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Letter to the Editor: Assignment of the ^{1}H, ^{15}N and ^{13}C resonances of the nucleocapsid-binding domain of the Sendai virus Phosphoprotein

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Biological context

Sendai virus (SeV) is the prototype virus of the paramyxoviruses. It infects the respiratory track of laboratory mice and causes pneumonia. This family contains human respiratory viruses, such as human parainfluenza viruses, that cause croup, bronchiolitis and pneumonia, but also measles and mumps viruses. The negative-stranded RNA genome of SeV is encapsidated by the nucleoprotein N. This N-RNA complex serves as the template for the RNA-dependent RNA polymerase of the virus, which is constituted by two proteins, the large (L) protein and the phosphoprotein (P). The L protein contains the polymerase activity as well as the capping and polyadenylation activities. The P protein binds to the C-terminal domain of N and positions the polymerase onto the template (Horikami and Moyer, 1995). The P protein acts as well as a chaperone for newly synthesised N, so called N^0 and prevents it from binding to non-viral RNA in the infected cell. SeV P protein (568 amino acid) seems to have a modular structure. The domains, from the N-terminus to the C-terminus are: The N^0 binding domain (residues 33–41), the oligomerisation domain (residues 320–446) which includes the L binding domain (residues 412–445) and the N-RNA binding domain (residues 479–568) (Curran et al., 1995). The high resolution X-ray structure of the oligomerisation domain (residues 320-433) has been solved recently revealing a homotetrameric coiled coil structure (Tarbouriech et al., 2000b). The C-terminal, N-RNA binding part of P (also called the X-protein) is produced in large amounts in infected cells from P mRNA by an internal ribosomal entry mechanism (Curran and Kolakofsky, 1988).

In order to better understand the role of the SeV phosphoprotein a structural investigation of the X-protein was initiated. Since the X-protein was found to be extremely soluble and no crystals were obtained after extensive trials, we chose NMR for solving its structure. In this present note, we report the ^{1}H, ^{15}N and ^{13}C resonance assignment of the X domain of the SeV Phosphoprotein. The assignment of this small protein has been made difficult due to the limited amount of regular secondary structure (the N-terminus before Pro58 is disordered) shown by chemical shift data and the absence of medium and long-range NOEs. This suggests that a flexible linker is needed between the oligomerisation domain/polymerase binding site and the N-RNA binding domain for biological activity.

Methods and experiments

An isotopically $^{15}N/^{13}C$ double-labelled sample of the X protein spanning amino acids 474 to 568 of the phosphoprotein (accession number swiss-prot P04859) (corresponding respectively to residue 15 and 109 in our numbering scheme) was obtained from 4 L *E. coli* BL21 (DE3) culture in M9 minimal medium containing 1 g/l $^{15}NH_4Cl$ and 1 g/l $^{13}C_6$-glucose as sole nitrogen and carbon sources. For purification purposes the construct contained 6 additional histidine residues and a factor Xa cleavage site at its N-terminus. Details on the expression and purification procedures are already published (Tarbouriech et al.,

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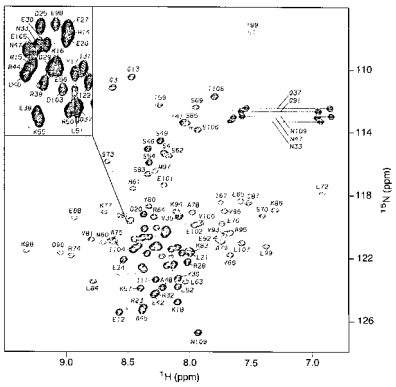

Figure 1. 2D ^{1}H-^{15}N HSQC spectrum of uniformly ^{15}N/^{13}C-enriched X domain of the Sendai virus Phosphoprotein recorded on a 600 MHz Varian Inova spectrometer at 298 K. The side chain NH_2 resonances of asparagine and glutamine residues are connected by horizontal bars.

2000a). The sample used for resonance assignments (protein concentration $\approx$ 1.2–1.5 mM) was prepared in potassium phosphate buffer (50 mM pH 6.0) with 0.5 M NaCl, 10 mM DTT, protease inhibitor cocktail (Complete, Boehringer Mannheim), 0.02% NaN_3, and 10% D_2O. The NMR tube was then sealed under argon gas.

NMR spectra were acquired at 298 K on a 600 MHz Varian Inova spectrometer equipped with a triple resonance (^{1}H, ^{13}C, ^{15}N) probe including shielded z-gradients. For sequential backbone assignment, two triple resonance reduced dimensionality 3D experiments were used which connect the H^N, N, CO, C^α resonances. The 3D MQ-HNCOCA experiment (Brutscher et al., 1995) connects the H^N(i), N(i), CO(i$-$1) and C^α(i$-$1) nuclei whereas in the 3D MQ-COHNCA experiment (Szyperski et al., 1995), magnetization is simultaneously transferred *via* one-bond scalar couplings to CO(i$-$1) and C^α(i) via a two-bond connectivity to C^α (i$-$1). This approach allows the unambiguous correlation of the four resonances even in cases where the H^N and N resonances are degenerated. This information was completed by HNCA, CBCANH, CBCA(CO)NH, HNCO, (HCA)CO(CA)NH triple resonance experiments and the sequential connectivities were built using the in-house program ALPS (Assignment Of Labelled Protein Spectra, Morelle et al., 1995). Aliphatic ^{1}H and ^{13}C sidechain assignment was performed using the following experiments: 3D H(CC)(CO)NH-TOCSY, 3D (H)CC(CO)NH-TOCSY and 3D HC(C)H-TOCSY. The (H)C(CO)NH-TOCSY spectrum also allowed unambiguous assignment of the $^{1}H^N$ and ^{15}N side chain resonances of Asn and Gln. All triple resonance experiments except the two reduced-dimensionality experiments used the pulse sequences provided by the Varian protein pack (available at ftp site : http://www.varianinc.com/). Parameters for all experiments can be obtained from the authors. All data were processed with Felix 2000 (Molecular Simulations Inc.). Proton chemical shifts were reported with respect to the H_2O signal (4.773 ppm at 25 °C). The ^{15}N and ^{13}C chemical shifts were referenced indirectly using the ^{1}H/X frequency ratios of the zero-point: 0.101329118 (^{15}N) and 0.251449530 (^{13}C) (Wishart et al., 1995).

Extent of assignments and data deposition

With the exception of residues M1, H6 to H9 (polyhistidine tag), 98% of non-prolyl backbone ^{15}N and backbone amide ^{1}H, 96.9% of non-labile ^{1}H, 98% of protonated ^{13}C (the aromatic ring atoms of aromatic residues are not included) and 98% of backbone carbonyl ^{13}C have been assigned. The chemical shift values of ^{1}H, ^{15}N and ^{13}C of the C-terminal domain of the X domain of Sendai virus phosphoprotein have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under accession number BMRB-4999.

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