Induced Fit in HIV-Neutralizing Antibody Complexes: Evidence for Alternative Conformations of the gp120 V3 Loop and the Molecular Basis for Broad Neutralization[†,‡]

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ABSTRACT: Human monoclonal antibody (mAb) 447-52D neutralizes a broad spectrum of HIV-1 isolates, whereas murine mAb 0.5β, raised against gp120 of the X4 isolate HIV-1_{IIIB}, neutralizes this strain specifically. Two distinct gp120 V3 peptides, $V3_{MN}$ and $V3_{IIIB}$, adopt alternative β-hairpin conformations when bound to 447-52D and 0.5β, respectively, suggesting that the alternative conformations of this loop play a key role in determining the coreceptor specificity of HIV-1. To test this hypothesis and to better understand the molecular basis underlying an antibody's breadth of neutralization, the solution structure of the $V3_{IIIB}$ peptide bound to 447-52D was determined by NMR. $V3_{IIIB}$ and $V3_{MN}$ peptides bound to 447-52D exhibited the same N-terminal strand conformation, while the $V3_{IIIB}$ peptide revealed alternative N-terminal conformations when bound to 447-52D and 0.5β. Comparison of the three known V3 structures leads to a model in which a 180° change in the orientation of the side chains and the resulting one-residue shift in hydrogen bonding patterns in the N-terminal strand of the β-hairpins markedly alter the topology of the surface that interacts with antibodies and that can potentially interact with the HIV-1 coreceptors. Predominant interactions of 447-52D with three conserved residues of the N-terminal side of the V3 loop, K312, I314, and I316, can account for its broad cross reactivity, whereas the predominant interactions of 0.5β with variable residues underlie its strain specificity.

Human immunodeficiency virus type 1 (HIV-1)[1] utilizes two membrane-bound molecules to gain entry into cells: CD4 and one of several types of chemokine receptors. Most HIV strains can utilize either the receptor for CC chemokines, CCR5, or the receptor for CXC chemokines, CXCR4, and are thus termed R5- and X4-tropic viruses, respectively. A minority of HIV strains that use both of these receptors are termed dual-tropic (*1*). The third hypervariable region of envelope glycoprotein gp120 (V3 loop, residues 303−340) is directly involved in the binding of gp120 to the chemokine receptors (*2*, *3*). Analysis of the HIV-1 genome demonstrated that the V3 loop contains major determinants responsible for the phenotype of the virus and its cell tropism and that the V3 sequence determines whether the virus binds to CCR5 or CXCR4 (*4*). Results of alanine-scanning mutagenesis led to the conclusion that Lys305, Ile307, Arg313, and Phe315 are involved in CCR5 utilization (*5*). [These residues are numbered Lys312, Ile314, Arg322, and Phe324 according to the numbering system used in this paper (*6*).] A single mutation in the V3 loop, D329R, transforms an R5 virus into an X4 virus (*7*). Exchange of the V3 loop of the IIIB strain (an X4 virus) with the V3 loop of an R5 virus creates an R5-like virus with infectivity that is inhibited by chemokines specific for CCR5 (*8*).

Many HIV-1-neutralizing antibodies in infected individuals or in immunized animals are directed against the V3 loop, which was designated, accordingly, the "principal neutralizing determinant" (PND) of HIV-1 (*9*). HIV-neutralizing antibodies against V3 are thought to prevent the binding of gp120 to either CCR5 or CXCR4, thus abolishing viral fusion with its target cell (*3*, *10*). HIV-1 has developed a number of highly effective mechanisms for evading the immune system and especially for escaping neutralization by anti-V3 antibodies (*11*, *12*). Study of chimeric gp120s from R5 and X4 strains and sequence analyses revealed that neutral-

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[‡] The coordinates have been deposited in the Protein Data Bank as entries 1U6U (average structure) and 1U6V (ensemble of low-energy structures).

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[1] Abbreviations: HIV-1, human immunodeficiency virus type 1; gp120, envelope glycoprotein of HIV-1; V1−V3, first, second, and third hypervariable regions of gp120, respectively; CXCR4, CXC chemokine receptor 4; CCR5, CC chemokine receptor 5; NMR, nuclear magnetic resonance; PND, principal neutralizing determinant; CDR, complementarity-determining region; $V3_{IIIB}$, V3 loop peptide from the IIIB strain of HIV-1; $V3_{MN}$, V3 loop peptide from the MN strain of HIV-1; mAb, monoclonal antibody.

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ization resistance involves the major variable loops, V1/V2 and V3 (*13*−*16*).

The structures of the gp120 core of both an X4 laboratory-adapted virus and of an R5 primary isolate in complex with a CD4 fragment and with the Fab fragment of a gp120-specific antibody have been determined (*17*). However, crystals could be obtained only for gp120 polypeptides lacking the first three variable loops, including V3, and the structures of V4 and V5 have not been defined. Despite dramatic antigenic differences between the primary R5 and the laboratory-adapted X4 isolates, the structure of their gp120 core is very similar (*18*). Thus, while being a major accomplishment, the determination of the X-ray structure of the gp120 core could not explain HIV-1 coreceptor selectivity. As an alternative to the direct study of the V3 conformation of intact gp120 molecules, complexes of V3 peptides with antibodies elicited against gp120 or HIV-1 can be investigated.

The 447-52D human mAb, derived from the cells of an HIV-1-infected patient, is directed against a determinant located within the V3 loop of gp120. 447-52D binds to a broad spectrum of highly divergent V3 peptides from six clades (A, B, D, and F−H) (*19*) with association constants of 2×10^5 to 10^8 M^{-1}, the highest of which is only 1 order of magnitude below its affinity for the corresponding gp120 protein (*20*). 447-52D neutralizes a broad spectrum of R5 and X4 viruses, including primary isolates, which are more resistant than laboratory-adapted strains to antibody neutralization (*21*, *22*). How this antibody can neutralize both R5 and X4 viruses has remained obscure.

Murine mAb 0.5β is a potent strain-specific HIV-1-neutralizing antibody that was raised against the entire gp120 of the X4 virus HIV-1_{IIIB} (*23*). The V3 peptide P1053 (^{311}RKSIRIQRGPGRAFVTIG^{328}) comprises the complete 0.5β epitope and binds to the antibody with almost the same affinity as RP135, a peptide that is three residues longer than P1053 at each end (*24*). Neutralization of the free virus was achieved at a 0.5β concentration of 100 ng/mL, whereas full neutralization of infected cells (measured by syncytium inhibition) required antibody concentrations of 50 μg/mL (*23*).

Studies of complexes of HIV-1-neutralizing antibodies with V3 loop peptides have provided unique insight into the conformation of the V3 loop and about the immune response against this important HIV-1 determinant (*25*−*29*). Our approach has been to use NMR to study V3 peptides in complex with antibodies elicited against native conformations of the V3 loop in either gp120 or the intact virus (*30*−*33*). The two antibodies that have been studied, 0.5β and 447-52D, bind strongly to synthetic V3 peptides with affinities comparable to those exhibited for native gp120 (*20*, *34*). Since these linear peptides are unstructured when free in solution (*35*, *36*), we assume that the peptides bind to such antibodies by an induced fit mechanism and adopt a conformation that mimics that against which these antibodies were elicited.

The structures of the $V3_{MN}$ peptide bound to the Fv fragment of 447-52D and of the $V3_{IIIB}$ peptide bound to 0.5β Fv (*23*) were determined previously using NMR (*30*, *37*). Both structures are β-hairpins with two antiparallel β-strands. Notably, the structure of the $V3_{MN}$ peptide was found to have similarities in conformation and sequence to β-hairpins in CCR5 ligands, RANTES, MIP-1α, and MIP-1β (*37*), while the structure of the $V3_{IIIB}$ peptide bound to the 0.5β antibody resembles a β-hairpin in the CXCR4 ligand SDF-1 (*37*).

Since, in the previous NMR studies, complexes were composed of different combinations of Fv fragments and peptides ($V3_{MN}$ bound to 447-52D Fv and $V3_{IIIB}$ bound to 0.5β Fv), we chose to extend these studies by examining a complex of $V3_{IIIB}$ (^{310}TRKSIRIQRGPGRAFVTIGK^{329}) bound to the Fv of 447-52D, and have obtained results that allow an analysis of the conformation of the $V3_{IIIB}$ peptide in complex with two different antibodies. At the same time, comparison could be made to the structure of the $V3_{MN}$ peptide in complex with 447-52 Fv. These comparisons have led to insights into the molecular interactions that determine whether an antibody is broadly neutralizing or strain-specific for HIV-1. They also reinforce our conclusion that alternative conformations are assumed by V3 loops and suggest that these conformational variations determine the target cell selectivity of the virus.

MATERIALS AND METHODS

Sample Preparation. The 20-residue $V3_{IIIB}$ peptide $^{310-329}$gp120_{IIIB} (TRKSIRIQRGPGRAFVTIGK) was expressed as a fusion protein in *Escherichia coli*, cleaved, and purified as previously described (*38*). However, since the efficiency of CNBr cleavage in formic acid when a threonine residue follows methionine is very low (*39*), the cleavage was performed in 70% TFA. The 447-52D Fv was expressed in the BL21(DE3)pLysS strain as described elsewhere (*40*). The Fv−peptide complex (28.3 kDa) was prepared by the addition of a 20% molar excess of the peptide to a dilute Fv solution (~0.04 mM), and the resulting Fv−peptide complex was concentrated to 0.4−0.5 mM by membrane filtration using vivaspin (Vivascience) with a 10 kDa cutoff. All samples contained 10 mM d_4-acetic acid buffer at pH 5 and 0.05% NaN_3.

NMR Spectroscopy and Structure Calculations. Isotope-edited NMR spectra using a uniformly ^{15}N-labeled or ^{13}C- and ^{15}N-labeled peptide in complex with unlabeled 447-52D Fv were acquired, processed, and analyzed as described previously (*37*). A two-dimensional homonuclear TOCSY spectrum (25 ms mixing time) in D_2O was measured to identify the spin systems of the aromatic residues of the Fv. NOESY spectra were acquired with a mixing time of 150 ms. Seven ψ angles for residues ^{IIIB}K312− ^{IIIB}I316, ^{IIIB}A323, and ^{IIIB}F324 were included in the calculations on the basis of the analysis of the ^{1}Hα, ^{13}Cα, ^{13}Cβ, and ^{15}N chemical shifts using TALOS (*41*). The structure was calculated using standard procedures (*37*). Hydrogen bonds that formed in more than 70% of the structures were used as constraints in later stages of refinement.

RESULTS

Solution Structure of the Antibody-Bound $V3_{IIIB}$ Peptide. Nearly complete backbone and side chain assignments (96 and 84%, respectively) were obtained for the Fv-bound $V3_{IIIB}$ peptide. Side chain assignment was especially problematic for ^{IIIB}R318, ^{IIIB}P320, and ^{IIIB}R322 for which only the Cβ and Hβ protons could be assigned. The structure of the $V3_{IIIB}$ peptide bound to 447-52D Fv was determined using 370 NMR-derived distance (75 long- and medium-range), 21

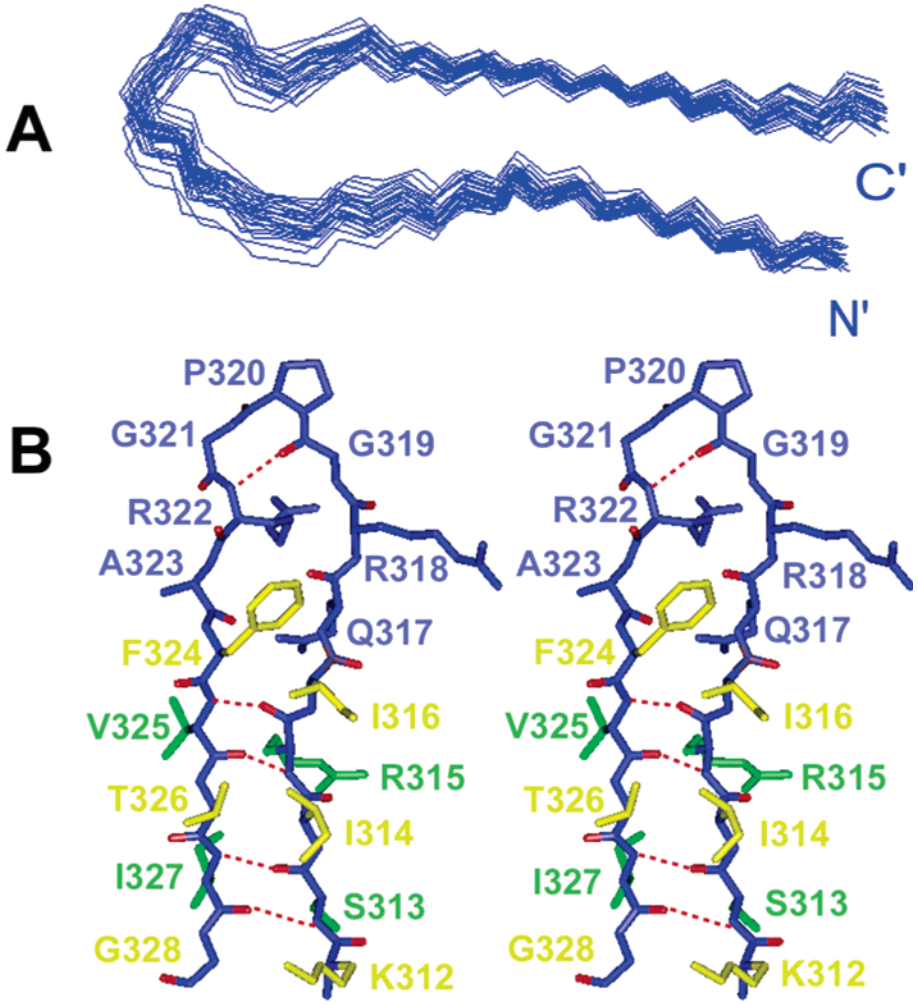

FIGURE 1: Solution structure of the $V3_{IIIB}$ peptide ($^{312-328}gp120_{IIIB}$) bound to the 447-52D Fv. (A) Backbone superposition of the 30 lowest-energy structures. (B) Stereo representation of $V3_{IIIB}$ bound to the 447-52D Fv showing hydrogen bonds within the peptide (red). Side chains pointing out from the page are colored yellow, side chains pointing inward green, and side chains of the loop residues blue.

dihedral angle, and five hydrogen bond constraints. The superposition of the 30 lowest-energy structures that satisfy the experimental restraints with no NOE violations larger than 0.4 Å is shown in Figure 1A. The bound peptide forms a well-defined β-hairpin consisting of two antiparallel β-strands made of residues ^{IIIB}K312−^{IIIB}I316 and ^{IIIB}F324−^{IIIB}G328. The loop region consisting of residues Q317−A323 was not as well defined as the β-strands. The root-mean-square deviation (rmsd) values for the entire epitope ($^{312-328}$gp120) are 0.41 and 1.07 Å for the backbone and heavy atoms, respectively. The structural statistics and rmsd values are presented in Table 1 in the Supporting Information. The Ramachandran plot (not shown) of the mean structure of the $V3_{IIIB}$ peptide bound to 447-52D Fv suggests that the φ and ψ angles of all peptide residues (except glycines) occupy allowed regions.

NOE interactions characteristic of a β-hairpin conformation were observed between backbone atoms of the N- and C-terminal halves of the peptide. These interactions include ^{IIIB}R315 H^N−^{IIIB}V325 H^N, ^{IIIB}I316 Hα−^{IIIB}V325 H^N, ^{IIIB}Q317 H^N−^{IIIB}F324 Hα, ^{IIIB}K312 Hα−^{IIIB}G328 Hα, ^{IIIB}I314 Hα−^{IIIB}T326 Hα, and ^{IIIB}I316 Hα−^{IIIB}F324 Hα interactions. The expected ^{IIIB}S313 H^N−^{IIIB}I327 H^N and ^{IIIB}R315 H^N−^{IIIB}T326 Hα NOE interactions could not be assigned because of resonance overlap. $^3J_{HNH\alpha}$ coupling constants higher than 7.7 Hz, typical of a β-strand, were measured for ^{IIIB}S313, ^{IIIB}I314, ^{IIIB}R315, ^{IIIB}I316, ^{IIIB}V325, ^{IIIB}T326, and ^{IIIB}I327.

The side chains of residues ^{IIIB}S313, ^{IIIB}R315, ^{IIIB}V325, and ^{IIIB}I327 form the lower face of the β-hairpin, while the side chains of ^{IIIB}K312, ^{IIIB}I314, ^{IIIB}I316, ^{IIIB}F324, and ^{IIIB}T326 form the upper face. Numerous NOE interactions are indicative of this topology.

Interactions with 447-52D Fv and Side Chain Topology of $V3_{IIIB}$. The N-terminal segment ^{IIIB}K312−^{IIIB}I316 was found to contribute approximately 60% of the $V3_{IIIB}$ peptide NOE interactions with the 447-52D Fv. The pattern of intermolecular NOE interactions in the $V3_{IIIB}$−447-52D-Fv complex was found to be similar to that observed previously for the $V3_{MN}$ peptide bound to the same antibody Fv (*37*). The dominance of the interactions of the N-terminal strand (residues 312−316) of V3 peptides with HIV-1-neutralizing antibodies was observed also in the crystal structure of a $V3_{MN}$−447-52D Fab complex (*25*) and in the NMR structure of a $V3_{IIIB}$−0.5β Fv complex (*31*). The N-terminal residues that have the most extensive interactions with 447-52D are K312, I314, and I316 in the $V3_{IIIB}$−447-52D complex and I314 and I316 in the $V3_{MN}$−447-52D complex (*25*). This pattern of interactions differs from that observed for the $V3_{IIIB}$ complex with 0.5β in which R315 and Q317 are the N-terminal residues interacting most extensively with the

Table 1: Dominant N-Terminal Interacting Residues, the Postulated Conformation, and the Actual Sequence for V3 in the Three Complexes Studied by NMR

	$V3_{IIB}-0.5\beta$	$V3_{IIIB}-447$	$V3_{MN}-447$
dominant N-terminal interacting residues	R^{315},Q^{317}	I^{314},I^{316}	I^{314},I^{316}
postulated conformation	X4	R5	R5
actual sequence	X4	X4	X4

antibody as summarized in Table 1.

The ^{13}C-separated NOESY spectrum reveals several interactions between residues in the N-terminal strand and the turn of the $V3_{IIIB}$ peptide and aromatic residues of the antibody Fv. The presence of aromatic residues in the binding site is also reflected in the unusually high-field chemical shift observed for the protons of ^{IIIB}G319 and ^{IIIB}P320, caused by local ring current fields induced by aromatic amino acid residues. Similar NOE interactions were also observed in $V3_{MN}$ complexed with 447-52D, and almost identical Fv proton chemical shifts were observed for 25% of these NOE interactions. On the basis of this similarity, the $V3_{IIIB}$ peptide is believed to interact with the same aromatic residues as does $V3_{MN}$. To identify the type of aromatic amino acid involved in the interaction with the V3 peptide, according to the spin system of the side chain, we measured a two-dimensional homonuclear TOCSY spectrum. We found that ^{IIIB}Ile316 and ^{IIIB}Gly319 most likely interact with tyrosine residues, while ^{IIIB}Pro320 and ^{IIIB}Gly321 most likely interact with tryptophan residues. Since there are at least six tyrosine residues ($Tyr^{H100g-k}$ and Tyr^{L32}) as well as three tryptophan residues (Trp^{H33}, Trp^{L91}, and Trp^{L96}) in the antibody binding site, we cannot assign these interactions to a specific antibody residue. Our results are consistent with the crystal structure of 447 Fab in complex with $V3_{MN}$ determined recently (*25*). The crystal structure reveals that Pro320 and Gly321 interact with Trp^{L91} and Trp^{L96} residues and Ile316 interacts with Tyr^{H100i} and Tyr^{L32}. On the other hand, Gly319 appears to interact with a tryptophan residue in the crystal structure, while in NMR analysis, it interacts with a tyrosine residue. The latter observation can be explained by the different location of this residue in the Fv binding site resulting from the QR insertion preceding this glycine residue in $V3_{IIIB}$.

As a result of the different pattern of interactions of the $V3_{IIIB}$ peptide in complex with 447-52D and 0.5β, the orientation of the N-terminal strand side chains differs by 180° in the two complexes (Figure 2A,B). The surface formed by the side chains of ^{IIIB}K312, ^{IIIB}I314, and ^{IIIB}I316 in the 0.5β complex is almost a mirror image of the surface formed by the same residues in $V3_{IIIB}$ in the 447-52D complex. In contrast, the side chains of K312, I314, and I316 are similarly oriented in the $V3_{IIIB}$ and $V3_{MN}$ complexes with 447-52D (Figure 2A,C). Accordingly, in both 447-52D Fv-bound peptides, the overall topologies of the surfaces presented by the N-terminal strand are very similar.

Despite the difference in the N-terminal strand topology, the surface presented by the side chains of the $V3_{IIIB}$ C-terminal β-strand, ^{IIIB324}FVTIG^{328}, is similar in the $V3_{IIIB}$ complexes with 0.5β and 447-52D (Figure 2D,E). The side chain orientation of the C-terminal strand residues of $V3_{IIIB}$ in complex with both Fv's differs from that of $V3_{MN}$ in complex with 447-52D (panels D and E of Figure 2 versus panel F).

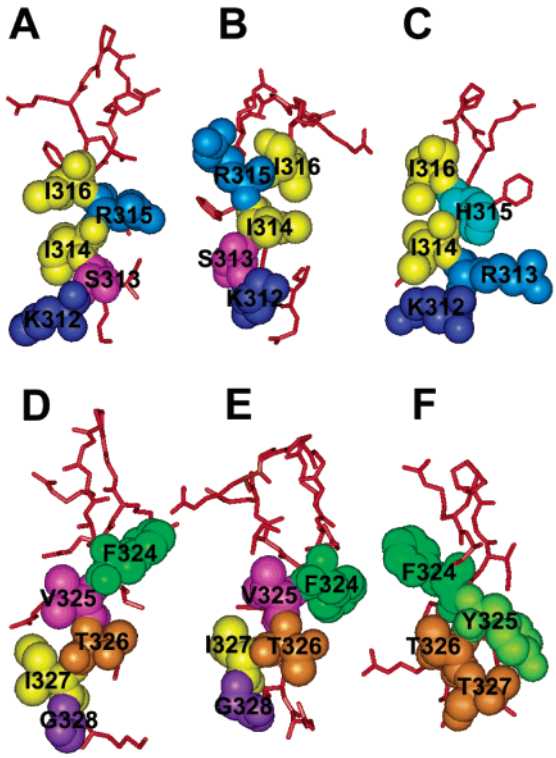

FIGURE 2: Space-filling representation of V3 peptides bound to HIV-1-neutralizing antibodies. Views showing the surface presented by the N-terminal β-strand of (A) a $V3_{IIIB}$ peptide bound to 447-52D Fv, (B) $V3_{IIIB}$ bound to 0.5β Fv (*31*), and (C) $V3_{MN}$ bound to 447-52D Fv (*37*). Views showing the surface presented by the C-terminal β-strand of (D) $V3_{IIIB}$ bound to 447-52D Fv, (E) $V3_{IIIB}$ bound to 0.5β Fv (*31*), and (F) $V3_{MN}$ bound to 447-52D Fv (*37*).

Hydrogen Bond Network. The NMR structure shows that the β-hairpin of the $V3_{IIIB}$ epitope ($^{312-328}$gp120) bound to 447-52D is stabilized by a network of hydrogen bonds between the two strands (Figures 1B and 3A). Two pairs of hydrogen bonds form between ^{IIIB}S313 and ^{IIIB}I327 and between ^{IIIB}R315 and ^{IIIB}V325. This pattern differs from that observed in $V3_{IIIB}$ complexed with 0.5β in which ^{IIIB}K312, ^{IIIB}I314, and ^{IIIB}I316 form the intrapeptide hydrogen bonds with ^{IIIB}I327, ^{IIIB}V325, and ^{IIIB}A323, respectively (Figure 3B). Thus, a one-register shift in the hydrogen bond forming residues is observed in the N-terminal strand of $V3_{IIIB}$ when the D and 2E of the two antibodies' complexes are compared (Figure 3A,B).

The N-terminal strand of the $V3_{IIIB}$ peptide bound to 447-52D, however, shows remarkable similarity in side chain topology and hydrogen bonding to the $V3_{MN}$ peptide bound to the same antibody (*37*). In both peptides, residues 313 and 315 form hydrogen bonds with the C-terminal strand of the β-hairpin (Figure 3A,C). However, while in $V3_{IIIB}$ bound to 447-52D ^{IIIB}I327 and ^{IIIB}V325 form the hydrogen bonds with the N-terminal strand, in $V3_{MN}$ bound to this antibody residues ^{MN}T326 and ^{MN}F324 form these bonds.

Conformation of the Central Loop Linking the β-Strands. P320 and R322 of the $V3_{IIIB}$ peptide show only a small number of interactions with 447-52D, which contrasts with the more extensive web of intermolecular interactions of these same residues in the crystal structure of the $V3_{MN}-$447-52D Fab complex (*25*) and in the NMR structure of the $V3_{IIIB}-0.5\beta$ Fv complex (*31*). The fewer interactions result from a lack of resonance assignment for most of the side

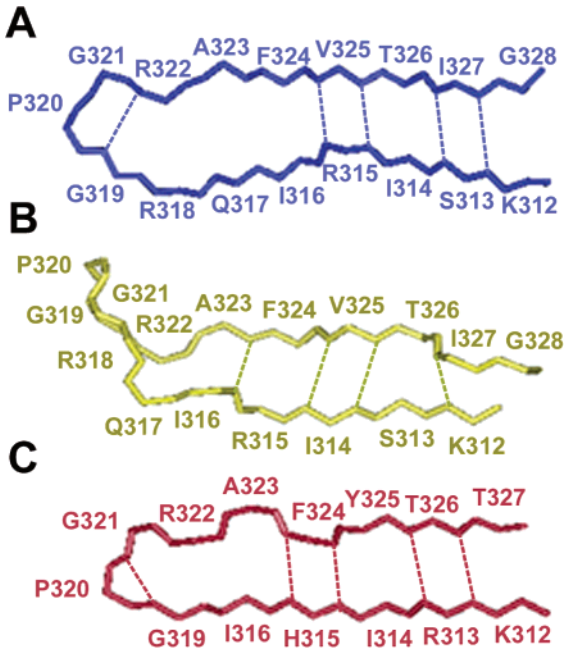

FIGURE 3: NMR structures of V3 peptides bound to HIV-1-neutralizing antibodies and the hydrogen bond network in the V3 β-hairpins. The backbone conformation and hydrogen bonds (represented by dashed lines) network of (A) $V3_{IIIB}$ bound to 447-52D Fv (blue) determined in this study, (B) $V3_{IIIB}$ bound to 0.5β Fv (yellow) (*31*), and (C) $V3_{MN}$ bound to 447-52D Fv (red) (*37*) are shown.

chain protons of these two $V3_{IIIB}$ residues. The rare QR insertion, which does not interact with the 447-52D Fv, lengthens the loop region of $V3_{IIIB}$ peptide, possibly disturbing the fit of the peptide in the antibody binding site and causing greater conformational flexibility leading to the weaker signal.

In the NMR-derived structure, the $V3_{IIIB}$ peptide in complex with the 447-52D Fv forms a seven-residue loop which links the two $V3_{IIIB}$ β-strands and comprises residues ^{IIIB}Q317−^{IIIB}A323. This seven-residue loop is one residue longer than the loop formed by the same peptide bound to 0.5β. In the 0.5β complex, the proline was in a cis conformation and a type VI β-turn was formed by the RGPG segment (*30*). This unusual turn conformation was stabilized by a hydrogen bond between ^{IIIB}R318 and ^{IIIB}G321. Because of the rare two-residue insertion, the loops formed by the $V3_{IIIB}$ peptide in both antibody complexes are longer than the four-residue loop observed in the $V3_{MN}$ peptide bound to 447-52D. The conformation of the $V3_{IIIB}$ loop bound to 447-52D Fv is stabilized by an $i,i+3$ hydrogen bond between the carbonyl oxygen of ^{IIIB}G319 and the amide proton of ^{IIIB}R322 (Figures 1B and 3A). Twenty of the 30 lowest-energy structures were found to adopt a type II β-turn for the GPGR segment. The structure of the loop, however, is not as well defined as that of the β-strands. In all accepted structures, P320 was found to be in the trans conformation. The strong NOE peak between α^{IIIB}P320 and α^{IIIB}G319, typical for a *cis*-proline conformation, has not been observed in the NOESY spectra. Moreover, when a *cis*-proline was introduced into the initial templates and the proline was constrained to a cis conformation, no structures were accepted during the calculations. If these constraints were relaxed, the *cis*-proline turned into the trans configuration.

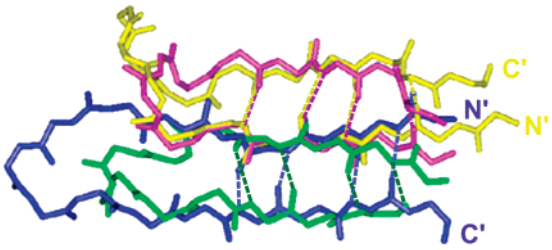

FIGURE 4: Structures of the $V3_{IIIB}$ peptide bound to 447-52D Fv and 0.5β Fv (*31*) compared with the structure of the β-hairpin formed by strands β2 and β3 in MIP-1α (*46*) and SDF-1 (*45*). Backbone superposition of the $V3_{IIIB}$ peptide in complex with the 447-52D Fv (blue) or 0.5β Fv (yellow) N-terminal segment ($^{312-316}$gp120) with the corresponding segments ($^{38-42}$MIP-1α and $^{38-42}$SDF-1) of MIP-1α (green) and SDF-1 (pink). The hydrogen bonds between the N- and C-termini (represented by dashed lines) are shown.

The 447-52D-Bound $V3_{IIIB}$ Structure Is Homologous to the β-Hairpin Linking the β2 and β3 Strands in CCR5 Chemokines. The three-dimensional structures of SDF-1 and the CCR5 chemokines (MIP-1α, MIP-1β, and RANTES) are similar, and all four manifest a hairpin structure involving the β2 and β3 strands (*44*). The orientation of side chains of strand β3 in the CCR5 chemokines and in SDF-1 is similar, and the same residues form intrapeptide hydrogen bonds (*45*, *46*). On the other hand, strand β2 of SDF-1 and the CCR5 chemokines differ by a one-register shift in their surface-exposed residues and also in the residues involved in hydrogen bonding, as calculated from their published structures (*45*, *46*) using INSIGHT. This shift is similar to that observed between the N-terminal strand conformations of the $V3_{IIIB}$ peptide bound to 447-52D Fv and 0.5β Fv.

Superposition of the $V3_{IIIB}$ structures in the 447-52D and 0.5β complexes with the chemokines' $\beta2-\beta3$ hairpin (Figure 4) shows that the conformation of $V3_{IIIB}$ in the 447-52D complex is quite similar to the β-hairpin of MIP-1α, and the same hydrogen bond network is formed within the β-hairpin (compare green and blue structures). When the ^{40}IFL^{42} and ^{48}QVCA^{51} segments of MIP-1α are superimposed on the ^{IIIB314}IRI^{316} and ^{IIIB324}FVTI^{327} segments of $V3_{IIIB}$, an rmsd of 1.48 Å is calculated. In addition, the conformation of the $V3_{IIIB}$ peptide bound to 0.5β Fv is strikingly similar to the β-hairpin of SDF-1, and both have the same hydrogen bond network (compare yellow and pink structures). The rmsd between $V3_{IIIB}$ bound to 0.5β and SDF-1 is 0.47 Å when the ^{IIIB312}KSIRIQR^{318} and ^{IIIB322}RAFVT^{I327} segments of $V3_{IIIB}$ are superimposed over the ^{38}IVARLKN^{44} and ^{46}NRQVCI^{51} segments of SDF-1, respectively (*37*).

DISCUSSION

Alternative Conformations of the V3 Loop Are Formed by an Induced Fit to Neutralizing Antibodies. This study was designed to probe the possible conformations of the V3 loop of gp120 and to understand the molecular basis for the broad neutralizing activity of the 447-52D human monoclonal antibody. We compared the conformations of a $V3_{IIIB}$ peptide bound to the Fv fragments of two different antibodies, 447-52D and 0.5β, and the conformation of a $V3_{MN}$ peptide bound to 447-52D Fv. In all three complexes, the V3 loop formed a β-hairpin with a central reverse turn comprising the GPG segment. In comparison with the C-terminal strand, the

$V3_{IIIB}$ NNTR**KSIRIQRGPGRAFVTIG**KIG
$V3_{MN}$ YNKR**KRIHI--GPGRAFYTT**KNIIG
$V3_{JRFL}$ NNTRKSIHI--GPGRAFYTTGEIIG
(consensus R5)

FIGURE 5: Sequence alignment of three different V3 peptides, $V3_{IIIB}$, $V3_{MN}$, and $V3_{JR-FL}$, the last of which represents the consensus sequence of clade B R5 viruses. The residues comprising the epitope recognized by the 447-52D antibody are in bold letters.

N-terminal strand interacted more extensively with these antibodies (*31*, *37*), underscoring the importance of the N-terminal strand in interactions with HIV-1-neutralizing antibodies. The side chain orientation of the N-terminal strand residues of the $V3_{IIIB}$ peptide bound to 447-52D Fv was found to differ by approximately 180° from that of the same peptide bound to 0.5β Fv. On the other hand, the side chain orientation and the hydrogen bond pattern of the N-terminal strand (^{IIIB312}KSIRI^{316}) of the $V3_{IIIB}$ peptide bound to 447-52D Fv were similar to those in a corresponding segment of the $V3_{MN}$ peptide bound to 447-52D Fv, despite the 40% difference in sequence and the two-residue insertion in $V3_{IIIB}$ (Figure 5). Since the same orientation of the N-terminal strand residues' side chains is found in two different V3 peptides, $V3_{IIIB}$ and $V3_{MN}$, bound to 447-52D and a different orientation is found in the same peptide ($V3_{IIIB}$) bound to two different antibodies, we conclude that the conformation of the antibody-bound V3 peptide is dictated not merely by the sequence of the peptide but rather by an induced fit to the specific antibody.

The difficulties in crystallizing the entire gp120 molecule, coupled with the successful crystallization of gp120 molecules devoid of variable region V3 (as well as V1 and V2), suggest that these regions are flexible (*17*, *18*). The observation that 0.5β and 447-52D recognize a β-hairpin conformation indicates that, despite the inferred flexibility of the V3 loop, its secondary structure is conserved even though the side chain orientation and therefore the hydrogen bond network within the loop may vary. The existence of dual-tropic viruses suggests that interconversion between the conformations recognized by CCR5 and CXCR4 is possible. Thus, the flexibility of the V3 loop could facilitate conversion between the two V3 conformations by an induced fit upon binding to a broadly neutralizing antibody such as 447-52D (*47*).

Comparison of the structures of the V3 peptides bound to monoclonal antibodies 447-52D and 0.5β has led us to formulate a model in which a 180° change in the orientation of the side chains and the resulting one-residue shift in the hydrogen bonding pattern in the N-terminal strand of the β-hairpins markedly alter the topology of the surface that interacts with different monoclonal antibodies and, by extension, with different chemokine coreceptors. In effect, this topological change creates two distinct binding sites despite the essential conservation of sequence. Our past and present studies establish structural homology between the V3 loop of gp120 and the $\beta2-\beta3$ hairpins of CC and CXC chemokines (*37*). Knowing that (1) antibody 0.5β was raised against gp120 from the X4 virus $HIV\text{-}1_{IIIB}$ and (2) the homology between the structures of $V3_{IIIB}$ bound to this antibody and SDF-1, we suggest that the $V3_{IIIB}$ conformation in the 0.5β complex resembles the "X4 conformation" of the V3 loop (Table 1). On the basis of the foregoing and a similarity with the conformation of CCR5 chemokines, we propose that the conformation of the N-terminal strand of both $V3_{MN}$ and $V3_{IIIB}$ bound to the Fv fragment of the broadly neutralizing antibody 447-52D represents the "R5 conformation" of the V3 loop (Table 1).

The $\beta2-\beta3$ hairpin structure of SDF-1 and the CCR5 chemokines is also known as the "40s loop", a term which is somewhat misleading since this region includes amino acids 37−51 in SDF-1 and 37−52 in RANTES, MIP-1α, and MIP-1β. The $\beta2-\beta3$ hairpin is one of the chemokine domains responsible for receptor binding. Thus, mutations in the $\beta2-\beta3$ hairpins of MIP-1β and MIP-1α cause a significant decrease in the level of receptor binding (*48*, *49*). A RANTES peptide ^{31}SGKCSNPAVVFV^{42} including strand $\beta2$ (underlined) (as well as additional peptides from the N-terminus of RANTES) was shown to bind CCR5 (*50*). Thus, like the V3 loop, the structurally homologous $\beta2-\beta3$ hairpin of various chemokines is involved in binding to chemokine receptors (*51*, *52*). Moreover, NMR studies of other chemokines in complex with N-terminal peptides of their corresponding receptors clearly indicate that the chemokines' N-terminus and N-loop as well as the 40s loop (the $\beta2-\beta3$ hairpin) interact with their corresponding receptors (*51*, *52*). Since all chemokines have a similar three-dimensional structure, it has been suggested that other chemokines share the same mode of interaction (*51*, *52*). In view of this, it is plausible that the $\beta2-\beta3$ hairpin in CCR5 chemokines and probably also in SDF-1 is involved in interactions with CCR5 and CXCR4, respectively.

The Broadly Neutralizing Antibody 447-52D Recognizes an Exposed Surface Consisting of Conserved Residues. Despite the differences in the V3 sequence of $HIV\text{-}1_{IIIB}$ and $HIV\text{-}1_{MN}$ (Figure 5), antibody 447-52D neutralizes both HIV-1 strains, although a 3-fold higher concentration of antibody is needed to neutralize $HIV\text{-}1_{IIIB}$ (*54*). Unlike 447-52D, antibody 0.5β specifically neutralizes $HIV\text{-}1_{IIIB}$ alone (*23*). To understand the molecular basis for the broad neutralization of HIV-1 by certain antibodies, the interactions of 447-52D Fv and 0.5β Fv with the same $V3_{IIIB}$ were compared. We found that these antibodies showed different patterns of interactions with the N-terminal strand of $V3_{IIIB}$. 447-52D had the largest number of interactions with ^{IIIB}I316, extensive interactions with ^{IIIB}I314 and ^{IIIB}K312, fewer interactions with ^{IIIB}S313 and ^{IIIB}R315, and almost no interactions with ^{IIIB}Q317 which is part of the two-residue insertion (Table 1). In contrast, 0.5β interacted extensively with residues ^{IIIB}R315 and ^{IIIB}Q317, had fewer interactions with ^{IIIB}I314, and had almost no interactions with ^{IIIB}I316 (Table 1). Clearly, the two antibodies differ markedly in their interactions with the N-terminal strand of the V3 β-hairpin. Comparison of the crystal structure of the $V3_{MN}$−447-52D Fv complex (*25*) with the NMR structure of the $V3_{IIIB}$−0.5β Fv complex (*31*) reveals that in the former complex, residues ^{MN}I314, ^{MN}I316, ^{MN}P320, and ^{MN}R322 have the largest contact surface with the antibody, while in the latter complex, ^{IIIB}R315, ^{IIIB}Q317, ^{IIIB}P320, and ^{IIIB}R322 are the major contributors to antibody−peptide contacts. Thus, the existence of those structures underscores the importance of (a) the conserved $^{IIIB/MN}$I314 and $^{IIIB/MN}$I316 residues in V3 interactions with the broadly neutralizing antibody 447-52D and (b) the dominance of variable residues ^{IIIB}R315 and ^{IIIB}Q317 in interactions with strain-specific antibody 0.5β.

The dominant $V3_{IIIB}$ and $V3_{MN}$ residues that interact with 447-52D, $^{IIIB/MN}$K312, $^{IIIB/MN}$I314, and $^{IIIB/MN}$I316 (Table 1), are conserved in clade B HIV-1 viruses and are also very common in R5 and X4 viruses of clades A, C, and F (http://www.hiv.lanl.gov/content/hiv-db/COMPENDIUM/1998/III/V3.pdf)). Isoleucine appears at positions 314 and 316 in 96 and 69% of the isolates, respectively. Isoleucine or other bulky hydrophobic amino acids appear in more than 99% of the isolates at these positions. Lysine appears at position 312 in 83% of clade B isolates, and lysine or arginine appears at position 312 in 98% of the isolates. Interestingly, K312 and I314 were implicated in CCR5 binding (*6*). Thus, the V3 loop of most clade B isolates could interact similarly with 447-52D. On the other hand, 0.5β interacts strongly with ^{IIIB}R315 and ^{IIIB}Q317. Arginine appears at position 315 in only 2% of clade B isolates, and none of the isolates has lysine at this position. Moreover, position 315 is one of the most variable in V3. ^{IIIB}Q317 is part of the rare two-residue insertion in $V3_{IIIB}$. Thus, the V3 loop of only very few isolates could interact with 0.5β. We therefore postulate that it is the dominant interactions with the conserved triad of K312, I314, and I316 that make antibody 447-52D broadly neutralizing, whereas dominant interactions with residues at variable positions 313 and 315 and the interaction with the (rare) insertion underlie the strain specificity of antibody 0.5β. The hydrophobic interactions of ^{MN}I314 and ^{MN}I316 with 447-52D and the possible electrostatic interactions between ^{MN}K312 and residue E100e of 447-52D heavy chain CDR3 probably stabilize the observed intermolecular β-sheet between this V3 peptide and 447-52D (*25*). The predominance of backbone−backbone interactions between the V3 peptide and the antibody (*25*) allows replacement of the isoleucine with other bulky hydrophobic residues, thus contributing to the broad cross reactivity of 447-52D.

Possible Role of the Conserved GPG Sequence. As a result of the two-residue insertion in $V3_{IIIB}$, the RGPG segment of this peptide, when bound to the 0.5β antibody, adopts a type VI β-turn with proline in a cis conformation. In the $V3_{MN}$ peptide bound to 447-52D Fv, the GPG segment forms a γ-turn. As noted above, in the case of $V3_{IIIB}$ bound to 447-52D, the GPGR segment forms a type II β-turn. The apparent flexibility of the loop region of the V3 β-hairpin structure allows shifts in the hydrogen bond pattern in the β-strands without creating strain in the reverse turn. Moreover, diversity in the loop conformation enables the V3 loop to accommodate deletions and insertions while still maintaining the β-hairpin. The ability of this loop to adopt different types of turns was also observed by crystallography, where, in four different antibody complexes, each loop had a different turn: type II for GPG in complex with the 50.1 antibody, a double turn consisting of type II and type I turns for GPGRAF in complex with the 59.1 antibody, a double turn consisting of two consecutive type I turns for GPGRFY in complex with the 58.2 antibody, and type IV for GPGR in complex with the 83.1 antibody. The flexibility of the GPG segment may enable the interconversion between an R5 conformation and an X4 conformation without creating a strain in the loop, while the proline residue promotes formation of the turn responsible for the fundamental β-hairpin conformation.

CONCLUSIONS

Two alternative conformations of the same V3 peptide are created by an induced fit to two different kinds of V3-specific antibodies. A common N-terminal strand conformation is created when two highly divergent V3 peptides bind to the same antibody. The existence of these alternative β-hairpin conformations explains how gp120 molecules with very similar V3 sequences can bind selectively to one chemokine receptor or another, CCR5 or CXCR4. Analysis of the interactions of the $V3_{IIIB}$ peptide with two different antibodies also provides a structural explanation for the difference between strain-specific and broadly neutralizing antibodies. A broadly neutralizing antibody is characterized by dominant interactions with an N-terminal surface of V3, consisting of highly conserved residues; indeed, a goal of anti-HIV immunotherapy would be a generation of human mAbs with this desirable property. For the development of more effective HIV-1 vaccines, our findings argue for inclusion of both R5 and X4 conformations of the V3 loop.

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SUPPORTING INFORMATION AVAILABLE

Structural statistics and rmsd values of the structure of $V3_{IIIB}$ (Table 1). This material is available free of charge via the Internet at http://pubs.acs.org.

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