Backbone ^{15}N relaxation analysis of the N-terminal domain of the HTLV-I capsid protein and comparison with the capsid protein of HIV-1

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Abstract

Human T-cell leukemia virus type 1 (HTLV-I) is an oncogenic retrovirus that exhibits specific tropism for human T-cells. The capsid (CA) proteins of retroviruses share highly conserved secondary and tertiary structures. However, they can form quaternary structures (assembled cores) that are conical (e.g., the lentivirus subgroup, including HIV) or spherical (e.g., the oncovirus subgroup, including HTLV). The intrinsic features that drive these differences are not understood. So far, only structural studies have been used as a basis for comparison. Dynamics may play a role in particle formation. High-resolution nuclear magnetic resonance (NMR) ^{15}N relaxation data (T_1 , $T_{1\rho}$, and NOE) have been used to characterize the backbone dynamics of the N-terminal domain (NTD) of the oncovirus HTLV-I and to compare with the CA NTD of HIV-1. Large variations in the ^{15}N heteronuclear NOEs and transversal relaxation rates for individual residues are consistent with the bundle RMSD of the previously calculated NMR structures. The β -hairpin and CyP-A loop exhibit different mobility in HTLV-I and HIV-1. The overall hydrodynamic property of the HTLV-I capsid NTD is quite distinct from the HIV-1.

Keywords: HTLV-I; capsid protein; retrovirus; NMR spectroscopy; relaxation; mutant; CyP-A

Recently we published a structural study of the N-terminal domain of the retroviral capsid (CA) protein from the human T-cell leukemia virus type I (HTLV-I; Cornilescu et al. 2001), an oncogenic retrovirus with tropism for T-cells.

Despite the low sequence conservation in the N-terminal domain (NTD), the mostly helical structure is highly similar among different retroviral capsid NTDs. The N-terminal 134-amino-acid fragment (CA₁₃₄) contains a central six-helix core packed through (extensive) hydrophobic contacts and a β -hairpin. The potential to form an N-terminal Pro 1–Asp (Glu) salt bridge that links Asp (Glu) in the helical core to the N terminus to form the β -hairpin is highly conserved in the retroviral family.

In all mature retrovirus particles, the CA protein forms the shell of an inner core structure that encases the diploid RNA genome and the replicative enzymes. Whereas the noninfectious, immature particles look alike in the electron microscope, the mature particles are morphologically distinct and are characterized by the shape of the capsid core structure. The mature virion of HIV-1 and other lentiviruses is conical, spherical for HTLV-I, and an irregular polyhe-

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Abbreviations: HTLV-I, human T-cell leukemia virus type-I; HIV-1, human immunodeficiency virus type-I; CA, capsid; NTD, N-terminal domain; CyP-A, cyclophilin-A; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; RSV, Rous sarcoma virus; MLV, murine leukemia virus; T_1 , spin–lattice relaxation time; $T_{1\rho}$, spin–spin relaxation time; CPMG, Carr–Purcell–Meiboom–Gill; SD, standard deviation; psec, picosecond; τ_c , overall correlation time; S^2 , order parameter.

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dron for Rous Sarcoma Virus (RSV) and Murine Leukemia Virus (MLV). The N-terminal β -hairpin orientation may contribute to the various condensed core assemblages, as the CA NTD undergoes refolding after proteolysis from a structural precursor polyprotein (Gag), and this event influences capsid assembly (von Schwedler et al. 1998; Tang et al. 2002). Supporting this notion, we found that the conserved Asp residue that defines the orientation of the β -hairpin (by forming the salt bridge with residue Pro 1) contacted the helical core such that there was an $\sim 120^\circ$ difference in the β -hairpin orientation in the HTLV-I and HIV-1 CA NTDs. However, this structural difference alone cannot be attributed with certainty to the differences in the conical versus spherical core assemblages. The flexibility of this segment may also be a factor. So far, no comparisons of the dynamic properties of these two capsids have been carried out.

The HIV-1 CA domain binds human cyclophilin-A (CyP-A; Luban et al. 1993). The binding site is on a flexible, proline-rich loop situated between the least conserved region of the CA (between the fourth and the last helices of the core) showing variations in length and orientation among prototypes of the retroviral subgroups (HTLV-I, HIV-1, RSV, EIAV). Although the Ala-Gly-Pro-Leu/Ile motif in this loop of HTLV-I appears to be more exposed in the CA₁₃₄ structure than in the 16-residue-deleted structure CA₁₆₋₂₁₄ (Khorasanizadeh et al. 1999), we found that the HTLV-I CA does not bind CyP-A. We suggested that the length of the CyP-A-binding loop and the slow *cis-trans* transitions of residue Pro 90 in HIV-1 might be more important factors in the CyP-A-binding activity. The possible contributions to functional differences in the CA proteins from flexibility of the backbone and/or side chains may provide additional insight into the CA assembling mechanism.

In this study, we performed quantitative ^{15}N NMR relaxation measurements together with NOE build-up rate measurements to assess the HTLV-I CA NTD backbone dynamic properties. A similar study on HIV-1 CA concluded that several regions including the β -hairpin and CyP-A-binding loop exhibit a high degree of conformational mobility. A comparison of our results with previous findings reported for HIV-1 revealed differences in their backbone dynamic profile that might be important in understanding the differences in assembly of their mature particles.

Results

Backbone ^{15}N relaxation measurements

The longitudinal relaxation time, T_1 , the transverse relaxation time, $T_{1\rho}$, and the steady-state ^{15}N - $[^1\text{H}]$ nuclear Overhauser effect (NOE), were obtained for 99 of the 121 protonated backbone ^{15}N nuclei by analysis of the two-dimensional ^1H - ^{15}N correlation spectra. Data for residues Gly 7,

Met 17, Val 67, Gln 115, Ser 127, and Asp 130 could not be fitted satisfactorily because of either significant overlapping or signal broadening. Residues 1, 5, 9, 10, 14, 32, 35, 50, 92, 96, 104, 125, and 131 are prolines.

The experimental values of T_1 , $T_{1\rho}$, and NOE are plotted versus the residue number in Figure 1. The value of T_1 for the rigid part of the molecule (residues 1–125) does not change dramatically with the sequence position and has an average value of 707.5 (± 11.3) msec. The averages of the $T_{1\rho}$ values for residues 1 to 125, which are well defined in the solution structure, are 91.8 (± 1.8) msec. Statistically significant larger $T_{1\rho}$ values were observed for residues 126 to 134 in the highly disordered C-terminal region, indicating that this region is more flexible than the rest of the protein on the picosecond/nanosecond time scale. Residues Gln 29 and Thr 88 have $T_{1\rho}$ values smaller than the average. The average backbone ^{15}N $T_{1\rho}$ value of 80 msec is consistent with the size of monomeric HTLV-I in solution. All our samples containing 0.3, 0.6, and 0.8 mM protein exhibited similar linewidths, and the $T_{1\rho}$ values measured for these samples are indistinguishable. Therefore, we can conclude that the NTD of HTLV-I CA protein is monomeric in solution. In the HIV-1 case, Gitti et al. (1996) observed that concentrations higher than 1.3 mM result in broader resonances, indicating oligomerization.

The NOE values vary from ~ 0.65 to 0.85 for the ordered residues 1–125 to ~ 0.7 in the highly disordered C-terminal region. The decrease in the NOE follows the pattern obtained for the $T_{1\rho}$, indicating increased flexibility for this region on the picosecond/nanosecond time scale.

Internal motion analysis

For proteins the size of CA₁₃₄, NOEs are very sensitive to fluctuations in the internal dynamics of the individual NH vector as well as the overall rotational diffusion of the molecule. NOE values exceeding the maximum theoretical value show highly restricted internal motion, whereas NOE values smaller than 0.65 indicate significant internal motion. The averages of the NOE values at the C terminus (residues 126–134) are 0.02, indicating rapid internal motion for the C-terminus tail. The averages of the NOE values for the secondary structure elements are $\beta 1 = 0.80 \pm 0.04$, $\beta 2 = 0.75 \pm 0.02$, $\alpha 1 = 0.75 \pm 0.07$, $\alpha 2 = 0.73 \pm 0.14$, $\alpha 3 = 0.79 \pm 0.06$, $\alpha 4 = 0.76 \pm 0.07$, $\alpha 5 = 0.77 \pm 0.06$, and $\alpha 6 = 0.79 \pm 0.04$. The NOE values' averages for the loops are 0.36 ± 0.62 for residues 5–9, 0.82 ± 0.17 for residues 13–16, 0.81 ± 0.14 for residues 31–34, 0.76 for residues 50–51, 0.79 ± 0.03 for residues 63–64, 0.71 ± 0.07 for residues 86–96, 0.71 ± 0.08 for residues 103–106, and 0.18 ± 0.46 for residues 123–134. The loop that connects $\beta 1$ to $\beta 2$ (residues 5–9) exhibits intermediate values and the C terminus (residues 123–134) the smallest values. The NOE values for residues from the CyP-A-binding loop (residues

Figure 1. Experimental ^{15}N backbone amide relaxation parameters for CA_{134} at 600 MHz: (A) The longitudinal relaxation time, T_1 ; (B) the transverse relaxation time, $T_{1\rho}$; and (C) the steady-state ^{15}N - ^1H nuclear Overhauser effect (NOE). The secondary structure elements represented in panel B are as follows: β -strand $\beta 1$ (residues Val 2–His 4), β -strand $\beta 2$ (residues Pro 10–His 12), α -helix $\alpha 1$ (residues Met 17–Ala 30), α -helix $\alpha 2$ (residues Pro 35–Asp 49), α -helix $\alpha 3$ (residues Ala 52–Leu 62), α -helix $\alpha 4$ (residues Ser 65–Arg 85), α -helix $\alpha 5$ (residues Leu 97–Asn 102), and α -helix $\alpha 6$ (residues Gln 107–Ala 122).

86–96) are slightly lower, but not significant, than the average.

The shape of CA_{134} ($49 \times 47 \times 36$ Å) indicates that the molecule is nonspherical. The relative lengths of the principal axes of the inertia tensor for CA_{134} are 1.00:0.85:0.56. This implies that the rotational diffusion tensor is either axially symmetric or fully anisotropic. For anisotropic diffusion, the ^{15}N T_1/T_2 ratio depends on the orientation of the NH bond vector relative to the rotational diffusion tensor, $\mathbf{D}$ (Woessner 1962). T_1/T_2 ratios are maximum when the NH bond vectors are parallel to the long axis of $\mathbf{D}$ and minimum when the NH bond vectors are perpendicular to the long axis. The ^{15}N T_1/T_2 ratio is, to a first approximation, independent of the rapid internal motions and magnitude of the chemical shift anisotropy. The presence of very rapid internal motions causes almost the same fractional increase in T_1 and T_2 . Thus, T_1/T_2 ratios can be used to derive the rotational diffusion tensor. One must make sure that residues with a significant contribution from internal motion to the observed T_1/T_2 ratios are eliminated. First, residues with $\text{NOE} < 0.65$ must be excluded because their change in T_1 values is much bigger than the T_2 variation. Second, residues that exhibit conformational exchange on a microsec-

ond or millisecond time scale will shorten the T_2 values. Such residues are recognized by a shorter T_2 without a simultaneous increase of T_1 . To exclude the residues with slower internal motions, for any residue n the following criteria have been imposed:

$$\langle T_2 \rangle - T_{2,n} > \text{SD},$$

$$(T_{2,n} - \langle T_2 \rangle) / T_{2,n} > 3 \times (\langle T_1 \rangle - T_{1,n}) / T_{1,n},$$

where $\langle T_1 \rangle$ and $\langle T_2 \rangle$ are the averages over the residues that have $\text{NOE} > 0.65$ and SD is the standard deviation of the T_2 . The residues that do not fulfill these criteria experience additional line broadening, commonly described by the exchange term R_{ex} (Clare et al. 1990). Using the above criteria, Gln 29 and Thr 88 have been excluded from the fittings.

The NH bond vectors of an α -helix are nearly parallel to the helix axis and their T_1/T_2 ratios are uniform. Different helices exhibit different average T_1/T_2 values corresponding to the orientation relative to the rotational diffusion tensor (Fig. 2). The observed average T_1/T_2 values for the different helices in CA_{134} have characteristic values of $\alpha 1 = 7.78 \pm 0.65$, $\alpha 2 = 8.46 \pm 0.30$, $\alpha 3 = 8.15 \pm 0.38$,

Figure 2. Plot of the T_1/T_2 ratios versus the residue number. Secondary structure elements are shown on top.

$\alpha 4 = 7.58 \pm 0.28$, $\alpha 5 = 7.50 \pm 0.20$, and $\alpha 6 = 8.50 \pm 0.38$, whereas average values of 7.91 ± 0.60 and 7.59 ± 0.02 were obtained for the $\beta 1$ and $\beta 2$ strands, respectively. For a given molecule, assuming a uniform hydration shell, the diffusion tensor should be collinear with the inertia tensor (the axis of fastest diffusion, D_z , corresponds to that of minimal inertia). The actual angles between helix $\alpha 6$, the closest to the orientation of the minor principal axis of the inertia tensor, and the helix axes of helices $\alpha 1$ through $\alpha 5$ are 40° , 21° , 19° , 44° , and 59° , respectively. The average T_1/T_2 values of the six α -helices correlate well with their orientation relative to the diffusion tensor.

Slow motion

Representative examples of the experimental relaxation CPMG curves are presented in Figure 3A for resonances Ala 8, Arg 42, Gln 105, and Lys 129. Figure 3B shows the differences between $R_2^{\text{disp}}(\tau_{\text{cp}})$ obtained for τ_{cp} values of 10 and 0.5 msec. The difference $\Delta R_2^{\text{disp}}(\tau_{\text{cp}})$ does not change dramatically with the sequence position. We conclude that the NTD of HTLV-I CA protein does not exhibit a high degree of conformational mobility on the millisecond/second time scale.

Diffusion tensor

The rotational diffusion tensor was calculated from the T_1/T_2 ratios for 91 residues. For a fully asymmetric diffusion

tensor, there are six unknown independent variables: the principal components of the diffusion tensor (D_x , D_y , and D_z), the polar coordinates θ and ϕ that describe the orientation of D_z , and the φ angle that defines the orientation of D_y , where $D_z > D_y > D_x$. As previously described by Tjandra et al. (1996), the above parameters are obtained by minimizing the difference, E , between the observed and the predicted T_1/T_2 ratios:

$$E = \sum_n \frac{[(T_1^{\text{obs}}/T_2^{\text{obs}}) - (T_1^{\text{pred}}/T_2^{\text{pred}})]^2}{\Delta^2},$$

where the summation is over all residues used in the fitting and Δ is the estimated error in the measured T_1/T_2 ratio. The principal components of the diffusion tensor (D_x , D_y , and D_z) are uniquely defined by the correlation time, $\tau_c = [2\text{Tr}(\mathbf{D})]^{-1}$, anisotropy $D_{\parallel}/D_{\perp}$ (with $D_{\perp} = [D_x + D_y]/2$ and $D_{\parallel} = D_z$) and asymmetry D_x/D_y .

To estimate the correlation time, $\tau_c = (6D_z)^{-1}$, the isotropic model, $D_x = D_y = D_z$, was first assumed. This one-dimensional Powell minimization generated a $\tau_c = 8.43$ nsec and a normalized error function $E/N = 7.7$. Second, the data were fitted into the axially symmetric model, $D_x/D_y = 1$, resulting in a significant reduction of the normalized error function relative to the isotropic model. The use of the fully asymmetric model, six-parameter fit, results in a further reduction of E/N . The results of the fitting to different rotational diffusion models are presented in Table 1.

We used the F -test (Tjandra et al. 1996) to evaluate if the decrease in the error function obtained with an increase in

Figure 3. (A) CPMG dispersion curves, R_2^{disp} as a function of τ_{cp} for Ala 8 (●), Arg 42 (○), Gln 105 (●), and Lys 129 (○). (B) The differences $\Delta R_2^{\text{disp}}(\tau_{\text{cp}}) = R_2^{\text{disp}}(10 \text{ msec}) - R_2^{\text{disp}}(0.5 \text{ msec})$ versus the CA₁₃₄ residue number.

the number of parameters is statistically significant. The probability that the improvement in the error function occurs by chance, P_F , is a measure for statistical significance of the additional parameters in the fit. A P_F value $<5\%$ is considered statistically significant. When the diffusion tensor is constrained to the axially symmetric model, the improvement in the error function is clearly significant: $F = 25.6 \pm 2.7$ and $P_F = P(F, 3, 92) = 2.3 \pm 0.4 \times 10^{-6}$. However, adding another two degrees of freedom, for the fully asymmetric model, the improvement is not statistically

significant. In this case, the use of the F -test results in $F = 1.7 \pm 0.7$ and $P_F = P(F, 2, 92) = 0.15 \pm 0.19$.

The principal components of the axially symmetric diffusion tensor are $D_z = 2.34 \times 10^7 \text{ sec}^{-1}$ and $D_x = D_y = 1.88 \times 10^7 \text{ sec}^{-1}$. The symmetry axis of the diffusion tensor is oriented within 30° of the principal axis of the inertia tensor.

Internal motion parameters S^2 and τ_e were obtained by minimizing the difference between the calculated and measured T_1 , T_2 , and NOE for each residue (Tjandra et al. 1996). During this minimization the global diffusion parameters were fixed to the statistically significant values found from the T_1/T_2 optimization. The results are shown in Figure 4A,B. The square of generalized order parameter (S^2) has average values of 0.85 for the whole molecule, neglecting the C-terminal region. In the cone model (Brainard and Szabo 1981), this would correspond to motion within a semiangle of 18° . The following residues exhibit lower S^2 values: 0.73 for Trp 15, 0.72 for Gly 95, 0.71 for Gly 126, 0.59 for Ala 128, 0.45 for Lys 129, 0.46 for Trp 133, and 0.23 for Ala 134. With the cone model of Brainard and Szabo (1981), these values correspond to motion of the NH bond vectors in cones of semiangles 26° , 26° , 27° , 33° , 39° , 42° , 42° , and 53° , respectively. Effective correlation time (τ_e) values for internal motions are below 100 psec and range as far as 210 psec for residue Phe 37. The sites with higher τ_e values also have lower S^2 values, with the exception of residues Ser 34 and Leu 97, which exhibit high τ_e (160 and 110 psec, respectively) and S^2 values within the average (0.90 and 0.87, respectively). The values of order parameters are completely independent of secondary structure.

Discussion

In this work, the dynamical properties of the backbone ^1H - ^{15}N bond vectors of the N-terminal domain of HTLV-I CA protein have been characterized from ^{15}N relaxation measurements. Analysis of the T_1/T_2 ratios, together with the known 3D structure of the protein, yielded a correlation time, τ_e , of 8.2 nsec, and the molecule tumbles with an axially symmetric diffusion tensor ($D_{\parallel}/D_{\perp} = 1.25$).

Table 1. Calculated diffusion parameters for N-terminal domain of the HTLV-I capsid

Model	τ_e (nsec)	$2D_z/(D_x + D_y)$	D_x/D_y	θ ($^\circ$)	ϕ ($^\circ$)	φ ($^\circ$)	E/N	F	P_F
Isotropic	8.43 (0.02)	1	1				7.7 (0.3)		
Axially symmetric	8.20 (0.02)	1.25 (0.01)	1	69 (2)	180 (2)		4.1 (0.3)	25.6 (2.7)	$2.3 (0.4) \times 10^{-6}$
Fully asymmetric	8.19 (0.02)	1.25 (0.01)	1.08 (0.02)	70 (3)	180 (2)	50 (8)	3.9 (0.3)	1.7 (0.7)	0.15 (0.19)

The Euler angles θ , ϕ , and φ are defined with respect to the PDB coordinate frame of the N-terminal HTLV-I capsid structure (PDB access code 1G03). A total of 91 residues were used in the analysis. Uncertainties in these parameters are indicated in parentheses, and they were estimated from their rms distribution when repeating the optimization procedures 20 times, each time randomly deleting 20% of the data points.

Figure 4. Internal motion parameters. (A) Worm representation of the S^2 parameter, with color designation: Proline (white), no data (gray), 0.60 or less (yellow), 0.60–0.80 (light orange), 0.81–0.85 (orange), 0.86–0.89 (dark orange), 0.90–0.93 (red). (B) Worm representation of τ_e , with color designation: Proline (white), no data (gray), 1–60 psec (green), 60–100 psec (cyan), 100–150 psec (light blue), 150 psec and greater (blue). (C) S^2 and τ_e as a function of the residue number. Estimated errors for the S^2 and τ_e are within the size of the symbols.

A comparison between the diffusion tensor's principal axes of HTLV-I and HIV-1 is presented in Figure 5. The structural studies of the N-terminal domain of HIV-1 CA protein (Campos-Olivas and Summers 1999) have concluded that its highly asymmetric shape correlates well with its inertia tensor principal axes (of relative magnitudes of 1.00:0.76:0.46), which, in turn, correlate with the diffusion tensor such that the axis of the fastest diffusion is nearly collinear with the minimal inertia (they form a 22° angle). In HIV-1, the CyP-A-binding loop contributes to the magnitude of the largest axis of the diffusion tensor by pointing outward along that axis. In HTLV-I, the same loop is shorter and folded toward the helical cluster, therefore shortening the respective axis of the diffusion tensor. Moreover, helix α_4 is tilted outward along the intermediate diffusion axis, bringing its magnitude closer to the "largest" axis. The shortest axis is slightly extended relative to the HIV's value, owing to the β -hairpin pointing out from the helical core. The relative ratio of the inertia tensor's components for HTLV-I is 1.00:0.85:0.56. The diffusion tensor calculations correspond to an axially symmetric model, as described in the Results section, that correlates with the shape of the molecule and with the inertia tensor. The angle between the largest axis of the inertia tensor and the axis of the fastest

diffusion is roughly 30° , whereas for the HIV-1, this angle is 22° . These angles might at first seem large; however, earlier comparisons made between the diffusion tensor calculated from NMR relaxation data and extensive hydrodynamic computations indicated that a deviation of $\sim 12^\circ$ between the two tensors can be expected (Tjandra et al. 1995, 1996). These studies also pointed out that the deviation of the NMR relaxation-derived diffusion tensor from the inertia tensor was even larger. Relatively high-resolution X-ray structures were used in these analyses; thus, imperfect local geometry or "structural noise" was not a big factor and their results therefore provided the lower limit for the angular deviation between these tensors. Note that small changes in the local geometry involving the relaxation dipoles (NH bonds) can change the overall orientation of the tensor dramatically. Even though we did not find any consistent "outlier" data point, we still cannot completely rule out a contribution from structural noise in our analysis. Furthermore, we used HYDRONMR (de la Torre et al. 2000) to try to estimate from the bead method what the expected diffusion tensor would be for these capsid proteins. Interestingly, the results of these calculations consistently showed the intermediate inertia axis to be the fastest diffusion axis for both HTLV-I and HIV-1 NTD capsids. This is in disagreement

Figure 5. (A) The diffusion tensor of the NTD of HTLV-I capsid protein (red) drawn in the inertia tensor frame of the molecule. (B) The diffusion tensor of the NTD of HIV-1 capsid protein (blue). Both molecules are oriented such that their inertia frames are collinear. All ribbon and worm diagrams were created using the program MolMol (Koradi et al. 1996).

with both our result and a previously published dynamic analysis of the HIV-1 capsid (Campos-Olivas and Summers 1999).

As noted in Figure 2, the helices $\alpha 2$ and $\alpha 6$ have the largest average values of the T_1/T_2 ratios, and their helix axes are the most aligned with the axis of fastest diffusion. In contrast, the $\beta 1$ and $\beta 2$ strands have the lowest average T_1/T_2 values with the exception of the disordered C-terminal region. This indicates that the NH bond vectors in the β -hairpin must be perpendicular to the principal axis of diffusion.

The orientation of the β -hairpin in CA₁₃₄ (away from the helical core) was consistent with all our generated NMR structures. This is mostly caused by the NH and C $_{\alpha}$ H $_{\alpha}$ measured dipolar couplings that define accurately the relative orientations of protein fragments to the molecular alignment frame and, therefore, to one another. If true, this hairpin's fixed orientation would imply a reduced mobility on both slow and fast time scales. The dynamic measurements $T_{1\rho}$ and NOE have confirmed the rigidity of this structural element with values virtually identical to the rest of the well-structured protein backbone, in contrast with the obvious pattern of local mobility (on a fast time scale) exhibited by the β -hairpin in HIV-1 in the same type of experiments (Campos-Olivas and Summers 1999). Mutation, deletion, and addition studies of the β -hairpin region of HIV-1 capsid

have shown that this region is essential for capsid assembly (Campbell and Vogt 1995; Gross et al. 1998, 2000; von Schwedler et al. 1998). Changes in this region can alter the shape of the mature particles. This region has been hypothesized to form the capsid-capsid interface in the mature viral particle. Our study revealed that differences in backbone dynamics of the β -hairpin of retroviruses might also govern their respective capsid assembly by modulating the overall flexibility of the capsid-capsid interface.

Packaging of host CyP-A is important for HIV-1 infectivity. The real function of CyP-A in the context of HIV-1 infectivity is still not known. Several recent results indicated that CyP-A might facilitate capsid particle disassembly (Rothman and Schmid 1986; Thali et al. 1994; Braaten et al. 1996; Ackerson et al. 1998). Although the binding sites in both proteins are exposed and oriented away from the main core, only HIV-1 capsid binds CyP-A. The loops between helices $\alpha 4$ and $\alpha 5$ ($\alpha 6$ in HIV-1), which contain the Ala-Gly-Pro-Leu/Ile motif for binding to the human CyP-A, differ in length, number of helices, and proline isomerization state (Cornilescu et al. 2001). The CyP-A loop in HIV-1 exhibits high mobility (Campos-Olivas and Summers 1999), whereas the same loop in HTLV-I shows less flexibility. This is another distinguishing feature between these loops in the two proteins. Thus the high degree of conformational mobility as well as the proposed Pro 90 *cis*-

and *trans*-isomerization may facilitate CyP-A binding to the HIV-1 CA. This, in turn, may differentiate how the core particles are disassembled in these two retroviruses.

In summary, our results identify clear differences in the backbone dynamic properties of the HTLV-I CA NTD, as compared with the HIV-1 structure. The fact that formation of at least one region (the N-terminal β -hairpin) is a critical step in the viral capsid maturation process indicates that the flexibility of the elements that comprise the structure is also likely to be an important determinant of correct core assembly. The mobility of the loop containing the CyP-A-binding motif is different in the two capsid proteins. Perhaps a more flexible loop, such as in the HIV-1 capsid, is needed to allow the *cis*–*trans*-isomerization of the proline in this region, thus promoting CyP-A interaction. The difference in the hydrodynamic shapes between the HTLV-I and HIV-1 capsids that is reflected in their varying diffusion tensors must play an important role in their quaternary structures. Based on the hard-sphere interaction argument alone, one can already see that the packing of these proteins to assemble their associated core particles must be different. Clearly, having access to both local backbone dynamic and global hydrodynamic properties of retroviral capsid proteins is crucial in understanding the molecular basis of their functions.

Materials and methods

Sample preparation

DNA encoding residues Pro 1 to Ala 134 of HTLV-I CA (CA₁₃₄) protein was cloned into the *NdeI/SapI* sites of the pTYB1 plasmid vector (New England Biolabs). The recombinant plasmid was transformed into *Escherichia coli* ER2566 strain for protein expression. The purified CA₁₃₄ was obtained by the same method as described previously (Cornilescu et al. 2001).

NMR data collection

All NMR experiments were carried out at 27°C on Bruker AVANCE 600, operating at the ¹H frequency of 600 MHz, equipped with a 5-mm shielded triple-gradient triple resonance probe. The 250- μ L NMR sample contained 0.8 mM uniformly ¹⁵N-labeled protein in 92.5% H₂O/7.5% ²H₂O and 10 mM Tris-acetate, 0.1 mM NaN₃ (pH 6.0). The ¹H was positioned at the water frequency (4.78 ppm), and the ¹⁵N carrier was at 116.50 ppm for all experiments. The spectra were processed using the NMR-Pipe package (Delaglio et al. 1995) and analyzed with PIPP/STAPP (Garrett et al. 1991). The indirect dimension data were zero-filled prior to Fourier transformation.

Backbone ¹⁵N relaxation measurements

Spin–lattice (*T*₁) and spin–spin (*T*_{1ρ}) relaxation times for the backbone ¹⁵N nuclei were measured using conventional pulse sequences (Barbato et al. 1992), adapted to include the Watergate scheme (Piotto et al. 1992), pulsed field gradient (Bax and Po-

chapsky 1992), and a semiconstant time evolution period in *t*₁ (Grzesiek and Bax 1993a). The *T*_{1ρ} experiment used a continuous ¹⁵N spin-lock field strength of 2.5 kHz (Peng et al. 1991).

All experiments were acquired as 512*(*t*₂) × 128*(*t*₁) data sets with 64, 96, and 128 scans per *t*₁ point for *T*₁, *T*_{1ρ}, and NOE, respectively. The *T*₁ relaxation decay was sampled at eight different time points: 12, 84, 204, 324, 404, 564, 804, and 1004 msec. For the *T*_{1ρ} data, eight spectra were recorded in an interleaved manner (to minimize any effects of spectrometer drift and sample heating) using the following ¹⁵N relaxation delays: 4.9, 14.5, 26.5, 38.5, 60.1, 88.9, 112.9, and 139.3 msec. ¹⁵N-[¹H]-NOE values were measured using the water flip-back NOE pulse sequence described by Grzesiek and Bax (1993b) with a relaxation delay of 3.8 sec.

All *T*₁, *T*_{1ρ}, and NOE experiments were performed twice, more than 1 wk apart, to calculate the pairwise root mean square difference (RMSD). The resulting random errors (half the pairwise RMSD values) used in the calculations were: 1.6% (*T*₁), 2.0% (*T*₂), and 7.8% (NOE). Relaxation times were calculated by nonlinear fittings of the delay-dependent peak intensities to an exponential decay function with a peak intensity baseline of zero.

¹⁵N-[¹H]-NOE values were calculated as the intensity ratios of the ¹⁵N–¹H correlation peaks from pairs of spectra acquired with and without ¹H presaturation during the recycle time. The saturated and unsaturated experiments were interleaved; the absence of saturation was obtained by shifting the proton frequency off-resonance by ~3 MHz during the recycle time; 128 scans per *t*₁ point were accumulated. ¹⁵N-[¹H]-NOE values were corrected as previously described to compensate for the effects of incomplete ¹H magnetization recovery (Grzesiek and Bax 1993b).

Relaxation dispersion data were obtained using a modified proton-detected ¹⁵N Carr-Purcell-Meiboom-Gill (CPMG) spin-echo pulse sequence (Loria et al. 1999) performed in a constant time (CT) manner (Tollinger et al. 2001) with a CT delay of 80 msec. Each 2D spectrum was acquired as 512*(*t*₂) × 128*(*t*₁) data sets with 64 scans per *t*₁ point. Relaxation decay curves were measured for nine values of τ_{cp} : 0.5, 0.67, 1, 1.25, 2, 2.5, 3.33, 5, and 10 msec, where τ_{cp} is the delay between pulses in the spin-echo pulse train.

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