Characterization of the Conformational State and Flexibility of HIV-1 Glycoprotein gp120 Core Domain

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gp120 is key to the human immunodeficiency virus type 1 viral core entry. Knowledge of the detailed conformational states of gp120 is crucial to intervention, yet the unbound form is still resistant to structural characterization probably because of its flexibility. Toward this goal, we performed molecular dynamics simulations on the wild type gp120 core domain extracted from its ternary crystal structure and on a modeled mutant, S375W, that experimentally has a significantly different phenotype from the wild type. Although the mutant retained a bound-like conformation, the wild type drifted to a different conformational state. The wild type β strands 2 and 3 of the bridging sheet were very mobile and partially unfolded, and the organization among the inner and outer domains and β strands 20 and 21 of the bridging sheet, near the mutation site, was more open than in the bound form, although the overall structure was maintained. These differences were apparently a result of the strengthening of the hydrophobic core in the mutant. This stabilization further explains the experimentally significantly different thermodynamic properties between the wild type and the mutant. Taken together, our results suggest that the free form, although different from the bound state, shares many of the bound structural features. The observed loss of freedom near the binding site, rather than the previously hypothesized more dramatic conformational transition from the unbound to the bound state, appears to be the major contributor to the large entropy cost for the CD4 binding to the wild type.

The human immunodeficiency virus type 1 virus continues to be the main cause for AIDS infection affecting millions of people every year. The persistence of the infection is believed to be related to the "wise" evolution of the structure of the envelope glycoprotein gp120 on the virion surface that can efficiently evade the immune system. Contributing elements include the heavy glycosylation on the surface (1), residue variation (2, 3), oligomerization (4, 5), and conformational alterations (6, 7). The structure of the gp120 core domain revealed in a co-crystal with CD4 and a Fab from the neutralizing antibody 17b (6), along with information derived from sequence alignments (8, 9) of different primary immunodeficiency viruses, shows a conserved core domain (see Fig. 1) with five long variable loops V1–V5 interspersed over the surface of the core domain. The molecule has been shown to be able to adopt multiple conformations along the pathway of the infection. Upon binding to CD4, the principal receptor in the course of the infection (10, 11), variable loops V1 and V2 move out of the way allowing transient exposure of the otherwise shielded conserved residues (12). CD4 binding then induces a conformational change that poses the necessary epitopes for its coreceptor association, usually CCR5 (6, 13–15), another major receptor of gp120 (16–20). Analysis of the crystal structure revealed a peculiar three-dimensional organization within the core domain with a deeply recessed CD4 binding pocket shaped by the inner domain, the outer domain, and the bridging sheet. As Kwong et al. have argued (6), the hydrophobic core that holds together the three subdomains and sits at the bottom of the recessed pocket might be sensitive to a change in the environment because of the unique organization among the interacting residues. The flexibility of the association between the inner and outer domains is thought to be responsible for the low immunogenicity due to the spread of the epitope residues over separate regions (21). This highly flexible nature of the gp120 has necessarily made it difficult to characterize the detailed conformational features of the free form. Yet, such a characterization of the gp120 would be extremely valuable to the understanding of its biology and intervention, particularly the inhibition of the gp120–CD4 association, which is of primary interest yet has met only limited success (22–24).

Despite the lack of direct structural determination, a large body of experimental work relating to the CD4–gp120 association, including thermodynamics (25), mutagenesis (26–32), and crystallography (21, 33–36), has provided considerable insight into the gp120 structure and flexibility. Of particular note is the thermodynamics data (see Table I) (25), which revealed exceptionally large enthalpy and entropy changes, suggesting that the unbound form of the gp120 may be very flexible and significantly different from the CD4-bound state. This was further supported by the fact that the CD4 molecule shows little conformational change upon ligand binding (6, 33–35), and multiple binding partners for gp120 consistently indicated similar thermodynamic behavior (7). The similar thermodynamic behavior for both the full protein and the core domain of gp120 (see Table I) going from the free-form to the CD4-complexed state, confirms that it is the core domain that is largely
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**Table 1**

Experimental thermodynamic data for the CD4-gp120 binding

<table>
<thead>
<tr>
<th></th>
<th>ΔΔG</th>
<th>ΔΔH</th>
<th>ΔΔS</th>
<th>-TΔΔS</th>
<th>ΔΔC</th>
<th>Kd</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBD1 full-length gp120</td>
<td>-11.8 ± 0.3</td>
<td>-63 ± 8</td>
<td>51.2 ± 3</td>
<td>1.7 ± 0.4</td>
<td>190 ± 30</td>
<td>nm</td>
</tr>
<tr>
<td>Core gp120</td>
<td>-9.5 ± 0.1</td>
<td>-62 ± 8</td>
<td>52.5 ± 3</td>
<td>1.6 ± 0.4</td>
<td>190 ± 30</td>
<td>nm</td>
</tr>
<tr>
<td>Wild-type YU2 gp120 + sCD4</td>
<td>-10.52 ± 0.03</td>
<td>-52.1 ± 0.2</td>
<td>41.6 ± 0.2</td>
<td>38 ± 2</td>
<td>6.4 ± 0.9</td>
<td>nm</td>
</tr>
<tr>
<td>S375W gp120 + sCD4</td>
<td>-11.62 ± 0.08</td>
<td>-35.5 ± 0.2</td>
<td>23.0 ± 0.2</td>
<td>38 ± 2</td>
<td>6.4 ± 0.9</td>
<td>nm</td>
</tr>
</tbody>
</table>

The ga120-C4 complex were extracted from the ternary crystal structure (PDB 1gpm) (6). Those with the S375W mutations were modeled from the wild type crystal structure using CHARMM. The missing loop 4 was also modeled in by first building the corresponding 14-residue peptide (the central 12 residues were missing) and subjecting it to molecular dynamics simulation at a temperature of 300 K in the gas phase with the distance between the Ca atoms of the terminal residues restrained to the desired target, which corresponds to 24.3 Å in the crystal structure. The resulting conformations with these matched distances were selected and integrated into the core domain by matching the terminal residues of the Ca of the peptide with the corresponding atoms in the protein. A structure with no van der Waals overlap between loop 4 and the core domain was selected and minimized for 500 steps of the steepest decent algorithm. For the monomer simulations, the gp120 core domain was first solvated in a TIP3P (39) water box with dimensions of ~88 × 85 × 71 Å, with a minimum distance of 10 Å from any edge of the box to any protein atom, resulting in a system size of ~52,000 atoms. Minimizations were first performed for 500 steps with the steepest decent algorithm with the gp120 constrained and for additional 500 steps for the whole system. Six steps of a concerted solvent model was used instead, and a total of 4-ns duration of the trajectories was generated with a time step of 1 fs and a group-based electrostatic calculation.

**RESULTS**

**Structural Deviation from the Bound State**—The monomer core domain consists of the inner domain, outer domain, and the bridging sheet with its β-strands 20 and 21 folded against the inner-outer domain interface and β-strands 2 and 3 protruding out (Fig. 1). Such a unique monomer organization taken from the ternary complex structure was expected to undergo conformational changes to restore its unbound free form. Tables II and III summarize the core domain RMSD from all four simulations for the monomers. Over the 3-ns period at 300 K, the structural change was moderate with a Cα RMSD for the inner and the outer domains together (Table II) of 1.9 and 1.7 Å for the wild type and the mutant, respectively. When a portion of the bridging sheet, β20,21, was added, the RMSD was essentially unchanged (Table II). However, when the whole domain was included, the RMSD climbed to 2.6 Å for the wild type and only slightly changed for the mutant (Table II), indicating a larger movement of the β2,3 motif in the wild type than in the mutant. The RMSDs, however, did not show dramatic structural changes within each subdomain (inner and inner domain, β20,21, and β2,3). The results from the 325 K simulations were very similar to those from the 300 K simulations except that the difference between the wild type and the mutant was much smaller.

The difference in the structural deviations of the bridging sheet between the wild type and the mutant was further highlighted when the structures were aligned against the inner and outer domains (Table III). At both 300 and 325 K, the RMSD difference between the wild type and the mutant is much larger for the β2,3 and β20,21, whereas it is similar for the inner and the outer domains. These results indicate that the S375W mutation appears to stabilize the bridging sheet from drifting.
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Fig. 1. Ribbon representation of the gp120 core structure with subdomains color-coded, green (inner domain), cyan (outer domain), red (β-strands 20, 21 of the bridging sheet), and pink (β-strands 2, 3 of the bridging sheet). The orientations in A and B are perpendicular to each other to better view the geometrical relationship between the inner domain, the outer domain, and the protruding bridging sheet.

**Table II**

<table>
<thead>
<tr>
<th></th>
<th>Inner</th>
<th>Outer</th>
<th>β20,21</th>
<th>β2,3</th>
<th>Inner + Outer</th>
<th>Inner + outer + β20,21</th>
<th>Inner − outer + β2,3</th>
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</thead>
<tbody>
<tr>
<td>300K</td>
<td>1.3 ± 0.1</td>
<td>1.8 ± 0.2</td>
<td>0.9 ± 0.1</td>
<td>1.9 ± 0.3</td>
<td>1.9 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>S375W</td>
<td>1.1 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>1.8 ± 0.3</td>
<td>1.7 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>322K</td>
<td>1.5 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.1 ± 0.2</td>
<td>2.0 ± 0.4</td>
<td>1.8 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>S375W</td>
<td>1.6 ± 0.2</td>
<td>1.7 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>2.0 ± 0.4</td>
<td>1.9 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>2.3 ± 0.3</td>
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</table>

**Table III**

<table>
<thead>
<tr>
<th></th>
<th>Inner</th>
<th>Outer</th>
<th>β20,21</th>
<th>β2,3</th>
<th>Inner + Outer</th>
<th>Inner + outer + β20,21</th>
<th>Inner − outer + β2,3</th>
</tr>
</thead>
<tbody>
<tr>
<td>300K</td>
<td>1.8 ± 0.3</td>
<td>2.1 ± 0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S375W</td>
<td>1.4 ± 0.2</td>
<td>1.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>3.0 ± 0.6</td>
<td>3.1 ± 0.9</td>
<td>5.8 ± 1.3</td>
<td>4.8 ± 1.0</td>
</tr>
<tr>
<td>322K</td>
<td>1.7 ± 0.2</td>
<td>1.8 ± 0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S375W</td>
<td>1.9 ± 0.2</td>
<td>1.9 ± 0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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away from the inner and outer domains, which in turn shows that the bridging sheet is the most mobile region of the core domain for the wild type.

Analysis of Interdomain Motions—Above, our results have indicated that the bridging sheet, especially the β2,3 motif, was the most mobile in the core domain of the wild type. To detect additional potential motions between the inner and outer domains, two selected distances between the inner and the outer domains were measured. One distance is between the Cs of residues 231 and 360, and the other is between the Cs of residues 375 and the nearest heavy atom of residue 112 (Figs. 1B, and 2). Residues 231 and 360 are located at the centers of the well structured β sheets, and the distance between them was expected to be a good measure for the opening-closing motions between the inner and outer domains. Residues 112 and 375 were selected to monitor similar motions between the inner and outer domains. These residues are located at the narrower region of the heart-shaped inner domain-outer domain configuration (Fig. 1B). Because residue 375 was the mutation site in the S375W mutant, the mutational effect on the local conformations is also of interest.

The distance between residues 231 and 360 fluctuated between 39.5 and 40.5 Å for the wild type at 300 K (Fig. 2A). Compared with that in the crystal structure (39.5 Å), the expansion was very mild considering the thermal effect on the structure. The mutant behaved similarly. To increase the sampled conformational space, simulations were also performed at elevated temperature (325 K). However, no significant difference was observed for either the wild type or the mutant (Fig. 2A). Thus, it seems that the motions between the domains were quite limited by this measure.

The distance between residues 112 and 375 also increased for the wild type at 300 K (Fig. 2B). Starting from 9.4 Å in the bound state, it fluctuated between 10 and 12 Å after 1 ns of the simulation. Such a magnitude of fluctuation is more significant compared with the fluctuation for the distance between residues 231 and 360, indicating that this part of the structure might be more flexible and relatively more open in the free form. Interestingly, this distance for the mutant did not change as much and was consistently shorter than in the wild type (Fig. 2B). Such a difference between the wild type and the mutant was more significant when measured at 325 K (Fig. 2B). These results indicate that the effect of the S375W mutation was the stabilization of the bound-state association between the inner domain and the outer domain, consistent with the assertion based on the experimental observations (7).

To examine the conformational changes of the gp120 observed by molecular dynamics from a different perspective, we performed a normal mode analysis on both the monomer and the gp120-CD4 dimer with a method developed by Keskin et al. (40). This normal mode analysis method uses a coarse-grained model of the system and focuses on the lowest frequency modes.

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Results from the monomer analysis show that two of the three lowest frequency modes correspond to the β2,3 motif of the bridging sheet (Fig. 3, A and B), and the third lowest frequency mode involves the β4,5 motif (Fig. 3C). In addition, the magnitudes of two modes for β2,3 were significantly larger than the rest of the vibration modes. On the other hand, the magnitudes of these two motions were significantly reduced to a level that was comparable with the rest of the small amplitude motions in the gp120-CD4 dimer complex form (data not shown). This result agrees with our simulation, confirming that β2,3 is indeed the most flexible and might experience a large conformational change during the CD4 binding process.

S375W Mutation Reduces the Flexibility of gp120—The S375W mutation was designed to fill the partially occupied CD4 binding pocket (7) so that the stability or the conformation of gp120 would be altered. Because the mutation site is at the bottom of the CD4 binding pocket, and the pocket is surrounded by several hydrophobic/aromatic residues, this mutation was likely to perturb these interactions in its vicinity. Fig. 4 shows the constellations of the hydrophobic and aromatic residues near the mutation site in the average structures from the 300 K simulations (Fig. 4, A and B) and snapshots from 325 K simulations (Fig. 4, C and D). Compared with the wild type, the hydrophobic residues near the mutation site in the mutant shifted slightly toward each other within the pocket, resulting in a better packed hydrophobic cluster because of the presence of Trp-375. As a consequence, the distance between residues 112 and 375 was shortened (Figs. 2 and 4). Such a change in the constellation of the hydrophobic residues might have an effect on the stability of this region. To test whether the stability was affected, residue-wise root mean square fluctuations were compared between the wild type and the mutant (Fig. 5). Overall, residues that were nearby the hydrophobic cluster displayed lower fluctuations in the mutant than in the wild type (Fig. 5, B and C), whereas residues far from this site either became more flexible or stayed the same in the mutant. Interestingly, β2,3 of the bridging sheet was also stabilized, possibly because of the lower mobility of the helix α1 or of the β20,21 of the bridging sheet. Other structural effects resulting from the stabilization include the better retained helical structure of helix α5, especially at the C-terminal (data not shown).

Of particular note are the residues that were most stabilized upon mutation (Fig. 5, B and C in blue) that were also within 4.5 Å of CD4 in the complex (Fig. 5D). Residues within 4.5 Å of CD4 include 124 (P), 126 (C), 196 (C), 279–281 (DNA), 283 (T), 365–368 (SGGD), 370–371 (EU), 425–430 (NMWQKV), 455–469 (TRDGGN), 469 (R), 472–475 (GGDM), and 477 (D) (Fig. 5D). Most of these residues are from the outer domain and the bridging sheet except for Met-475 and Asp-477. Only three residues from β2,3 of the bridging sheet are within 4.5 Å of CD4, two of which are Cys-126 and -196, which form the disulfide bond (1) and the other is Pro-124, which may also be important in maintaining the structure. β20,21 of the bridging sheet contains the longest continuous sequence of residues (NMWQKV) that were in contact with CD4. The only residue (Val-430) that made hydrophobic contact with CD4 also residues in this part of the bridging sheet. The rest of the residues were mostly charged or polar. Interestingly, three segments are rich in Gly (Fig. 5D, red). For each of the Gly-rich segments, there is an Asp immediately next to the GG motif. Because the interaction between gp120 and CD4 is quite polar with mostly polar and charged residues within the 4.5 Å (6), these Asp residues are important for the interactions. Changes in Asp-368 and Glu-370, for example, will disrupt interactions with

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**Fig. 2.** Minimum distances between the Cα of residues 231 and 360 and between the Cα of residue 112 and heavy atoms of residue 376. Black and red are for the wild type (WT) and the S375W mutant at 300 K, respectively, and green and blue are for the wild type and the S375W mutant at 325 K, respectively. The locations of these atoms are shown in Fig. 1B.

**Fig. 3.** The three lowest motional modes generated from normal mode analysis. The first two modes (A and B) correspond to the motions of the β strands 2 and 3 of the bridging sheet, and the third mode corresponds to β strands 4 and 5. The structural motifs involved in the modes were colored red, and the directions and the relative magnitudes of the motions are shown with arrows. Note that the third mode has much smaller magnitude of motions.

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many CD4BS antibodies (41). However, to interact favorably with CD4, these residues need to be able to adjust their conformations. The Glys next to them make it possible. The stabilization of these segments upon mutation, however, will hinder the ability of these motifs to adjust their orientations with respect to CD4, resulting in the reduced enthalpy change as well as the entropy cost.

**Change of Solvent-accessible Surface Areas upon Mutation**—For the antibodies to bind, the protein needs to assume certain conformations that allow the access to the specific epitopes. The mutagenesis experiment shows that the S375W mutant binds CD4BS antibodies much weaker than the wild type and binds CD4 6-fold better (7). Thus, it is interesting to see whether the effect of the mutation is reflected in the solvent-accessible surface areas of the epitope residues. We calculated the solvent-accessible surface area difference between the wild type and the mutant for both the monomer and the gp120-CD4 complex (Fig. 6, A–C). For a direct comparison, the epitope residues for the CD4BS antibodies were mapped onto the structure (21) (Fig. 6D). Interestingly, we observed that most epitope residues for the CD4BS antibody binding became less accessible upon mutation in both the monomer and the complex simulations, with more dramatic changes in the complex simulations (Fig. 6). Mutagenic analysis indicates that many residues important for the binding of the CD4BS antibodies were not exposed on the surface of the
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CD4-bound gp120 (21). This result shows that part of the mutational effect on antibody binding is the change of the accessibility of the epitope residues. Furthermore, the more dramatic effect on the surface accessibility of these epitope residues when in the complex form, indicates that CD4 binding is also partially responsible for the conformational change that reduced the accessibility of the CD4BS antibody epitopes. Because the mutation did not affect the binding affinity of CD4, this again confirms that CD4 might have interacted with a different set of residues or with different configurations of the residues.

The Source of Entropy and Entropy Differences—The interesting part of the binding free energy of gp120 and CD4 is the significant difference for the wild type and the mutant (Table I). The entropy cost for the mutant is only about half that of the wild type and the enthalpy gain is −17 kcal/mol less favorable for the mutant. In addition to the large surface area burial and the conformational changes such as the folding of the bridging sheet as suggested earlier (6, 33), the reduction of the flexibility of residues near the mutation site was also responsible for the entropy changes. To compare the enthalpy changes, we calculated the interaction energy between gp120 and CD4, which is roughly proportional to the enthalpy change upon CD4 binding. Surprisingly, the calculated interaction energy was much smaller for the mutant than for the wild type after 1 ns of equilibration (Fig. 7A). The averages for the last 2 ns from the trajectories are −217 ± 16 kcal/mol and −175 ± 11 kcal/mol for the wild type and the mutant, respectively, which is −40 kcal/mol more favorable for the wild type. Thus, our calculated free energy profile was qualitatively in very good agreement with the experimental result.

The calculated difference in interaction energies between the wild type and the mutant is very significant, because the only difference between the wild type and the mutant is the S375W mutation. Further, the mutated residue was not in contact distance with CD4 in either the wild type or the mutant and therefore did not directly contribute to the interaction energy. Therefore, its impact must be on the conformations. A simple measure of the distances between the Ca of residue Phe-43 of CD4, a residue that pointed to the hydrophobic pocket of the CD4 binding site, and the Ca of residue 375 of gp120 shows that this distance is quite stable in the mutant, but it fluctuated much more in the wild type (Fig. 7B). The interpretation of this result is that the low flexibility of the mutant at the interface of the inner domain, outer domain, and the bridging sheet, because of the stabilization effect, imposed some rigidity to the molecule. Because the CD4 molecule is known to be quite rigid, the low flexibility of the gp120 conformation will limit the freedom of the binding partners to adjust their conformations to yield optimal interactions, resulting in the less favorable interaction energy. For the same reason, the entropy cost is largely reduced.
DISCUSSION

Unbound States of gp120 and Its Flexibility—Our simulation results have shown that the wild type and the mutant preferred different conformations. In particular, the mutant assumed a conformation that is more similar to the CD4-bound structure, whereas the wild type is relatively more open with respect to the inner and outer domains, with part of the bridging sheet (β2,3) partially unfolded. Such an observation is consistent with the previous speculations about the conformations of gp120 in its free form (7).

However, the putative open-state conformation observed in our simulations appears to be not as different from the bound state as was suggested previously (6). For example, it is proposed that the whole bridging sheet might be in an unfolded state in its free form and that the inner and outer domains may have larger flexibility with respect to each other. If so, our results show that the β2,3 part of the bridging sheet indeed experienced large conformational fluctuations and was partially unfolded. The β20.21 part of the bridging sheet, although also showing some mobility with respect to the inner/outer domains, was well structured and associated with the inner/outer domains throughout the trajectories. The inner and outer domains displayed only limited flexibility with respect to each other. The hydrophobic/aromatic cluster composed of residues Trp-112, Leu-116, and Phe-210 from the inner domain, Phe-382 and Tyr-384 from the outer domain, Ile-424, Trp-427, Tyr-435 from the bridging sheet, and Val-255 from the linker (Fig. 4), appeared to play an important role in the conformation stabilization. The hydroxyl group of Tyr-435 was also bonded to Lys-116 from helix α1, further enhancing the stability of the conformation. Breaking such a hydrophobic network needs sufficient energy to overcome the potentially high free energy barrier. In addition, although there is no other hydrophobic core along the inner/outer domain interface, there are extensive contacts between the two domains and they are connected by two fairly extended linkers (Fig. 1). Given these facts, one would not expect a much larger flexibility between the inner and outer domains than what is observed in the simulation. On the other hand, the β2,3 of the bridging sheet did not share a hydrophobic core with the rest of the molecule and was accordingly much more mobile. Thus, it is not unreasonable to believe that the unbound state still has a well-structured inner domain, outer domain, and part of the bridging sheet (β20.21) that are associated with each other, although we do not exclude the possibility that a more dramatic difference between the unbound and bound states may exist, given our limited simulation time. We further note that the flexibility of the core domains observed in this work may be changed in the context of the whole protein.

Conformational Features Versus Antibody Recognition—Mutanogenesis studies show that structural perturbation of the bridging sheet affected the binding of gp120 to the CD4, CCR5, and the CD4i antibodies but not to the CD4BS antibodies (7). This indicates that the CD4BS antibodies recognize conformations of gp120 that are different from those recognized by the CD4 and CD4i antibodies. Consistently, most of the CD4BS epitopes have been mapped onto the inner and outer domain (21). The fact that the S375W mutant did not bind to any of the CD4BS antibodies indicates a conformational alteration in the inner and outer domain regions. Our results suggest that these antibodies may prefer a conformation that is more open than that of the crystal structure particularly at the interface of the inner domain, outer domain, and the bridging sheet, because the mutant had a more contracted inner domain to outer domain distance. It is known that the antibodies are usually larger in size than CD4. Thus, the narrowed binding pocket induced by the S375W mutation explains perfectly the low binding affinity of this mutant to the CD4BS antibodies which were elicited against the potentially more open free form of gp120.

Implications for Drug Design—Anti-AIDS drugs are still in great need. However, there has been only limited success in the search for potent anti-AIDS agents even with the available structure of the gp120 in a complexed form. One of the reasons is that the prevailing conformation of gp120 in its free form may be very different from the CD4-bound state. Thus, targeting the bound-state structure that is not well populated is less likely to succeed. In addition, chemical agents targeting the CD4-bound form may act in a way similar to CD4 and thus possibly even trigger conformational changes needed for the chemokine receptor binding and consequently enhance the infection. It is known that several CD4 binding inhibitors are actually infection enhancers, and CD4 binding inhibitors are often known to be CCR5-binding activators (42). Thus, targeting the open form would be more efficient and possibly can avoid the CD4-binding inhibitor-infection-enhancing dilemma. With no available experimental structure of the gp120 in free form, a molecular-dynamics-generated open form structure provides another venue for the search for potent gp120 inhibitors. Encouragingly, our results suggest that the free form of gp120, although flexible as is true for many other proteins, assumes a well defined three-dimensional conformation that is targetable relative to the previously believed very mobile structure. In addition, multiple conformations of the backbone and side chains can be easily explored in this regard. Thus, with the use of our simulated models and the consideration of structural flexibility and multiple conformations, more potent and specific inhibitors can be expected from such structure-based efforts.

Conclusions—We have characterized the conformational features of the gp120 core domain in its free form via molecular dynamics simulations. Although the overall free-state structure is very similar to the bound-state, two specific regions have been identified to be conformationally different. First, when compared with the crystal CD4-bound form, β strands 2 and 3 of the bridging sheet in the free form were very flexible and partially unfolded. Second, the association among the inner domain, the outer domain, and β strands 20 and 21 of the bridging sheet was not as tight as in the bound form, resulting in a more expanded organization. Furthermore, analyzing the difference in stability and flexibility between the wild type and the mutant, we were able to show that the stabilization of the residues and the overall conformation in the S375W mutant account for an important part of the experimentally observed thermodynamic properties that differentiate between the wild type and the mutant. Overall, our results are in very good agreement with experiment (6, 7, 25), and explain the surprising experimental behavior of the S375W mutant. This mutant was designed with the goal of filling the space at the bottom of the CD4 binding pocket, which is not occupied by the CD4. Unexpectedly, the mutant bound CD4 tighter than the wild type did, and its affinity to antibodies elicited against the CD4BS was almost totally abolished (7). The conformational behavior shown here points toward the underlying factors that contribute to the altered phenotypes and highlights the differences between the CD4-bound and free states.

Hence, our results may further suggest that the reason that drugs designed toward the gp120-bound state have had limited success. The conformational features of the free-state gp120 model obtained in our simulations should therefore be of great value in efforts toward new inhibitor development.

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