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Letter to the Editor: Sequence-specific ^{1}H, ^{13}C and ^{15}N chemical shift assignment and secondary structure of the HTLV-I capsid protein

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Biological context

We have initiated NMR studies of the capsid protein (CA) from human T-cell leukemia virus type I (HTLV-I), a retrovirus linked with adult T-cell leukemia and myelopathy (Vogt, 1997). The capsid proteins condense during maturation to form the viral core particle. Whereas the HTLV-I core is spherical, the human immunodeficiency virus (HIV-1) core is conical (Vogt, 1997). These differences are intriguing in view of the 20% sequence identity between the HTLV-I and HIV-1 CAs. Knowledge of the 3D structure of HTLV-I CA is important for understanding retrovirus assembly mechanisms.

Methods and results

We have over-expressed and purified an N-terminal deletion mutant (Δ15) of HTLV-I CA that is monomeric in solution (M_r = 24.5 kDa). DNA encoding the HTLV-I CA residues 16–214 was subcloned into a pET-16b vector (Novagen) and was used to transform *E. coli* strain HMS174(DE3)pLysS (Novagen). This construct expresses an N-terminal polyhistidine tag [Gly^{-6}-(His)$_{10}$-Ser^{6}-Ser^{7}-Gly^{8}-His^{9}-Ile^{10}-Glu^{11}-Gly^{12}-Arg^{13}-His^{14}-Met^{15}] which helps efficient purification by nickel affinity chromatography (Qiagen). The protein is soluble at millimolar concentrations in the presence of NaCl, and upon addition of 5% 1,4-dioxane-d_8 (Aldrich) the quality of the HSQC spectrum improves. Thus, the NMR samples (U-^{15}N; U-^{15}N/^{13}C; and U-^{15}N/^{13}C/^{2}H) contained 1–1.5 mM protein in 50 mM sodium phosphate (pH 6.0), 300 mM NaCl, 0.1 mM DTT in H_2O:D_2O:dioxane (90:5:5), or D_2O:dioxane (95:5). NMR studies were carried out at 30 °C on a Bruker AVANCE 800 MHz spectrometer equipped with a z-gradient triple resonance probe. Data were processed with NMRPipe (Delaglio et al., 1995) and analyzed with NMRView (Johnson and Blevins, 1994). Data were typically apodized with 65°-shifted sine-squared (^{1}H) and cosine-squared (^{13}C and ^{15}N) functions. Chemical shifts were referenced to the internal water signal (4.725167 ppm at 30 °C).

Analysis of the triple-resonance HNCA, HN(CO)CA (Bax and Grzesiek, 1993), and deuterium-decoupled HN(CA)CB and HN(COCA)CB (Yamazaki et al., 1994) spectra, in conjunction with the 4D ^{15}N/^{15}N-edited NOESY (t_{mix} = 200 ms) (Grzesiek et al., 1995) recorded for a triply-labeled (U-^{2}H/^{13}C/^{15}N) sample, was sufficient to obtain unambiguous sequential assignment for > 90% of the protein signals. Assignments were subsequently made for a U-^{13}C/^{15}N-labeled sample by the analysis of HNCA, HNCO, HCACO, and CBCACONH spectra (Bax and Grzesiek, 1993). ^{1}H, $^{13}C^{\alpha/\beta}$ and $^{13}C'$ chemical shift indices (Wishart and Sykes, 1994) indicate that 61% of the sequence exists in an α-helical conformation (Figure 1).

The side chain resonance assignments were made mainly by analysis of 4D ^{15}N/^{13}C- and ^{13}C/^{13}C-edited NOESY spectra (t_{mix} = 100 ms) (Wüthrich, 1986; Clore and Gronenborn, 1991). Weak or broad resonances were assigned with 3D ^{15}N- or ^{13}C-edited NOESY spectra. In addition, 2D [^{1}H,^{1}H]-NOESY data obtained for a sample in D_2O were useful for the assignment of some of the aromatic side chain signals. Stereospecific assignments of the methyl groups of Val

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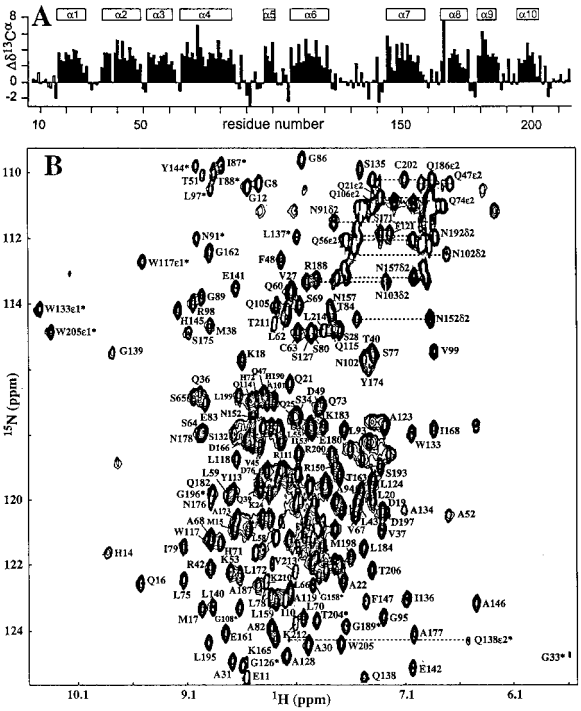

Figure 1. (A) Secondary $^{13}C^{\alpha}$ chemical shifts for HTLV-I $CA_{\Delta 15}$. Open bars are used for the non-native residues at the N-terminus. Rectangles represent helices as identified by the consensus chemical shift index obtained from $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and $^{13}C'$ analyses (Wishart and Sykes, 1994). (B) 2D [^{15}N,^{1}H]-HSQC spectrum of ^{15}N-labeled HTLV-I $CA_{\Delta 15}$. Spectral widths of 1344 Hz in ^{15}N and 12019 Hz in the ^{1}H dimensions were used along with 128 t_1 complex points. Backbone amide cross peaks in the central region of the spectrum are not labeled for clarity. The folded resonances in the ^{15}N dimension are marked with *. Upon addition of 5% dioxane, amides of residues 134 and 175 became detectable and the linewidths of residues 23, 44, 46, 82, 103, 126, 130, 132, 133, 155, 159, 169, 187 improved; in addition, ^{1}H and ^{15}N chemical shifts showed small changes of ca. −0.01 and 0.1 ppm, respectively.

and Leu were made by inspection of 2D [^{13}C,^{1}H]-HSQC data collected for a 10% ^{13}C-labeled sample (Senn et al., 1989). Intraresidual NOE information was used to obtain assignments for the side chain amides of asparagines and glutamines.

Extent of assignments and data deposition

Complete backbone assignments were made for the HTLV-I $CA_{\Delta 15}$ (except for Thr^{191}) as well as for some of the N-terminal tag (Figure 1B). The NH_2 resonances of 9 out of 22 glutamines (21, 46, 47, 56, 74, 100, 106, 138, 186) and 6 out of 8 asparagines (91, 102, 103, 152, 157, 192) were assigned. Assignments were made for the majority of the side chain ^{1}H-^{13}C pairs. One set of H^{δ} and H^{ϵ} resonances were detected for all Tyr and Phe residues. Stereospecific assignments were made for the methyl groups of all 6 Val and 28 Leu residues. The following ^{1}H-^{13}C pair resonances remained unassigned: CH_2^{γ} of Met^{38} and Met^{198}, CH_2^{δ} of Pro^{143}, CH_2^{γ} of Pro^{50}, $CH_2^{\gamma 1}$ of Ile^{79}, CH_2^{γ} of Glu^{142}, CH^{ϵ} of Phe^{147}, $CH\delta^2$ of His^{71}, CH_2^{γ} of Arg^{42}, CH_2^{δ} of Arg^{85}, Arg^{111} and Arg^{150}, and $CH_2^{\delta}/CH_2^{\epsilon}$ of Lys^{210} and Lys^{212}.

^{1}H, ^{13}C and ^{15}N chemical shifts for HTLV-I $CA_{\Delta 15}$ have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 4311. ^{15}N and $^{13}C^{\alpha/\beta}$ chemical shifts for the perdeuterated protein have also been deposited. Perdeuteration of the protein caused significant chemical shift changes that appeared to be independent of secondary structure. The mean differences and standard deviations in the chemical shifts of ^{15}N, $^{13}C^{\alpha}$, and $^{13}C^{\beta}$ are 0.20 ± 0.07, 0.31 ± 0.09, and 1.09 ± 0.26 ppm, respectively.

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