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Letter to the Editor: Assignment of ^{1}H, ^{13}C, and ^{15}N resonances of WT matrix protein and its R55F mutant from Mason-Pfizer monkey virus

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Biological context

Matrix protein (MA) is the N-terminal domain of the Gag polyprotein in retroviruses. It plays an important role in the transport of retroviral proteins to the site of assembly of immature viral particles as well as in their association with the cell membrane and in budding (Wills et al., 1991). These so called late phases of the retroviral life cycle have been potential targets for intervention that might lead to the development of a new type of drugs against HIV or other retroviruses. Therefore, a better understanding of the mechanism of immature viral capsid assembly is an inevitable prerequisite for developing an efficient strategy for affecting this phase of the retroviral life cycle.

Mason-Pfizer monkey virus (M-PMV) belongs to the family of betaretroviruses (formerly called D-type retroviruses) which are characterized by a different mechanism of immature capsid assembly compared with a more extensively studied class of lentiviruses (formerly C-type), where HIV-1 is the best known member. While M-PMV forms immature virions within cytoplasm of infected cells and the virions are then transported to the plasma membrane, polyprotein precursors of lentiviruses are directly transported to the cell membrane where the process of assembly takes place (Rhee et al., 1990). It was demonstrated that a replacement of arginine in the position 55 in the sequence of MA protein of M-PMV for a large hydrophobic amino acid residue, i.e. tryptophane or phenylalanine, results in a dramatically changed morphogenesis of M-PMV (Rhee et al., 1991). In this aspect the virus then behaves similarly as HIV-1, i.e. it assembles the immature virions at the plasma membrane. We have started a comparative structural study of WT MA and R55F mutant with the aim to reveal possible structural changes caused by the single point mutation. We present here the assignment of the backbone as well as side-chain resonances of both proteins.

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Methods and experiments

For preparation of the proteins we used a bacterial expression vector pETMAPPHis containing the genes encoding the wild type M-PMV matrix protein or the R55F mutant in fusion with part of phosphoprotein and a histidine tag. The fusion protein was expressed in *E. coli* strain BL 21 (DE3) under the control of bacteriophage T7 promoter. The cells were grown either in a double labeled ($^{13}C/^{15}N$) rich growth medium (OD2, Silantes, GmbH, in case of R55F) or in a minimal growth medium (containing $^{13}C_6$-D-glucose and ^{15}N ammonium chloride, Spectra Stable Isotopes, in case of WT) and induced by addition of IPTG. The cells were harvested 4 h after the induction and lysed using lysozyme, sodium deoxycholate and sonication. The fusion protein was purified using Ni–NTA agarose column and matrix protein

was liberated from the fusion by the specific action of purified recombinant M-PMV protease (Zábranský et al., 1998; Rumlová et al., 2001). Released MA protein was further purified by gel permeation chromatography on Sephacryl 100 column (HR 16/26, Pharmacia), concentrated by ultrafiltration to the final concentration of 1 mM (measured by UV absorption using the recalculated extinction coefficient of 23590 mM^{-1} cm^{-1} at 280 nm for both MA proteins) in NMR buffer (50/100 mM phosphate buffer, 100/200 mM NaCl, 10 mM DTT, 5% D_2O, pH 6).

NMR experiments were performed on a Bruker DRX-500 Avance spectrometer equipped with a triple-resonance probe with three-axes actively shielded gradient coils at 25 °C. Backbone and side-chain atom resonances were assigned using the following experiments: 2D ^1H-^{15}N HSQC and ^1H-^{13}C HSQC, 3D HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH, HBHA(CBCACO)NH, CC-TOCSY(CO)NH and H(C)CH-COSY. NMR data were processed with the NMRPipe software (Delaglio et al., 1995) and analyzed in Sparky (Goddard T.D. and Kneller D.G., SPARKY 3, University of California, San Francisco).

Extent of assignments and data deposition

Complete assignment of the backbone resonances of both molecules has been obtained for most of the non-proline residues. H_N-N signals were not detected for several residues from the ends of the helical motifs, presumably due to the chemical exchange of H_N protons with water. The carbonyl carbons were not assigned for R55F MA. The resonances of side chain atoms were assigned to the extent of 65 % (WT) and 55 % (R55F), considering all assignable atoms (^1H, ^{13}C, ^{15}N).

A comparison of a part of ^1H-^{15}N HSQC spectra of WT and R55F MA is shown in Figure 1. ^1H and ^{15}N chemical shifts are nearly identical for most residues. However, the chemical shifts differ substantially for the residues in the vicinity of the mutation point, probably due to large structural changes caused by the replacement of the hydrophilic side-chain of arginine 55 for a more hydrophobic phenylalanine.

The secondary structure of the proteins was predicted based on C_α, C_β, H_α and C' (for R55F without C') chemical shifts using the CSI program (Wishart and Sykes, 1994). The analysis revealed

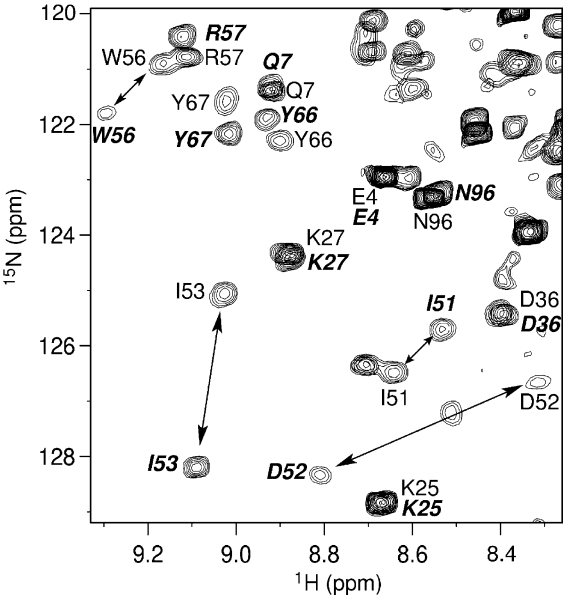

Figure 1. A part of overlayed 2D ^1H-^{15}N HSQC spectra of WT and R55F MA proteins. The assignments of the WT MA resonances are in regular font, the assignments of the R55F MA resonances in bold oblique. The arrows indicate the largest differences in signal positions between WT and R55F MA.

four α-helical regions in both, WT and R55F MA, which is a typical feature of retroviral matrix proteins (Turner et al., 1999). The assignments of both proteins have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession numbers 6400 (WT) and 6401 (R55F).

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