Resonance assignment of nsp7α from arterivirus

Cyril Gaudin · Ioannis Manolaridis · Paul A. Tucker · Maria R. Conte

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 arterviruses, has been identified within nsp7 giving rise to two sub-domains, nsp7α and 7β, even though immuno-precipitation 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the solution structure of nsp7α, which has a unique protein fold but possesses no recognizable functional motifs (unpublished data). In this note we report the 1H, 15N and 13C backbone and side chains resonance assignments for nsp7α.
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 nsp7α (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 nsp7α (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\(^{-1}\) \(^{15}\)N-ammonium chloride and 2 g L\(^{-1}\) \(^{13}\)C glucose. Protein expression was induced by adding 1 mM isopropyl \(\beta\)-D-thiogalactopyranoside (IPTG) to cells grown to OD\(_{600}\) *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 nsp7α 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 nsp7α (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 \(^{13}\)C/\(^{15}\)N labelled nsp7α in 20 mM potassium phosphate, 50 mM NaCl, 1 mM TCEP, pH 7.5 in D\(_2\)O or 90% H\(_2\)O/10% D\(_2\)O 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 \(^{15}\)N-edited TOCSY-HSQC and NOESY-HSQC (mixing time of 120 ms) (Fesik and Zuiderweg 1988), HCCH-TOCSY and \(^{13}\)C-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 Cβ 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% \(^1\)H and \(^{13}\)C 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 \(^1\)H,\(^{15}\)N HSQC spectrum of nsp7α. Analysis of the backbone chemical shift using the programme TALOS++ (Shen et al. 2009) and the characteristic NOE patterns from the \(^{15}\)N-edited NOESY-HSQC reveals that nsp7α contains 3 \( \alpha\)-helices and 5 \( \beta\)-strands as follows: \( \alpha_1 \) (11–21), \( \alpha_2 \) (38–54), \( \alpha_3 \) (58–70), \( \beta_1 \) (25–28), \( \beta_2 \) (81–86), \( \beta_3 \) (93–96) \( \beta_4 \) (99–107) and \( \beta_5 \) (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 \(^1\)H, \(^{15}\)N and \(^{13}\)C chemical shifts assignments of nsp7α have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu/) under accession number 16977.

![Fig. 1](image-url)
Acknowledgments The authors are grateful to the MRC Biomedical NMR Centre, Mill Hill, and its staff, for a generous allocation of NMR time and for expert technical assistance. We thank Luigi Martino for help in preparing the figure.

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

References

Barrette-Ng IH, Ng KK, Mark BL, Van Aken D, Cherney MM, Garen C, Kolodenko Y, Gorbalenya AE, Snijder EJ, James MN (2002) Structure of arterivirus nsp4. The smallest chymotrypsin-like proteinase with an alpha/beta C-terminal extension and alternate conformations of the oxyanion hole. J Biol Chem 277: 39960–39966

Bax A, Grzesiek S (1993) Methodological advances in protein NMR. Accounts Chem Res 26:131–138

Cavanagh D (1997) Nidovirales: a new order comprising Coronaviridae and Arteriviridae. Arch Virol 142:629–633

Delaglio F, Grzesiek S, Vuister GW, Zhu G, Pfeifer J, Bax A (1995) NMRPipe: a multidimensional spectral processing system based on UNIX pipes. J Biomol NMR 6:277–293

Fesik SW, Zuiderweg ERP (1988) Heteronuclear 3-dimensional NMR spectroscopy. A strategy for the simplification of homo-nuclear two-dimensional NMR spectra. J Magn Res 78:588–593

Goddard TD, Kneller DG. SPARKY 3. University of California, San Francisco

Huntington PJ, Forman AJ, Ellis PM (1990) The occurrence of equine arteritis virus in Australia. Aust Vet J 67:432–435

Johnson BA (2004) Using NMRView to visualize and analyze the NMR spectra of macromolecules. Methods Mol Biol 278: 313–352

Shen Y, Delaglio F, Cornilescu G, Bax A (2009) TALOS+: a hybrid method for predicting protein backbone torsion angles from NMR chemical shifts. J Biomol NMR 44:213–223

Snijder EJ, Meulenberg JJ (1998) The molecular biology of arteriviruses. J Gen Virol 79(Pt 5):961–979

Snijder EJ, van Tol H, Pedersen KW, Raamsman MJ, de Vries AA (1999) Identification of a novel structural protein of arteriviruses. J Virol 73:6335–6345

Tijms MA, Nedialkova DD, Zevenhoven-Dobbe JC, Gorbalenya AE, Snijder EJ (2007) Arterivirus subgenomic mRNA synthesis and virion biogenesis depend on the multifunctional nsp1 autoprotease. J Virol 81:10496–10505

Timoney PJ, McCollum WH (1993) Equine viral arteritis. Vet Clin North Am Equine Pract 9:295–309

van Aken D, Zevenhoven-Dobbe J, Gorbalenya AE, Snijder EJ (2006) Proteolytic maturation of replicase polyprotein pp1a by the nsp4 main proteinase is essential for equine arteritis virus replication and includes internal cleavage of nsp7. J Gen Virol 87: 3473–3482

Ziebuhr J, Snijder EJ, Gorbalenya AE (2000) Virus-encoded proteinases and proteolytic processing in the Nidovirales. J Gen Virol 81:853–879

Resonance assignment of nsp7α from arterivirus