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Oligomerization of the Hydrophobic Heptad Repeat of gp41

HELENE B. BERNSTEIN,[1] SIMON P. TUCKER,[2] SAMBIT R. KAR,[1] SYLVIA A. MCPHERSON,[1] DAVID T. MCPHERSON,[1] JOHN W. DUBAY,[1] JACOB LEBOWITZ,[1] RICHARD W. COMPANS,[3] AND ERIC HUNTER[1]*

Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama,[1] Infectious Disease Section, Searle Research and Development, St. Louis, Missouri,[2] and Department of Microbiology and Immunology, Emory University, Atlanta, Georgia[3]

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The transmembrane protein of human immunodeficiency virus type 1 (HIV-1) contains a leucine zipper-like (hydrophobic heptad) repeat which has been predicted to form an amphipathic α helix. To evaluate the potential of the hydrophobic heptad repeat to induce protein oligomerization, this region of gp41 has been cloned into the bacterial expression vector pRIT2T. The resulting plasmid, pRIT3, expresses a fusion protein consisting of the Fc binding domain of monomeric protein A, a bacterial protein, and amino acids 538 to 593 of HIV-1 gp41. Gel filtration chromatography demonstrated the presence of oligomeric forms of the fusion protein, and analytical centrifugation studies confirmed that the chimeric protein formed a higher-order multimer that was greater than a dimer. Thus, we have identified a region of HIV-1 gp41 which is capable of directing the oligomerization of a fusion protein containing monomeric protein A. Point mutations, previously shown to inhibit the biological activity of the HIV-1 envelope glycoprotein, have been engineered into the segment of gp41 contained in the fusion protein, and expressed mutant proteins were purified and analyzed via fast protein liquid chromatography. A point mutation in the heptad repeat, which changed the central isoleucine to an alanine, caused a significant (>60%) decrease in oligomerization, whereas changing the central isoleucine to aspartate or proline resulted in almost a complete loss of oligomerization. Deletions of one, two, or three amino acids following the first isoleucine also resulted in a profound decrease in oligomerization. The inhibitory effects of the mutations on oligomer formation correlated with the effects of the same mutations on envelope glycoprotein-mediated fusion. A possible role of the leucine zipper-like region in the fusion process and in an oligomerization event distinct from assembly of the envelope glycoprotein complex is discussed.

The envelope glycoprotein of the human immunodeficiency virus (HIV) is responsible for the binding of the virus to the cellular receptor, CD4 (20, 22, 23, 30), and participates in the CD4-dependent cell fusion exhibited by HIV (28). The envelope glycoprotein is synthesized as a polyprotein precursor (gp160), which is cleaved in the Golgi complex by cellular proteases to form the transmembrane (gp41) and surface (gp120) subunits of the envelope glycoprotein (10, 13, 16, 27, 34). The mature, cleaved form of the envelope glycoprotein remains associated through noncovalent interactions and is present on the surface of HIV-infected cells. The surface subunit is responsible for the interaction of the virus with CD4 (24), and the transmembrane protein is involved in the fusion process (14). The HIV type 1 (HIV-1) envelope glycoprotein is oligomeric and is likely to exist as either a trimer or tetramer (12, 26, 29, 33, 36).

The leucine zipper motif was first described for DNA-binding proteins including c-Myc, c-Jun, and the yeast gene regulatory protein GCN4 (19). A leucine zipper contains repeats of leucine at every seventh amino acid residue (termed the first or a position). In addition to the heptad repeat of leucines, there tend to be nonpolar amino acids at the fourth or d position within the α helix. The secondary structure of a leucine zipper is predicted to be a coiled coil, and X-ray crystallography as well as X-ray scattering studies have supported this structural prediction (25, 32). In the case of DNA-binding proteins, these motifs have been demonstrated to be responsible for dimerization, and point mutations to the heptad repeats of the leucine zipper have prevented oligomerization and DNA binding (18, 31). A leucine zipper-like motif has since been identified in other types of proteins including viral envelope glycoproteins (2, 9), and a similar structural domain has been identified in the transmembrane glycoprotein of HIV-1 between amino acids 559 and 587 (9). However, unlike a classical leucine zipper, the hydrophobic heptad repeat of this region includes valine and isoleucine residues in addition to the leucine residues. In addition, there are several hydrophobic amino acids including leucine, isoleucine, and valine within the heptad repeat at the fourth position (d) in each of the heptads. The arrangement of these hydrophobic residues within a putative α helix suggested that this region might form a coiled coil (15), and experimental analyses by circular dichroism of peptides corresponding to this region have confirmed this prediction (38). These peptides were capable of inhibiting cell fusion as well as HIV-1 infection of peripheral blood mononuclear cells. The antiviral effect of the peptides appeared to require the ability to form a coiled coil, since amino acid substitutions which resulted in a destabilized solution structure led to the elimination of antiviral activity (37, 38).

Mutational analyses of gp41 have demonstrated that glycoproteins containing point mutations within the hydrophobic heptad repeat region of gp41 are transported to the cell surface and can be incorporated into virions. However, such glycoproteins were defective in mediating cell fusion, and virions containing glycoproteins with mutations of the central isoleucine

* Corresponding author. Mailing address: Department of Microbiology, University of Alabama at Birmingham, BBRB256, 845 19th St. South, Birmingham, AL 35294-2170. Phone: (205) 934-4321. Fax: (205) 934-1640.

(amino acid 573) within the heptad repeat were not capable of entering cells or initiating an infection (7, 11). Substituting proline for other isoleucines and leucines within the heptad repeats yielded similar results (6, 7). This finding indicates that the intact leucine zipper-like repeat of gp41 is not required for the assembly or release of HIV-1 but is required for virus entry and/or fusion.

To better understand the function of this hydrophobic heptad repeat, we have undertaken to examine the ability of this region of gp41 to drive a monomeric protein into an oligomeric structure independent of residual transmembrane (TM) glycoprotein components. In addition, we have examined the effects of point mutations in the central isoleucine (residue 573 of the HIV TM), and of deletions within this region, on the ability of this domain to oligomerize. Fast protein liquid chromatography (FPLC), gel exclusion chromatography, and analytical ultracentrifugation analyses show that this region can induce polypeptide oligomerization and that mutations within the domain drastically reduce this property. These results are discussed in the context of glycoprotein complex assembly and virus fusion.

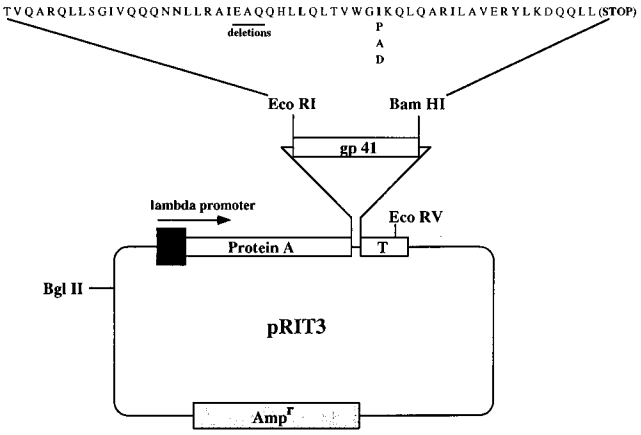

FIG. 1. Diagram of the pRIT3 bacterial expression plasmid. The Pharmacia vector pRIT2T was digested with *Eco*RI and *Bam*HI and ligated with a PCR-amplified fragment of gp41 which had been cut with *Eco*RI and *Bam*HI. The resulting plasmid, pRIT3, is under the control of the λ promoter, and induction conditions are via a temperature shift to 42°C in transformed bacteria containing a temperature-sensitive λ repressor.

MATERIALS AND METHODS

Construction of plasmids. A segment of the HIV-1 *env* gene corresponding to nucleotides 7834 to 8001 was amplified from plasmid pSRHP (11) via PCR using the primers 5′ CCGAATCCACGGTACAGGCCAGA 3′ and 5′ CCGGATCC AATCAGGAGCTGTTGATCC 3′. These primers created an upstream *Eco*RI site and a downstream *Bam*HI site into the fragment amplified, as well as a stop codon immediately preceding the *Bam*HI site. A single base was added after the *Eco*RI site to preserve the reading frame. PCR-amplified DNA was digested with the restriction endonucleases *Eco*RI and *Bam*HI, gel purified, and ligated into the polylinker region of the bacterial expression plasmid pRIT2T (Pharmacia, Piscataway, N.J.). The resulting expressed fusion protein, PA/gp41, consisted of the N-terminal region of protein A followed by the above-mentioned region of gp41. Mutants were verified by dideoxysequencing using Sequenase (U.S. Biochemical Corp., Cleveland, Ohio). Transformations and large-scale plasmid preparations were done in *Escherichia coli* N99cI$^+$ by using standard protocols (21).

Mutations in the heptad repeat region of the HIV-1 envelope glycoprotein have been described previously (11) and were constructed by site-directed mutagenesis using the Altered Sites mutagenesis system (Promega, Madison, Wis.) according to the protocol supplied by the manufacturer. Briefly, a 1-kb fragment of DNA containing the 3′ half of the *env* gene was cloned into the pSELECT1 vector, and single-stranded DNA was produced by using M13KO7 helper phage. Single-stranded DNA (0.1 μg per reaction) was used in a mutagenesis reaction mixture that contained 1.25 pmol of the mutagenic oligonucleotide and 0.25 μg of ampicillin repair oligonucleotide. The primers were annealed and elongated with T4 DNA polymerase in the presence of T4 DNA ligase, and the reaction mix was transformed onto *E. coli* BMH 71-18mutS and subsequently onto strain DH-1. All mutants were confirmed by dideoxy sequencing and cloned into the pSHRP vector.

Protein expression. Plasmids were transformed into *E. coli* N4830-1, which contains a temperature-sensitive λ *c*I857 repressor. Bacteria were grown to middle logarithmic phase (optical density at 600 nm of 0.8 to 1.0) in Luria-Bertani medium. Protein expression was then induced by a rapid temperature shift from a permissive temperature (30°C) to a nonpermissive temperature (42°C) followed by incubation for 2 h. Cells were then pelleted and lysed via sonication in lysis buffer containing 0.1 M $NaPO_4$, 0.05% Tween 20, and 20μg of phenylmethylsulfonyl fluoride per ml (pH 7.0).

Protein purification. Proteins were purified from clarified cell lysates via affinity chromatography using immunoglobulin G-Sepharose 6 (Pharmacia). Lysates were applied to 5-ml columns and washed overnight in sonication buffer to remove unbound proteins. Further washing with a low-salt buffer (5 mM ammonium acetate [pH 5.0]) was then performed. Protein was eluted with 0.1 M glycine (pH 3.0) and adjusted to pH 7.0 with 1.5 M Tris (pH 8.8). Eluted protein was then dialyzed overnight against 0.1 M sodium phosphate (pH 7.0) and concentrated in a Centricon-10 concentrator (Amicon, Beverly, Mass.). Bradford protein quantitation assays (Bio-Rad, Melville, N.Y.) were used to determine protein concentration, and aliquots of protein were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to determine the apparent molecular weight of the cloned fusion protein. Although yields of mutant fusion proteins were less than wild-type yields, following purification, both mutant and wild-type forms of the protein were stable under the conditions of the assays described below.

FPLC. Gel filtration chromatography was performed with a Pharmacia FPLC system equipped with a Superose 12 column. Protein samples at a concentration of 1 to 2 mg/ml were passed over the Superose 12 column and eluted with Tris-buffered saline (10 mM Tris [pH 7.0], 150 mM NaCl) at a flow rate of 0.5 ml/min (8). Elution was measured spectrophotometrically at a wavelength of 214 nm. An oligomer-to-monomer ratio was calculated by measuring the areas under both the oligomer and monomer peaks, and percent oligomerization was calculated directly from this ratio. The results represent averages of three independent experiments performed with different protein concentrations.

Analytical ultracentrifugation. Sedimentation studies were performed on a Beckman Spinco model E analytical ultracentrifuge equipped with scanning UV absorption optics. Sedimentation coefficients were determined by both band and boundary sedimentation velocity analysis (4). Boundary data were collected from a series of moving boundary tracings taken at 4-min intervals during centrifugation at a speed of 56,000 rpm, and the radial displacement of the midpoint of the moving boundary was used to calculate the sedimentation coefficient. The proteins analyzed were at concentrations of 1.5 to 3.0 mg/ml in 0.1 M $NaPO_4$. Band sedimentation velocity measurements were performed by layering a thin layer of protein solution (20 μl) onto 1 M KCl–0.1 M $NaPO_4$ buffer. Data were collected from tracings of the moving protein peak taken at 4-min intervals at a speed of 56,000 rpm. Centrifugal analyses were performed at three different protein concentrations, and the results shown represent mean sedimentation coefficients ± the standard deviations.

RESULTS

Construction of plasmids. To examine the potential of the hydrophobic heptad repeat of gp41 to oligomerize, we have expressed it as a part of a fusion protein by using the bacterial expression plasmid pRIT2T. This plasmid encodes the N-terminal region of protein A, including the Fc binding sites, followed by a multiple cloning site and a translation termination signal. Expression is under the control of a temperature-sensitive λ repressor. The resulting plasmid, pRIT3, encoded a protein consisting of the N-terminal portion of protein A fused to 56 amino acids from HIV-1 gp41 (Fig. 1). Since protein A is a monomer, the ability of the hydrophobic heptad repeat of gp41 to drive oligomerization of the fusion protein can be determined. This type of system has been used previously to examine the oligomeric structure of the leucine zipper region of c-Myc by using FPLC and chemical cross-linking (8).

Expression and purification of fusion proteins. Plasmids were used to transform *E. coli* N4830-1 cells, and the induction of protein expression and subsequent purification were performed as described in Materials and Methods. Aliquots of protein A and PA/gp41 were analyzed via SDS-PAGE to determine the apparent molecular weights of the recombinant

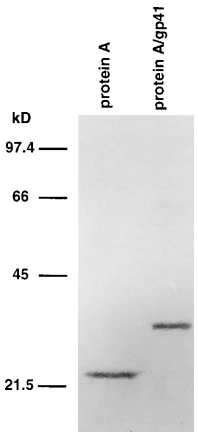

FIG. 2. SDS-PAGE of truncated protein A and PA/gp41. Aliquots of purified protein A and PA/gp41 were subjected to SDS-PAGE (12% polyacrylamide gel) to determine the apparent molecular weights and purity of the cloned fusion proteins.

proteins. The truncated form of protein A expressed by plasmid pRIT2T had an apparent molecular weight of 31,000 determined by SDS-PAGE (Fig. 2), compared with the calculated molecular weight from the predicted amino acid sequence of 29,400. The fusion protein, PA/gp41, had an apparent molecular weight of 37,000 determined by SDS-PAGE, compared with the predicted molecular weight of 34,800.

FPLC analysis of fusion proteins. FPLC has been used to observe oligomeric forms of proteins (8). To determine whether oligomeric species were present, we analyzed purified protein A and PA/gp41 by gel filtration chromatography using a Superose 12 size exclusion column. The truncated protein A eluted as a single, sharp peak with an elution volume of 11.75 ml (Fig. 3A). This rate of elution, which was close to that of the bovine serum albumin standard) was much faster than that expected from its molecular weight of 30,000. This observation may be accounted for by the shape of the protein A molecule, which has been determined to be grossly asymmetrical (1). PA/gp41 segregated into three well-defined peaks, one with an elution volume of 11.75 ml which coeluted with truncated protein A, a larger, faster-moving peak which eluted at 9.08 ml, corresponding to a molecular weight in excess of 200,000, and a third peak which marked the void volume of the column and which presumably resulted from a minor population of aggregated protein (Fig. 3B). The presence of the two major peaks in the FPLC analysis of the fusion protein indicates that two forms of PA/gp41 exist, a monomeric form and an oligomeric form.

Mutant fusion proteins. To evaluate the specificity of the interaction between the fusion proteins, we expressed constructs containing changes in the amino acid sequence of the protein. Point mutations were incorporated into the coding region for the gp41-derived portion of the fusion protein in order to evaluate the importance of the central hydrophobic isoleucine residue (amino acid 573) within the heptad repeat for oligomerization of the fusion protein. These mutations had previously been shown to have no discernible effect on the sedimentation properties of the intact envelope glycoprotein but interfered significantly with the biological activity of the glycoprotein complex. The mutant fusion proteins were expressed at levels similar to that of the wild type and following purification on immunoglobulin G columns yielded proteins of the expected molecular weight on SDS-PAGE and with a purity of greater than 80% (data not shown). Oligomerization of

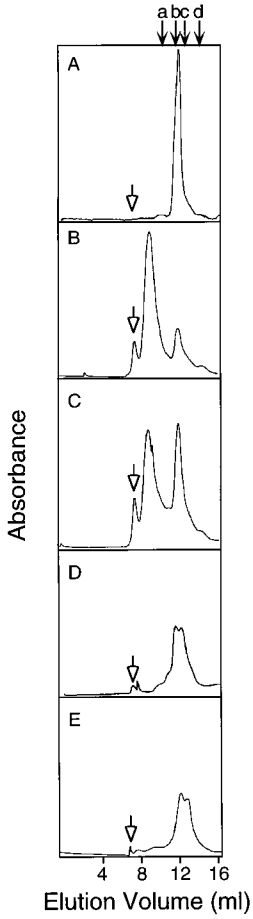

FIG. 3. FPLC analysis of protein A and PA/gp41. Aliquots of purified protein A and PA/gp41 were injected onto a Superose 12 size exclusion column. Samples were eluted at a flow rate of 0.5 ml/min, using Tris-buffered saline as an eluent, and UV absorption was measured at a wavelength of 214 nm. (A) Protein A; (B) PA/gp41; (C) PA/gp41[I573A]; (D) PA/gp41[I573P]; (E) PA/gp41del3. Molecular weight standards are as follows: a, 200,000; b, 66,000; c, 45,000; d, 14,200. Each open arrowhead marks the void volume of the column.

the mutant fusion proteins was compared with that of the wild type by determining the fraction of mutant protein in the monomer and oligomer peak following gel filtration chromatography (Fig. 3C and D; Table 1). Substituting alanine for the middle isoleucine (PA/gp41[I573A]) resulted on average in a 63% decrease in oligomerization as compared with the wild-type fusion protein (Fig. 3C and Table 1). Substituting either

TABLE 1. Oligomerization of fusion proteins

Construct	Oligomer/monomer[a]	Oligomerization (%)[b]
PA/gp41	2.09	100
Protein A	0	0
PA/gp41[I573A]	0.79	37.7
PA/gp41[I573D]	0.22	10.5
PA/gp41[I573P]	0.06	2.9
PA/gp41del1	0.07	3.3
PA/gp41del2	0.02	1.0
PA/gp41del3	0.09	4.3

[a] Area under the oligomer peak divided by area under the monomer peak.
[b] Relative to the oligomerization of the wild-type fusion protein, determined by using the following formula: % oligomerization = (oligomer/monomer) sample/(oligomer/monomer) PA/gp41.

TABLE 2. Hydrodynamic measurements of fusion proteins

Protein	Analysis	Sedimentation coefficient (S)	SD	Mol wt[a]
PA/gp41	Band sedimentation	6.60	0.08	122,100
PA/gp41	Boundary sedimentation	7.02	0.06	109,800
Protein A	Boundary sedimentation	1.94	ND[b]	15,300
PA/gp41[I573P]	Boundary sedimentation	2.37	0.06	21,700

[a] Calculated as follows: $M^{2/3} = s\bar{v}^{1/3}/0.01\ (1 - \bar{v}\rho)$.
[b] ND, not done.

aspartic acid (PA/gp41[I573D]; Table 1) or proline (PA/gp41[I573P]; Fig. 3D and Table 1) for the same isoleucine resulted in an almost complete loss of oligomerization. These results indicate that the central isoleucine plays a key role in mediating the oligomerization of the fusion protein.

To evaluate the importance of the five heptad repeats in the oligomerization of the fusion protein, small deletions were made in the coding region for the gp41-derived portion of the fusion protein. We constructed clones which coded for deletions of one, two, or three amino acids beyond the first isoleucine of the hydrophobic heptad repeat and analyzed these fusion proteins by FPLC. A profound decrease in the oligomerization of fusion proteins containing these deletions was observed (Fig. 3E and Table 1), indicating that an intact series of heptad repeats is required for oligomerization of the fusion protein.

Analytical ultracentrifugation of fusion proteins. To further define the oligomeric structure of the protein A/gp41 fusion protein, sedimentation velocity studies were carried out on truncated protein A, PA/gp41, and PA/gp41[I573P]. Band and boundary sedimentation analysis were used to determine the sedimentation coefficients of the fusion proteins. Boundary sedimentation velocity analysis was used to experimentally determine the sedimentation coefficient of truncated protein A, which exists as a monomer. The sedimentation coefficient ($s_{20,w}$) of this molecule was determined to be 1.94S and is comparable to that obtained for full-length protein A, which has an apparent molecular weight of 42,000 and a sedimentation coefficient of 2.1S (1). Similarly, the sedimentation coefficient for the PA/gp41[I573P] mutant fusion protein, in which the central isoleucine had been replaced with proline, was experimentally determined following boundary analysis to be 2.38S. The low sedimentation coefficient confirms that PA/gp41[I573P] exists as a monomer and provides a sedimentation coefficient for a monomeric form of the fusion protein. The molecular weight of this protein, 21,700, calculated from the sedimentation data, while clearly lower than that expected from its amino acid composition, is consistent with the molecular weight for the truncated protein A molecule (15,300) calculated from the same series of experiments. The wild-type PA/gp41 fusion protein, by contrast, had a significantly higher sedimentation coefficient. The $s_{20,w}$ of PA/gp41, as calculated from band sedimentation velocity patterns, was determined to be 6.6S and by boundary analysis was 7.0S, consistent with an oligomer molecular weight of 109,800 to 122,100 (Table 2). The small difference in the sedimentation coefficients for PA/gp41 obtained by two independent velocity methods at different protein concentrations is within experimental error for determination of $s_{20,w}$ values and indicates that the oligomeric complex is stable under high-salt conditions. Additionally, the discrete nature of the sedimenting band for each of the constructs ruled out the possibility of nonspecific aggregation or multiple oligomeric forms. On the other hand, because of the anomalous shape of the native protein A, it is not possible to directly calculate the valency of the oligomer from the monomer $s_{20,w}$. Nevertheless, the native state of the fusion protein is clearly too large to be a dimer and is likely to be a trimer or tetramer. Experiments to determine a native molecular weight of the oligomer by equilibrium centrifugation are currently under way.

DISCUSSION

To evaluate the potential of the hydrophobic heptad repeat of gp41 to direct protein oligomerization, we have cloned this region of HIV-1 gp41 into the bacterial expression vector pRIT2T, a plasmid which encodes the N-terminal region of staphylococcal protein A followed by a multiple cloning site. Protein A is a monomeric protein, and the leucine zipper domain from c-Myc has been shown to cause its oligomerization (1, 8). Therefore, it was of interest to determine whether a fusion protein consisting of protein A and the hydrophobic heptad repeat of gp41 could oligomerize. Evidence supporting the presence of an oligomeric form of the fusion protein was derived from FPLC analysis using a Superose 12 size exclusion column. Truncated protein A eluted in a single peak, and the fusion protein, PA/gp41, segregated into two major peaks, indicating the presence of both monomeric and oligomeric forms of the fusion protein. Similar results have been obtained with use of this system to analyze the leucine zipper region of c-Myc (8). Analytical ultracentrifugation was used to further define the oligomeric state of PA/gp41. A sedimentation coefficient of 6.6S was obtained from band velocity sedimentation analysis, and a sedimentation coefficient of 7.0S was obtained from boundary sedimentation analysis. The sedimentation coefficient of the protein A/gp41 fusion protein was substantially higher than the sedimentation coefficient of truncated protein A. On the basis of a sedimentation coefficient of 1.94S, the apparent molecular weight of truncated protein A was calculated to be 15,300, a value which is substantially lower than the molecular weight of 29,800 calculated from the deduced amino acid sequence. This discrepancy results from the necessity to assume that the molecule has a spherical shape for the molecular weight calculations from sedimentation coefficients. This is clearly not the case (1), and because of their high frictional coefficients, asymmetric molecules typically yield an abnormally low native molecular weight when calculated by this method. This finding indicates that mathematical calculations of the molecular weights of our fusion proteins on the basis of their sedimentation coefficients will likely result in abnormally low numbers. From sedimentation coefficients from boundary and band runs (7.0S and 6.6S, respectively), the native molecular weight of the fusion protein can be calculated to be 109,800 to 122,100 (35). However, considering the disparity between the molecular weight estimations of truncated protein A, the native molecular weight of PA/gp41 is likely to be higher than is estimated by this method. Therefore, we conclude that this region of gp41 is capable of inducing protein A to form either a trimer or a tetramer.

We have defined a region of gp41 which on its own is a monomeric protein. This domain of gp41, which is capable of directing the oligomerization of a fusion protein containing protein A, has been shown to contain an amino acid sequence similar to sequences of leucine zipper motifs (9). However, unlike a classical leucine zipper, the heptad repeats of this region include valine and isoleucine residues in addition to the leucine residues. This arrangement of hydrophobic residues is consistent with this region forming a coiled coil (15), and circular dichroism analyses of peptides corresponding to this domain support this hypothesis (37, 38). A recent report has

described a switch between two-, three-, and four-stranded coiled coils in the GCN4 leucine zipper (17). These different oligomeric forms of the leucine zipper were obtained by changing the core amino acids of the heptad repeats which lie at the a and d positions of the α helix from leucines to either isoleucines or valines. Similarly, the isoleucines and valines within this region of gp41 may be responsible for the formation of the higher-ordered, trimer or tetramer structures that we have observed here. Our findings and the results obtained with the GCN4 leucine zipper support the notion that this region of gp41 functions as a structural domain, which has a high potential to form a multimeric hydrophobic heptad repeat-containing coiled coil, rather than a traditional dimeric leucine zipper.

The specificity of the interaction between PA/gp41 subunits was determined via mutations within the leucine zipper region of the fusion protein. FPLC analysis of a fusion protein in which the central isoleucine of the leucine zipper has been changed to alanine (PA/gp41[I573A]) resulted in a two-thirds decrease in oligomerization. Changing the central isoleucine to either aspartic acid or proline resulted in even larger decreases in oligomerization, indicating that the central isoleucine is required for oligomerization of the fusion protein. In addition, the PA/gp41[I573P] mutant had a sedimentation coefficient of 2.37S, as determined by boundary sedimentation analysis, confirming that it exists as a monomer. Proteins which contained limited deletions of one, two, or three amino acids beyond the first isoleucine of the heptad repeat also yielded proteins which were profoundly deficient in oligomerization. These results demonstrate that oligomerization requires the heptad repeats of hydrophobic amino acids within this domain of gp41.

Potential functions of this domain of gp41 have been addressed by prior work using synthetic peptides and site-directed mutagenesis of the envelope glycoprotein (6, 7, 11, 37, 38). Envelope glycoproteins, in which the middle isoleucine (amino acid 573) of the heptad repeat had been changed to alanine, resulted in viruses with reduced cell fusion and infectivity. Viruses containing aspartic acid and proline substitutions for the central isoleucine of this domain of gp41 were not viable, indicating that an intact hydrophobic heptad repeat is required for cell fusion and infectivity. Interestingly, the substitutions made to full-length viral glycoprotein did not prevent the formation of glycoprotein oligomers, transport to the cell surface, or incorporation into virus (7, 11). These data argue strongly against the oligomerization potential of the heptad repeat domain playing a significant role in assembly oligomerization of the native viral glycoprotein. A possible solution to this paradox has come from peptide studies. Peptides containing 36 amino acids from this domain of gp41 have a solution structure, as analyzed by circular dichroism, predictive of a coiled-coil and are able to inhibit both cell-cell fusion and infection of peripheral blood mononuclear cells by HIV-1. In contrast, a synthetic peptide containing proline in place of the middle isoleucine of the heptad repeat did not have a solution structure predictive of a coiled coil, nor did it exhibit antifusion or antiviral activity (38). Moreover, the antiviral activities of peptides containing alanine or serine substitutions correlated closely with the stability of their coiled-coil structure (37). The results obtained here with the fusion proteins, taken together with those from the synthetic peptides and the virus mutants, strongly suggest that this structural domain of gp41 is involved in fusion and virus entry and that the propensity to form a coiled-coil structure correlates closely with this function. In contrast, the same mutations which abrogate oligomer formation of the fusion protein have no detectable effect on assembly-oligomerization of the intact glycoprotein complex, indicating that the propensity to form a coiled-coil structure is not the driving force of this process. It is possible, therefore, that the hydrophobic heptad repeat domain does not associate into an oligomeric structure until a point during the stage of virus-mediated membrane fusion.

A synthetic peptide from the HA glycoprotein of influenza virus has recently been identified as having the propensity to form a coiled coil in solution. This sequence corresponds to a loop region in the three-dimensional structure of native HA2 and is presumably constrained from forming a coiled coil by the glycoprotein complex. It has been hypothesized that under acidic conditions, this region of HA forms a coiled coil in the fusogenic conformation of HA, causing the entire ectodomain of HA2 to form an extended coiled coil, thus relocating the fusion peptide of HA2 100 Å (10 nm) toward the target membrane (5). This model is supported by the recently reported three-dimensional structure of the fusogenic conformation of the HA2 glycoprotein (3). If the hydrophobic heptad repeat of HIV-1 is an analogous structure, the structural domain of gp41 that we have analyzed may be instrumental in the assumption of a fusion-competent state by the HIV-1 envelope glycoprotein. The facts that a peptide corresponding to this region of gp41 has been shown to be capable of oligomerization and form a coiled coil in solution (38), as was demonstrated for the HA2-derived peptide, and that the ability to form a coiled coil correlates with the ability of peptides from this region to inhibit the fusion process (37) provide additional support for this hypothesis. We conclude that this structural domain of gp41 plays a key role in the entry of HIV-1 into cells and that this function is dependent on the ability of this region to oligomerize through the formation of coiled coils.

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