Conformational Preferences of a Chimeric Peptide HIV-1 Immunogen from the C4−V3 Domains of gp120 Envelope Protein of HIV-1 CAN0A Based on Solution NMR: Comparison to a Related Immunogenic Peptide from HIV-1 RF[†]

Hai M. Vu,[‡] Robert de Lorimier,[‡] M. Anthony Moody,[§] Barton F. Haynes,[§] and Leonard D. Spicer*[,‡,||]

Departments of Biochemistry, Immunology, Medicine, and Radiology, Duke University Medical Center, Durham, North Carolina 27710

Received November 8, 1995; Revised Manuscript Received February 13, 1996[⊗]

ABSTRACT: A critical problem to overcome in HIV vaccine design is the variability among HIV strains. One strategy to solve this problem is the construction of multicomponent immunogens reflective of common HIV motifs. Currently, it is not known if these motifs should be based primarily on amino acid sequence or higher-order structure of the viral proteins or a combination of the two. In this paper, we report NMR-derived solution conformations for a synthetic peptide taken from the C4 and V3 domains of HIV-1 CAN0A gp120 envelope protein. This peptide, designated T1-SP10CAN0(A), is compared to a recently reported C4−V3 peptide, T1-SP10RF(A) from the HIV-1 RF strain [de Lorimier *et al*. (1994) *Biochemistry 33*, 2055−2062], in terms of conformational features and immune responses in mice [Haynes *et al*. (1995) *AIDS Res. Hum. Retroviruses 11*, 211−221]. The T1 segment of 16 amino acids from the gp120 C4 domain is identical in both peptides and exhibits nascent helical character. The SP10 region, taken from the gp120 V3 loop, differs from that of T1-SP10RF(A) in both sequence and conformations. A reverse turn is observed at the conserved GPGX sequence. The rest of the SP10 domain is extended with the exception of the last three residues which show evidence for a helical arrangement. Modeling of the turn region of the T1-SP10CAN0(A) peptide shows exposure of a continuous apolar stretch of side chains similar to that reported in the crystal structure of a V3 peptide from HIV-1 MN complexed with a monoclonal antibody [Rini *et al*. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 6325−6329]. This hydrophobic patch is interrupted by a charged Lys residue in the T1-SP10RF(A) peptide. This observation suggests that the HIV-1 CAN0A and HIV-1 RF C4−V3 peptides can induce widely different anti-HIV antibodies, consistent with immunogenic results.

Recent work defining appropriate targets of anti-HIV[1] antibodies which inactivate or neutralize the virus has centered on the third variable (V3) and fourth constant (C4) domains of HIV-1 envelope glycoprotein gp120 (Palker *et al*., 1988, 1989; Harouse *et al*., 1991; Girard & Sheare, 1993; Letvin, 1993; Moore *et al*., 1993; Thali *et al*., 1993; Haynes *et al*., 1995a) and specific regions of HIV-1 envelope glycoprotein gp41 (Purtscher *et al*., 1994). Since peptide segments corresponding to certain epitopes of pathogens can induce antibodies that recognize original intact antigens and neutralize the virus (Lerner, 1984), Haynes *et al*. (1995a) have synthesized four chimeric peptides, each of which includes segments taken from the gp120 C4 domain and from the gp120 V3 loops of four HIV-1 variants. The two peptides under consideration here are C4−V3 CAN0A, whose primary sequence is KQIINMWQEVGKAMYATR-PHNNTRKSIHMGPGKAFYTTG, and C4−V3 RF, whose primary sequence is KQIINMWQEVGKAMYATRPNNN-TRKSITKGPGRVIYATG.

These peptides are of interest in that epitopes of the gp120 V3 region of the C4−V3 peptides induce potent anti-HIV neutralizing antibodies that inhibit the growth of HIV in T cell lines *in vitro* (Palker *et al*., 1989; Haynes *et al*., 1995a) and the C4 T helper region of the peptides activates peptide-primed T cells that recognize native HIV gp120 (Palker *et al*., 1989; Hart *et al*., 1990). The gp120 V3 segment of C4−V3 peptides also contains an HLA class I restricted cytotoxic T lymphocyte (CTL) epitope restricted by HLA-B7 (Safrit *et al*., 1994). Moreover, these four C4−V3 peptides are currently being used as a multicomponent (polyvalent) immunogen to boost anti-HIV immune responses in HLA-B7-typed, HIV-infected patients (Haynes, 1995b). Recent studies of this polyvalent HIV envelope C4−V3 peptide mixture in mice demonstrated that the V3 sequences of each C4−V3 peptide had separate and distinct immunogenic properties in that HIV RF and HIV CAN0A V3 peptides

[†] Supported in part by National Institutes of Health Grants GM 41829 (L.D.S.) and AI35351 (B.F.H.) and the Department of Defense, Army (DAMD 17-94-J-4467). H.M.V. and R.d.L. were supported by NIH Training Fellowships AI07392-06 and AI07217, respectively. B.F.H. is a Carter Wallace Fellow in Retroviral Research. The Duke NMR Center was established with partial support from the NIH, NSF, and North Carolina Biotechnology Center.

* Address correspondence to Leonard D. Spicer, Department of Biochemistry, Box 3711, Duke University Medical Center, Durham, NC 27710.

[‡] Department of Biochemistry.

[§] Departments of Immunology and Medicine.

[||] Department of Radiology.

[⊗] Abstract published in *Advance ACS Abstracts,* April 1, 1996.

[1] Abbreviations: HIV, human immunodeficiency virus; gp120, 120 kDa surface glycoprotein; gp41, 41 kDa transmembrane glycoprotein; C4, fourth constant domain of gp120; V3, third variable domain of gp120; C4−V3 peptide, synthetic 39-mer that begins with 16 residues from the C4 domain and ends with 23 residues from the V3 domain of HIV gp120; CTL, cytotoxic T lymphocytes; HLA, human leukocyte-associated antigen; CAN0A, T1-SP10CAN0(A); RF, T1-SP10RF(A); NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy; TOCSY, total correlation spectroscopy; DQF-COSY, double-quantum-filtered correlation spectroscopy.

0006-2960/96/0435-5158$12.00/0

induce different anti-HIV antibody responses (Haynes *et al*., 1995a). These data prompted us to undertake the analysis of the solution conformations of these C4−V3 peptides, using high-field NMR spectroscopy, in order to explore the contribution of structural features to their differential antigenicities.

Nuclear magnetic resonance is an effective method of studying conformational propensity of small immunogenic peptides in solution (Dyson & Wright, 1991, 1995; de Lorimier & Spicer, 1994). Thus, a nonapeptide derived from influenza virus haemagglutinin was shown to adopt a type II β turn in water (Dyson *et al*., 1985), whereas preference for helical conformations in an aqueous solution was demonstrated for a 22-amino acid T cell-stimulating peptide of sperm whale myoglobin (Waltho *et al*., 1989). In recent years, HIV-1 immunogenic peptides have become a subject of interest in this field. A stable α helix was reported for a segment of 25 residues from HIV-1 Tat that constituted the RNA-binding domain (Mujeeb *et al*., 1994). Dyson *et al*. (1992) observed helical turns in a 23-mer derived from a transmembrane protein of simian immunodeficiency virus of rhesus macaque origin.

Solution conformations of synthetic peptides corresponding to the major neutralizing determinants of various HIV-1 strains have also been reported. In one case, a sequence of 24 amino acids taken from the V3 region of the HIV-1 IIIB isolate was shown by two-dimensional NMR to exist in a nascent helical conformation in aqueous solution and as a more stable helix in trifluoroethanol (Zvi *et al*., 1992). Others have seen evidence for reverse β turns at the tips of the V3 loop peptides from HIV-1 MN gp120 (Chandrasekhar *et al*., 1991) and HIV-1 RF gp120 (de Lorimier *et al*., 1994). These turns were also observed by X-ray crystallography in HIV-1 MN V3 peptides bound to their antibodies (Rini *et al*., 1993; Ghiara *et al*., 1994). In addition, initial efforts to map a specific antibody binding epitope from an HIV-1 IIIB V3 peptide complex using NMR have recently been reported (Zvi *et al*., 1995). In order to identify conformational motifs that may confer immunogenic specificity, we describe here detailed studies of solution conformational preferences for a C4−V3 peptide, T1-SP10CAN0(A) (C4−V3 CAN0A), for comparison with a previously reported C4−V3 peptide, T1-SP10RF(A) (C4−V3 RF) (de Lorimier *et al*., 1994).

MATERIALS AND METHODS

Peptide Synthesis and Purification. The T1 peptide is a T helper epitope from the C4 HIV gp120 region, and the SP10 segment contains CTL, T helper, and B cell neutralizing antibody determinants from the gp120 V3 loop region (Cease *et al*., 1987; Palker *et al*., 1989; Takahashi *et al*., 1992). The C4−V3 CAN0A peptide was synthesized by the FMOC method on an ABI 431A peptide synthesizer, consistent with earlier reported methods (de Lorimier *et al*., 1994; Haynes *et al*., 1995a). The end product was N-terminal amine and C-terminal acid. High-performance reversed phase liquid chromatography was used to purify the product by running a gradient of 0.1% trifluoroacetic acid (TFA) in H_2O and 0.08% TFA in CH_3CN through a Vydac C18 column. The molecular weight of the purified peptide was determined by electrospray mass spectroscopy to be 4463.51 (calculated MW is 4464.19).

NMR Experiments. Peptide solutions of 4 mM concentration were prepared in 90% H_2O and 10% D_2O (pH 3.95). All spectra were obtained on a Varian Unity 500 MHz spectrometer at 5 °C with a 5 mm inverse probe optimized for proton detection. Two-dimensional proton double-quantum-filtered correlation spectra, DQF-COSY (Piantini *et al*., 1982; Rance *et al*., 1983), total correlation spectra, TOCSY (Bax & Davis, 1985), and nuclear Overhauser effect spectra, NOESY (Jeener *et al*., 1979), were acquired with mixing times of 60 and 85 ms for the TOCSY and 300 ms for the NOESY experiments. The water peak was suppressed by presaturation during the relaxation delay and the mixing period. For the TOCSY experiment, 256 free induction decays (64 scans each) of 1024 complex points per increment were recorded (512 FIDs for NOESY) in a spectral width of 10 ppm in each dimension. Temperature-shift coefficients of 35 amide protons were obtained from seven TOCSY spectra (60 ms mixing time) over the range of 278−296 K using a linear least squares method to fit the temperature dependence of the measured chemical shifts.

Data Analysis. Felix 2.1 (Biosym Technologies Inc.) was used to process data on an SGI Indigo workstation. Time domain data in the directly acquired dimension were zero-filled to 2048 points, phase-shifted by 90° (75° for NOESY), and multiplied by a sinebell-squared window function before Fourier transformation. Zero- and first-order phase adjustments and a polynomial baseline correction were applied to the F2 dimension before the second transformation. Spectra were referenced against the methyl protons of the sodium salt of 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid which were set at 0 ppm.

Resonance assignments were analyzed using standard methods (Wüthrich, 1986). Proton resonances were assigned to their spin systems using scalar-coupling data from the DQF-COSY and TOCSY spectra. The sequential arrangements of the amino acids were in turn sorted out by NOESY spectra. The patterns of short- and medium-range NOE connectivities were used to determine conformations (Wüthrich, 1986; Dyson & Wright, 1991).

Molecular Modeling. Insight II (Biosym Technologies Inc.) was used to create CPK models for segments from the tip of the V3 loop: RKSIHMGPGKAFY for C4−V3 CAN0A and RKSITKGPGRVIY for C4−V3 RF. The conformational features and secondary structural elements represented by the NOE correlation patterns for short- and medium-range NOESY peaks were used to construct the backbone configurations. For C4−V3 CAN0A, residues RKSIHM and AFY were assigned with an extended β conformation, whereas a type II β turn was defined for the sequence GPGK. Similarly, RKSITK and VIY in C4−V3 RF were assigned with an extended β conformation, while GPGR was given a type I β turn. The coordinates of the initial conformers were exported to Discover 3.1 (Biosym Technologies Inc.), and parameters from a consistent valence force field were assigned to all atoms. Potential energies were calculated by a quadratic function and minimized first by the steepest descent and then by the Newton−Raphson algorithm. After the first 100 iterations, cross terms and Morse potentials were included in the calculation. Nonbond energy cutoffs for Coulombic and van der Waals interactions were 9.50 Å, and the dielectric constant was set to 1. The energy-minimized structure was used as a starting point to sample other conformational space by molecular dynamics (Discover 3.1). The system was equilibrated at a target temperature of 298 K in the initialization phase. An NVT

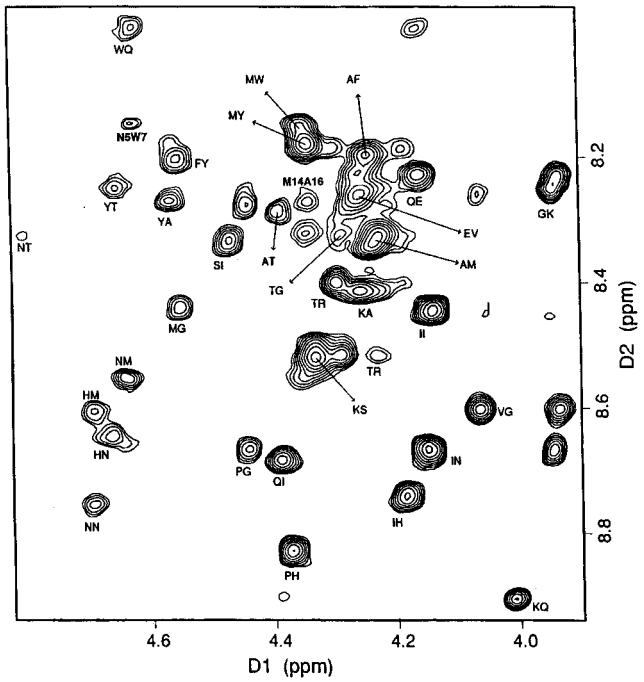

FIGURE 1: NOESY spectrum (300 ms mixing time) of the fingerprint region of T1-SP10 C4−V3 peptide from HIV CAN0A gp120. Interresidue correlations between the α protons of residues *i* and the amide protons of residues *i*+1 are labeled. Two dαN (*i*, *i*+2) NOEs, Asn[5]−Trp[7] and Met[14]−Ala[16], were also identified as additional evidence for a nascent helical conformation.

ensemble (constant volume, constant temperature) with a direct velocity scaling method was used to control the temperature. Dynamic trajectories were calculated by the velocity−Verlet algorithm every 1 fs for 300 ps, during which period energy minimization was performed at 5 ps intervals. The reported models were those of the molecular dynamics lowest-energy conformers after they were subjected to a final energy minimization.

RESULTS

Peptide Resonance Assignment and Overall Conformation. The assignments of the proton chemical shifts for C4−V3 CAN0A are available as supporting information. C4−V3 RF assignments have previously been reported (de Lorimier *et al*., 1994). Cross-peaks between the main chain amide and α-carbon protons in C4−V3 CAN0A are well-dispersed in the fingerprint region of the COSY and TOCSY spectra (not shown) and the NOESY spectrum as shown in Figure 1. Sequential assignments were made on the basis of NOEs between the α-carbon protons of residue *i* and the amide protons of residue *i*+1 [dαN (*i*, *i*+1)] and were confirmed by dβN (*i*, *i*+1) and dNN (*i*, *i*+1) NOEs.

The N-terminal C4 gp120 region, termed T1, of C4−V3 CAN0A is identical in sequence to the C4 (T1) sequence in the C4−V3 peptide from HIV RF (de Lorimier *et al*., 1994), and the corresponding proton chemical shifts for this 16-amino acid segment are nearly the same in the two C4−V3 peptides except for Ala[16]. This residue is located at the junction of the T1 region with the gp120 V3 domain, termed SP10, which starts with Thr[17] in CAN0A. In C4−V3 RF, the first residue in the SP10 domain is Cys[17] which has been deleted from the T1-SP10 junction of the C4−V3 CAN0A peptide to avoid the potential for intermolecular cross-linking. This cysteine deletion did not affect the immunogenicities of the peptides (Palker *et al*., 1989; Haynes *et al*., 1995). Chemical shift values for main chain protons of residues 17−39 (17−40 for C4−V3 RF) in the SP10 domains of the two peptides vary considerably as expected due to differences in the amino acid sequences, and as illustrated below, NOE patterns show corresponding differences in preferred conformers in these regions. Analysis of the individual chemical shifts according to Wishart *et al*. (1991, 1992, 1994) suggests that the C4−V3 CAN0A peptide does not satisfy the criteria for stable secondary structures. As shown in Figure 2, C4−V3 CAN0A exhibits alternating and generally small shift differences from the residue specific average values determined from a large number of proteins. This implies that the peptide assumes an ensemble of conformations that are mostly random, a result similar to that reported earlier for the C4−V3 RF peptide (de Lorimier *et al*., 1994).

Conformation of the HIV CAN0A gp120 C4 Region, T1. The NOE connectivities for the C4−V3 CAN0A peptide in aqueous solution at 5 °C and pH 4 are summarized in Figure 3. Although long-range proton−proton NOEs are not detected, short- and medium-range interactions are observed which can be used to identify peptide conformational features of the two domains. The presence of strong dαN (*i*, *i*+1) NOEs and the absence of dNN (*i*, *i*+1) as well as other medium-range NOEs between the same residues are indicative of backbone dihedral angles in the β region of ϕ,ψ space (Dyson *et al*., 1988a) and are diagnostic of an extended conformation (Dyson & Wright, 1991). The first three residues of the T1 region in the C4−V3 CAN0A peptide, Lys[1]−Ile[3] , display this pattern as shown in Figure 3. The next 13 residues from Ile[4] to Ala[16] show NOEs that are characteristic of a nascent helix (Dyson *et al*., 1988a, 1991). These include two uninterrupted stretches of medium-strength dNN (*i*, *i*+1) NOEs illustrated by the data in Figure 4 and the dαN (*i*, *i*+1) NOEs shown in Figure 1. Two other types of medium-range NOEs that are signatures of a nascent helix, dNN (*i*, *i*+2) and dαN (*i*, *i*+2) (Dyson *et al*., 1988b), are also identified in this region of T1. Thus, dNN (*i*, *i*+2) NOEs are observed for Ile[4]−Met[6], Asn[5]−Trp[7], Met[6]−Gln[8], Gln[8]−Val[10], and Gly[11]−Ala[13] as shown for the Ile[4]−Met[6] and Gly[11]−Ala[13] pairs in Figure 5A. In addition, dαN (*i*, *i*+2) NOEs are detected for Ile[4]−Met[6], Asn[5]−Trp[7], Glu[9]−Gly[11], and Met[14]−Ala[16], illustrated by the Asn[5]−Trp[7] and Met[14]−Ala[16] NOEs in Figure 1. Longer-range dαN (*i*, *i*+3) and dαβ (*i*, *i*+3) NOEs which are indicative of full helical turns may also be present but could not be unambiguously assigned in this region. Also in this region, protection of the Gln[8] backbone amide proton is suggested by an unusually low temperature-shift coefficient of 2.74×10^{-3} ppm/K. A similar low temperature-shift coefficient was observed at Gln[8] in C4−V3 RF (de Lorimier *et al*., 1994) and is suggestive of hydrogen bond formation (Wright *et al*., 1988).

Evidence for some cis conformations is also detected, mainly in the carboxyl end of the T1 region, as indicated by interresidual correlations between sequential α protons of Val[10] and Gly[11], Gly[11] and Lys[12], and Ala[13] and Met[14]. The NOEs indicating cis peptide bonds are weak, suggesting that the trans conformation dominates at these peptide bonds. Similar evidence for cis bonds between Ile[4] and Asn[5], Val[10] and Gly[11], and Gly[11] and Lys[12] was previously reported in the T1 region of the C4−V3 RF peptide (de Lorimier *et al*., 1994). A kink in the T1 region of C4−V3 CAN0A which is not detected in C4−V3 RF is suggested by a dβN (*i*, *i*+2)

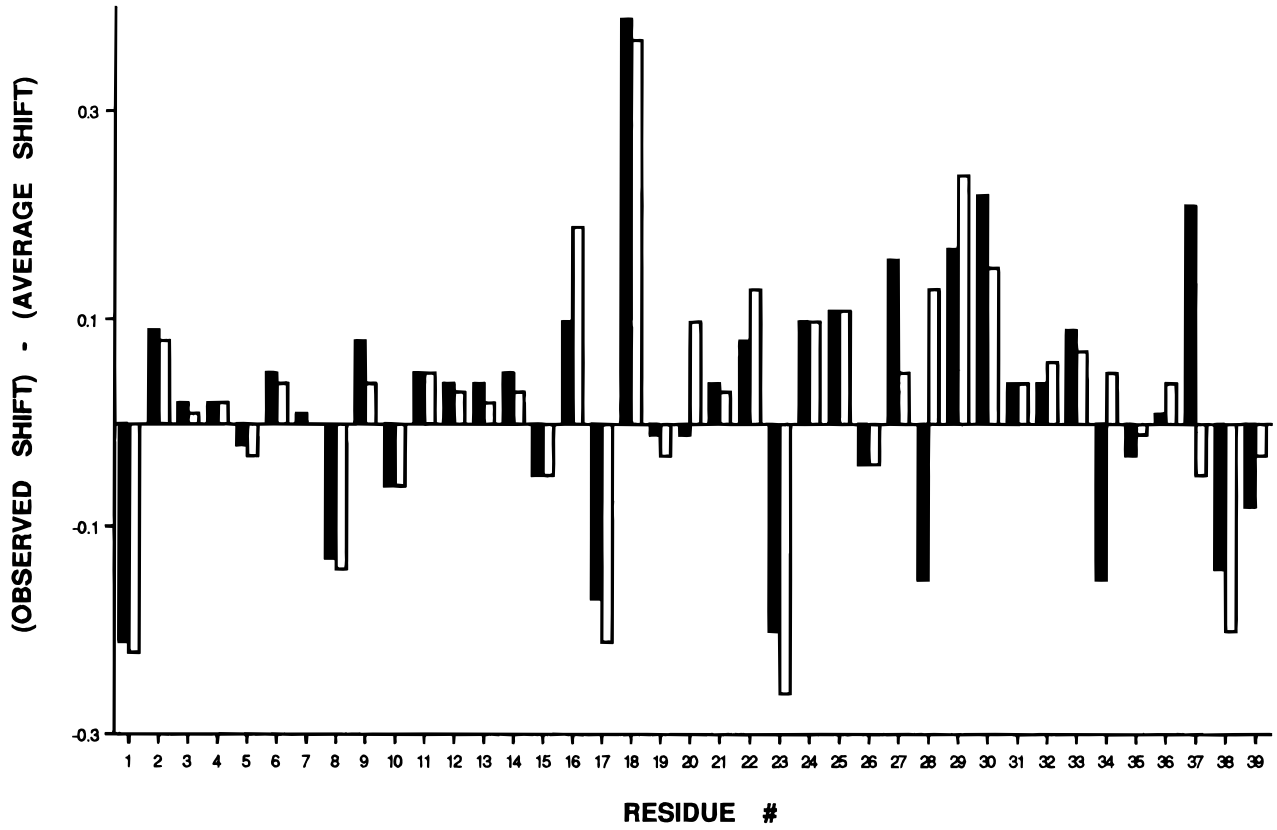

FIGURE 2: Deviation between the observed α proton chemical shifts of T1-SP10 C4−V3 peptide from HIV CAN0A gp120 and the average chemical shifts of the corresponding amino acids whose values were taken from Wishart *et al*. (1991). The values reported for the RF peptide are also included for comparison, but the chemical shift of Cys^{17} was omitted so that corresponding residues in the two SP10 regions can be aligned. Solid bars denote T1-SP10 C4−V3 peptide from HIV RF gp120, and open bars are for T1-SP10 C4−V3 peptide from HIV CAN0A gp120.

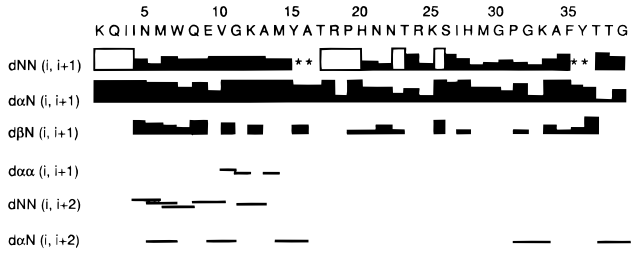

FIGURE 3: NOE connectivity patterns of T1-SP10 C4−V3 peptide from HIV CAN0A gp120. Open boxes denote unambiguously absent NOEs; black boxes and their heights show the presence of NOEs and their relative intensities. An asterisk signifies ambiguity of NOE assignment due to peak overlap.

NOE (Osterhout *et al*., 1989) between a β proton of Trp^7 and the amide proton of Glu^9.

Conformation of the HIV CAN0A gp120 V3 Loop Region, SP10. The N-terminal sequence of the SP10 region of C4−V3 CAN0A tends to exhibit an extended β conformation as shown by the absence of dNN (i, i+1) and presence of dαN (i, i+1) connectivities for residues Thr^{17}−Pro^{19}, Asn^{22}, and Lys^{25} (Figure 3). In addition, all measurable vicinal spin−spin coupling constants between the amide and α proton in this region range from 9 to 10 Hz which further imply a β conformation (Wagner *et al*., 1986). However, the most prominent conformational feature over the full SP10 domain is a reverse β turn comprised of residues Gly^{30}−Lys^{33}. This sequence is part of the V3 neutralizing determinant in gp120 of HIV-1. Previous reports have both predicted reverse turns in this region (La Rosa *et al*., 1990) and presented evidence for turn structures within fragments of corresponding V3

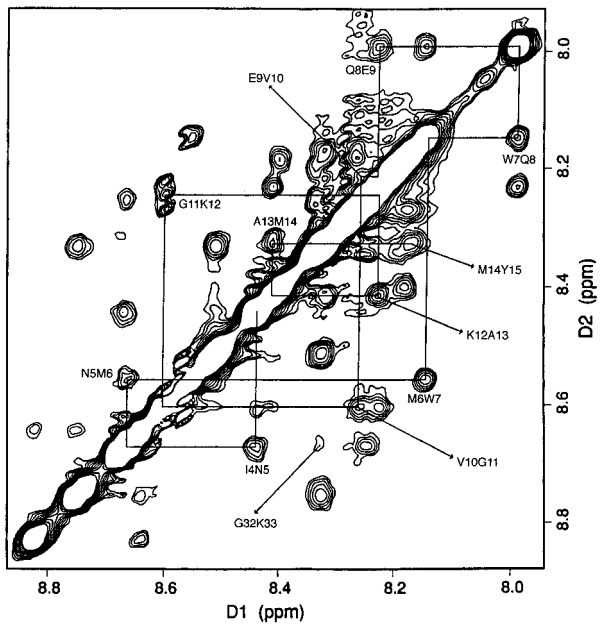

FIGURE 4: dNN (i, i+1) NOEs in the T1 region of T1-SP10 C4−V3 peptide from HIV CAN0A gp120. The sequential connectivity trace begins with Ile^4 and ends at Tyr^{15}. Also shown is a dNN (i, i+1) peak between Gly^{32} and Lys^{33} in the SP10 portion of the peptide. The spectrum was obtained with a 300 ms mixing time.

loop sequences derived from the HIV MN (Chandrasekhar *et al*., 1991; Rini *et al*., 1993; Ghiara *et al*., 1994), HIV IIIB/LAI (Zvi *et al*., 1992), and HIV RF (de Lorimier *et al*., 1994) HIV-1 strains. Within this sequence of the CAN0A peptide, the following connectivities are detected: a weak dNN (i,

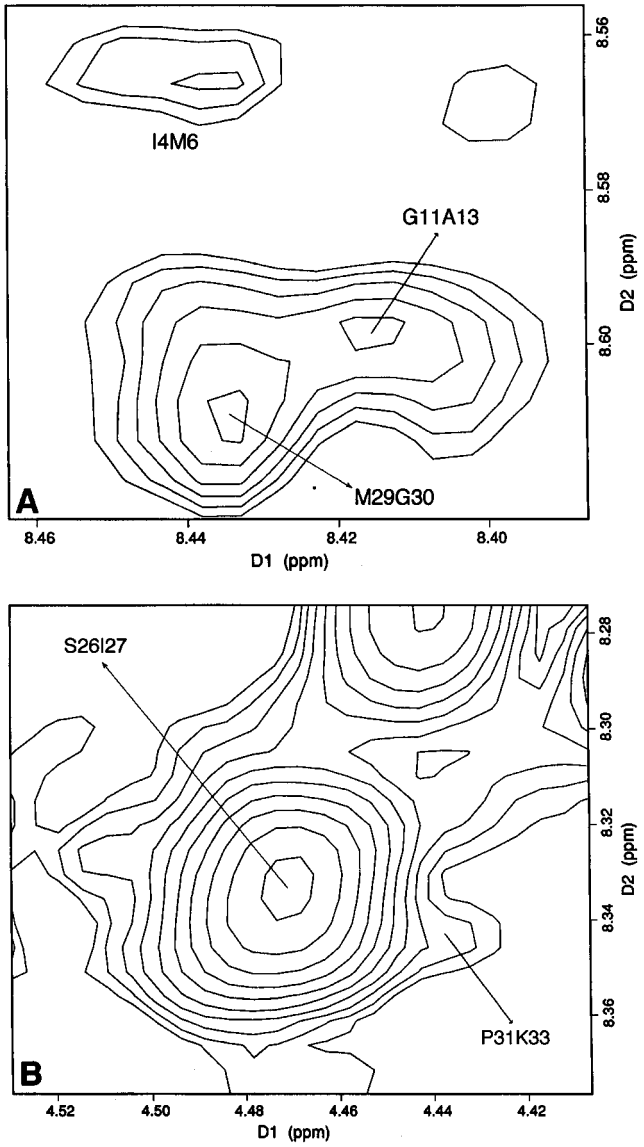

FIGURE 5: NOESY spectrum (300 ms mixing time) showing medium-range NOEs. (A) Two dNN (*i*, *i*+2) cross-peaks that arise from Ile[4]−Met[6] and Gly[11]−Ala[13] correlations which are indicative of a nascent helical conformation. (B) The dαN (*i*, *i*+2) NOE between Pro[31] and Lys[33] is shown here as part of the evidence for a type II β turn at the tip of the V3 loop.

i+1) NOE between the third (Gly[32]) and fourth (Lys[33]) residues of the turn shown in Figure 4, a medium to strong dαN (*i*, *i*+1) NOE between the second (Pro[31]) and third (Gly[32]) residues shown in Figure 1, and a weak dαN (*i*, *i*+2) NOE between the second and fourth residues (Pro[31]−Lys[33]) shown in Figure 5B. This specific combination of NOEs is diagnostic of a β turn (Dyson *et al*., 1988a). The nature of the (*i*, *i*+2) NOE between the second and fourth residues of a β turn indicates its type. In this case, the fact that a dαN (*i*, *i*+2) NOE is observed together with the unambiguous absence of a dδN (*i*, *i*+2) between Pro[31] and Lys[33] suggests that the sequence GPGK comprises a type II β turn. Immediately C-terminal to this turn is an extended region from Phe[35] to Thr [37] which is followed by a nascent helical or turn conformation at the C-terminal end of C4−V3 CAN0A as suggested by a weak dαN (*i*, *i*+2) NOE between Thr[37] and Gly[39]. The overall composite sequence of observed conformations for the T1-SP10CAN0(A) peptide is illustrated in Figure 6 along with the corresponding preferred conformations reported earlier for the C4−V3 RF peptide (de Lorimier *et al*., 1994).

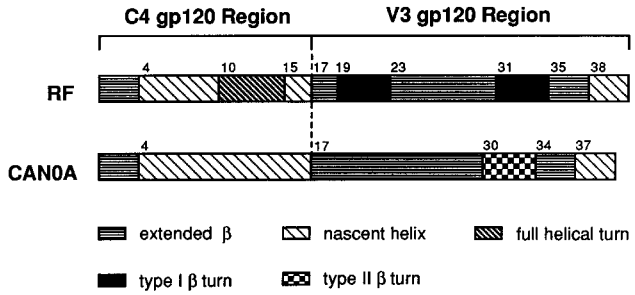

FIGURE 6: Secondary structural conformer comparison between the T1-SP10 C4−V3 peptides from HIV CAN0A and RF gp120. Numbers indicate the first amino acid residues from a specified conformational region.

DISCUSSION

Previous studies have shown that the chimeric C4−V3 peptides designated T1-SP10 induce anti-HIV neutralizing antibodies for HIV grown in T cell lines and induce anti-HIV T helper lymphocytes and cytotoxic T lymphocytes (Palker *et al*., 1989). These peptides have also recently been characterized for their effectiveness as a multicomponent, polyvalent immunogen (Haynes *et al*., 1995a). Results indicate differential antigenicities from each component with respect to priming and boosting responses as well as cross-reactivity characteristics toward antibody production aimed at specific HIV-1 strains. In particular, the T1-SP10CAN0-(A) and T1-SP10MN(A) C4−V3 peptides appear to be more broadly reactive than either T1-SP10RF(A) or T1-SP10EV91-(A) peptides. Our interest in studying conformational characteristics for these peptides represents an initial effort to establish likely conformations for related immunogenic sequences from C4−V3 peptides and to compare conformational features between particular peptides in this series to gain insight into structural contributions to specific immunogenic behavior. We have previously reported a conformational analysis of the T1-SP10RF(A) peptide (de Lorimier *et al*., 1994), to which the results of this study of T1-SP10CAN0(A) are compared and discussed below in the context of immunogenic differences between the two peptides (Haynes *et al*., 1995a).

The T1 segments of the C4−V3 CAN0A and C4−V3 RF peptides are taken from the fourth constant domain of HIV-1 gp120 which is a component of the HIV gp120 CD4 binding site (Capon & Ward, 1991) and also is a potent T helper epitope (Cease *et al*., 1987; Palker *et al*., 1989). The T1 regions in the C4−V3 peptides from HIV CAN0A and RF are identical in primary amino acid sequence and are shown here to be similar in their solution conformations. They both exhibit evidence for nascent helix conformations as indicated by the presence of dNN (*i*, *i*+2) and dαN (*i*, *i*+2) NOEs (Figure 3; de Lorimier *et al*., 1994). The observation of dαN (*i*, *i*+3) and dαβ (*i*, *i*+3) NOEs within this region of C4−V3 RF indicates a full helical turn conformation from Val[10] to Met[14]. Such NOEs could not be unambiguously assigned in the case of C4−V3 CAN0A due to the degeneracy of chemical shift values.

The SP10 portions of the C4−V3 CAN0A and C4−V3 RF peptides are derived from the V3 loop regions of gp120 in HIV-1 CAN0A and HIV-1 RF, respectively. This loop

is highly variable in sequence in various strains of HIV-1 gp120 and consists of 35 amino acids flanked by two cysteine residues that form a disulfide bridge (Leonard *et al*., 1990). It contains a potent neutralizing determinant of gp120 (Javaherian *et al*., 1989; Zwart *et al*., 1991). Consequently, it has been a target for numerous attempts at designing vaccines against HIV-1 (Emini *et al*., 1992; Haynes *et al*., 1995a). Parallel efforts have been made to elucidate structural features of this region to better understand the basis for its reactivity. The predicted structural elements of the V3 loop of HIV-1 gp120 based on a neural network approach is Cys-β strand-reverse β turn-β strand-α helix-Cys in that order (La Rosa *et al*., 1990). According to this prediction, the reverse β turn is located roughly at the center of the loop and includes a highly conserved Gly-Pro-Gly tripeptide. X-ray crystal structures of the corresponding GPGR sequence from the HIV-1 MN isolate when bound to Fab fragments of antibodies, 50.1 (Rini *et al*., 1993) and 59.1 (Ghiara *et al*., 1994), indicated a type II β turn. Nuclear magnetic resonance studies of free peptides derived from this region of the HIV-1 MN, IIIB, and RF strains in aqueous solution also suggested a reverse β turn within this sequence at the tip of the V3 loop (Chandrasekhar *et al*., 1991; Zvi *et al*., 1992; de Lorimier *et al*., 1994).

We report here that conformational preference for a reverse β turn also exists in the V3 loop of HIV-1 CAN0A gp120. The corresponding GPGK sequence in the SP10 portion of the C4−V3 CAN0A peptide tends to form a type II β turn from Gly^{30} to Lys^{33} as indicated by a combination of dαN (Pro^{31}−Gly^{32}), dαN (Pro^{31}−Lys^{33}), and dNN (Gly^{32}−Lys^{33}) NOEs. For HIV RF, due to a dδN (*i*, *i*+1) NOE between Pro^{32} and Gly^{33} (second and third residues of the turn), de Lorimier *et al*. (1994) suggested a type I β turn for the sequence GPGR. Thus, while the preferred type of reverse turn and therefore the ϕ and ψ main chain bond angles may vary in the solution conformers observed for this domain of different HIV-1 strains, it appears that both free peptides tend to form reverse turn conformations consistent with the general topology found in the HIV MN V3 loop peptide complexed with Mab 50.1 (Rini *et al*., 1993). This turn conformation may be important for direct antibody and B cell receptor recognition and also may provide an effective method of presenting hydrophobic residues required for interaction and binding (Rini *et al*., 1993).

An extended β strand conformation N-terminal to the reverse turn is observed for C4−V3 CAN0A from Thr^{17} to Met^{29} as illustrated in Figure 6. This segment of the V3 loop of C4−V3 RF, which is also generally extended in conformation, exhibits evidence for an additional type I β turn within residues Arg^{19}-Pro^{20}-Asn^{21}-Asn^{22}. The asparagine residue at position 21, which is correlated with a high propensity for β turns (Dyson *et al*., 1988a), is replaced by histidine in C4−V3 CAN0A (TRPNNN in C4−V3 RF versus TRPHNN in C4−V3 CAN0A). This substitution may contribute to the abrogation of a reverse β turn at this position and will likely have influence on the immunogenicity of this B7-restricted CTL epitope of HIV gp120 (Safrit *et al*., 1994). The C-termini of the SP10 regions of both C4−V3 CAN0A and C4−V3 RF show NOEs characteristic of an extended β conformation immediately following the central turn and end with a three-amino acid stretch of nascent helix.

Taken together, the NMR-derived solution conformations of the C4−V3 CAN0A and C4−V3 RF peptides show similarities in the C4 regions but differ considerably in the V3, SP10 peptide segments. The solution conformation of the SP10 portion of C4−V3 CAN0A V3 loop includes an extended β segment from Thr^{17} to Met^{29}, a type II β turn from Gly^{30} to Lys^{33}, a second extended structure from Ala^{34} to Tyr^{36}, and a nascent helix from Thr^{37} to Gly^{39}. By comparison, the C4−V3 RF peptide exhibits two sequences where type I β turns are preferred conformations, the second of which is some nine residues N-terminal from the central turn (de Lorimier *et al*., 1994). Furthermore, the V3 portion of the C4−V3 RF peptide shows preferred conformations in the sequence SIT that are not detected in the corresponding region of C4−V3 CAN0A.

Initial studies of the immunological activities of C4−V3 RF and C4−V3 CAN0A peptides have determined the ability of each peptide to prime and/or boost antibody responses to itself and other peptides in the polyvalent mixture in Balb/c mice (Haynes *et al*., 1995a). In addition, [^{3}H]thymidine incorporation into splenocytes from immunized mice was assayed to evaluate the relative proliferative responses induced by each peptide *in vitro*. Results indicated that sera from mice immunized with the C4−V3 CAN0A peptide induced an anti-HIV CAN0A antibody titer that was 20 times greater than that induced by the C4−V3 RF peptide for corresponding anti-HIV RF antibodies. Mice primed and boosted with C4−V3 CAN0A peptide also stimulated significant antibody production directed toward C4−V3 RF and other C4−V3 HIV peptides, whereas the C4−V3 RF peptide immunogen alone produced antibodies almost exclusively to HIV RF and not to HIV CAN0A (Haynes *et al*., 1995a). The fact that the V3 region of RF peptide exhibits conformations not detected in CAN0A or MN (unpublished data; Chandrasekhar *et al*., 1991) suggests that the immunogenic specificity of RF arises from these conformational features. Studies are underway to test this hypothesis.

It seems unlikely that the T1 regions alone contribute differently to the overall immunogenicities of the T1-SP10 peptides since their primary amino acid sequences are identical and the NMR-detected conformational features of this segment are very similar between the C4−V3 CAN0A and C4−V3 RF peptides (de Lorimier *et al*., 1994). It is possible however that both sequence and conformational variations of the two SP10 V3 segments of the C4−V3 peptides determine their differential immunogenic behavior and are responsible for different levels of antibody production and T cell proliferation induced by HIV CAN0A and RF C4−V3 peptides. Indeed, it has been suggested that the type specificities of the antibodies generated to various HIV strains are defined by residues flanking the reverse β turn in the V3 loop that vary from one strain to another (Meloen *et al*., 1989).

To better understand the interplay of primary and secondary structural elements of C4−V3 HIV peptides, we have constructed molecular models of the antibody-binding sites from the SP10 domains of CAN0A and RF C4−V3 peptides on the basis of NMR results as outlined above. The models were subjected to molecular dynamics for a total of 300 ps followed by energy minimization. The space-filling CPK models generated for the tips of the V3 loops of HIV CAN0A and RF are depicted in Figure 7. By developing the conformational model for a single short domain of the peptide, we avoid any implication that other regions of the

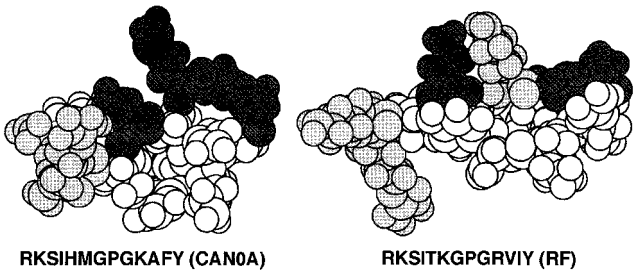

FIGURE 7: CPK models of the tips of the V3 loops of CAN0A and RF. Important basic residues are shown in gray, and the nonpolar residues of the apolar patches are darkened. Note the protrusion of a positively charged lysine residue in the middle of the hydophobic region in RF which differentiates it from CAN0A.

molecule have time-correlated conformers. Indeed, for largely unstructured biopolymers, the conformational elements of independent domains experimentally observed in solution NMR studies may not be present at the same time in the same molecules. Crystallographic studies of the cocrystal of the comparable V3 segment (16 residues) of gp120 from the HIV-1 MN strain complexed with its monoclonal antibody 50.1 show extensive contacts between the antigen-binding pocket of Fab 50.1 and the side chains of residues located immediately N-terminal to the tip of the V3 loop (Rini *et al*., 1993). For MN, these residues are Ile (P314), Ile (P316), Gly (P319), and Pro (320) and appear to form a continuous hydrophobic surface that not only interacts with the Fab fragment but also is required for high-affinity binding in mutagenesis studies. In the C4−V3 CAN0A and C4−V3 RF peptides compared here, the amino acids corresponding to these four hydrophobic residues are I, M, G, and P and I, K, G, and P, respectively, shown darkened in Figure 7. Like the X-ray crystal structure of the antibody-bound MN sequence, C4−V3 CAN0A exhibits an apolar patch that includes residues Ile^{27}, Met^{29}, Gly^{30}, and Pro^{31}. Our models show that the presence of methionine in place of isoleucine does not alter the geometry of the apolar patch in that it appears to be flat, much like that in the crystal structure of MN (Figure 7; Rini *et al*., 1993). For the C4−V3 RF peptide, however, this extended stretch of amino acids is interrupted by a positively charged residue, a lysine at the second position of the patch (gray in Figure 7). Thus, the V3 region of HIV CAN0A, but not of RF, may contain a structural motif similar to that of HIV MN. As mentioned above, Haynes *et al*. (1995a) demonstrated that, while HIV MN and CAN0A are immunologically similar to each other in that they both induce cross-reactive antibodies, HIV RF is very type-specific in antibody responses induced. That is, whereas antibodies raised against C4−V3 RF peptide do not recognize antigens from other HIV strains, those raised against C4−V3 CAN0A or C4−V3 MN peptides can cross-react to each other and to the HIV RF V3 loop as well. From these models, we therefore suggest that the selectivity and restriction to cross-reactivity of anti-HIV RF V3 loop antibodies may be due to the presence of the positively charged lysine residue, the side chain of which is conformationally presented in the center of the hydrophobic region. This feature likely induces antibodies specific for its recognition. The presence and continuity of an apolar surface in proximity to the GPGX turn region of MN and CAN0A V3 peptides may be important for antibody cross-reactivity.

ACKNOWLEDGMENT

We acknowledge Drs. Anthony Ribeiro and Ronald Venters for helpful assistance and Dr. David Myers for sharing his preliminary spectra and analysis of CAN0A.

SUPPORTING INFORMATION AVAILABLE

One table of the proton resonance assignments of the T1-SP10CAN0(A) C4−V3 peptide (1 page). Ordering information is given on any current masthead page.

REFERENCES

Bax, A., & Davis, D. (1985) *J. Magn. Reson. 65*, 355−360.
Capon, D., & Ward, R. (1991) *Annu. Rev. Immunol. 9*, 649−678.
Cease, K., Margalit, H., Cornette, J., Putney, S., Robey, W., Ouyang, C., Streicher, H., Fischinger, P., Gallo, R., DeLisi, C., & Berzofsky, J. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 4249−4253.
Chandrasekhar, K., Profy, A., & Dyson, H. (1991) *Biochemistry 30*, 9187−9194.
Clapham, P., McKnight, A., Simmons, G., & Weiss, R. (1993) *Philos. Trans. R. Soc. London, Ser. B 342*, 67−73.
Clerici, M. (1993) *AIDS 7*, S135−S140.
Cordonnier, A., Montagnier, L., & Emerman, M. (1989) *Nature 340*, 571−574.
de Lorimier, R., & Spicer, L. (1994) *Tech. Protein Chem. V*, 423−430.
de Lorimier, R., Moody, M., Haynes, B., & Spicer, L. (1994) *Biochemistry 33*, 2055−2061.
Dyson, H., & Wright, P. (1991) *Annu. Rev. Biophys. Biophys. Chem. 20*, 519−538.
Dyson, H., & Wright, P. (1995) *FASEB J. 9*, 37−42.
Dyson, H., Cross, K., Houghten, R., Wilson, I., Wright, P., & Lerner, R. (1985) *Nature 318*, 480−483.
Dyson, H., Rance, M., Houghten, R., Lerner, R., & Wright, P. (1988a) *J. Mol. Biol. 201*, 161−200.
Dyson, H., Rance, M., Houghten, R., Wright, P., & Lerner, R. (1988b) *J. Mol. Biol. 201*, 201−217.
Dyson, H., Norrby, E., Hoey, K., Parks, E., Lerner, R., & Wright, P. (1992) *Biochemistry 31*, 1458−1463.
Emini, E., Schleif, W., Numberg, J., Conley, A., Eda, Y., Tokiyoshi, S., Putney, S., Matsushita, S., Cobb, K., Jett, C., Eichberg, J., & Murthy, K. (1992) *Nature 355*, 728−730.
Feinberg, M., & Greene, W. (1992) *Curr. Opin. Immunol. 4*, 466−474.
Fenouillet, E., Gluckman, J., & Jones, I. (1994) *Trends Biochem. Sci. 19*, 65−70.
Ghiara, J., Stura, E., Stanfield, R., Profy, A., & Wilson, I. (1994) *Science 264*, 82−85.
Girard, M., & Shearer, G. (1993) *AIDS 7*, S115−S116.
Goudsmit, J., Debouck, C., Meloen, R., Smit, L., Bakker, M., Asher, D., Wolff, A., Gibbs, C., & Gajdusek, D. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 4478−4482.
Guo, C., Jardetzky, T., Garrett, T., Lane, W., Strominger, J., & Wiley, D. (1992) *Nature 360*, 364−366.
Harouse, J., Bhat, S., Spitalnik, S., Laughlin, M., Stefano, K., Silberberg, D., & Gonzalez-Searano, F. (1991) *Science 253*, 320−323.
Harrison, S., Wang, J., Yan, Y., Garrett, T., Liu, J., Moebius, U., & Reinherz, E. (1992) *Cold Spring Harbor Symp. Quant. Biol. 62*, 541−548.
Hart, M., Palker, T., Matthews, T., Langlois, A., Lerche, N., Martin, M., Scearce, R., McDanal, C., Bolognesi, D., & Haynes, B. (1990) *J. Immunol. 145*, 2677−2685.
Haynes, B. (1995b) *Division of AIDS Treatment Resource Initiative (DATRI) Protocol 010, A Phase I Trial of C4-V3 Polyvalent Peptide Vaccine in HIV-1 Infected Persons*, Division of AIDS, NIAID, NIH, Bethesda, MD.
Haynes, B., Moody, A., Heinley, C., Korber, B., Millard, W., & Searce, R. (1995a) *AIDS Res. Hum. Retroviruses 11*, 211−221.
Holley, H., & Karplus, M. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 152−156.

Javaherian, K., Langlois, A., McDanal, C., Ross, K., Eckler, L., Jellis, C., Profy, A., Rusche, J., Bolognesi, D., Putney, S., & Matthews, T. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 6768−6772.

Jeener, J., Meier, B., Bachman, P., & Ernst, R. (1979) *J. Chem. Phys. 71*, 4546−4553.

Johnston, M., & Hoth, D. (1993) *Science 260*, 1286−1293.

LaRosa, G., Davide, J., Weinhold, K., Waterbury, J., Profy, A., Lewis, J., Langlois, A., Dressman, G., Boswell, R., Shadduck, P., Holley, L., Karplus, M., Bolognesi, D., Matthews, T., Emini, E., & Putney, S. (1990) *Science 249*, 932−935.

Leonard, C., Spellman, M., Riddle, L., Harris, R., Thomas, J., & Gregory, T. (1990) *J. Biol. Chem. 265*, 10373−10382.

Lerner, R. (1984) *Adv. Immunol. 36*, 1−44.

Letvin, N. (1993) *N. Engl. J. Med. 329*, 1400−1405.

Madrenas, J., Wange, R., Wang, J., Isakov, N., Samelson, L., & Germain, R. (1995) *Science 267*, 515−518.

Meloen, R., Liskamp, R., & Goudsmit, J. (1989) *J. Gen. Virol. 70*, 1505−1512.

Moore, J., Thali, M., Jameson, B., Vignaux, F., Lewis, G., Poon, S., Charles, M., Fung, M., Sun, B., Durda, P., Åkerblom, L., Wahren, B., Ho, D., Sattentau, Q., & Sodroski, J. (1993) *J. Virol. 67*, 4785−4796.

Mujeeb, A., Bishop, K., Peterlin, M., Turck, C., Parslow, T., & James, T. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 8248−8252.

Osterhout, J., Baldwin, R., York, E., Stewart, J., Dyson, H., & Wright, P. (1989) *Biochemistry 28*, 7059−7064.

Palker, T., Clark, M., Langlois, A., Matthews, T., Weinhold, K., Randall, R., Bolognesi, D., & Haynes, B. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 1932−1936.

Palker, T., Matthews, T., Langlois, A., Tanner, M., Martin, M., Scearce, R., Kim, J., Berzofsky, J., Bolognesi, D., & Haynes, B. (1989) *J. Immunol. 142*, 3612−3619.

Piantini, U., Sorensen, O., Bodenhausen, G., & Ernst, R. (1982) *J. Am. Chem. Soc. 104*, 6800−6801.

Prutscher, M., Trkoln, A., Gruber, G., Buchacher, A., Predl, R., Steindl, F., Tauer, C., Berger, R., Barrett, N., & Jungbauer, A. (1994*) AIDS Res. Hum. Retroviruses 10*, 1651−1658.

Putney, S. (1992) *Trends Biochem. Sci. 19*, 65−70.

Rammensee, H., Falk, K., & Rötzschke, O. (1993) *Curr. Opin. Immunol. 5*, 35−44.

Rance, M., Sorensen, O., Bodenhausen, G., Wagner, G., Ernst, R., & Wüthrich, K. (1983) *Biochem. Biophys. Res. Commun. 117*, 479−485.

Rini, J., Stanfield, E., Stura, E., Salinas, P., Profy, A., & Wilson, I. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 6325−6329.

Safrit, J., Lee, A., Andrews, C., & Koup, R. (1994) *J. Immunol. 153*, 3822−3830.

Sattentau, Q., & Moore, J. (1993) *Philos. Trans. R. Soc. London, Ser. B 342*, 59−66.

Steinaa, L., Sorensen, A., Nielsen, J., & Hansen, J. (1994) *Arch. Virol. 139*, 263−271.

Takahashi, H., Nakagawa, Y., Pendleton, C., Houghten, R., Yokomuro, K., Germain, R., & Berzofsky, J. (1992) *Science 255*, 333−336.

Thali, M., Moore, J., Furman, C., Charles, M., Ho, D., Robinson, J., & Sodroski, J. (1993) *J. Virol. 67*, 3978−3988.

Venet, A., & Walker, B. (1993) *AIDS 7*, S135−S140.

Wagner, G., Neuhaus, D., Wörgötter, E., Vasák, M., Kägi, J., & Wüthrich, K. (1986) *J. Mol. Biol. 187*, 131−135.

Waltho, J., Feher, V., Lerner, R., & Wright, P. (1989) *FEBS Lett. 250*, 400−404.

Wishart, D., & Sykes, B. (1994) *J. Biomol. NMR 4*, 171−180.

Wishart, D., Sykes, B., & Richards, F. (1991) *J. Mol. Biol. 222*, 311−333.

Wishart, D., Sykes, B., & Richards, F. (1992) *Biochemistry 31*, 1647−1651.

Wright, P., Dyson, H., & Lerner, R. (1988) *Biochemistry 27*, 7167−7175.

Wüthrich, K. (1986) *NMR of Proteins and Nucleic Acids*, John Wiley & Sons, New York.

Zvi, A., Hiller, R., & Anglister, J. (1992) *Biochemistry 31*, 6972−6979.

Zvi, A., Kustanovich, I., Feigelson, D., Levy, R., Eisenstein, M., Matsushita, S., Richalet-Sécordel, P., Regenmortel, M., & Anglister, J. (1995) *Eur. J. Biochem. 229*, 178−187.

Zwart, G., Langedijk, H., van der Hoek, L., de Jong, J., Wolfs, T., Ramantarsing, C., Bakker, M., de Ronde, A., & Goudsmit, J. (1991) *Virology 181*, 481−489.

BI952665X