cell number dropped to less than 25% after 1 h of induction. The behavior of fragments B and C was less drastic than that of fragment D, but clearly the expression of these fragments also has a negative effect on cell growth. The expression of other membrane-active proteins from animal viruses in *E. coli* BL21(DE3) cells devoid of pLys is much less lytic, suggesting that destabilization of the membrane by a membrane-active protein allows the passage of the T7 lysozyme to the periplasmic space, where lysis of the bacterial cell wall occurs (34). This may be the reason for the acute cell lysis observed when fragments D and F were expressed.

Membrane permeabilization by gp41 and its fragments. Different tests have been used to analyze alterations of membrane permeability (10). To measure the efflux of compounds, the release of radioactivity from uridine-loaded cells was tested, while the increased entry of compounds was performed with the hygromycin B test (12, 17). Bacteria were loaded with [^{3}H]uridine, and the release of radioactivity was analyzed after induction of gp41 and its fragments (Fig. 5). This radioactivity is not precipitable by trichloroacetic acid, suggesting that it corresponds to low-molecular-weight compounds. Fifty percent of the loaded radioactivity was released after 1 h of in-

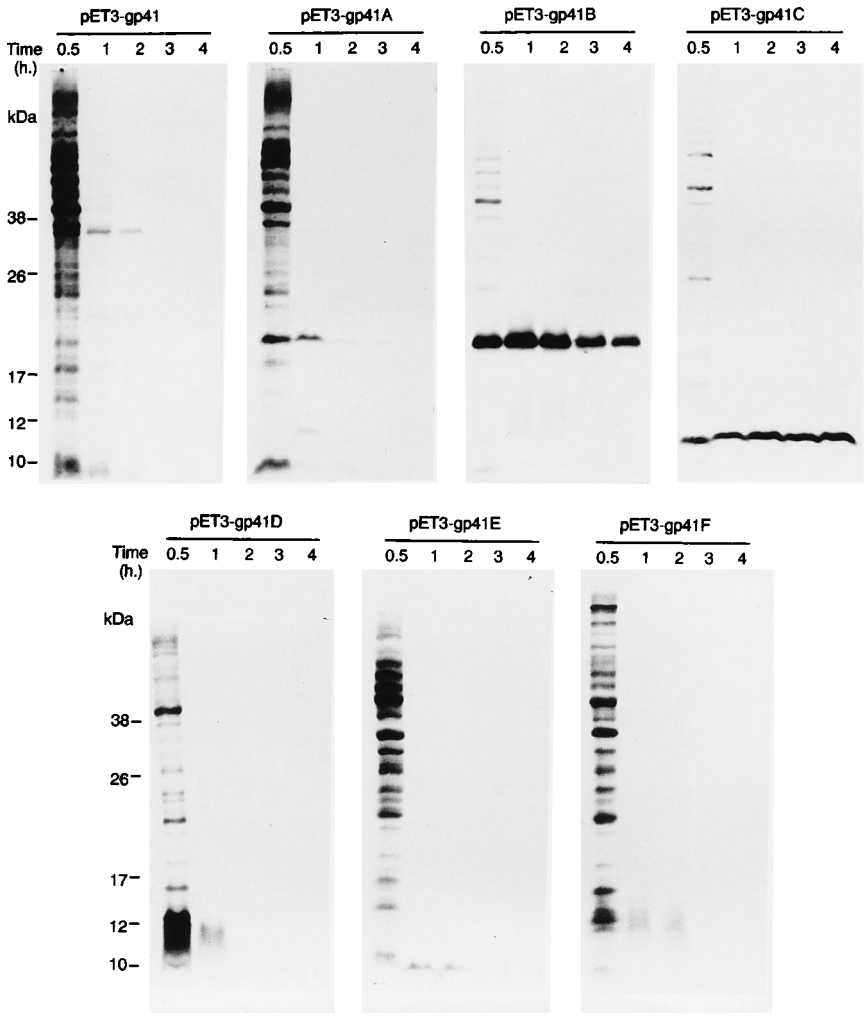

FIG. 3. Kinetics of protein synthesis after induction of the different gp41 constructs. Bacteria containing plasmid pET3a-gp41 (gp41) and plasmids pET3a-gp41A, pET3a-gp41B, pET3a-gp41C, pET3a-gp41D, pET3a-gp41E, and pET3a-gp41F (fragments A to F from gp41) were induced with 1 mM IPTG and labeled at different times postinduction; 150 μg of rifampin was added 30 min after induction with IPTG. Electrophoresis of cell extracts was carried out in SDS–20% polyacrylamide gels.

duction of fragment D synthesis. At this time, only 20% of total radioactivity was released by induction of fragment F; 3 h after induction of fragments D and F, most of the loaded radioactivity was released to the medium. Fragment C also produced some efflux of radioactivity, although with a more delayed kinetics. A significant but small efflux was also observed with fragment B.

A number of translation inhibitors do not block protein synthesis in intact cells because of the permeability barrier due to the cell membrane (12). These compounds readily enter into virus-infected cells or in cells treated with membrane-active compounds (1, 8, 17). Hygromycin B shows a high selectivity for blocking translation only in cells in which membrane permeability has been altered, irrespective of whether these cells are prokaryotic or eukaryotic (17, 35). To our knowledge, this is one of the most sensitive tests available for analyzing the increased influx of compounds into cells. Figure 6 shows the inhibition of protein synthesis by hygromycin B in control *E. coli* cells bearing plasmid pET-3a or in cells induced to synthesize gp41 or its fragments. Clearly hygromycin B entry is not detected in control cells that synthesize high levels of the control protein xa (a 260-aa fragment of the T7 protein encoded in gene 10) (36, 51). A similar behavior was observed only with fragment E, which contains 85 residues of the amino terminus of gp41. It seems, therefore, that the presence of the fusion peptide in this fragment that in principle interacts with membranes (25, 42) does not alter membrane permeability. Total inhibition of protein synthesis is observed with fragments D, B, and F, which most effectively permeabilize the membrane to the aminoglycoside antibiotic. Entry of hygromycin B is also detected with gp41 and fragments A, C, and F, particularly when they are induced by IPTG in the presence of rifampin and much less when they are induced with IPTG alone (results not shown). The lower degree of permeabilization to the antibiotic obtained with gp41 and fragment A after induction with IPTG alone may be due, at least in part, to a lower level of synthesis of these two products. However, we consider this possibility less likely than the idea that these products are less permeabilizing than other fragments, since fragment D is also synthesized at low levels and is highly lytic and permeabilizing.

Flow cytometry analysis of *E. coli* cells expressing gp41 and its fragments. Flow cytometry enables measurements of a variety of cellular parameters, including membrane permeability

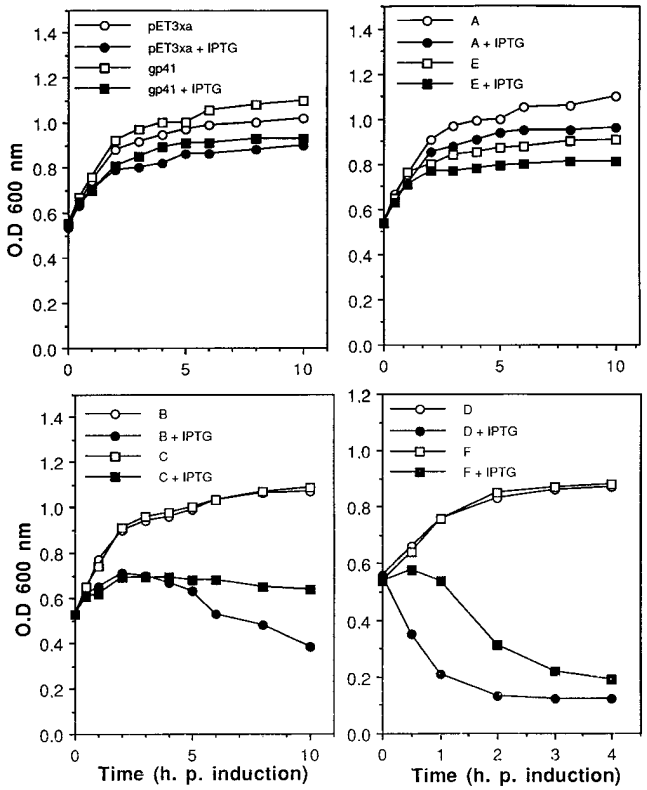

FIG. 4. Growth of *E. coli* BL21(DE3)pLysS cells expressing different proteins. The optical densities at 600 nm (O.D. 600 nm) of induced cultures of *E. coli* cells carrying the pET-3xa, pET3a-gp41, pET3a-gp41A, pET3a-gp41B, pET3a-gp41C, pET3a-gp41D, pET3a-gp41E, and pET3a-gp41F constructions were measured at the indicated times (hours) postinduction (h. p. induction). Cells carrying plasmid pET-3xa were used as controls.

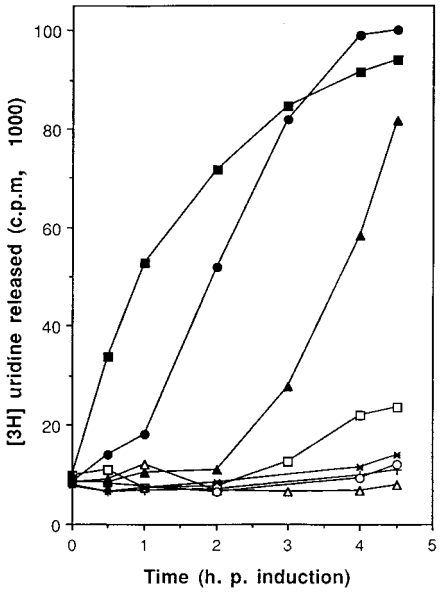

FIG. 5. [^{3}H]uridine release in *E. coli* cells expressing Xa, gp41, or the different gp41 fragments. *E. coli* BL21(DE3)pLysS cells carrying pET-3xa (control) pET3a-gp41 (○), pET3a-gp41A (+), pET3a-gp41B (□), pET3a-gp41C (▲), pET3a-gp41D (■), pET3a-gp41E (×), and pET3a-gp41F (●) were preloaded with [^{3}H]uridine as described in Materials and Methods and induced by addition of 1 mM IPTG, and radioactivity released to the medium was measured at the times (hours) postinduction (h. p. induction) shown.

in bacteria (32). Since measurements are made on individual cells, this technique permits quantification of the heterogeneity of the cell population with respect to the measured parameter. PI fluoresces only when linked to double-stranded nucleic acids and can be incorporated only into cells that have lost their selective permeability (19). Flow cytometry analysis of the uptake of PI by *E. coli* BL21(DE3)pLysS cells induced to express the gp41 protein or the particular gp41 fragments at several postinduction times is shown in Fig. 7. *E. coli* cells that synthesize gp41, fragment A, or fragment E behave similarly to control cells with respect to PI entry; i.e., no entry is observed even after 3 h postinduction. However, modification of membrane permeability to PI is observed upon expression of the other fragments. A strong signal for PI is detected 1 h after induction of fragments D, F, and C. Even higher signals are seen with fragments D and F than with fragment C after 3 h of induction. Fragment B was the least active in modifying the selective permeability to PI. Curiously, in the case of fragments B and C, a clear cellular heterogeneity is observed 3 h after induction with IPTG, with a percentage of the cells that allow higher PI entry and a cell population with a lower accumulation of PI, similar to cells expressing these fragments 1 h postinduction. In general, the results obtained with flow cytometry agree well with the findings on cellular lysis and permeability changes. Thus, fragments D and F are again the most active, while gp41 and fragments A and E had almost no effect. Fragments B and C show a clear permeabilizing action but lower than that of fragments D or F. In addition, these results point to the usefulness of flow cytometry analyses to measure permeability changes, avoiding the use of radioactive compounds.

DISCUSSION

The modification of membrane permeability by animal viruses has important consequences for the infected cells, including interference with many metabolic processes and the development of the cytopathic effect (10, 11). During the infection of patients by HIV, the appearance of more pathogenic vari-

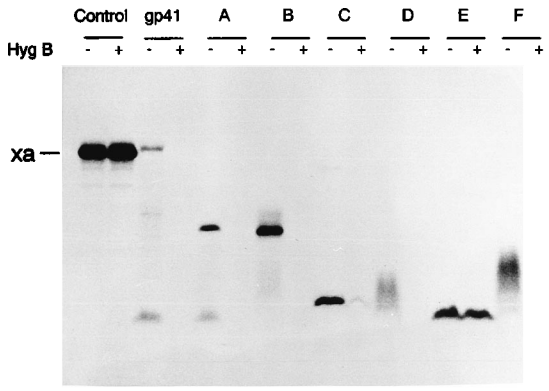

FIG. 6. Permeability to hygromycin B induced by the expression of different proteins. Cultures of *E. coli* BL21(DE3)pLysS cells carrying pET-3xa, pET3a-gp41, pET3a-gp41A, pET3a-gp41B, pET3a-gp41C, pET3a-gp41D, pET3a-gp41E, and pET3a-gp41F were induced with 1 mM IPTG and treated with rifampin as described in Materials and Methods. At 40 min after induction, 1 mM hygromycin B was added to an aliquot of the culture. After 30 min of incubation in the presence (+) or absence (−) of 1 mM hygromycin B (Hyg B), the proteins were labeled for 10 min with [^{35}S]methionine and analyzed in an SDS–20% polyacrylamide gel.

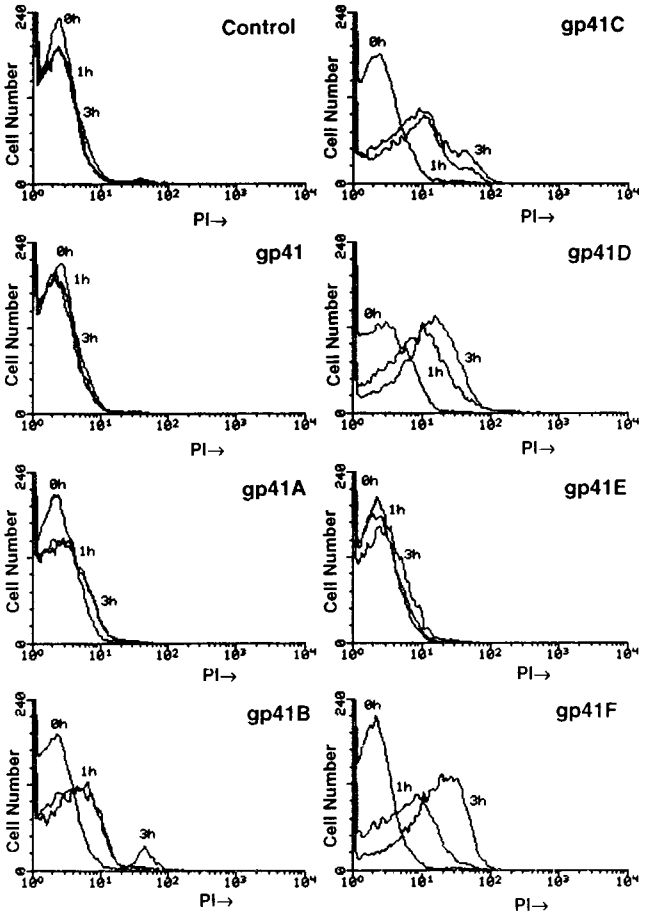

FIG. 7. Flow cytometry analysis of *E. coli* permeability to PI. Several hours after induction with IPTG, *E. coli* BL21(DE3)pLysS cells expressing gp41 and gp41 fragments were labeled with 0.005% PI in PBS for 10 min at 37°C. After labeling, cells were analyzed in a Becton Dickinson flow cytometer as described in Materials and Methods.

ants that replicate faster in culture cells may influence the course of the disease (2, 38, 55). Therefore, it is important to understand the viral proteins involved and the nature of these variations at the molecular level. Genetic studies have characterized gp41 as one important determinant of the cytopathology produced by HIV (7, 33). Two regions can be clearly distinguished in gp41, one located at the amino terminus, which is involved in cell fusion and syncytium formation (25), and another, located at the other end of the molecule, that interacts with membranes (29), compromising their integrity (40). Most of the evidence supporting these conclusions has been obtained with mutant HIV isolates or with synthetic peptides (2). However, to our knowledge no studies using the whole HIV TM domain to analyze its effects on membrane permeability have been carried out. Our present findings indicate that the fragment containing the initial 35 aa located at the amino terminus does not modify permeability, at least in the system used for this work. This result is of interest because it shows that although the HIV fragment bearing the fusion peptide interacts with natural and artificial membranes (25, 42), it does not trigger permeability changes. Our results not only identify the carboxy region (residues 685 to 855) as a domain responsible for increasing membrane permeability but also show that the TM region of gp41 plays a more important role in this process. The HIV-1 TM fragment with the highest lytic activity and that most drastically altered the membrane barrier was fragment D (residues 684 to 705), containing the membrane-spanning domain of gp41. Therefore, the TM region is a good candidate for the protein region responsible for individual cell killing. The HIV gp41 forms oligomers, most likely tetramers, that may lead to pore formation in membranes (45, 53). Inspection of the TM region of HIV shows the presence of a conserved arginine residue in this domain. This is very unusual in glycoproteins; in fact, bacteriorhodopsin is the only case known to us of a protein with an Arg residue in the TM region (54). The fact that this Arg residue is very conserved among the different HIV and SIV TM regions analyzed argues in favor of an important function for this basic residue (43). It could be speculated that this Arg plays a role in modifying membrane permeability, contributing to the formation of a hydrophilic pore. Moreover, many toxins that disorganize membranes contain an Arg-rich sequence close to the hydrophobic sequence that interacts with the membrane (4, 9, 20). This sequence rich in basic amino acids plays a part in disorganizing the membrane. The TM region of HIV or SIV contains two or three conserved basic (Arg or Lys) residues close to the hydrophobic region. Therefore, it seems likely that the membrane-spanning region plus this adjacent sequence rich in basic residues is responsible for the membrane-active properties of fragment D. Mutational analyses of these residues would indicate the extent to which they influence virus viability as distinct from cell fusion (43) and contribute to the modification of membrane permeability that develops during the replication of HIV (16).

Another aspect of our work is the efficiency of expression of gp41 or its fragments in *E. coli* cells. From our initial attempts to express gp41 in the inducible system used in this work, we noticed that gp41 expression was not very efficient. Our present findings suggest that there is a region located at the amino terminus (perhaps corresponding to the fusion peptide) which is responsible for this lower rate of synthesis of gp41, since both fragments A and E are poorly expressed. This effect is unrelated to the permeability changes or lytic potential of the products synthesized, because fragment E is devoid of these capacities. Perhaps the potential fusion activity of these fragments (A and E) is toxic for cells, particularly when bacterial gene expression is blocked by rifampin. A practical conclusion from our study is that the initial amino acid residues of the amino terminus should be removed if high-level expression of gp41 is desired in *E. coli* cells. Other fragments, particularly fragment D, were highly lytic for *E. coli* cells, thus hampering efficient expression in bacteria. Curiously, this fragment was not as lytic or toxic when expressed as part of a larger molecule, as with the entire gp41 protein or fragment A, both of which include the TM domain. Fragment D did produce changes in cell permeability to hygromycin B, however, reflecting the high sensitivity of this assay. A similar situation was observed with fragment B, which contains the TM domain and the amphipathic helices but it is not as lytic as fragment D. These findings suggest that there are conformational constraints for these fragments to increase membrane permeability in bacteria.

Theoretically, it is possible that the interaction of gp41 with some cellular proteins could trigger permeability changes in mammalian cells. The absence of these proteins in bacteria may hamper this increase in permeability. Attempts to express gp41 and its fragments in mammalian cell to assay for their membrane-active effects are now in progress in our laboratory.

In conclusion, at least two different regions involved in modifying membrane permeability can be identified in HIV gp41, one located at the carboxy terminus and the most active one

corresponding to the TM domain. We speculate that the arginine residues present in this domain may be important in increasing membrane permeability as seen for other membrane-active proteins (9).

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