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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C assignments of a monomeric N-terminal deletion mutant of the Rous sarcoma virus protease

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Biological context

In the Rous Sarcoma Virus (RSV), the C-terminus of the Gag polyprotein contains the protease (PR), which must proteolytically liberate itself to realize full activity. In mature virions, approximately 7% of the PR population is missing the first three N-terminal residues (Pepinsky et al., 1996). This truncated form (RSVPR ΔLAM) arises from an alternate autocleavage site three amino acids (Leu-Ala-Met) downstream of the primary site, and exhibits markedly reduced proteolytic activity (Schatz et al., 1997, 2001). Crystal structures of uncomplexed wild-type RSV PR dimer (Jaskolski et al., 1990) and specificity-engineered RSV S9 PR dimer in complex with a peptide inhibitor (Wu et al., 1998) have been determined. As with other retroviral PRs, the N- and C-termini of the mature enzyme form a four-stranded intermolecular β-sheet that is essential for dimer stability (as reviewed by Vogt, 1996). The RSVPR ΔLAM N-terminal deletion is expected to abolish this important β-sheet and consequently destabilize the dimer. Dimer formation is widely believed to be the rate limiting step in the activation of PR, necessary for virion maturation. Consequently, structural insight into monomeric PR is of great interest in elucidating the mechanism of protease activation in the virus life cycle as well as from a drug-design perspective. Here we present the backbone and side-chain assignments of RSVPR ΔLAM.

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Methods and experiments

RSVPR ΔLAM is a 121 amino acid protein with a molecular weight of 13.5 kDa after purification and proteolytic cleavage. RSVPR ΔLAM was expressed in *E. coli* BL21 cells, harboring a pET11 (Novagen) derived vector. ^{15}N/^{13}C-labeled samples were purified from cultures grown in ^{13}C and ^{15}N enriched MT-9 CN medium (Martek), and ^{15}N-labeled samples were prepared from M9 medium containing ^{15}N NH_4Cl (Martek). After induction with 1 mM IPTG, cultures were incubated overnight. Inclusion bodies were collected from sonicated cell lysates, washed and then dissolved in 20 mM Tris, pH 7.5, 7 M urea, 10% glycerol, 5 mM EDTA. Denatured protein was refolded by dilution with 20 mM Tris, pH 8, 20 mM NaCl, 5 mM EDTA, 10% glycerol, to a final urea concentration of 1 M, clarified by centrifugation and was passed over a DEAE column. The flow through, containing the RSVPR ΔLAM, was collected and dialyzed into NMR buffer (20 mM Na phosphate, pH 6, 100 mM NaCl, 1% glycerol, 0.4 M urea, 10 mM DTT). After removal of precipitate by centrifugation, the supernatant was concentrated by ultrafiltration (Millipore) to a typical concentration of 3 mg/mL and supplemented with 7% v/v D_2O and 0.25 mM AEBSF (Sigma). The relatively low concentration of protein and the presence of urea and glycerol in the sample were necessary for optimum solubility and to retain predominantly monomeric protein as assayed by dynamic light scattering. The ^{15}N/^{13}C-labeled sample was exchanged into deuterated NMR buffer by dialysis after all amide proton-observed experiments had been completed. All NMR experiments were performed at 298 K on a Varian Inova 600 spectrometer. The ^{15}N-labeled samples were used

to collect ^{1}H-^{15}N HSQC, 2D homonuclear NOESY, 2D homonuclear TOCSY, ^{1}H-^{15}N NOESY-HSQC and ^{1}H-^{15}N TOCSY-HSQC spectra. The ^{13}C/^{15}N double labeled sample was used to collect ^{1}H-^{13}C HSQC, HNCA, HNCACB (optimized for Cβ), CCC-TOCSY, HCC-TOCSY, HNCO, HCACO, CBCACONH, ^{13}C-separated NOESY and HCCH-TOCSY spectra. The backbone was principally assigned using Cα and Cβ resonances (from CBCACACONH, HNCA, HNCACB and CCC-TOCSY), and verified using ^{15}N NOESY in conjunction with ^{15}N-TOCSY and HCC-TOCSY spectra. Known ^{1}H-^{13}C correlations were used as starting points to assign spin systems in the HCCH-TOCSY. Aromatic ring systems were assigned using the 2D TOCSY and NOESY spectra and information from β-strips of ^{13}C NOESY spectra, Trp ε1-strips of ^{15}N NOESY and the aromatic ^{13}C HSQC. Carbonyl resonances were assigned using HNCO and HCACO spectra. Detailed descriptions of these experiments have been reviewed elsewhere (Cavanagh et al., 1996). Spectra were processed with NMRPipe/NMRDraw (Delaglio et al., 1995) and analyzed with PIPP (Garret et al., 1991).

Extent of assignments and data deposition

Backbone assignments are complete with the exception of N-terminal residues T4 and M5, as well as S29, Y31, D92 and R93, all presumably absent due to chemical exchange. Figure 1A shows the ^{1}H-^{15}N HSQC spectrum of RSVPR ΔLAM. Only one of the side-chain NH_2 resonance pairs was successfully identified (N123). Due to the relatively low concentration of the sample (0.22 mM), not all residues gave rise to discernible signals in many of the double- and triple-resonance experiments. Consequently, side-chain resonances are not as complete, with ∼81% of protonated ^{13}C and ∼79% of nonlabile ^{1}H resonances unambiguously assigned. Consensus CSI predictions (Wishart and Sykes, 1994), as shown in Figure 1B, include portions of every secondary structural feature found in the wild-type crystal structure (Jaskolski et al., 1990), excepting the N- and C-terminal β-strands. The consensus CSI contains a gap in the β-strand prediction for the two residues prior to the β-bulges found at residues D78 and R93 in the crystal structure. RSVPR ΔLAM assignments have been deposited in the BioMagResBank (accession number 4839).

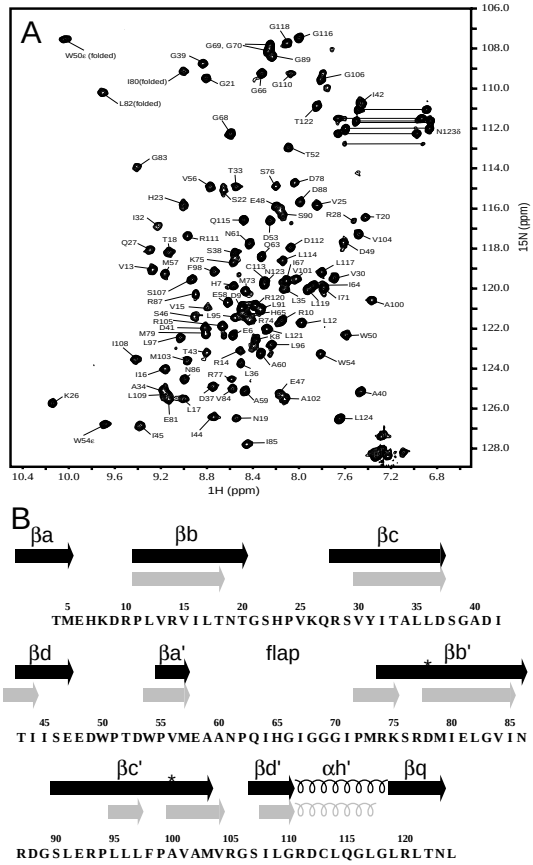

Figure 1. (A) 2D ^{1}H-^{15}N HSQC spectrum and assignments of RSVPR ΔLAM. G69 and G70 cannot be unambiguously differentiated (B) Consensus CSI predictions (gray) and wild-type secondary structure (black). Standard retroviral PR secondary structure nomenclature is noted. β-bulges found in wild-type structure are denoted by asterisks (*).

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