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Immunology

Crystal structure of a human immunodeficiency virus type 1 neutralizing antibody, 50.1, in complex with its V3 loop peptide antigen

(human immunodeficiency virus type 1 neutralization/gp120/anti-peptide antibody/peptide conformation)

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ABSTRACT **The crystal structure of the Fab fragment of a human immunodeficiency virus type 1 (HIV-1) neutralizing monoclonal antibody Fab has been determined at 2.8 Å resolution in complex with a linear 16-residue peptide from the third hypervariable region (V3) of gp120. The first 9 residues of the peptide are ordered in the electron density maps, and their conformation is in partial agreement with the β-strand–type II β-turn structure predicted for this portion of the V3 loop. Notably, several of the peptide residues that are well conserved among different HIV-1 isolates contact a nonpolar 25-Å-long groove in the antibody-combining site. The largely extended structure of the peptide differs from the β-turns seen as the primary determinants in other published anti-peptide Fab structures. Analysis of the specific Fab–peptide interactions only partially explains the MN isolate specificity shown by this antibody.**

The principal neutralizing determinant of the human immunodeficiency virus type 1 (HIV-1) is located in the third hypervariable region (V3) of the envelope glycoprotein gp120 (1, 2). Natural and synthetic immunogens containing V3 sequences elicit neutralizing immune responses in experimental animals (1–3). The epitopes of several HIV-1 neutralizing monoclonal antibodies have been mapped to the V3 region, and one of these monoclonal antibodies was shown to effect passive protection of chimpanzees (4).

Unfortunately, the development of vaccines based on the V3 determinant has been hindered by a tendency for anti-V3 antibodies to neutralize the virus in a type-restricted fashion. Although exceptions have been reported (5), in general, only isolates that show a high sequence homology to the original immunogen are neutralized. Analysis of the V3 sequences of 245 HIV-1 isolates has confirmed that, although certain positions in the V3 loop are relatively well conserved, others vary considerably (6). Recognition of these more variable residues by neutralizing antibodies may account for the observed type specificity. Alternatively, it has been suggested that subtle differences in V3 conformation may also affect recognition (7).

To better understand the nature of HIV-1 neutralization, we have determined the x-ray crystal structure of a complex between a V3-derived synthetic peptide (MP1; Cys-Lys-Arg-Ile-His-Ile-Gly-Pro-Gly-Arg-Ala-Phe-Tyr-Thr-Thr-Cys) and the Fab fragment of an isolate-specific, V3-directed monoclonal antibody.¶ Antibody R/V3-50.1 (50.1) was derived from a mouse immunized with a synthetic peptide corresponding to the disulfide-linked V3 loop of HIV-1 gp120 (MN isolate) (Fig. 1) (8). This antibody binds with high avidity to MN-derived synthetic peptides coated on plastic, neutralizes HIV-1 MN at low concentration in two *in vitro* assays, and prevents the fusion of cells infected with a recombinant vaccinia virus expressing the HIV-1 MN envelope protein (8). The MN peptide and five homologous peptides containing similar sequences compete with 50.1 in the peptide binding and fusion inhibition assays, but other peptides corresponding to more divergent isolates, including the HIV-1 BH10, SF2, and WMJ2 isolates, do not compete at concentrations up to 10 μg/ml. Furthermore, competition experiments using alanine-substituted peptides suggest that 50.1 recognizes the sequence Arg-Ile-His-Ile-Gly (8).

EXPERIMENTAL PROCEDURES

Fab Production. Monoclonal antibody 50.1 was generated after immunization of ASW mice with a cyclic 40-residue synthetic peptide (RP70) (Fig. 1) corresponding to the V3 region of HIV-1 MN gp120 (8). Large amounts of antibody 50.1 were produced in ascitic fluid of BALB/c mice and purified using an immobilized protein A column. Fab was produced by papain cleavage of the IgG2a and purified by size-exclusion and cation-exchange chromatography.

Immunochemical Specificity. To determine the binding specificity of antibody 50.1, an array of 266 different synthetic peptides, each containing a single amino acid substitution in the sequence Lys-Arg-Lys-Arg-Ile-His-Ile-Gly-Pro-Gly-Arg-Ala-Phe-Tyr, was prepared using the spot synthesis technique of Frank (9). Peptides recognized by the antibody were detected by autoradiography.

Crystallization. Crystals were grown by vapor diffusion from solutions containing 7.5 mg of Fab per ml, 2.5 mg of MP1 peptide per ml (the cysteine residues were acetamidomethylated), 0.2 M imidazole malate (pH 5.5), and 14% PEG 10,000 (10). The crystals grow in the monoclinic space group $P2_1$ with unit cell parameters a = 130.3 Å, b = 52.6 Å, c = 82.0 Å, and β = 97.5° and contain two Fab molecules per asymmetric unit, giving a solvent content of 57%. Data were collected on a Siemens (Iselin, NJ) multiwire area detector mounted on an Elliott GX-18 rotating anode, equipped with a 100-μm focal cup, Franks focusing mirrors (11), and a

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Abbreviations: HIV, human immunodeficiency virus; L1, L2, L3, H1, H2, and H3, first, second, and third hypervariable regions of antibody light and heavy chains; V3, third hypervariable region of HIV-1 gp120.

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¶The atomic coordinates have been deposited in the Protein Data Bank, Chemistry Department, Brookhaven National Laboratory, Upton, NY 11973 (reference 1GGI).

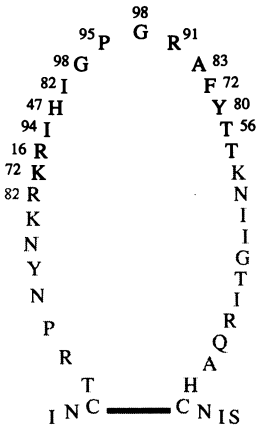

FIG. 1. V3 loop sequence of the MN isolate of HIV-1 gp120. The amino acid sequence of the cyclic peptide (RP70) used in immunization is shown. The peptide MP1 used in the crystallization consisted of the 14 residues shown in uppercase bold type with the addition of acetamidomethylated cysteine residues at the N- and C-terminal positions. Residues that are highly conserved in HIV-1 isolates (6) are shown with their percent identity (out of the 245 isolates). (A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr).

helium cone. Due to a lack of isomorphism between crystals, data were obtained from a single crystal (0.4 × 0.4 × 0.3 mm) that had been enlarged by macroseeding. The data were reduced with the XENGEN program package (12) to give a data set with an Rsym on intensities of 10.0%, which is 81% complete to 2.8 Å resolution.

Structure Determination. The structure was determined by molecular replacement with Fab 17/9 (13) as a starting model, using previously described methods (14). The Crowther fast rotation function (15) was used to orient the variable and constant regions of both Fabs in the asymmetric unit (8.0–4.0 Å, 23.0 Å Patterson cutoff radius). As only the variable regions gave good solutions, the procedure was repeated with the intact Fab. The orientations and relative domain dispositions of the intact Fabs, which were consistent with the variable solutions, were then optimized with the INTREF (16) procedure as described (14). The shift in orientation and relative translational separation for the variable and constant regions was 1.9° and 4.9° and 1.3 Å for molecule 1 and 3.7° and 12.4° and 2.5 Å for molecule 2. The large adjustments for molecule 2 were due almost entirely to differences in the elbow angle from the Fab 17/9 structure. The INTREF-refined models were then used independently in the X-PLOR (17) translation search (8.0–4.0 Å, 0.5 Å grid), yielding very clean solutions for both molecules. Using X-PLOR, both molecules were placed on a common origin by running a one-dimensional search along the *b* axis for molecule 2, including the scattering contribution from molecule 1, at each of the four choices of relative origin. The resulting model was then optimized by rigid body refinement using X-PLOR, with refinement of the individual variable and constant domains (V_L, C_L, V_H, and C_H1) of both molecules (eight domains) in the final stages. The resultant model gave an R value of 0.41 for the 8.0–4.0 Å data. The known sequence information was then incorporated into the model, which was refined by simulated annealing and positional refinement with X-PLOR (18, 19) followed by manual rebuilding from 10% omit maps using FRODO (20). The current model has an R value of 0.18 for all data in the 10.0 to 2.8 Å resolution range, with restrained atomic temperature factors, and r.m.s. deviations on bond lengths and angles of 0.018 Å and 3.9°, respectively. The Fab molecules are numbered according to standard convention (21) and the peptide is numbered with reference to the BH10 isolate sequence (22) (Cys-Lys-Arg-Ile-His-Ile-Gly-Pro-Gly; 311–316, 319–321). The light chain, heavy

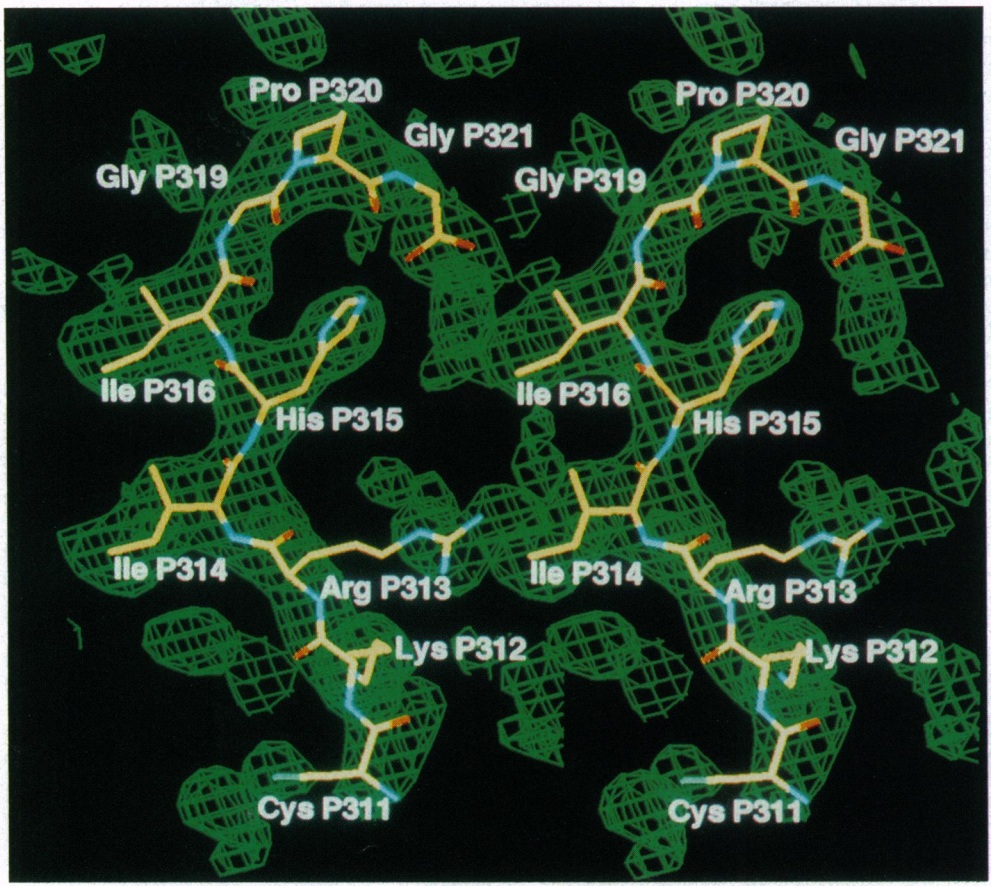

FIG. 2. Fo–Fc omit electron density for the bound peptide. Electron density for the peptide buried in the Fab combining site of molecule 1 is shown. The peptide residues were removed from the model and positional refinement with X-PLOR (18) was performed before phases were calculated. The map is contoured at 2.5 σ.

Table 1. Hydrogen bonds and salt bridge interactions in the 50.1 Fab–peptide complex

Peptide atom	Fab atom	Distance, Å	
		In molecule 1	In molecule 2
Lys^{P312} Nζ	Asp^{54} Oδ1 (H2)	3.24	3.33
Lys^{P312} Nζ	Asp^{54} Oδ2 (H2)	2.91	3.02
Lys^{P312} Nζ	Asp^{56} Oδ1 (H2)	2.76	3.25
Lys^{P312} O	Arg^{58} NH2 (H2)	2.91	2.63
His^{P315} N	Glu^{95} Oε1 (H3)	2.78	3.14
His^{P315} O	Gly^{96} N (H3)	2.79	2.70
Gly^{P319} N	Gly^{96} O (H3)	2.95	2.87

Distances are between peptide atoms and atoms in Fab molecule 1 and Fab molecule 2. These were calculated using the program CONTACSYM written by S. Sheriff. (25).

chain, and peptide residue numbers are preceded by the letters L, H, and P, respectively.

RESULTS AND DISCUSSION

With the exception of the elbow angle (164° for molecule 1 and 177° for molecule 2), both molecules in the asymmetric unit are very similar. Both Fabs show interpretable electron density (Fig. 2) for the first nine residues of the 16-mer MP1 peptide. Peptide residues P311–P316 adopt an extended conformation with backbone torsion angles in the β region of ϕ–ψ space, whereas the last three residues of the epitope (P319–P321) form a turn-like structure stabilized by an intrapeptide hydrogen bond. The peptide is bound in a long groove (≈25.0 Å long, 8–9 Å wide and 8 Å deep) that spans the top surface of the variable region forming a narrow channel between the V_L and V_H domains. The shape of this binding site differs significantly from that found in two other Fab–peptide complexes, where the binding sites form pockets that accommodate β-turns in the bound peptide (13, 23).

The peptide interacts primarily with the hypervariable loops H2, H3, L3, and L1 (Fig. 3), making, on average, 22, 21, 7, and 5 contacts with each, respectively. There are no contacts with H1 in either complex and only one contact with L2 (in molecule 1). In addition to a salt bridge interaction, four hydrogen bonds are made between the peptide and the Fab, involving three peptide and three Fab residues (Table 1). All four hydrogen bonds are made with Fab main chain atoms. On average, the peptide makes a total of 63 contacts with 19 Fab residues (Table 2).

The extent and nature of the Fab–peptide interactions give some insight into the relative importance of each peptide residue in the formation of the complex. Perhaps most striking and unexpected are the interactions made by the side chains of the two isoleucine residues, which in both cases are localized in well-defined pockets formed by Fab 50.1. The side chain of Ile P314 interacts extensively with the Fab residues Trp H47, His H50, Phe H52, and Leu L96, but

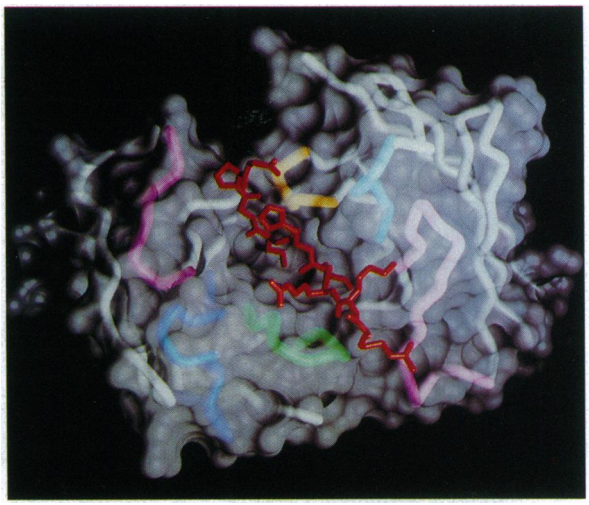

FIG. 3. Antigen binding site of Fab 50.1. The solvent accessible surface around the combining site of Fab 50.1 is shown as a translucent mask over the Cα trace. Shown in red are the nine epitope residues of peptide MP1 ordered in the electron density maps. The complementarity determining region loops are color coded as follows: L1, dark blue; L2, magenta; L3, green; H1, cyan; H2, pink; H3, yellow. The extended groove between V_L and V_H accommodates the bound peptide. The figure was generated with the program MCS using a 1.7 Å probe radius (24).

neither its main chain amide nitrogen nor its carbonyl oxygen is hydrogen bonded to the Fab. Similarly, the specific interactions of Ile P316 with the Fab are mediated exclusively through its side chain, which in this case is sandwiched between Tyr H97 and His L34. The next three residues (Gly P319-Pro P320-Gly P321) form a turn-like structure and constitute the C-terminal end of the epitope. Of these, Gly P319 and Pro P320 also make extensive van der Waals contact with the Fab, interacting with Tyr L49, Ile L55, and Ile H101. The Gly P319 amide is hydrogen bonded to the carbonyl oxygen of Gly H96. This conformation of the bound peptide places the side chains of Ile P314 and Ile P316 on the same side of the peptide (Fig. 4) and along with Gly P319 and Pro P320 form a continuous apolar face that contacts the Fab. The corresponding Fab contact residues, His L34, Leu L46, Ile L55, Leu L96, Trp H47, His H50, Phe H52, Tyr H97, and Ile H101, line the extended groove between the V_L and V_H domains.

As in some other Fab complexes (13, 23, 26), salt bridge formation plays a role in antigen recognition. In this case, Lys P312 interacts with Asp H54 and Asp H56. In addition, an intrapeptide hydrogen bond is formed between the side chain nitrogen of His P315 and the carbonyl oxygen atom of Pro P320. As a result of the histidine side chain folding back on its own peptide backbone, the solvent-exposed surface area

Table 2. van der Waals contact residues in the Fab–peptide complex

Peptide residues	Fab contact residues
Cys^{P311}	
Lys^{P312}	Phe^{H52} (H2), Trp^{H53} (H2), Asp^{H54} (H2), Asp^{H56} (H2), Arg^{H58} (H2)
Arg^{P313}	Phe^{H52} (H2)
Ile^{P314}	Leu^{L96} (L3), Trp^{H47}, His^{H50} (H2), Phe^{H52} (H2)
His^{P315}	Glu^{H95} (H3), Gly^{H96} (H3)
Ile^{P316}	His^{L34} (L1), Tyr^{L36}, Leu^{L46}, Gln^{L89} (L3), Ser^{L91} (L3), Leu^{L96} (L3), Gly^{H96} (H3), Tyr^{H97} (H3)
Gly^{P319}	Leu^{L46}, Tyr^{L49}, Gly^{H96} (H3), Ile^{H101} (H3)
Pro^{P320}	Tyr^{L49}, Ile^{L55} (L2), Ile^{H101} (H3)
Gly^{P321}	

The contacts were evaluated with CONTACSYM (25) with a cutoff of up to 4.11 Å for van der Waals contacts depending on atom type. Complementarity-determining region loops (L1–L3, H1–H3) are defined according to standard convention (21).

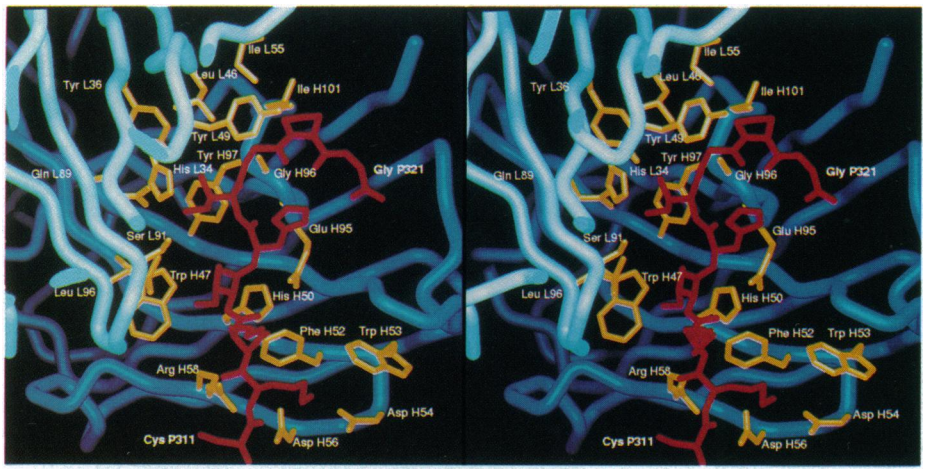

FIG. 4. Stereoview of the interactions between Fab 50.1 and peptide MP1. Fab side chains within van der Waals contact distance of the peptide are shown in yellow. The Cα backbone of the Fab light chain is colored in cyan and the heavy chain is colored in blue. Ile P314 and Ile P316 are clearly buried in well-defined pockets. Also evident are the close contacts of Gly P319 and Pro P320 with the Fab as well as the salt bridges between Lys P312 and Asp H54 and Asp H56.

of the peptide is reduced. However, as His P315 can be replaced by Ala, Asp, Lys, and Pro in amino acid substitution analysis, this intrapeptide hydrogen bond is obviously not an absolute requirement for binding. In other peptide–Fab complexes, the peptide-buried surface area also increases when peptides assume β-turn conformations in the antibody-combining site (13, 23, 27, 28).

The Fab and peptide surface areas buried upon binding are 530 and 475 Å^2, respectively, for molecule 1 and 529 and 480 Å^2 for molecule 2 (Fig. 5). Although these values are comparable to those found in two other Fab–peptide complexes (13, 23), the percentage contributed by the heavy chain (55%) is considerably lower (Fab B13I2, 78%; Fab 17/9, 74%) but similar to those in other Fab–antigen complexes (26). The importance of van der Waals interactions and the apolar nature of the Fab 50.1 binding site is reflected in significantly fewer antibody–antigen hydrogen bonds (4 vs. 10–15) than the peptide complexes of Fab B13I2 or 17/9.

Since an effective antibody-based approach to HIV-1 neutralization depends on the ability of the immune system to recognize a wide range of viral isolates, it is of particular interest to understand the structural basis for type specificity. The relative affinities of different V3 sequences for antibody 50.1 (8) are, in general, consistent with the Fab–peptide interactions observed in the crystal structure. Residues Ile P314, Ile P316, Gly P319, and Pro P320 were found to be an absolute requirement for high-affinity binding. Substitution of one of these residues, as seen in the V3 sequences of the WMJ2 (8), NY5, and RF isolates, leads to a dramatic reduction in binding affinity (unpublished). As discussed above, these residues form several van der Waals contacts with the apolar groove in the Fab surface, and the complementarity of fit appears to severely limit their substitution. Certain V3 sequences, however, contain these four residues but are not recognized by antibody 50.1. Other factors must therefore contribute to isolate specificity.

Recognition of V3 sequences by antibody 50.1 also seems to require the presence of a positively charged residue at position P312 or P313 (8). The canonical MN sequence contains Lys and Arg residues at these positions. Substitution of Lys P312 with Gly or Asn leads to an order of magnitude reduction in antibody binding affinity. This is consistent with the loss of the interactions with Asp H54 and Asp H56. Substitution of Arg P313 leads to an even greater

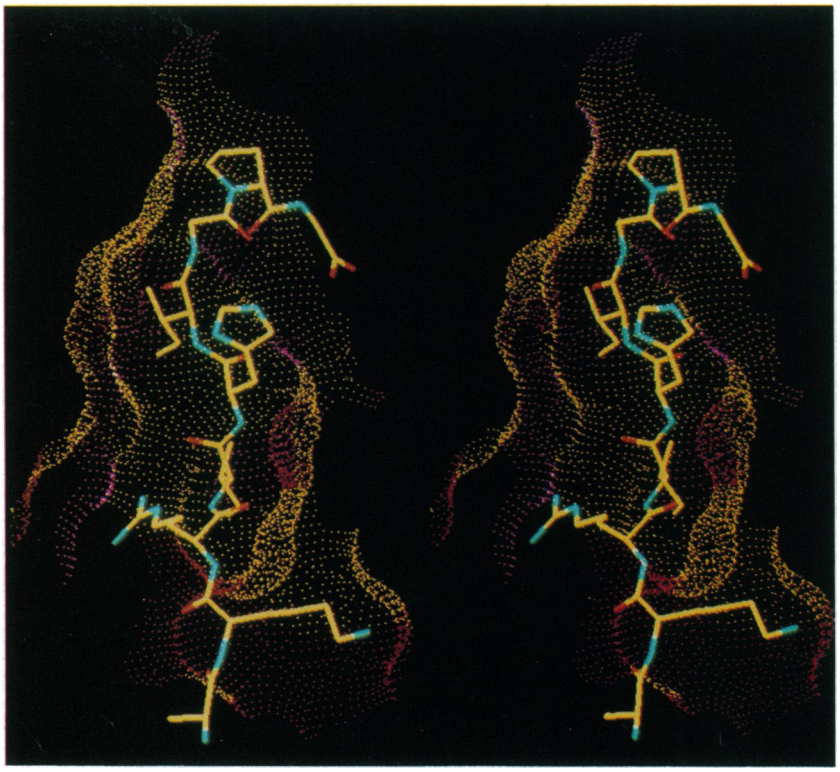

FIG. 5. Stereoview of the Fab 50.1 surface buried upon peptide binding. The solvent accessible surface is color coded in terms of the Fab atom type. Yellow, magenta, and red correspond to nonpolar (carbon), polar noncharged, and polar charged atoms, respectively. Although the majority of the surface is nonpolar, there is a substantial negatively charged region complementary to Lys P312. It is interesting to note that, at the base of both Ile pockets, there are polar noncharged atoms, which may coordinate water in the unliganded Fab. The buried surface was calculated with the program MS (24) using a 1.7 Å probe sphere and standard atomic radii (29).

reduction in binding affinity, but the structural basis for this is not readily apparent.

To better understand the effect of single amino acid changes on antigen recognition by antibody 50.1, an array of peptides, each containing a single substitution in the sequence Lys-Arg-Lys-Arg-Ile-His-Ile-Gly-Pro-Gly-Arg-Ala-Phe-Tyr, was prepared. Residues outside of the determinant Lys-Arg-Ile-His-Ile-Gly-Pro can be replaced by all other amino acids with no effect on binding, but residues within the determinant show little tolerance for substitution. Of particular importance is the observation that any substitution of Ile P314 eliminates binding and that Ile P316 can only tolerate conservative replacement by Val. Similarly, Gly P319 cannot be substituted, consistent with the van der Waals contacts between its Cα atom and the side chain of Tyr L49. Also, Gly P319 has main chain torsional angles ($\phi = 103°$, $\psi = 172°$ for molecule 1 and $\phi = 127°$, $\psi = -161°$ for molecule 2; the epsilon region of ϕ–ψ space) that are usually restricted only to glycine residues or the $i + 1$ residue in a type II′ β-turn. Pro P320, which also makes extensive van der Waals contacts with the Fab, can only be substituted by glutamic acid. In contrast, His P315 can be replaced by all common amino acids except Ala, Asp, Lys, and Pro, consistent with its lack of side chain interaction with the Fab. The importance of the salt bridge interactions between Lys P312 and Fab residues Asp H54 and Asp H56 is confirmed, as substitution of Lys P312 by only Arg or His is tolerated. Arg P313 can be replaced only by Lys or Met, even though this residue is solvent exposed and quite mobile in the structure. The significance of this result is unclear, but the preference for a relatively long, unbranched side chain may in some way favor the bound conformation of the peptide.

The gp120 V3 loop has been predicted to assume a β-strand–type-II β-turn–β-strand–α-helix conformation with the turn at Gly-Pro-Gly-Arg (6). Analysis of 59 nonhomologous proteins shows that Pro, Gly, and Arg are preferred at positions $(i + 1)$, $(i + 2)$, and $(i + 3)$ in type II β-turns (30). NMR analyses of the V3 loop peptides, RP142 and RP70, show evidence for the presence of a significant population of conformations containing a β-turn at the sequence Gly-Pro-Gly-Arg (31). The x-ray structure of the bound peptide partially supports these predictions as residues P311–P316 have dihedral angles in the β region of ϕ–ψ space and Pro P320–Gly P321 have ϕ_2, ψ_2, and ϕ_3 angles in the range found for type II β-turns. However, since the epitope ends in the middle of the proposed turn, firm evidence for the turn and the turn type must await the analysis of other Fab–peptide complexes in which the epitope spans this region. In addition, the peptide main chain preceding the turn interacts with the Fab, suggesting that recognition of the V3 loop does not depend on the formation of a hydrogen bonded β-hairpin-type structure.

CONCLUSION

The binding of Fab 50.1 to the V3 loop appears to be dominated by apolar interactions, primarily involving highly conserved residues Ile P314, Ile P316, Gly P319, and Pro P320. Perhaps the V3 apolar face, which has been exploited by the immune system for antibody binding, forms a structural motif important in mediating virus infectivity via membrane fusion (32). Based on our previous results with two other anti-peptide antibodies (refs. 13 and 23; R.L.S. and I.A.W., unpublished data), we expect that when intact virus or gp120 binds to the antibody, the conformation of the epitope will be similar to that in the Fab–peptide complex described here. By examining a number of Fab–peptide complexes involving different V3 loop epitopes and isolate specificities, we hope to obtain a consensus for the structural features responsible for antibody binding and neutralization to aid in the design of improved peptide-based HIV-1 vaccines and monoclonal antibodies for passive immunotherapy.

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