# The complete Consensus V3 loop peptide of the envelope protein gp120 of HIV-1 shows pronounced helical character in solution

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**Abstract The disulfide bridge closed cyclic peptide corresponding to the whole Consensus V3 loop of the envelope protein gp120 of HIV-1 was examined by proton 2D-NMR spectroscopy in water and in a 20% trifluoroethanol/water solution. In water, NOE data support a β-turn conformation for the central conservative GPGR region and point towards partial formation of a helix in the C-terminal part. Upon addition of trifluoroethanol, a C-terminal helix is formed. This is evidenced by NOE data, α-proton chemical shift changes and changes in the $J_{N\alpha}$ vicinal coupling constants. The C-terminal helix is amphipathic and also occurs in other examined strains. It could therefore be an important feature for the functioning of the V3 loop.**

*Key words:* NMR; Consensus sequence V3 loop; Conformation in solution; Amphipathic helix; gp120; HIV-1

## 1. Introduction

The crucial proteins involved in the first steps of infection of CD4-positive cells by the human immunodeficiency virus type I (HIV-1) are the HIV-1 envelope glycoprotein gp120 and its associated transmembrane glycoprotein gp41 [1,2]. Binding of gp120 tp CD4 induces structural changes in gp120 [3,4], thereby disrupting the heterodimeric gp120–gp41 complex and exposing fusogenic domains at the N-terminal region of gp41 [5]. This ultimately results in the fusion of the lipid bilayers of the viral envelope and the target cellular membrane [6,7]. The third variable region (V3) of gp120 is also involved in this fusion step [8,9], but its specific role in cell penetration has not yet been fully clarified.

The V3 region is a surface accessible loop formed by a disulfide bridge between two invariant cysteines at positions 303 and 338 of gp120 [10] and is the part of HIV-1 which is most subject to antibody response [11,12]. Antibodies directed towards the V3 loop do not affect gp120–CD4 binding, but do prevent the subsequent cell infection [13]. The amino acid sequence of V3 loops is highly variable among different isolates, especially in the regions flanking the highly conserved central part [14]. This variability ensures that most antibodies which are elicited against the V3 loop of one strain are not effective against V3 loops of other strains. Antibodies directed against the highly conserved central GPGR region, however, do respond to a range of HIV-1 strains [14–16]. Elucidating the structure of V3 loops can thus provide insight in the importance of the central region. Determinants for viral tropism and syncytium inducing capacity are also situated within this loop [17–22]. Comparing the conformations preferred by the V3 loops of different strains could therefore point out important structural features associated with viral tropism.

Previous experimental work on the complete V3 loops of the MN [23,24], the Chang Mai [25] and the RF (Vranken et al., submitted) strains proved their respective central GPGX sequence to prefer a β-turn conformation, while the remainder of the loop was subject to considerable conformational averaging in water. The studies on the MN and RF V3 loops showed the C-terminal part to incline toward helical structures, especially in trifluoroethanol (TFE)/water mixtures, while in the Chang Mai peptide two extended β-strand regions flanking the central turn were apparently present.

The Consensus V3 loop sequence derived from 245 V3 loop sequences [14] corresponds to existing macrophagetropic strains (BAL1, JRFL, LRCSF, ADA) [19]. For this Consensus sequence, a neural network approach predicted an N-terminal β strand followed by a type II β-turn (incorporating the conserved GPGR fragment), a second β-strand and finally a C-terminal α-helix [14].

In the present paper we report the characterization of a disulfide-bridged peptide corresponding to the Consensus sequence of the HIV-1 V3 loop in water solution and in 20% TFE/water mixture by two-dimensional proton nuclear magnetic resonance.

## 2. Materials and methods

The disulfide bridge closed cyclic peptide corresponding to the whole Consensus V3 loop of the envelope protein gp120 of HIV-1 was purchased as the 'Universal' V3 loop of gp120 from Neosystem Laboratoire (Strasbourg, France). The purity of the peptide was 93% by HPLC. An electrospray mass spectrum shows the peptide to have a molecular weight of 3895.3 Da, corresponding to the correct disulfide bridge closed peptide. The presence of the disulfide bridge in solution is confirmed by NOE contacts between sidechain protons of $Cys^1$ and $Cys^{35}$ (data not shown).

### 2.1. Sample preparation

The initial NMR sample was a 4.0 mM solution of the peptide in a mixture of 90% $H_2O$ and 10% $D_2O$ at a pH of 2.5. The solution was not buffered. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was added as an internal chemical shift reference. The 20% v/v TFE/water solution was obtained by adding the appropriate amount of deuterated TFE to the water sample.

### 2.2. NMR measurements

All proton NMR spectra were acquired on a Bruker AM-500 spec-

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*Abbreviations:* 2D, two-dimensional; COSY, *J*-correlated spectroscopy; CT-NOE, constant time NOE; NOESY, nuclear Overhauser spectroscopy; ROESY, rotating frame nuclear Overhauser and exchange spectroscopy; TOCSY, total correlation spectroscopy.


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trometer. Two-dimensional NMR experiments performed on the water solution Consensus sample at 275 K were a magnitude COSY [26], a TOCSY (spin-lock time $\tau_{SL}$ = 75 ms) [27], two NOESYs (mixing times $\tau_m$ = 300 and 150 ms) [28], a ROESY ($\tau_{SL}$ = 150 ms) [29,30] and two CT-NOE spectra ($\tau_m$ = 300 ms, with constant time delay $\tau_e$ set for 6.0 and 7.9 Hz couplings) [31].

Spectra of the 20% TFE solution of Consensus were recorded at several temperatures. A TOCSY ($\tau_{SL}$ = 60 ms) and NOESY ($\tau_m$ = 300 ms) were recorded at 280 K, a TOCSY ($\tau_{SL}$ = 60 ms), two NOESYs ($\tau_m$ = 300 ms and $\tau_m$ = 150 ms) and two CT-NOE spectra ($\tau_m$ = 300 ms, with constant time delay $\tau_e$ set for 6.0 and 7.9 Hz couplings) at 290 K, and a TOCSY ($\tau_{SL}$ = 60 ms) at 300 K. The $H_2O$ peak was suppressed by selective irradiation during the 1.2 s relaxation delay [32,33]. The irradiation was continued during the mixing time in the NOESY spectra. The spin-lock used in the TOCSY spectra, with a field strength of 8.4 kHz, was an MLEV-17 sequence preceded by a 1.5 ms trim pulse. In the ROESY spectra continuous irradiation with a field strength of 3.8 kHz was used for spin-locking. The spectra were acquired in the F2 dimension with 2048 real data points and in F1 with 512 $t_1$-increments. Time proportional phase incrementation (TPPI) [34] was used for phase- sensitive acquisition. The data were processed on a Bruker X32 workstation using the UXNMR program. A squared cosine window function was used in the F2 dimension, a $\pi/3$ shifted sine function in the F1 dimension. The Fourier-transformed spectra had 2k data points in both dimensions, giving a digital resolution of 3.4 Hz/pt. A polynomial baseline correction was performed in the F2 dimension. Spectral analysis was done manually on plotted spectra and on a Silicon Graphics Crimson workstation with the program PRONTO [35].

## 3. Results

### 3.1. Assignments

The proton resonances were assigned according to the established sequential strategy [36] using 2D COSY and TOCSY spectra to recognize amino acid spin systems and 2D ROESY and NOESY spectra to identify sequential connectivities. Low chemical shift dispersion resulted in extensive overlap of the resonances in both water and TFE/water solutions. Additional problems due to line broadening were encountered in the 20% TFE spectrum, making the extraction of chemical shift values at 280 K and the extraction of NOE data at 290 K necessary. Table 1A shows the complete assignment of all protons with their chemical shift values as extracted from the 2D NOESY spectrum at 275 K in water. Table 1B shows the assignments as extracted from the 2D NOESY spectrum at 280 K in 20% TFE. All observed sequential NOE connectivities ($d_{\alpha N}(i,i+1)$, $d_{\beta N}(i,i+1)$ and $d_{NN}(i,i+1)$) are summarized in Fig. 1A and B.

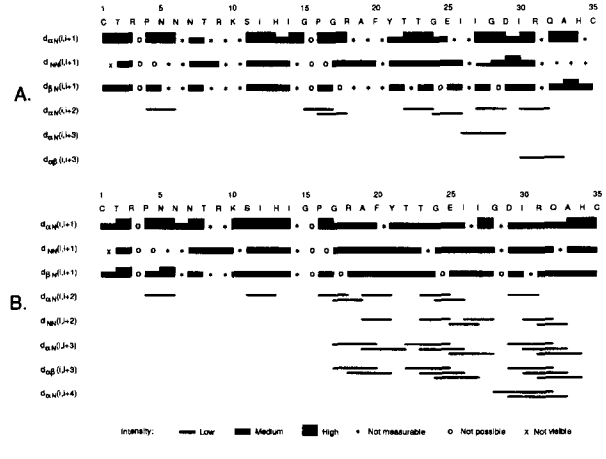


Fig. 1. Diagrams showing the sequential NOE connectivities and the NOE connectivities indicating secondary structure observed in the water (A) and the 20% v/v TFE/water (B) solutions of the Consensus V3 loop peptide.


Fig. 2. Diagrams showing the CT-NOE intensities of the NH resonance lines in F1 of the Consensus V3 loop peptide in water (A) and a 20% v/v TFE/water (B) solution. The CT-NOE spectra recorded had zero amplitude modulation for 6.0 and 7.9 Hz couplings. Negative intensity indicates a $^3J_{Na}$ coupling lower than the value shown on the left, positive intensity indicates a $^3J_{Na}$ coupling higher than the value shown on the left.

Sequential NOE connectivities of the $\alpha$ protons of $Arg^3$ and $Gly^{15}$ with the $\delta$ protons of $Pro^4$ and $Pro^{16}$, respectively, evidence that both Xxx-Pro amide bonds have the *trans* configuration in the major isomer. A few weak signals from a minor component could not be assigned unambiguously to a *cis* isomer.

### 3.2. Conformation in water

In water solution the peptide still exists as a mixture of conformations. This is experimentally evidenced by the backbone and side chain chemical shift values, which closely approach typical random coil values [37] (Fig. 3), and by the vicinal $J_{N\alpha}$ coupling constants observed via two CT-NOE spectra* (Fig. 2A), which range between 6.0 and 7.9 Hz for almost all residues. The values below 6.0 Hz for $Ala^{19}$ and $Ala^{33}$ are not unusual and have been measured before for alanine residues in random coil peptides [37].

The observed NOE connectivities (Fig. 1A) also confirm the disordered state of the V3 Consensus peptide in water. The consistent presence of both strong $d_{\alpha N}(i,i+1)$ and relatively strong $d_{NN}(i,i+1)$ NOEs indicates that the peptide samples a broad range of conformations. A series of consecutive NOE contacts that are diagnostic for regular secondary structures (a well-defined $\beta$-sheet (long-range $d_{\alpha\alpha}(i,j)$ or $d_{\alpha N}(i,j)$ NOEs) or an ordered $\alpha$-helix ($d_{\alpha\beta}(i,i+3)$, $d_{\alpha N}(i,i+3)$ and $d_{\alpha N}(i,i+4)$ NOEs)) could not be observed in the water solution.

Although no regular secondary structure is present, the occurrence of relatively strong $d_{NN}(i,i+1)$ NOEs throughout the V3 Consensus peptide, in combination with the presence of several $D_{\alpha N}(i,i+2)$ contacts ($Pro^4$-$Asn^6$, $Gly^{15}$-$Gly^{17}$, $Pro^{16}$-$Arg^{18}$, $Thr^{22}$-$Gly^{24}$, $Gly^{24}$-$Ile^{26}$, $Ile^{27}$-$Asp^{29}$ and $Ile^{30}$-$Gln^{32}$) suggest that some segments have a preference for the $\alpha$ region of

*In 2D CT-NOE spectra, only the chemical shift is $t_1$-dependent, while the coupling constants, together with a constant time $\tau_e$, modulate the amplitude of the cross-peaks in F1. By choosing $\tau_e$ properly one can accomplish that, for example, all couplings greater than 7 Hz generate positive cross peaks in F1, while couplings smaller than 7 Hz generate negative cross-peaks in F1. This also means that unambiguous conclusions about the coupling a proton experiences can only be obtained from the amplitude if the proton experiences only one coupling. This makes the method ideal for an evaluation of $^3J_{N\alpha}$ couplings for all residues (except glycine) when overlap or line width makes use of the conventional methods difficult.

Table 1 (A)
$^1$H Resonance assignments for the V3 Consensus peptide in water at pH = 2.5 and $T$ = 275 K

| Residue | Chemical shift (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | NH | $C^\alpha H$ | $C^\beta H$ | $C^\gamma H$ | $C^\delta H$ | Others |
| $Cys^1$ | | 4.48 | 3.37, 3.14 | | | |
| $Thr^2$ | 9.11 | 4.44 | 4.13 | 1.23 | | |
| $Arg^3$ | 8.79 | 4.63 | 1.86, 1.76 | 1.70 | 3.21 | 7.31 ($N^\varepsilon H$) |
| $Pro^4$ | | 4.41 | 2.29, 1.91 | 2.02 | 3.84, 3.65 | |
| $Asn^5$ | 8.75 | 4.68 | 2.85, 2.81 | | | 7.79, 7.08[a] ($N^\delta H_2$) |
| $Asn^6$ | 8.67 | 4.73 | 2.87, 2.79 | | | 7.79, 7.07[a] ($N^\delta H_2$) |
| $Asn^7$ | 8.65 | 4.77 | 2.89, 2.81 | | | 7.75, 7.06 ($N^\delta H_2$) |
| $Thr^8$ | 8.26 | 4.31 | 4.28 | 1.22 | | |
| $Arg^9$ | 8.47 | 4.33 | 1.86, 1.79 | 1.65, 1.63 | 3.19 | 7.26 ($N^\varepsilon H$) |
| $Lys^{10}$ | 8.50 | 4.34 | 1.84, 1.76 | 1.47, 1.42 | 1.68 | 2.99 ($C^\varepsilon H_2$), 7.64 ($N^\zeta H_3$) |
| $Ser^{11}$ | 8.47 | 4.47 | 3.83 | | | |
| $Ile^{12}$ | 8.33 | 4.19 | 1.82 | 1.34, 1.13 | 0.83 | 0.85 ($C^\gamma H_3$) |
| $His^{13}$ | 8.77 | 4.73 | 3.18, 3.10 | | 7.23 | 8.57 ($C^\varepsilon H$) |
| $Ile^{14}$ | 8.44 | 4.23 | 1.82 | 1.45, 1.14 | 0.83 | 0.91 ($C^\gamma H_3$) |
| $Gly^{15}$ | 8.54 | 4.12 | | | | |
| $Pro^{16}$ | | 4.47 | 2.28, 1.99[a] | 2.03 | 3.67, 3.64 | |
| $Gly^{17}$ | 8.72 | 3.97 | | | | |
| $Arg^{18}$ | 8.30 | 4.26 | 1.80, 1.70 | 1.57 | 3.15 | 7.23 ($N^\varepsilon H$) |
| $Ala^{19}$ | 8.43 | 4.24 | 1.25 | | | |
| $Phe^{20}$ | 8.26 | 4.56 | 2.99 | | 7.29 | 7.12 ($C^\varepsilon H_2$), 7.29 ($C^\zeta H$) |
| $Tyr^{21}$ | 8.26 | 4.68 | 3.03, 2.89 | | 7.08 | 6.78 ($C^\varepsilon H_2$) |
| $Thr^{22}$ | 8.20 | 4.43 | 4.25 | 1.21 | | |
| $Thr^{23}$ | 8.35 | 4.29 | 4.24 | 1.25 | | |
| $Gly^{24}$ | 8.64 | 4.01, 3.92 | | | | |
| $Glu^{25}$ | 8.23 | 4.33 | 2.04, 2.02 | 2.43 | | |
| $Ile^{26}$ | 8.47 | 4.15 | 1.86 | 1.50, 1.16 | 0.85[a] | 0.87[a] ($C^\gamma H_3$) |
| $Ile^{27}$ | 8.48 | 4.10 | 1.84 | 1.50, 1.17 | 0.84 | 0.91 ($C^\gamma H_3$) |
| $Gly^{28}$ | 8.63 | 3.95 | | | | |
| $Asp^{29}$ | 8.41 | 4.73 | 2.93, 2.84 | | | |
| $Ile^{30}$ | 8.28 | 4.15 | 1.92 | 1.49, 1.21 | 0.87 | 0.91 ($C^\gamma H_3$) |
| $Arg^{31}$ | 8.56 | 4.31 | 1.84, 1.78 | 1.67, 1.61 | 3.20 | 7.31 ($N^\varepsilon H$) |
| $Gln^{32}$ | 8.44 | 4.31 | 2.10, 1.97 | 2.37 | | 7.68, 7.05 ($N^\varepsilon H_2$) |
| $Ala^{33}$ | 8.52 | 4.27 | 1.36 | | | |
| $His^{34}$ | 8.62 | 4.75 | 3.30, 3.27 | | 7.35 | 8.63 ($C^\varepsilon H$) |
| $Cys^{35}$ | 8.66 | 4.60 | 3.34, 3.04 | | | |

(B)
$^1$H Resonance assignments for the V3 Consensus peptide in 20% v/v TFE/water solution at $T$ = 280 K

| Residue | Chemical shift (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | NH | $C^\alpha H$ | $C^\beta H$ | $C^\gamma H$ | $C^\delta H$ | Others |
| $Cys^1$ | | 4.53 | 3.41, 3.22 | | | |
| $Thr^2$ | 9.03 | 4.50 | 4.21 | 1.29 | | |
| $Arg^3$ | 8.55 | 4.70 | 1.92, 1.78 | 1.74 | 3.27 | 7.35 ($N^\varepsilon H$) |
| $Pro^4$ | | 4.47 | 2.31, 1.96 | 2.09[a], 2.04[a] | 3.85, 3.69 | |
| $Asn^5$ | 8.58 | 4.77 | 2.93 | | | 7.79, 7.03 ($N^\delta H_2$) |
| $Asn^6$ | 8.64 | 4.71 | 2.87 | | | 7.75, 7.01 ($N^\delta H_2$) |
| $Asn^7$ | 8.63 | 4.78 | 2.90[a] | | | 7.72, 7.00 ($N^\delta H_2$) |
| $Thr^8$ | 8.20 | 4.32 | 4.27 | 1.28 | | |
| $Arg^9$ | 8.33 | 4.28 | 1.93, 1.88 | 1.74, 1.68 | 3.24 | 7.29 ($N^\varepsilon H$) |
| $Lys^{10}$ | 8.21 | 4.35 | 1.91, 1.85 | 1.54, 1.48 | 1.73 | 3.03 ($C^\varepsilon H_2$), 7.70 ($N^\zeta H_3$) |
| $Ser^{11}$ | 8.16 | 4.49 | 3.91 | | | |
| $Ile^{12}$ | 7.93 | 4.18 | 1.84 | 1.45, 1.15 | 0.83[a] | 0.86 ($C^\gamma H_3$) |
| $His^{13}$ | 8.48 | 4.78 | 3.24, 3.12 | | 7.27 | 8.59 ($C^\varepsilon H$) |
| $Ile^{14}$ | 8.22 | 4.30 | 1.87 | 1.49, 1.17 | 0.88[a] | 0.94 ($C^\gamma H_3$) |
| $Gly^{15}$ | 8.19 | 4.12 | | | | |
| $Pro^{16}$ | | 4.48 | 2.29, 2.04[a] | 2.06 | 3.71, 3.62 | |
| $Gly^{17}$ | 8.58 | 4.08, 3.97 | | | | |
| $Arg^{18}$ | 8.21 | 4.29 | 1.89, 1.83 | 1.66 | 3.22 | 7.27 ($N^\varepsilon H$) |
| $Ala^{19}$ | 8.31 | 4.29 | 1.34 | | | |
| $Phe^{20}$ | 8.09 | 4.51 | 3.08 | | 7.31 | 7.12 ($C^\varepsilon H_2$), 7.31 ($C^\zeta H$) |
| $Tyr^{21}$ | 7.95 | 4.64 | 3.13, 2.96 | | 7.12 | 6.84 ($C^\varepsilon H_2$) |
| $Thr^{22}$ | 8.12 | 4.40 | 4.46 | 1.32 | | |
| $Thr^{23}$ | 8.42 | 4.01 | 4.21 | 1.30 | | |
| $Gly^{24}$ | 8.34[a] | 3.89, 3.79 | | | | |
| $Glu^{25}$ | 7.93 | 4.15 | 2.32, 2.18 | 2.53, 2.42 | | |
| $Ile^{26}$ | 8.05 | 3.83 | 2.03 | 1.71, 1.14 | 0.83 | 0.95 ($C^\gamma H_3$) |
| $Ile^{27}$ | 8.43 | 3.81 | 1.91 | 1.72, 1.24 | 0.85 | 0.93 ($C^\gamma H_3$) |
| $Gly^{28}$ | 8.16[a] | 3.93[a], 3.83[a] | | | | |
| $Asp^{29}$ | 8.16 | 4.59 | 3.20, 2.90 | | | |
| $Ile^{30}$ | 8.48 | 3.83 | 2.05 | 1.85, 1.16[a] | 0.86 | 0.95 ($C^\gamma H_3$) |
| $Arg^{31}$ | 8.60 | 4.15 | 1.99, 1.96[a] | 1.93[a], 1.69 | 3.19 | 7.24 ($N^\varepsilon H$) |
| $Gln^{32}$ | 8.04 | 4.30 | 2.24, 2.21 | 2.55, 2.48 | | 7.51, 6.91 ($N^\varepsilon H_2$) |
| $Ala^{33}$ | 7.96 | 4.31 | 1.49 | | | |
| $His^{34}$ | 8.20 | 4.74 | 3.46, 3.29 | | 7.43 | 8.61 ($C^\varepsilon H$) |
| $Cys^{35}$ | 8.35 | 4.64 | 3.41, 3.17 | | | |

[a] Chemical shift value could not be accurately measured because of overlap.

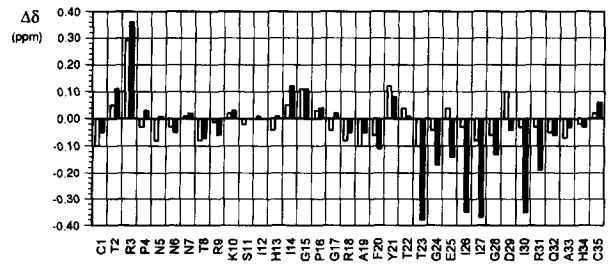


Fig. 3. Diagram showing the deviation ($\Delta\delta = \delta_{obs} - \delta_{r.c.}$) from random coil (r.c.) values of the chemical shift ($\delta$) of the $\alpha$ protons of the Consensus V3 loop peptide in water (□) and in a 20% v/v TFE/water solution (■).

($\varphi$,$\psi$) space. Without the presence of other helix-indicating NOEs, this state is often referred to as a nascent helix [38]. In the C-terminal region, however, evidence for a strong tendency toward helical structure is present in terms of an Ile$^{26}$-Asp$^{29}$ $d_{\alpha N}(i,i+3)$ and an Ile$^{30}$-Ala$^{33}$ $d_{\alpha\beta}(i,i+3)$ NOE contact (Fig. 1A). Also, the vicinal $J_{N\alpha}$ coupling constant for Thr$^{23}$ is lower than 6.0 Hz in water.

Experimental NMR evidence from previous studies on the complete V3 loops of the MN [23,24], the Chang Mai [25] and the RF (Vranken et al., submitted) strains, and on linear partial V3 loop peptides from the IIIB [39] and the RF [40] strains, as well as X-ray structures of complexes between antibodies and a peptide corresponding to the central region of the V3 loop of the MN strain [41,42] revealed the presence of a tight turn in the conserved GPGR region. The observed NOE contacts for the Consensus V3 loop peptide support this conclusion. According to our MNR data, both a type I or a type II $\beta$-turn conformation are possible for the central region of the Consensus peptide: the relatively strong Gly$^{17}$-Arg$^{18}$ $d_{NN}(i,i+1)$ NOE and Pro$^{16}$-Arg$^{18}$ $d_{\alpha N}(i,i+2)$ NOE are common features of both turns, while additional NOE contacts between the Gly$^{17}$ NH proton and both $\delta$ protons of Pro$^{16}$ and the $\alpha$ proton of Gly$^{15}$ suggest a significant population of a type I $\beta$-turn. The strong Pro$^{16}$-Gly$^{17}$ $d_{\alpha N}(i,i+1)$ NOE, however, indicates that a type II $\beta$-turn can also be present. Contacts involving side chain protons from Ile$^{12}$ and Ile$^{14}$to Tyr$^{21}$ (data not shown) imply that the region encompassing the turn forms a relatively structured loop.

The separate Pro$^{4}$-Asn$^{6}$ $d_{\alpha N}(i,i+2)$ combined with a strong Pro$^{4}$-Asn$^{5}$ $d_{\alpha N}(i,i+1)$ NOE indicates the presence of another $\beta$-turn at this position. The Asn$^{6}$ residue is a known glycosylation site [10], and the $\beta$-turn is possibly important to expose this residue for glycosylation [24].

### 3.3. *Conformation in the 20%* TFE/water solution

Upon addition of TFE a regular $\alpha$-helix is formed in the C-terminal part of the peptide. Various NOE contacts indicative of helix formation (a group of $d_{\alpha N}(i,i+2)$, $d_{NN}(i,i+2)$, $d_{\alpha N}(i,i+3)$, $d_{\alpha\beta}(i,i+3)$ NOEs and two $d_{\alpha N}(i,i+4)$ NOEs) are observed from Gly$^{17}$ to His$^{34}$ (Fig. 1B). The $J_{N\alpha}$ vicinal coupling constants for the residues in the Thr$^{23}$-Arg$^{31}$ region, as measured by two CT-NOE spectra, have shifted from above 6.0 Hz in water to below 6.0 Hz in the 20% TFE solution (Fig. 2B). This change is also indicative of helix formation. Finally, the $\alpha$ protons from Thr$^{23}$ to Arg$^{31}$ (except Gly$^{28}$) have chemical shift values in 20% TFE which are over 0.1 ppm lower than the corresponding random coil values [37] (Fig. 3). Since intrinsic changes in chemical shifts of $\alpha$ protons as a function of TFE concentration in random coil peptides are negligible [37], these upfield shifts are consistent with the formation of a helical fragment [43]. Overall, the data gathered from these three different sources strongly supports the actual presence of a helix from at least Thr$^{23}$ to Arg$^{31}$.

## 4. Discussion

In water solution the main part of the peptide adopts a broad range of conformations and is mainly random coil. Some structural features nonetheless seem to be present: two $\beta$-turns (one at the glycosylation site, one at the conserved central GPGR part), and a strong tendency toward helical conformations in the C-terminal part. In a 20% TFE solution, the C-terminal Thr$^{23}$-Arg$^{31}$ part is predominantly helical. The $\beta$-turn at the glycosylation site seems undisturbed upon addition of TFE, but conformational changes at the central $\beta$-turn cannot be excluded in view of the appearance of Gly$^{17}$-Phe$^{20}$ and Arg$^{18}$-Tyr$^{21}$ $d_{\alpha\beta}(i,i+3)$ NOEs and a Gly$^{17}$-Phe$^{20}$ $d_{\alpha N}(i,i+3)$ NOE.

This V3 Consensus peptide seems, out of all previously studies complete V3 loop peptides in TFE/water solution, to have the strongest tendency for formation of an $\alpha$-helix in the C-terminal part. The V3 MN peptide showed an almost similar helical preference (less helix indicating NOEs were observed), but was studied in 30% TFE [24]. The V3 RF peptide in 20% TFE (Vranken et al., submitted) also adopts C-terminal $\alpha$-helical conformations, but helix formation is not as pronounced as in the V3 Consensus peptide.

The appearance of a C-terminal helix from nascent helix (in water) when the polarity of the environment decreases (in TFE/water) emerges as a common feature for apparently most HIV-1 strains. The C-terminal helices of these studied V3 loop peptides are amphipathic (Vranken et al., submitted), and this suggests that they are to interact favorably with biological interfaces. It is well known that the V3 loop is actively involved in infection [13], and it has been suggested that cleavage of the V3 loop between (for Consensus) Arg$^{18}$ and Ala$^{19}$ is an essential step in this process [44,45]. Proximity of the cellular membrane after CD4–gp120 binding could assist the formation of an amphipathic C-terminal $\alpha$-helix in the V3 loop, and since amphipathic helices C-terminal to an 'active site' can enhance binding of the active site to a receptor [46], the helix formation could improve the presentation of the cleavage site to a cell surface protease, thereby assisting infection. Alternatively, this C-terminal amphipathic helix might, after cleavage of the V3 loop, cooperate with fusogenic domains of gp120 and/or gp41 and thus help to fuse the membranes of host and virus. The major determinant of viral tropism seems to be situated in the C-terminal part of the V3 loop [22], and viral tropism also correlates strongly with side chain electric charges at other specific positions in the C-terminal part of the V3 loop [18]. Since both T-cell tropic strains (MN and RF) and the macrophagetropic Consensus strain show C-terminal helicity in NMR studies, this feature seems not to be a major determinant of viral tropism. It rather appears that mutations in both classes preserve the helical tendency. However, the positioning of the charged residues on a C-terminal helix, combined with the influence of helix formation on the structure of the rest of the V3 loop, might well be important in determining interactions of the V3 loop with

host proteins. Improved characterizations of the three-dimensional structures of entire V3 loops are required to accurately interprete the data on viral tropism. Such attempts are presently in progress in this laboratory.

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