Resonance assignment of nsp7 α from arterivirus

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Abstract The ^1H , ^{15}N and ^{13}C resonance assignment of nsp7 α , a non-structural protein of unknown function from the equine arteritis virus, is reported.

Keywords Equine arteritis virus · *Arteriviridae* · Equine viral arteritis · nsp7 · NMR resonance assignment

Biological context

Equine arteritis virus (EAV) is the prototype virus of the enveloped positive-stranded RNA viral family *Arteriviridae* (Snijder and Meulenberg 1998). This family also includes lactate dehydrogenase-elevating virus, porcine reproductive and respiratory virus, and simian hemorrhagic

fever virus. Together with coronaviruses and roniviruses, arteriviruses belong to the order *Nidovirales* (Cavanagh 1997).

EAV is the causative agent of equine viral arteritis (EVA) that is globally distributed in equine populations, and even though it is mostly asymptomatic or sub-clinical, it has been reported that some infected horses exhibit clinical symptoms, such as persistent infection (Huntington et al. 1990; Timoney and McCollum 1993).

The EAV genome is approximately 12.7 kb long and consists of 9 open reading frames (ORFs) (Snijder and Meulenberg 1998; Snijder et al. 1999). As in all nidoviruses, the EAV replicase gene is comprised of two large ORFs, namely ORF1a and -1b, which are post-translationally processed by three ORF1a proteinases into at least 13 non-structural proteins (nsps) (Barrette-Ng et al. 2002; Tijms et al. 2007; van Aken et al. 2006; Ziebuhr et al. 2000). These nsps are essential for viral replication and transcription and, even though a number of nsp functional domains have been identified and characterized, many nsps remain to be studied in detail.

Nsp7 is the proteolytic product generated by the main arterivirus protease (nsp4) but its role in the viral life cycle remains unknown. No homolog of nsp7 has so far been identified in other viral systems (van Aken et al. 2006). Recently, a novel nsp4 cleavage site, conserved among arteriviruses, has been identified within nsp7 giving rise to two sub-domains, nsp7 α and 7 β , even though immunoprecipitation analyses could only identify the former (van Aken et al. 2006). In order to further our understanding of the role of nsp7, we have determined the solution structure of nsp7 α , which has a unique protein fold but possesses no recognizable functional motifs (unpublished data). In this note we report the ^1H , ^{15}N and ^{13}C backbone and side chains resonance assignments for nsp7 α .

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Methods and experiments

Cloning, expression and purification

The cDNA encoding for nsp7 was kindly provided from Dr B. Coutard (AFMB Marseille). The coding sequence corresponding to nsp7 α (Ser1453–Glu1575) was amplified and subcloned into a pETM-11 expression vector (Novagen) *via* NcoI and HindIII restriction enzymes. The resulting N-terminal hexahistidine tag construct was verified by DNA sequencing, showing a single amino acid substitution, from Ser1453 to Gly1453. The residues of nsp7 α (1453–1575) were renumbered 1–123 hereafter for convenience.

The recombinant protein was expressed in *E. coli* strain BL21(DE3)RIL grown in minimal media containing 0.8 g L⁻¹ ¹⁵N-ammonium chloride and 2 g L⁻¹ ¹³C glucose. Protein expression was induced by adding 1 mM isopropyl β -D-thiogalactopyranoside (IPTG) to cells grown to OD₆₀₀ ~0.8 and then left overnight at 15°C. Cells were harvested by centrifugation, resuspended in buffer A (50 mM Tris–HCl pH 8.0, 150 mM NaCl and 2% (v/v) glycerol) and disrupted by sonication.

His-tagged nsp7 α was purified by affinity chromatography on a Ni–NTA resin (Qiagen) as per the protocol of the manufacturer. The His-tag was removed overnight at 4°C in the presence of TEV (Tobacco Etch Virus) protease (EMBL–Hamburg) at 1:50 protease:protein. The native nsp7 α (plus three N-terminal pETM-11 amino acids, namely, glycine, alanine and methionine), was then further purified on a Superdex 75 (16/60) gel filtration column (GE Healthcare Life Sciences), in buffer A.

The final NMR sample contained 0.8 mM of uniformly ¹³C/¹⁵N labelled nsp7 α in 20 mM potassium phosphate, 50 mM NaCl, 1 mM TCEP, pH 7.5 in D₂O or 90% H₂O/10% D₂O as appropriate.

NMR spectroscopy

NMR spectra were acquired at 298 K on a Varian Inova spectrometers operating at 18.8 T and on Bruker Avance spectrometers operating at 14.1 and 16.4 T equipped with triple resonance cryoprobes. Data were processed using NMRPipe/NMRDraw (Delaglio et al. 1995) and analysed in SPARKY (Goddard and Kneller) and NMRView (Johnson 2004).

For backbone sequence specific resonance assignment the following standard triple resonance experiments were employed: HNCA, HN(CO)CA, CBCANH, CBCA(CO)NH and HNCO (Bax and Grzesiek 1993). Side-chain resonance assignments were obtained using collectively ¹⁵N-edited TOCSY-HSQC and NOESY-HSQC (mixing time of 120 ms) (Fesik and Zuiderweg 1988), HCCH-TOCSY and ¹³C-edited NOESY-HSQC (mixing time of 100–120 ms).

Extent of assignment, secondary structure and data deposition

Following a standard sequential assignment procedure, complete backbone and C β assignment was achieved for all residues, with the exception of Ser38 and Asp87. The CO signal for Val102, which is followed by a proline, was also not obtained. Side chain resonances were manually assigned to near completion, including 94% ¹H and ¹³C assignment for the aromatic residues. Furthermore, all the asparagine and glutamine NH₂ groups could be unambiguously identified. The resonance assignment for the non-native residues derived from the vector sequence (see above) is not reported.

The backbone NH assignment is reported in Fig. 1, which displays the well-resolved ¹H–¹⁵N HSQC spectrum of nsp7 α . Analysis of the backbone chemical shift using the programme TALOS+ (Shen et al. 2009) and the characteristic NOE patterns from the ¹⁵N-edited NOESY-HSQC reveals that nsp7 α contains 3 α -helices and 5 β -strands as follows: α_1 (11–21), α_2 (38–54), α_3 (58–70), β_1 (25–28), β_2 (81–86), β_3 (93–96) β_4 (99–107) and β_5 (114–121). The signals of the 10 most N-terminal residues appear weaker compared to the rest of the protein, indicative of conformational exchange in this part of the molecule. This topology does not resemble any other known protein, as also evinced from the solution structure (unpublished data).

The ¹H, ¹⁵N and ¹³C chemical shifts assignments of nsp7 α have been deposited in the BioMagResBank (<http://www.bmrb.wisc.edu/>) under accession number 16977.

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Conflict of interest The authors declare that they have no conflict of interest.

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