The human immunodeficiency virus (HIV) envelope (Env) glycoprotein (gp) 120 is a highly disulfide-bonded molecule that attaches HIV to the lymphocyte surface receptors CD4 and CXCR4. Conformation changes within gp120 result from binding and trigger HIV/cell fusion. Inhibition of lymphocyte surface-associated protein-disulfide isomerase (PDI) blocks HIV/cell fusion, suggesting that redox changes within Env are required. Using a sensitive assay based on a thiol reagent, we show that (i) the thiol content of gp120, either secreted by mammalian cells or bound to a lymphocyte surface enabling CD4 but not CXCR4 binding, was 0.5-1 pmol SH/pmol gp120 (SH/gp120), whereas that of gp120 after its interaction with a surface enabling both CD4 and CXCR4 binding was raised to 4 SH/gp120; (ii) PDI inhibitors prevented this change; and (iii) gp120 displaying 2 SH/gp120 exhibited CD4 but not CXCR4 binding capacity. In addition, PDI inhibition did not impair gp120 binding to receptors. We conclude that on average two of the nine disulfides of gp120 are reduced during interaction with the lymphocyte surface after CXCR4 binding prior to fusion and that cell surface PDI catalyzes this process. Disulfide bond restructuring within Env may constitute the molecular basis of the post-receptor binding conformational changes that induce fusion competence.

Although structural changes within Env following interaction with the cellular receptors are mostly attributed to the intrinsic properties of the viral envelope, they occur in the context of proteolytic and other catalytic cell surface activities that are indispensable for membrane fusion to occur (7–9). One such activity is cell surface protein-disulfide isomerase (PDI), which has been implicated in the process of HIV infection (9, 10).

PDI is a member of the thioredoxin superfamily. It catalyzes reduction, oxidation, and thiol-disulfide interchange reactions and has important roles in the folding of secretory proteins in the biosynthetic pathway (11, 12). On the surface of the cell, it has been shown to cause structural modifications of exofacial proteins involved in biological process (11, 13–15). Clustered on the lymphocyte surface in the vicinity of CD4-enriched regions (9), PDI may influence the conformational modifications that occur during the interaction of HIV Env with the target cell surface receptors through a partial reorganization of the network of the disulfide bonds of the viral protein. Alternatively, it may influence the thiol-disulfide content of other cell surface antigens involved in the HIV/cell fusion process. Experimental data support both possibilities because PDI inhibition interferes with the virus/lymphocyte fusion process post-CD4 binding (9).

Besides the observation that PDI inhibition blocks the HIV-replicative cycle (9, 10), the plausible involvement of redox changes of Env as part of the HIV-lymphocyte interaction is suggested by the observation that an unusually dense cluster of disulfide bonds occurs close to the receptor binding surfaces (16). As the precise molecular basis of the Env conformational changes that take place upon fusion remains enigmatic, we undertook a detailed study of the relationship between cell surface-associated receptor binding and the thiol-disulfide content of HIV Env. We focused solely on the redox state of the surface protein gp120 as it contains 9 of the 10 disulfide bonds of EnvLai (17).

Here, using a sensitive thiol reagent, we show that on average two Env disulfide bonds are reduced during interaction with the lymphocyte surface immediately prior to fusion. We provide evidence that a reductase activity belonging to the PDI family is involved. We propose that disulfide bond restructuring constitutes the molecular basis of the post-receptor binding conformational changes that induce fusion competence.

**EXPERIMENTAL PROCEDURES**

Reagents—The impermeant thiol reagent 3-(N-maleimidylpropionyl)-biocytin (MPB) was purchased from Molecular Probes (Eugene, OR). Reagents including thyroglobulin (Tg), the PDI inhibitors 3,3′,5′-triodothyronine (T3) (18) and bacitracin (10) were purchased from Sigma. EnvLai and EnvLai (reference batches) were supplied by the EVA Medical Research Council AIDS Reagent Program (Peters Bar, United
Kingdom) and the ANRS (Paris, France), respectively. Purified recombinant soluble CD4 (sCD4) was supplied by the EVA Medical Research Council AIDS Reagent Program and was labeled (30 μCi/μg) using iodogen before purification by Sepharose G₂₅ chromatography as described previously (9). SDF1-α was purchased from Peprotech (London, United Kingdom) and was labeled (150 μCi/μg) using iodoacetamide before purification by Sepharose G₂₅ chromatography as described previously (19, 20). Sheep polyclonal antibody D7324 (Alto, Dublin, Ireland) (19) is an anti-peptide antibody directed against the C terminus of gp120 (APTKAKRRVVQREKR sequence). Rabbit polyclonal antibody SPA-890 (Stressgen) (15) is directed against bovine PDI. Vaccinia virus (VV) 9-1 (kindly provided by M. F. Kieny) (21) and VB3D (kindly provided by R. Collman and R. Doms through the National Institutes of Health (NIH)) (22) are VV vectors coding for streptavidin-conjugated Envᵥᵥᵥᵥ and Envᵥᵥᵥᵥ, respectively. Following secretion into the cell supernatant, the viral antigens were concentrated and processed for binding experiments as described in previous (19).

**Cell Infections and Env Production**—Human CD4⁺ lymphoid cells (CEM) (10⁶ cells/ml) and CD4⁺ baby hamster kidney 21 cells (10⁵ cells/ml) were cultured and infected as described previously (9, 23). For Env expression, cells were infected using VV vectors (CEM cells): 3–5 plaque-forming units/cell; baby hamster kidney 21 cells: 5–10 plaque-forming units/cell in serum-free medium to enable further supernatant concentration (30 times) using the Ultrafree 15 device system (30-kDa cutoff, Millipore, St Quentin en Yvelines, France).

**MPB Labelling**

**Thiol Content of Purified Antigens**—Samples containing either Tg or Env diluted in phosphate-buffered saline (PBS), pH 7.4, were dot-blotted onto a nitrocellulose filter (Schleicher & Schuell). After blocking with PBS, 2% casein, filters were incubated with MPB (0.1 mM, 30 min at 25°C). Excess reagent was blocked using glutathione (0.6 mM, 10 min at 25°C), and the remaining sulphydryl groups in the sample on the nitrocellulose filter, blocking with PBS, 2% casein, and subsequent processing.

**Thiol Content of Surface-associated Env**—CEM cells (5 × 10⁵) were either treated using 1 mM bactinactin or mock-treated for 2 h. They were then incubated for 2 h at 37°C in CO₂ atmosphere with gp120 (30 μg/50 μl) produced by baby hamster kidney 21 cells infected using VY vectors. Cells were washed and treated with NaN₃ (0.1%) to inhibit further surface remodeling (20). MPB was added to the cell pellet (0.3 mM, 30 min at 25°C). Excess reagent was blocked using glutathione (0.6 mM, 10 min at 25°C), and the remaining sulhydryl groups in the system were quenched with iodoacetamide (1.2 mM, 10 min at 25°C) (24). Cells were washed twice and incubated in acid buffer (MES/HC l 10 mM, NaCl 150 mM, pH 5.0) for 10 min to dissociate surface-bound Env as described previously (25, 26). The eluate was adjusted at pH 7.0 using NaOH, and Env was immunoprecipitated for 4 h at 4°C using D7324 covalently coupled to CNBr-Sepharose CL4B (Amersham Biosciences) as described previously (27). Gp120 present in the original concentrated cell supernatant or in medium corresponding to supernatant containing gp120 subsequently to incubation with CEM cells (see above) was processed similarly. After elution from Sepharose-bound D7324 using 1% SDS, the purified envelope samples were dot-blotted onto nitrocellulose filter and processed as above to determine the corresponding thiol content. In parallel, dot-blot quantitation of the amount of gp120 present in the eluate after immunoprecipitation using D7324 was achieved by incubation with a pool of anti-HIV-1 polyclonal antibody D7324-coated microtiter plates was incubated with 125I CD4 (2 × 10⁶ cpm/well) for 2 h. After washing, radioactivity was counted. Background binding was measured as the signal generated by using similar plastic wells lacked Env. Unlabeled CD4 (150 ng) was used to determine nonspecific binding. To assess the β-mercaptoethanol treatment did not modify the subsequent capacity of Env to bind antibody-coated wells, the binding of 125I Env to microtiter plates was investigated for each β-mercaptoethanol concentration used as reported previously (29).

**CD4 Binding Assay**

The CD4 binding assay was performed as described previously (29). Env (100 ng) bound to D7324-coated microtiter plates was incubated with 125I CD4 (2 × 10⁶ cpm/well) for 2 h. After washing, radioactivity was counted. Background binding was measured as the signal generated by using similar plastic wells lacked Env. Unlabeled CD4 (150 ng) was used to determine nonspecific binding. To assess that the β-mercaptoethanol treatment did not modify the subsequent capacity of Env to bind antibody-coated wells, the binding of 125I Env to microtiter plates was investigated for each β-mercaptoethanol concentration used as reported previously (29).

**CXCR4 Binding Assay**

The CXCR4 binding assay was performed as described previously (19, 20). Env (2 μg/50 μl) was added to living CEM cells (3 × 10⁶) for 2 h at 37°C in culture medium. Cells were then treated by 0.1% NaN₃ for 10 min and further incubated with 125I SDF1-α (5 × 10⁶ cpm) for 1 h at 25°C in buffer (RPMI 1640 medium, 10 mM HEPES, 5% bovine serum albumin, and 0.1% NaN₃). Cell-associated and free radioactivity were separated using the dibutylphthalate/bis(2-ethylhexyl)phthalate two-phase system. Unlabeled SDF1-α (200 ng) was used instead of Env to determine nonspecific binding.

**RESULTS**

**Titration of Thiol**—To examine small changes in the thiol content of Env, a quantitative assay was developed using MPB, a membrane-impermeant compound coupled to biotin whose reaction with the thiols of proteins can be detected using streptavidin peroxidase. To establish the assay, we employed Tg as this glycoprotein exhibits a similar “cysteine to molecular weight” ratio to gp120 (17, 30). Preliminary experiments showed that (i) all the disulfide bonds of Tg are reduced using 1% β-mercaptoethanol and (ii) similar results were obtained when incubation with MPB was performed prior to or after immobilization of the protein onto nitrocellulose. Densitometry of reduced Tg (0.2–200 ng) blotted and processed using MPB produced a linear dose response between 2 and 40 ng. Taking into account its molecular mass (330 kDa) and Cys content (122 Cys residues/molecule) (30), we determined the thiol content for each protein sample. This provided a standard curve and allowed the development of an assay detecting thiols with an absolute sensitivity of 0.3 pmol (Fig. 1A) and a signal/background ratio of 50.
Increasing amounts of either gp120 (Env) or Tg treated (Tg) or not treated (Tg) by β-mercaptoethanol were blotted onto a nitrocellulose filter and stained using MPB. Spot intensity was quantified by densitometry and used to determine a standard curve (n = 6, means ± S.D. are presented). B, MPB reactivity of Env and Tg. Increasing amounts of either gp120 (Env) or Tg treated (Tg) or not treated (Tg) by β-mercaptoethanol were blotted onto a nitrocellulose filter and processed as described above (n = 3, means ± S.D. are presented).

Using these conditions, we verified that MPB reacted with native Tg in a dose-dependent manner and determined that its thiol content per molecule was 13 (Fig. 1B). Commercially available gp120 (data not shown) exhibited a low reactivity with MPB, which corresponded to 0.5−1 thiol/molecule. Thus, in native folded and secreted gp120, essentially all Cys residues are involved in disulfide bonds. The specificity and linearity of dosage observed for Tg were also observed with gp120 (Fig. 1B).

**Relationship between the Thiol Content of gp120 and Its Receptor Binding Capacity**—We then determined the relationship between the thiol content of Env and its capacity to bind lymphocyte receptors. Env was treated with various concentrations of β-mercaptoethanol, and the corresponding thiol content was determined (Fig. 2A). Alternatively, after reaction with β-mercaptoethanol, the sample was incubated with iodoacetamide, the reducing agent was removed by lyophilization, and the resulting Env was assayed for CD4 binding as described previously (29). We observed that reduction of one disulfide bond was tolerated but the reduction of more than one prevented CD4 binding (Fig. 2B).

β-Mercaptoethanol-treated Env was tested for its CXCR4 binding capacity (19, 20). Env and the natural CXCR4 ligand, SDF1-α, share a binding site on CXCR4 that allows Env binding to be measured indirectly through competition with SDF1-α (31), although lower receptor affinity limits maximum inhibition using Env to −50% (19, 32, 33). The reduction of a single disulfide bond impaired the capacity of gp120 to compete with 125I SDF1-α binding at the lymphocyte surface (Fig. 3A). The treatment of the cell surface by bacitracin did not block SDF1-α binding inhibition by Env (Fig. 3B), indicating that Env binding to CXCR4 did not require PDI activity. As a control, 125I SDF1 binding was inhibited by SPC3 (10−5 M), a V3-derived peptide that interacts with the binding site of Env on CXCR4 (19, 20, 33).

**Development of Env Disulfide Bonds during the Course of Membrane Fusion**—MBP was first used to biochemically demonstrate the presence of PDI on the CEM cell surface. Cells were incubated with MBP or a mock. Samples were washed, lysed, and incubated with streptavidin-agarose before the eluates were separated by SDS-PAGE and blotted with anti-PDI antibodies (28). A single band was detected migrating with the apparent molecular mass of PDI that was significantly enriched by the MBP labeling when compared with the mock-treated sample (Fig. 4A). Thus, PDI on the CEM cell membrane is accessible to the exogenous reagent. We next examined the capacity of bacitracin to alter the reductive activity associated on the CEM cell surface. Treatment with 1 mM bacitracin reduced MBP reactivity by 50% (Fig. 4B). This result is consistent with inhibition of surface PDI and is similar to the reduction in the thiol pool of the cell surface obtained using anti-PDI antisense phosphorothioates (34). For specificity, cell preincubation with thiol reagents was shown to dramatically reduce MBP reactivity.

We then addressed the thiol content of Env after its interaction with either CD4 or both CD4 and CXCR4. Gp120 was incubated with CEM cells as follows: (i) cells and gp120 incubated without prior incubation with SDF1-α and sCD4 (SDF/CD4); (ii) cells preincubated with SDF1-α (2 × 10−8 M to block CXCR4) before incubation with gp120 (SDF/CD4); and (iii) cells preincubated with SDF1-α and Env preincubated with sCD4 (0.5 × 10−8 M) before the addition of gp120 to cells (SDF/CD4). Concentrations of SCD4 and SDF1-α were saturating (19, 27). After incubation with Env, thiol labeling of the cell
bacterial (BCT) (1 mM) or mock-treated (C). They were then incubated with MPB and then with streptavidin peroxidase. The activity associated with the lymphocyte surface was assessed using orthophenylene diamine (n = 4, a representative experiment is shown). C, immunoprecipitation of gp120 associated with the lymphocyte surface. After incubation of CEM cells with gp120 and washing, the envelope protein was acid-dissociated from the cell surface, purified using Sepharose-coupled D7324 antibody, and analyzed by SDS-PAGE and protein staining. D, inhibition of gp120s –e disulfide bond cleavage following lymphocyte binding. CEM cells were treated using T3 (T3) (100 μM), bacitracin (BCT) (1 mM) or mock-treated (C). They were then incubated with gp120s –e before MPB labeling. Env was then acid-dissociated and immunoprecipitated before thiol content assessment (SH/gp120). The thiol content of secreted gp120s –e (Scrtd) was determined in parallel.

The thiol content of the immunopurified samples was determined as before, and the amount of gp120 was assessed to determine the thiol/gp120 ratio. The thiol content of gp120 associated with the lymphocyte surface in conditions where both CD4 and CXCR4 were accessible was found to be 4-fold higher than that of either immunopurified Env from the original supernatant or of gp120 present in the cell supernatant of the SDF+/CD4+ sample (Table I). In the SDF+/CD4+ sample, there was no significant change in the thiol content of cell-associated Env when compared with gp120 in the original supernatant.

The significance of lymphocyte surface PDI activity on the redox changes observed for lymphocyte-associated Env was addressed using 1 mM bacitracin. The inhibitor prevented the increase of the thiol content observed in the SDF+/CD4+ condition (Table I), whereas it neither interfered with Env binding to CD4 (9) and CXCR4 (see above) nor the reaction of MPB with thiols of proteins (data not shown).

Bacitracin reacts with the redox active CXXC sequences of PDI to block the catalyst (10, 13) and is a specific and potent PDI inhibitor (34). T3 is a weaker inhibitor that exerts its activity through binding the PDI/substrate interaction domain (18). To independently confirm both the PDI dependence of Env reduction and its physiopathological relevance, we did experiments using 200 μM T3, a dose that inhibits PDI activity (18), and gp120s –e, an envelope derived from a dual-tropic primary isolate (22). We observed that EnvS9 –6 was reduced upon interaction with the cell surface and that bacitracin and, to a lesser extent, T3 inhibited EnvS9 –6 reduction (Fig. 4D). We conclude that the thiol content of Env increases after interaction with a lymphocyte surface competent for CXCR4 binding and that this change depends upon a surface PDI activity.

To confirm this data using a biological system mimicking the HIV/lymphocyte interaction, we made use of EnvLAI expression on the surface of human lymphocytes. In this system, cell densities of greater than 10⁶ cells/ml permit syncytium formation, whereas those below 10⁶ cells/ml do not (9, 23). MPB labeling was done on the surface of the same number of Env-expressing cells cultured at either of the two densities, and cell surface Env was isolated as before. The thiol content and the amount of purified gp120 were assessed to determine the thiol/gp120 ratio. We found that Env associated with the surface of the dense culture (syncytium forming) had an MPB reactivity that was 3-fold higher than that associated with the surface of cells maintained at low cell density (Table I). Because various PDI inhibitors prevent syncytium spread in this system (9), we conclude that changes in the thiol content of lymphocyte surface-associated Env are obligatory for fusion.

**DISCUSSION**

HIV Env is an unusually highly disulfide-bonded molecule with 9 of a total of 10 disulfide bonds occurring within the outer membrane domain of gp120 (17). Gp120 is functionally complex as it interacts with at least three ligand surfaces to trigger HIV entry: 1) CD4, the primary receptor, 2) CCR, the secondary receptor, and 3) gp41, the fusogenic partner (3, 6). In addition, in the course of fusion, this heavily glycosylated protein (35) must be sufficiently pliable to not sterically inhibit the confor-

### Table 1

<table>
<thead>
<tr>
<th>THIOL CONTENT OF ENV FOLLOWING INTERACTION WITH A HUMAN LYMPHOCYTE SURFACE</th>
<th>-bacitracin (SH/gp120)</th>
<th>+bacitracin (SH/gp120)</th>
</tr>
</thead>
<tbody>
<tr>
<td>gp120s –e</td>
<td>0.81SH/gp120 ± 0.16</td>
<td>N.D.</td>
</tr>
<tr>
<td>SDF+/CD4+</td>
<td>9.2SH/gp120 ± 0.54</td>
<td>1.07SH/gp120 ± 0.21</td>
</tr>
<tr>
<td>SDF+/CD4+</td>
<td>1.47SH/gp120 ± 0.27</td>
<td>1.15SH/gp120 ± 0.27</td>
</tr>
<tr>
<td>SDF+/CD4+</td>
<td>0.93SH/gp120 ± 0.21</td>
<td>0.99SH/gp120 ± 0.13</td>
</tr>
<tr>
<td>Individual cells</td>
<td>0.61SH/gp120 ± 0.21</td>
<td>N.D.</td>
</tr>
<tr>
<td>Syncytia</td>
<td>1.73SH/gp120 ± 0.24</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

*The thiol content of gp120s –e was assessed using MPB (pmol SH/pmol gp120; SH/gp120).

* CEM cells were treated with bacitracin (+) or not (−) and then incubated with gp120s –e in various conditions: (i) cells and gp120 were incubated in the absence of SDF1–α and sCD4 (SDF+/CD4+); (ii) cells were preincubated with SDF1–α and then incubated with gp120 (SDF+/CD4+); (iii) cells were preincubated with SDF1–α and Env with sCD4 before the addition of gp120 to cells (SDF+/CD4+). The thiol content of Env either bound to cells (CD4+/CD4+/SDF+/SDF+) or present in the supernatant (CD4+/SDF+) was assessed.

* Alternatively, the thiol content of Env associated with either the surface of individual lymphocytes (Individual cells) or syncytium formations (Syncytia) was studied as described above.
nizational changes required for virus-cell fusion (2, 5). Here, we demonstrate that the later stages of the process leading to fusion are enabled by the reduction of disulfide bonds of Env by a lymphocyte surface-associated reductase activity.

Two articles suggested that changes in the redox status of the disulfides of Env post-synthesis may occur in relation to HIV entry into the target cell (9, 10). Ryser et al. (10) reported that HIV infection of human lymphocytes was markedly inhibited by 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), bacitracin, and antibodies directed against PDI. More recently, we showed that these inhibitors altered the HIV receptor-dependent gp41- and antibodies directed against PDI. More recently, we showed that these inhibitors altered the HIV receptor-dependent gp41- and antibodies directed against PDI. More recently, we showed that these inhibitors altered the HIV receptor-dependent gp41- and antibodies directed against PDI. More recently, we showed that these inhibitors altered the HIV receptor-dependent gp41- and antibodies directed against PDI. More recently, we showed that these inhibitors altered the HIV receptor-dependent gp41-

We then examined the redox state of gp120 as part of its interaction with a CD4- human lymphocyte surface. Firstly, we found that the thiol content of gp120 after interaction with a native cell surface displayed about 4 thiols per molecule compared with <1 thiol in the original population of soluble antigen. This observation was done both for a gp120 derived from a laboratory-adapted HIV strain or from a dual-tropic primary isolate. Using high concentrations of SDF1-α and/or sCD4, we obtained conditions that specifically impaired Env binding to either one or both surface antigens (19, 27). A comparison of the data obtained when cells were preincubated or not with SDF1-α showed that changes in the redox status of gp120 required the availability of the binding site for Env on surface CXCR4. Secondly, Env associated with the surface of the dense (syncytium-forming) culture infected using VV9-1 had a thiol content that was 3-fold higher than that associated with the surface of cells maintained at low cell density. These results and our previous report that PDI inhibition prevented syncytium spreading but not CD4 binding (9) allow us to conclude that changes in the thiol content of Env are a requirement of the fusion reaction.

We previously reported that PDI is clustered at the lymphocyte surface in the vicinity of CD4-enriched regions and that some colocalization occurs (9). We showed here that PDI is labeled on the cell surface by the impermeant thiol probe MPB, further demonstrating its accessibility on the outer membrane of the lymphocyte cell line. As both PDI-specific inhibitors and anti-PDI antibodies inhibit HIV/lymphocyte fusion post-CD4 binding (9), it seems probable that a member of this class of catalysts may be the mediator of Env reduction. A direct evidence supports this conclusion because the use of PDI inhibitors prevented the increase in thiol content of lymphocyte surface-associated gp120 in the SDF-1/CD4+ sample. Our data also show that the oxidizing nature of the cell surface still permits the persistence of thiols resulting from disulfides reduction of proteins, in agreement with a recent report (36).

Because the increase in the thiol content of monomeric soluble gp120 after its interaction with the cell surface was similar to that observed for oligomeric gp120 associated with syncytia, we conclude that changes in redox state do not depend on Env oligomeric status or on the presence of gp41. Our capacity to detect an increase in the thiol content of Env associated with

Fig. 5. A. HIV/lymphocyte interaction. The main steps of the HIV/lymphocyte interaction process are shown. Based on the data presented here, the step where a reductase activity belonging to the PDI family takes place is indicated. B. Gp120 structure. The image was rendered from the coordinates of the three-dimensional structure of gp120 (16) using RASMOl (40). The molecular surfaces involved in binding the primary receptor, CD4, and the secondary receptor, CXCR4, are shown. Cys residues are shown in cyan, and disulfide linkages are shown in red. Of particular note is the cluster of disulfide bonds that occurs at the base of variable loop 4 (as indicated) and in close proximity to the CCR binding site. Disulfide bond reduction at this location would be consistent with the increase in thiol dosage documented in the text.
Env Reduction and HIV/Lymphocyte Fusion

could reduce a number of bonds at one time and could bring production post-CXCR4 binding, but we note that a cluster of CXCR4/H18528/CD4.

We speculate that the Env reduction process and the disruption of the Env disulfide network it promotes assist post-receptor binding Env conformational changes and are necessary for acquisition of conformation competent to trigger membrane fusion (4, 6). Indeed, if the surface-associated form of PDI can act as a redox-driven chaperone to unfold proteins after disulfide (4, 6). Indeed, if the surface-associated form of PDI can act as a redox-driven chaperone to unfold proteins after disulfide bond reduction, as has been shown for its endoplasmic reticulum counterpart (38), it may directly catalyze the conformational changes occurring within Env required to ultimately unmask the gp41 fusion peptide. Recently, the reduction of the second domain of CD4 was reported to be an obligatory step in CD4-dependent fusion (39). Our results raise the possibility that redox changes observed within CD4 may be the consequence of thiol/disulfide interchanges occurring within the CD4-CXCR4-Env complex after Env reduction by PDI.

Our data do not address which disulfide bonds undergo reduction post-CXCR4 binding, but we note that a cluster of bonds occurs in gp120 at the base of the V4 region important for Env bioactivity (16). Disulfide bond reduction at this location could reduce a number of bonds at one time and could bring about considerable conformational change (Fig. 5B).

Acknowledgments—We are indebted to Dr. Alexandre Mezghrani for helpful discussions and critical readings of this article and to Pr. Roberto Sitia for much helpful advice on the analysis of PDI. We thank Drs. Edward Berger, Robert Doms, Marie-Paule Kiéyé, Marie-Jeanne Papandréou, Hervé Rochat, and Jean-Marc Sabatier for the gift of reagents, discussions, or support. R. Barbouré acknowledges the kind support of Drs. Aghleb Bartegi and Ahmed Hellal and the help found at the Institut Supérieur de Biotechnologie de Monastir (Monastir, Tunisia). The help of the ANRS, of the United Kingdom EVA Medical Research Council AIDS Reagent Program (Dr. H. Holmes) and of the National Institutes of Health AIDS Reagent Program (Dr. Y. Akyel) is acknowledged.

REFERENCES
