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Letter to the Editor: Assignments of ^{1}H, ^{15}N and ^{13}C resonances of the proline-rich matrix protein of Moloney Murine Leukemia Virus (MA MoMuLV)

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Biological context

The main role of viral matrix proteins is to bridge the nucleocapsids and the cytoplasmic parts of the glycoprotein spikes in the inner side of the virus envelope. All retroviral matrix proteins are essential for virus assembly and for the release of virus particles from cellular membranes (Soneoka et al., 1997; Hansen et al., 1990).

Although matrix proteins from different retroviruses perform similar functions, their primary sequences can be very different. Several three-dimensional structures of retroviral matrix proteins were determined up to the present time. In most cases, these are structures of closely related matrix proteins from human retroviral types A, C and D. All these matrix proteins have a high level of homology with the matrix protein of Human Immunodeficiency Virus (HIV-1) (Massiah et al., 1994) and the matrix protein of Simian Immunodeficiency Virus (SIV) (Rao et al., 1995). However, little is known about the structural organization of numerous others retroviral matrix proteins. The comparison of matrix protein structures from divergent retroviral classes may elucidate key residues and structural elements that are essential for general matrix protein functions. Therefore, MA MoMuLV (retroviral type G) has been cloned and isotopically labeled for subsequent NMR study. Assignments of ^{1}H, ^{15}N and ^{13}C resonances have been carried out using multidimensional NMR spectroscopy. MA MoMuLV has high proline content (24 prolines from a total of 131 amino acids). The majority of proline residues are located in the C-terminal part of MA MoMuLV (17 from 24). There is no experimental evidence for the functional and/or structural role of these units. Here we report the sequence-specific assignments of MA MoMuLV, including the poly-proline part. These assignments will be generally useful for mapping the binding sites of proteins or lipids, which interact with MA MoMuLV in the viral particle.

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Methods and experiments

NMR samples (1.0–1.2 mM) of purified isotopically labeled (^{15}N or ^{15}N, ^{13}C) MA MoMuLV were prepared in 50 mM lithium chloride, 10 mM sodium acetate (pH 5.5) containing 5% D_2O and 0.03% sodium azide. These conditions were found to be optimal for the combination of sample stability and spectra quality.

NMR data were acquired at 303 K using Bruker Avance DMX500 or DMX600 type spectrometers equipped with a three-axis gradient 5 mm triple resonance probe. Proton chemical shifts were referenced relative to internal DSS, whereas the ^{15}N and ^{13}C chemical shifts were referenced using the gyromagnetic ratios.

A set of 3D triple-resonance [^{15}N,^{1}H]-TROSY-HNCO, (HCA)CO(CA)NH and [^{15}N,^{1}H]-TROSY-HNCACB spectra has been collected for the sequential backbone resonance assignments, which was addition-

ally verified with intra-residual correlations from the CBCACOHA spectrum. Side-chain resonance assignments have been achieved using the following experiments: HBHA(CBCA)(CO)NH, 3D H(CC)(CO)NH-TOCSY, (H)C(C)(CO)NH-TOCSY and ^{15}N-edited 3D TOCSY-HSQC. The assignments were confirmed and completed with ^{15}N-edited NOESY-HSQC (100 ms mixing time) and ^{13}C-edited NOESY-HSQC spectra (70 ms mixing time). The later spectra have been recorded in three different versions – optimized for H^{α}/H^{β} (3D NOESY-[^{13}C, ^{1}H]-HSQC), for methyl protons (3D NOESY-(ct)-[^{13}C,^{1}H]-HSQC) and for the aromatic protons (3D NOESY-(ct)-[^{13}C,^{1}H]-TROSY).

Spectra for the correlations of aromatic $^{1}H^{\varepsilon 1}/^{15}N^{\varepsilon 1}$ or $^{1}H^{\delta 2}/^{13}C^{\delta 2}$ with $^{13}C^{\beta}$ resonances of tryptophan and histidine residues (2D [^{15}N,^{1}H]-TROSY-H(NCDCG)CB, 2D [^{15}N,^{1}H]-TROSY-H(N)C^{ar} spectra for tryptophans (Löhr et al., 2002) and 2D H(CDCG)CB (Löhr et al., 2002) for histidines) have been obtained in addition to the above mentioned standard sets of NMR spectra for backbone and side-chain resonance assignments.

A spectrum for the correlations of intra- and inter-residual $^{1}H^{\alpha}/^{13}C^{\alpha}$, ^{15}N resonances (HCAN, (Kanelis et al., 2000; Yamazaki et al., 1997) have been recorded and analyzed in order to perform resonance assignments in proline-rich regions of MA MoMuLV.

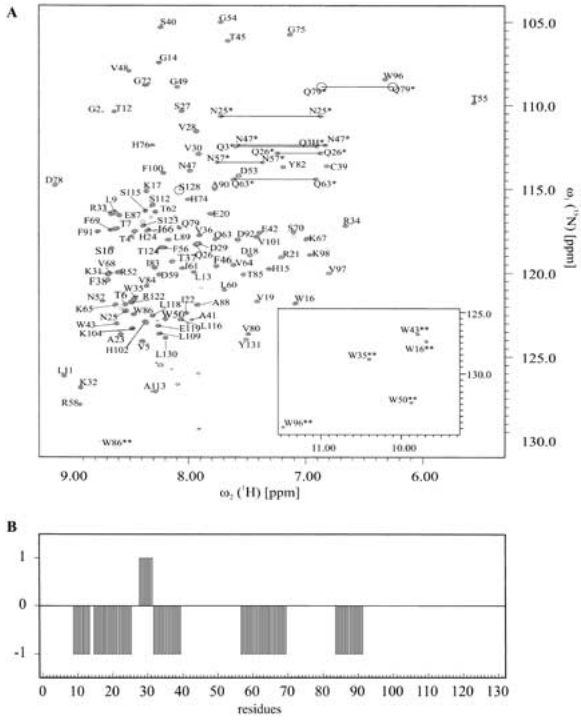

Figure 1. (A) ^{1}H, ^{15}N–HSQC spectrum and assignments of MA MoMuLV. Side-chain amide resonances of Asn and Gln are indicated by * and the resonances of Trp by **. (B) CSI consensus plot for MA MoMuLV as evaluated from $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and $^{13}C'$ chemical shifts values.

Extent of assignments and data deposition

The ^{1}H-^{15}N HSQC spectrum of MA MoMuLV at pH 5.5 and 303 K is shown in Figure 1.

An almost complete backbone resonance assignment of MoMuLV MA has been achieved, the exceptions being Met1 and Pro94. Most side-chain resonance assignments have been carried out for the non-proline part of MA MoMuLV ($H^{\varepsilon 1,2}$ of Gln3 and $H^{\delta 1}$ of Ile66 were not assigned). The C-terminal part (the proline rich part) of MA MoMuLV, however, has been assigned only partially. The side-chain resonances of Lys98, Lys104, Leu109, Leu118, Arg127 and Leu130 could not be assigned (preceding proline or weak signal intensities). Backbone resonance assignments of prolines in the C-terminal part have been obtained but side-chain resonances were found to be unassignable due to spectral overlap.

CSI predictions of secondary structure elements (Wishart and Sykes, 1994) indicate a predominantly helical structure for MA MoMuLV (Figure 1). Short- and medium-range NOE connectivities observed in ^{1}H-^{15}N NOESY-HSQC and ^{1}H-^{13}C NOESY spectra are consistent with the predictions for an α-helical secondary structure (data not shown). MA MoMuLV assignments have been deposited in the BioMagResBank (accession number 5623).

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