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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N chemical shift assignments of the capsid protein from Rous sarcoma virus

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Biological context

Rous sarcoma virus (RSV) is the prototype of the avian C-type retrovirus genus (also referred to as avian sarcoma/leukosis viruses), one of the seven genera in the retroviriade family. Like all retroviruses, RSV contains a central capsid core particle, comprising ∼1500 capsid proteins (CA), that encapsulates the genomic RNA and the essential viral enzymes. Unlike other retroviruses, the capsid protein of RSV (CA_{RSV}) does not contain two highly conserved C-terminal cysteine residues that have been implicated in an oxidative capsid assembly mechanism (Khorasanizadeh et al., 1999). To gain insights into the potential mechanisms of retroviral capsid assembly we have initiated NMR studies of CA_{RSV}.

Methods and results

The gene coding for CA_{RSV} was amplified by PCR from an unintegrated DNA library of RSV (ATCC 45000) and inserted into plasmid pET16b (Novagen). Transformation of *E. coli* strain HMS174(DE3)pLysS (Novagen) and induction resulted in efficient overexpression of the histidine-tagged protein. In our 262-residue (28.4 kDa) construct, the 240-residue native CA_{RSV} sequence (Genbank g508278) is preceded by a 22-residue tag [G-$(H)_{10}$-$(S)_2$-G-H-I-E-G-R-H-N-M], that facilitates purification by nickel affinity chromatography. Cells were grown at 37 °C in H_2O/D_2O-based M9 minimal medium (with $^{15}NH_4Cl$ and ^{13}C-glucose supplemented as required) to an OD_{600} ∼0.7/0.4, induced with 1 mM IPTG, and harvested 4/8 h thereafter at a final OD_{600} ∼1.4/0.9. From a 2 L cell culture 25/15 (±30%) mg of pure CA_{RSV} was obtained. $MW_{calc} = 28408$; $MW_{obs} = 28405 \pm 8$.

NMR samples (unlabeled; U-^{15}N; U-^{15}N/^{13}C; U-^{15}N/^{13}C, 80% ^{2}H; U-^{15}N-V; U-^{15}N-L; U-^{15}N-I; U-^{15}N-C, D, E, F, N, Q, S, Y, W; and U-10% ^{13}C, U-^{15}N CA_{RSV}) contained ∼1.0 mM protein in 10 mM sodium phosphate (pH 6.0), 0.1 mM EDTA, 5 mM β-mercaptoethanol, 1 mg/L pepstatin-A, 0.1 mM PMSF, and 0.2 mM NaN_3, in H_2O:D_2O (92:8) or D_2O. Data were collected at 30 °C with a Bruker AVANCE 800 MHz spectrometer equipped with a 5-mm z-gradient triple resonance probe, processed with NMRPipe (Delaglio et al., 1995), and analyzed with NMRView3 (Johnson and Blevins, 1994).

Backbone assignments were obtained from analysis of 3D HNCA, HN(CO)CA (Bax and Grzesiek, 1993), deuterium-decoupled HN(CA)CB and HN(COCA)CB (Gardner and Kay, 1998), and 4D ^{15}N/^{15}N-edited NOESY ($t_m = 200$ ms) spectra, all recorded with the triply-labeled sample. The process was facilitated by the information obtained from four different samples enriched specifically using ^{15}N-labeled Val, Leu, Ile or Asp (Muchmore et al., 1989). As expected, the first three samples clearly afforded residue specific assignments, but scrambling of ^{15}N-Asp resulted in at least partial labeling of most C, D, E, F, N, Q, S, Y, and W residues. Assignments were subsequently transferred to the non-deuterated protein, and extended to Hα and CO signals, by analysis of HNCA, HNCOCA, HNCO, HCACO, and CBCACONH spectra. The central region of the ^{1}H-^{15}N spectrum of CA_{RSV} is shown in Figure 1.

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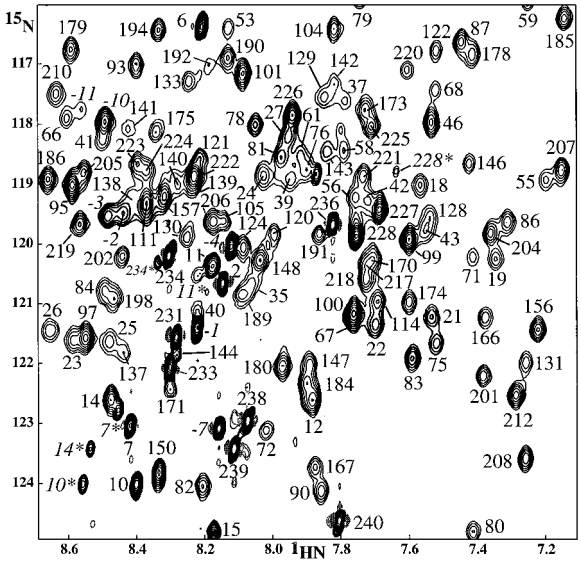

Figure 1. Central region of the 2D [^{15}N,^{1}H]-HSQC spectrum of triply labeled CA_{RSV} recorded at 800 MHz and 30 °C. Assigned peaks are labeled according to residue number, where the asterisk denotes minor species and negative numbers correspond to the non-native N-terminal tail (both in italics).

Side chain signal assignments were confounded by the fact that CA_{RSV} is highly helical, deficient in aromatic groups (containing 4 F, 2 Y, and 4 W residues), and contains a high number of residues with long side chains (12 E, 25 L, 23 P, 13 Q, 18 R). Because of the severe overlap in the ^{1}H-^{13}C correlation spectrum, side chain signals were primarily assigned by analysis of 4D ^{15}N/^{13}C- and ^{13}C/^{13}C-edited NOESY spectra (t_m = 100 and 85 ms) (Wüthrich, 1986; Clore and Gronenborn, 1991), often requiring identification of secondary and tertiary contacts for unambiguous assignment. The more sensitive 3D ^{15}N-NOESY data were used for analysis of weak or broad amide resonances and for identifying weak correlations in the corresponding 4D data. Stereospecific assignments of the methyl groups of Val and Leu were made from 2D CT-[^{13}C,^{1}H]-HSQC data collected for a 10% ^{13}C-labeled sample (Senn et al., 1989). The ^{13}CO-$^{15}NH_2$ moieties of N and Q residues were assigned from NHD scalar connectivities in HNCO, HN(CO)CA, HN(COCA)CB, and CBCA(CO)NH spectra, in combination with main-chain ^{13}Cα/β assignments, and by using intraresidual and tertiary NOE information. Aromatic signals were identified in HMQC spectra and assigned on the basis of NOEs. The conformation of X-P peptide bonds was determined by the presence of sequential Hα-Hδ (*trans*) or Hα-Hα (*cis*) NOEs, and was confirmed by the ^{13}Cγ/δ shifts.

Extent of assignments and data deposition

Assignment of the ^{1}H and ^{15}N shifts for all backbone amides was achieved with the exception of W69 and A139. Backbone ^{13}C′ resonances were obtained for all residues but L49. With the exception of S135, the ^{13}Cα-^{1}H shifts of all residues were assigned. Assignments were also made for the majority (>98%) of the aliphatic side chain ^{1}H and ^{13}C signals: only 10 β, 10 γ, and 2 δ groups (from 15 L, M, R, P, and S residues) remain unassigned and all 166 methyl resonances were assigned (stereospecifically in the case of Leu and Val groups). ^{1}H, ^{15}N, and ^{13}C′ side chain amide shifts were obtained for 5 of the 6 N and 11 out of the 13 Q (N116, Q120, and Q202 remain unassigned). Complete assignments were obtained for all aromatic residues (except H51). For 20 out of the 23 P residues (P50, P65, and P126 remain ambiguous) it was possible to identify an X-P *trans* conformation. The chemical shift assignments of CA_{RSV} have been deposited in the BioMagResBank under accession number 4384.

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