Expression, Purification, and Characterization of gp160e, the Soluble, Trimeric Ectodomain of the Simian Immunodeficiency Virus Envelope Glycoprotein, gp160*

Received for publication, June 6, 2000, and in revised form, August 14, 2000
Published, JBC Papers in Press, August 15, 2000, DOI 10.1074/jbc.M004905200

The envelope glycoprotein, gp160, of simian immunodeficiency virus (SIV) shares ~25% sequence identity with gp160 from the human immunodeficiency virus, type I, indicating a close structural similarity. As a result of binding to cell surface CD4 and co-receptor (e.g. CCR5 and CXCR4), both SIV and human immunodeficiency virus gp160 mediate viral entry by membrane fusion. We report here the characterization of gp160e, the soluble ectodomain of SIV gp160. The ectodomain has been expressed in both insect cells and Chinese hamster ovary (CHO)-Lec3.2.8.1 cells, deficient in enzymes necessary for synthesizing complex oligosaccharides. Both the primary and a secondary proteolytic cleavage sites between the gp120 and gp41 subunits of gp160 were mutated to prevent cleavage and shedding of gp120. The purified, soluble glycoprotein is shown to be trimeric by chemical cross-linking, gel filtration chromatography, and analytical ultracentrifugation. It forms soluble, tight complexes with soluble CD4 and a number of Fab fragments from neutralizing monoclonal antibodies. Soluble complexes were also produced of enzymatically deglycosylated gp160e and of gp160e variants with deletions in the variable segments.

The SIV1 and HIV envelope glycoproteins, known as gp160, bind to cell surface receptors, effect cell entry by fusion of viral and cellular membranes, and as major surface antigens, induce neutralizing antibodies in the host. gp160 is synthesized as a single chain precursor, which is cleaved after oligomerization by furin, or a similar enzyme, into the two chains gp120 and gp41. Cleavage occurs during transport to the plasma membrane (1, 2). In the cleaved gp120/gp41 molecule, gp120 and gp41 are associated noncovalently. The gp120 polypeptide contains the binding sites for the receptor CD4 (3–5) and the chemokine binding co-receptor (6–10). gp41 contains an N-terminal nonpolar fusion peptide, a nonpolar transmembrane anchor, and a C-terminal cytoplasmic domain (11–13).

The interaction of the gp120 polypeptide of gp120/gp41 with CD4 causes a conformational change in the viral glycoprotein, inducing altered antibody reactivity, increased proteolytic susceptibility, and the tendency in some viral strains for gp120 to dissociate from gp41 (14–16). The conformational change caused by CD4 binding apparently increases the affinity of gp120/gp41 for the chemokine-binding co-receptor (17–18). Interaction with the chemokine receptor activates the membrane fusion activity of gp120/gp41, probably by triggering a conformational change in gp41. This change is likely to expose the N-terminal fusion peptide and to refold gp41 into a helical hairpin, thereby placing the fusion peptides and transmembrane anchors of gp41 at the same end of a rod-shaped molecule (19–24).

There is a formal similarity among the SIV and HIV-1 gp160 glycoproteins, the envelope glycoproteins of other retroviruses such as Moloney murine leukemia virus, and HTLV-1, the Gp glycoprotein of filoviruses, and the hemagglutinin (HA) of influenza virus. Each is synthesized as a single chain precursor, which after cleavage results in an N-terminal subunit (gp120, SU, Gp1, HA1) with receptor binding activity and a C-terminal, membrane-anchored subunit (gp41, TM, Gp2, HA2) having a nonpolar, glycine-rich N-terminal fusion peptide followed by heptad repeats characteristic of α-helical coiled-coils (reviewed in Refs. 25 and 26). Evidence suggests that these viral glycoproteins are all trimeric (27–32), although some early studies proposed dimers or tetramers for gp160 (33–35). In each case, the N-terminal subunit (gp120, SU, Gp1, HA1) is monomeric when prepared in the absence of the transmembrane subunit, but oligomeric in its presence (36, 37). The C-terminal subunits are all trimeric when expressed in the absence of the N-terminal polypeptides, and they adopt similar structures dominated by an N-terminal, central triple-stranded α-helical coiled-coil and an outer layer of three helices or extended strands packing antiparallel along the central coiled-coil and connected to it by a loop with a reverse (reviewed in Ref. 26).

The three-dimensional structure of an enzymatically deglycosylated fragment of monomeric gp120 from HIV-1, with the variable segments V1, V2, and V3 deleted, along with 52 residues at the N terminus and 19 at the C terminus, has been determined by x-ray crystallography. The crystals contain a...
complex of truncated gp120 with a two-domain fragment of CD4 and an Fab fragment from the monoclonal antibody 17b (38). This structure contains about 60% of the gp120 residues (39, 40). It is in the CD4-induced conformation, which has enhanced affinity for the monoclonal antibody 17b (41). The structure of the gp120 trimer has not been determined, although it has been modeled (42), and the conformation of gp120 as it would appear on virus before CD4 binding is currently unknown.

We report the development of expression systems using insect and mammalian cells for production of soluble, oligomeric ectodomain, gp160e, from the SIV envelope glycoprotein. Purified oligomers are shown to bind CD4 and Fab fragments of neutralizing monoclonal antibodies. Experiments show that the molecule is trimeric. These observations should facilitate biochemical and structural characterization of the viral glycoprotein in its native, oligomeric form.

**EXPERIMENTAL PROCEDURES**

*SIV gp160e Expression in CHO Cells—* SIV gp160e from the SIV strain Mac32H pr35 (43) was expressed in CHO-Lec3.2.8.1 cells using pSIV-M (Fig. 1) digested with restriction enzyme NcoI previously (45). Briefly, 20 μg of SalI-linearized pSIV-M DNA was transfected into the Lec3.2.8.1 cells by calcium phosphate precipitation (Strategene). Transformants were selected in GMEM medium (Life Technologies, Inc.) containing 25 μg/ml methionine sulfoximine and assayed for secretion of soluble SIV gp160e by enzyme-linked immunosorbent assays. Immunol plates (Dynatech Laboratories Inc.) were coated with anti-SIV ENV monoclonal antibody KK41 (47) for 2 h at room temperature. After washing, plates were blocked with 5% nonfat dry milk and then incubated with either W6/32 (50 μg/ml) or biotinylated mAb KK41 used for pNES9D and pNES1D transfected into the Lec3.2.8.1 cells by calcium phosphate precipitation.

Lec3.2.8.1 cells, a shorter version of the tissue plasminogen activator (gp160e) supernatant (50 μg/ml solution overnight at 4 °C; the plates were then blocked with 1% bovine serum albumin in PBS at room temperature for 2 h. Cell culture supernatant (50 μl) from each transformed clone was added to the plates and incubated overnight at 4 °C. The plates were washed with 0.1% bovine serum albumin in PBS and incubated with 0.2 μg/ml of biotinylated mAb KK41 (47) for 2h at room temperature. After washing, the plates were developed with horseradish peroxidase-conjugated streptavidin (Sigma). The highest expressing cells were chosen for rescreening with the same assay. The expression of SIV gp160e was also confirmed by immunoprecipitation. Large scale production of SIV gp160e was carried out following the protocol previously described (45) in 175-cm² tissue culture flasks or 850-cm² roller bottles (Corning).

The protein expressed from pSIV-M in Lec3.2.8.1 cells was found to contain secreted SIV gp160e by Western blot and ELISA (48) using anti-SIV envelope antibodies. Secreted gp160e was purified by an immunoaffinity chromatography using a mAb 17A11 affinity column (5-ml bed volume), where the monoclonal antibody 17A11 was immobilized on Protein A-Sepharose (Pharmacia) and antigen was eluted from the columns with 0.5 M methionine sulfoximine and asparagine. Enzyme-linked immunosorbent assays (ELISA) were performed using sheep antisera against SIV envelope glycoprotein. The optimal amounts of virus and postinfection harvest time was determined by Plaque assay (5). The optimal harvest time was determined by Plaque assay (5).

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3 J. A. Hoxie, unpublished results.
crosslinked at 5 mg/ml to GammaBind Plus Sepharose (Amersham Pharmacia Biotech) with dimethyl pimelimidate (Pierce). The supernatants were passed through the column with a flow rate of about 0.5 ml/min. After extensive washing with PBS, the protein was then eluted with 100 mM glycine (pH 3.0), followed by immediate neutralization with 100 mM Tris-HCl (pH 8.0). The fractions were analyzed by SDS-PAGE. The fractions containing SIV gp160e were pooled, concentrated, and further purified by gel filtration chromatography on Superdex 200 or Superose 6 (Amersham Pharmacia Biotech) with an elution containing 25 mM Tris-HCl (pH 8.0) and 150 mM NaCl. SIV gp160eΔ(V1V2) and gp160eΔ(V1V2V3) proteins were purified following the same procedure.

SIV gp160e purified from insect cells was purified by metal chelate affinity chromatography with ProBond resin (Invitrogen). Concentrated insect cell culture supernatants were immediately changed into 1× column buffer (25 mM sodium phosphate (pH 8.0), 250 mM NaCl, 250 mM EGS (Pierce), 15 mM Tris-glycine (pH 7.0) and incubated at room temperature for 45 min. The cross-linked products were analyzed by SDS-PAGE. Cross-linked phosphorylation b (Sigma) was used as an SDS-PAGE molecular weight standard.

Analytical ultracentrifugation was performed on a Beckman XL-A analytical ultracentrifuge at 4 °C. Experiments were performed at concentrations of 1.6 μM, 3.3 μM, and 6.5 μM protein and centrifuged at a rotor speed of 6000 rpm. Data were fitted to a single-species model. The intermediate of the insect cell culture supernatants was immediately changed into 1× column buffer (25 mM sodium phosphate (pH 8.0), 150 mM NaCl, SIV gp160eΔ(V1V2V3) and gp160eΔ(V1V2V3) proteins were purified following the same procedure.

N-terminal analyses of purified proteins were carried out by the HHMI biopolymer facility.

**Chemical Cross-linking and Analytical Ultracentrifugation—** For chemical cross-linking experiments, SIV gp160e protein was dialyzed extensively against PBS. In 20-μl reactions, SIV gp160e (1 mg/ml) was incubated with ethylene glycol bis(succinimidylsuccinate) (EGS; Pierce) at concentrations of 0.06, 0.18, 0.55, 1.67, and 5 mM respectively, on ice incubated with 15 mM to reduce nonspecific binding to the nickel column. After centrifugation at 5000 rpm in a JA-14 rotor (Beckman), insect cell culture supernatants were immediately changed into 1× column buffer (25 mM sodium phosphate (pH 8.0), 250 mM NaCl, 250 mM EGS (Pierce), 15 mM Tris-glycine (pH 7.0) and incubated at room temperature for 45 min. The cross-linked products were analyzed by SDS-PAGE. Cross-linked phosphorylation b (Sigma) was used as an SDS-PAGE molecular weight standard.

Analytical ultracentrifugation was performed on a Beckman XL-A analytical ultracentrifuge at 4 °C. Experiments were performed at concentrations of 1.6 μM, 3.3 μM, and 6.5 μM protein and centrifuged at a rotor speed of 6000 rpm. Data were fitted to a single-species model. The protein specific volume and solvent density were calculated using previously described methods (45), and assuming that all 25 potential glycosylation sites are occupied, the partial specific volume of SIV envelop glycoprotein gp160e was calculated to be 0.70 ml/g.

**Binding of gp160e to CD4 and Antibody Fab Fragments—** Four-domain scCD4 expressed from CHO cells, affinity-purified, and sized by Superdex 75 gel filtration chromatography was kindly provided by Dr. Yi Xiong. Molecular weights were calculated based on a standard curve plotted from the elution volumes of known proteins. Peak fractions were verified to contain both gp160e and CD4 or Fab by SDS-PAGE.

**Circular Dichroism Spectroscopy—** CD spectra were recorded at 25 °C using SIV gp160e at a concentration of 0.56 mg/ml in PBS with a circular dichroism spectrometer model 62DS (Aviv). The molar ellipticity [θ]θ was monitored as the average of 12 scans with 0.5-nm bandwidth, and 1.0-nm wavelength increments from 195 to 260 nm. The spectra were corrected with a base line obtained using buffer alone under the same conditions.

Enzymatic Deglycosylation of SIV gp140—Endoglycosidase H (Endo H) digestion was carried out using 20 μg of the SIV gp160e expressed in CHO-Lec3.2.8.1 cells and 500 units of Endo H (New England Biolabs) for each reaction at room temperature under native and denaturing conditions with buffers supplied by the manufacturer. Digestion was then further optimized at various pH values, since low pH may interfere with complex formation of SIV gp160e with the Fabs that were used to prevent aggregation after deglycosylation. Digestion was found to be complete in 30 min at 25 °C under pH 6.5. To deglycosylate the complexes of SIV gp160e and Fab fragments, the purified SIV gp160e was incubated with 17A11, 9G3 Fab in PBS for at least 2 h at room temperature, followed by adjusting the pH to 6.5 with 100 mM PIPES (pH 6.5). The complexes were then treated with Endo H for 4 h and purified by a gel filtration chromatography on Superose 6. The peak fractions were pooled, concentrated, and analyzed by SDS-PAGE in an 8% gel.

**RESULTS**

Expression of SIV gp160e in Insect and CHO Lec3.2.8.1 Cells—The ectodomain of the SIV envelope glycoprotein, gp160e, was expressed as a secreted protein in both insect and CHO Lec3.2.8.1 cells, by adding a secretion signal (leader) sequences were confirmed by N-terminal sequencing of the expressed products and are indicated by arrows beneath the sequences. The sequences where the V1, V2, and V3 segments were deleted and replaced with a CAG linker (in italics) are shown for pNES1ΔV1V2V3 and pBSIVΔV1V2V3-Hsl1. The sequences where the transmembrane segment of gp41 was truncated are also shown for all constructs. Leader sequences are shown in normal type, and SIV gp160e sequences included in the expression constructs are in boldface type and underlined; sequences not included are printed with a strike-through.

Expression of SIV envelope glycoprotein in mammalian and insect cells.

Schematic representations for the expression constructs, SIV-M, pNES1ΔV1V2V3, pBSIV-Hsl1, and pBSIVΔV1V2V3-Hsl1, are shown. In all cases, the posttranslational cleavage sites (residues 512 and 523) between gp120 and gp41 have been mutated as shown to prevent cleavage and dissociation of gp120. pNES1ΔV1V2V3 and pBSIVΔV1V2V3-Hsl1, were shown. In all cases, the posttranslational cleavage sites (residues 512 and 523) between gp120 and gp41 have been mutated as shown to prevent cleavage and dissociation of gp120. pNES1ΔV1V2V3 and pBSIVΔV1V2V3-Hsl1, were shown. In all cases, the posttranslational cleavage sites (residues 512 and 523) between gp120 and gp41 have been mutated as shown to prevent cleavage and dissociation of gp120. pNES1ΔV1V2V3 and pBSIVΔV1V2V3-Hsl1, were shown.
The SIV gp160e proteins were purified from supernatant of CHO-Lec3.2.8.1 cells that expresses the gp160e by mAb 17A11 affinity chromatography (Fig. 1). Two basic residues at the primary (Lys-523) cleavage site and a secondary (Arg-512) cleavage site between gp120 and gp41 were replaced by glutamic acids, as described previously (44), to prevent cleavage of the gp160e precursor and to avoid shedding of gp120 that had been observed in native constructs. Variants were expressed with only the V1 and V2 segments deleted (not shown) or with the V1, V2, and V3 segments deleted (Fig. 1). Such deletion variants of HIV-1 gp120 have been shown to retain CD4 binding activity (1, 38–40).

SIV gp160e molecules were secreted into the culture media at levels of 0.5–1 mg/liter from Hi-5 insect cells and 2–4 mg/liter from CHO Lec3.2.8.1 cells. Purification and Size Determination by Gel Filtration Chromatography—SIV gp160e secreted from CHO Lec3.2.8.1 cells was purified from cell culture fluid by immunoaffinity chromatography using the monoclonal antibody 17A11 (a gift from J. Hoxie). The protein was eluted from the affinity column by low pH (3.0) and immediately neutralized. Peak fractions were concentrated and further purified by gel filtration chromatography. The purified gp160e eluted from the sizing column (Superose 6) with an apparent molecular weight of approximately 440,000, when compared with standard proteins (Fig. 2A). The relatively sharp gel filtration profile suggests that the molecule is reasonably monodisperse. Polydispersity of this sample was also approximately 20%, as measured by dynamic light scattering. Analysis by SDS-PAGE showing a single prominent protein band at an apparent molecular weight of approximately 120,000 indicates a high level of purity (Fig. 2B). Again, the ratio of the monomer molecular weight to the apparent molecular weight of the secreted molecular species indicates that the molecule is an oligomer.

CD spectra of SIV gp160e recorded at 25 °C indicate that the protein is folded and contains a mixture of secondary structures (Fig. 3).

Evidence from Chemical Cross-linking and Analytical Ultracentrifugation That gp160e Is Trimeric—The oligomeric structure of the soluble ectodomain of SIV gp160e was examined by both chemical cross-linking and sedimentation equilibrium by analytical ultracentrifugation. Purified SIV gp160e (1 mg/ml) was incubated for 30 min with the cross-linking reagent EGS at a series of EGS concentrations from 0.06 to 5 mM. The reaction products were analyzed by SDS-PAGE (Fig. 4A). At the lowest concentration of cross-linker, three protein bands are evident, migrating at apparent molecular weights corresponding to one, two, and three gp160e polypeptide chains (see legend to Fig. 4A). As the concentration of cross-linking reagent is increased, the apparent trimer band increasingly dominates. Some faint higher molecular weight bands are observed at higher cross-linker concentrations, but their mobility is too low to represent tetramers, and they presumably represent minor intertrimer cross-linking at high cross-linker concentrations.

The molecular weight of the soluble ectodomain of SIV gp160e was measured by sedimentation equilibrium. Measurements were made at three protein concentrations and one rotor speed. The results from one experiment are shown in Fig. 4B. No systematic deviations were found from a single-species model at the concentrations studied. The partial specific volume of gp160e, required for the buoyancy correction, was calculated from the amino acid composition and the carbohydrate content (see “Experimental Procedures”) (49). The resulting measured molecular weight of SIV gp160e of 340,000 is almost exactly 3 times the monomer molecular weight calculated (110,000) from the sequence and carbohydrate content or estimated by SDS-PAGE (120,000).

Complexes of gp160e with CD4 and Fab Fragments of Neutralizing Monoclonal Antibodies—The purified, soluble ectodomain of SIV gp160e was expressed from CHO-Lec3.2.8.1 and insect cells. The purified proteins were then resolved by gel filtration chromatography using a Superose 6 column. Molecular masses were calculated based on a standard curve plotted using known proteins, which include thyroglobulin (669 kDa), ferritin (440 kDa), catalase (232 kDa), aldolase (158 kDa), and bovine serum albumin (67 kDa). Peak fractions were pooled and analyzed by SDS-PAGE (inset). Similar experiments using an analytical Superdex 200 column gave the same results (not shown).
main of the HIV-1 receptor human CD4 (4-domain CD4), expressed as a secreted protein in CHO cells, was incubated with purified SIV gp160e to determine its receptor binding activity. Excess CD4 was removed from the gp160e-CD4 complex by gel filtration chromatography (Fig. 5). Analysis of the peak fractions from the gel filtration column by SDS-PAGE indicates that the pure gp160e binds CD4 (Fig. 5).

SIV gp160e binds 13 mAbs raised against SIV gp160 or against SIV-infected cells (mAb 17A11); the mAbs have been mapped to various epitopes: V1, V2, V3, and V4 on gp120 and others on gp41. Complexes of SIV gp160e with Fab fragments from either of the neutralizing mAbs KK9 (which recognizes the V3 and V4 segments of gp120) or 9G3 (which recognizes gp41) were prepared by incubating gp160e with a molar excess of the Fab and removing the excess Fab by gel filtration chromatography. Analysis of the peak fractions from the gel filtration column indicated that both Fabs bind the gp160e molecule. The change in apparent molecular weight estimated from the elution volume of molecular weight standards is approximately 150,000, suggesting that more than one and probably three Fab fragments bind per gp160e oligomer (Fig. 6).

Complexes of gp160eΔ(V1V2V3) with Fab Fragments of Neutralizing Monoclonal Antibodies—A SIV gp160e variant, gp160eΔ(V1V2V3), which has the V1, V2, and V3 segments deleted, also formed complexes with Fab fragments from neutralizing mAbs. The Fab complexes with gp160eΔ(V1V2V3) were purified from the excess Fabs by gel filtration chromatography (Fig. 7). Similar complexes of gp160eΔ(V1V2) with Fabs have been purified (data not shown).

Deglycosylation of SIV gp160e Expressed in CHO-Lec3.2.8.1 Cells—Endo H digestion at room temperature under native and denaturing conditions was used to remove sugar residues from SIV gp160e. Digestion was complete in 30 min at 25 °C, pH 5.5, as judged by the similar increase in mobility on SDS-PAGE of gp160e samples deglycosylated under native and denaturing conditions (Fig. 8A). Deglycosylated SIV gp160e was insoluble.

To prepare soluble, deglycosylated SIV gp160e required that complexes of SIV gp160e and Fab fragments (17A11 or 9G3) be incubated with endoglycosidase H for 4 h. This treatment yielded the same level of deglycosylation as digestion of free gp160e or denatured gp160e, as judged by mobility of the digested gp160e on SDS-PAGE (data not shown). The resulting deglycosylated gp160e-Fab complexes were soluble and monodisperse. These complexes were purified from the excess, unbound Fab by gel filtration chromatography (Fig. 8B).

**Fig. 3.** CD spectrum of SIV gp160e. CD spectra were recorded at 25 °C with a protein concentration of 0.56 mg/ml in PBS. The spectra were corrected with base-line spectra recorded from buffer alone under the same conditions.

**Fig. 4.** Chemical cross-linking and analytical ultracentrifugation indicate that SIV gp160e is trimeric. A, SIV gp160e protein was cross-linked at various concentrations of EGS (ethylene glycol bis(succinimidylsuccinate)) as indicated by numbers about the lanes. The cross-linked products were analyzed by SDS-PAGE in a 3.5% gel. The molecular weight standard for SDS-PAGE was cross-linked phosphorylase b (Sigma), which includes monomer (97 kDa), dimer (194 kDa), trimer (292 kDa), tetramer (389 kDa), pentamer (487 kDa), and hexamer (584 kDa) species. The molecular masses of monomer, dimer, and trimer bands (Fig. 4A) of SIV gp160e were determined from a plot of the logarithm of molecular mass of the standards against relative mobility (not shown). The bands marked monomer, dimer, and trimer in the 0.06 mM EGS lane are apparently 123 kDa, 234 kDa (246 calculated), and 346 kDa (369 calculated), respectively. B, analytical ultracentrifugation was performed on a Beckman XL-A analytical ultracentrifuge at 4 °C. The data shown were collected with the protein at concentration of 1.6 µg/ml and rotor speed of 6000 rpm. Data sets were fitted to a single species model, and protein partial specific volume and solvent density were calculated according to Laue et al. (49). The molecular mass determined is 340 kDa.

**DISCUSSION**

We have prepared a soluble oligomer of the envelope glycoprotein of SIV in the single chain, precursor state before cleavage into gp120/gp41, in an attempt to capture the native conformation of the envelope glycoprotein before CD4 receptor binding. The expression systems and purification developed provide biochemical quantities of pure protein for use in the study of the functions and structure of the glycoprotein. Following Rhodes and colleagues (44), we have used the tissue plasminogen activator leader sequence to induce secretion of the glycoprotein oligomer from mammalian cells, but we have used a variant cell line, CHO-Lec3.2.8.1 (50), which N-glycosylates with only a single, simple oligosaccharide, to reduce heterogeneity in the product and to permit full enzymatic deglycosylation by Endo H. This strategy was previously shown to
work well for other heavily glycosylated surface protein (45).

We have confirmed that the protein purified from the culture fluid of the CHO cells can be completely deglycosylated by enzyme treatment (Fig. 8A), and we have shown that deglycosylated oligomers can be stabilized against aggregation by forming complexes with Fab fragments from neutralizing mAbs (Fig. 8B).

We have also expressed the molecule in insect cells, using the honeybee melittin leader sequence to induce secretion. Both molecules purified from the culture fluids of mammalian and insect cells appear to be in a native conformation judging from their abilities to bind the soluble ectodomain of the viral receptor CD4 (Fig. 5; not shown for insect cells) and to be recognized by a number of conformationally sensitive neutralizing monoclonal antisera2 (Fig. 6). The gp160e from mammalian cells was purified by immunoaffinity chromatography requiring a low pH elution step, while a hexahistidine tag on the gp160e from insect cells permitted purification by metal chelation chromatography. Both methods produced proteins with the same properties, suggesting that the purified protein has a single conformation that is robust to such treatments.

Following Rhodes and colleagues (44), we eliminated the primary and secondary cleavage sites between the gp120 and gp41 segments by replacing two basic residues with glutamic acids. In experiments with SIV gp160e molecules produced in CHO cells without these mutations, we had observed spontaneous cleavage between gp120 and gp41 and some shedding of gp120 during purification.6 With the two mutations present, no cleavage between gp120 and gp41 has been observed (Fig. 2, inset), permitting the production of quantities of a stable oligomeric ectodomain.

The finding that the transmembrane anchor-containing subunits of the lenti- and retrovirus, HIV-1, SIV, MoMuLV, and HTLV-1 are all trimeric (21, 51–53) and some, but not all, of the earlier attempts to define the oligomeric state of HIV gp160 (27, 28) suggested that SIV gp160e was trimeric. We add evidence for a trimeric state from chemical cross-linking and from a direct molecular weight determination on the gp160e. Chemical cross-linking generates products that form three bands on SDS-PAGE, which migrate at apparent molecular weights that are 1, 2, and 3 times that of the gp160e monomer as determined by SDS-PAGE (120,000) (Fig. 4A). This “ladder” is a direct indication of the presence of a trimer of gp160e. Earlier chemical cross-linking experiments on full-length HIV gp160 have been interpreted to favor trimeric or tetrameric states. The

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6 A. Dessen, W. Weissenhorn, J. Skehel and D. Wiley, unpublished results.
results of the present study suggest that the aggregation of the SIV gp160e monomer may be regulated by a similar mechanism to that of HIV gp120-gp41 complex.

Acknowledgments—We thank Margaret Pietras for excellent technical assistance, Dr. K. Kent and C. Arnold (formerly of the NIBSC in United Kingdom) for provision of the monoclonal antibody reagents, and J. Hoxie (University of Pennsylvania) for providing monoclonal antibodies, and we acknowledge earlier contributions in our laboratory by Drs. Andrea Dessen and Winfried Weissennhorn to the study of SIV gp160e.

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FIG. 8. Enzymatic deglycosylation of SIV gp160e and purification of a complex of deglycosylated SIV gp160e and Fab fragments by gel filtration chromatography. A, Endo H digestion was carried using the SIV gp160e expressed CHO-Lec3.2.8.1 cells. Lane 1, untreated SIV gp160e; lane 2, the Endo H used in this assay; lane 3, SIV gp160e treated with Endo H under native condition; lane 4, SIV gp160e treated with Endo H under denaturing condition. B, purified SIV gp160e was incubated with 17A11 and 9G3 Frs generated by papain-digestion of 17A11 and 9G3 monoclonal antibodies, respectively. The complexes were treated with Endo H under native conditions and purified from the excess Frs by a gel filtration chromatography on a Superose 6 column. The peak fractions were pooled, concentrated, and analyzed by SDS-PAGE in an 8% gel. Lane 1, untreated gp160e control; lane 2, peak eluted at 12.5 ml; lane 3, peak eluted at 18 ml.
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