Published in final edited form as:

Biomol NMR Assign. 2008 ; 2(1): 81–84. doi:10.1007/s12104-008-9090-z.

NMR assignment of the arenaviral protein Z from Lassa fever virus

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Abstract

The arenavirus protein Z from Lassa fever virus was recently found to inhibit mRNA translation through direct interaction with eIF4E. Here, we report the NMR assignment of this RING-containing protein that was determined by triple resonance NMR techniques.

Keywords

LFV-Z; RING domain; Heteronuclear NMR; translational repressor

Biological context

Many arenaviruses cause fatal human hemorrhagic fevers. Infection by Lassa fever virus (LFV) or lymphocytic choriomeningitis virus (LCMV), two members of this arenavirus family, was studied in NIH3T3 cells by indirect immunofluorescence (Borden et al. 1998). The 11kDa arenavirus protein Z was found to directly bind the promyelocytic leukemia (PML) protein, an important regulator of mammalian cell growth. Upon infection, Z causes the relocation of PML nuclear bodies to the cytoplasm. This is likely a means to evade host cell apoptosis, given that PML promotes apoptosis. Also, the purified Z protein inhibits mRNA translation in reticulocyte lysates through direct interaction with the translation initiation factor 4E (eIF4E) (Kentsis et al. 2001). In eukaryotes, eIF4E recognizes the 5'-terminal cap structure of the mRNA, and recruits the large scaffold factor eIF4G to the mRNA. This mRNA-protein complex then binds the 40S preinitiation complex. In contrast to eIF4G and the 4E binding proteins (4E-BPs) that increase eIF4E affinity for the cap, Z was shown to decrease this affinity. Interestingly, all of these regulatory proteins bind the dorsal surface of eIF4E. Also, eIF4G and the 4E-BPs interact with eIF4E through a conserved α -helical recognition motif (YXXXXL ϕ , where X is variable and ϕ is a hydrophobic residue), while Z does not contain this motif. Instead, the Z protein contains a RING domain. The RING is a ~60-residue zinc-binding motif that usually uses conserved cysteines and a histidine to bind two zinc atoms. As a first step in providing the structural basis of translation repression by Z, we have undertaken NMR studies and report here the NMR assignments of LFV-Z. Understanding the structure of Z and its interactions with host cell proteins such as PML and eIF4E, may lead to novel therapeutic strategies for Lassa fever infection.

Further, Z is used as a model RING protein to study the self-assembly properties of these small domains. For instance, Z assembles into structures visible by electron microscopy (Kentsis et al. 2002a). Such super-molecular assembly of RING domains enhances their biochemical activities e.g. BRCA1/BARD1 and Mdm2 become more efficient E3 ligases when assembled while the Z bodies better inhibit the cap binding activity of eIF4E than Z monomers (Kentsis

et al. 2002b; Poyunoskvy et al. 2007). Thus, NMR studies could yield molecular insights into these assembly processes.

Methods and experiments

The full-length cDNA coding for LFV-Z was cloned into pGEX6p-1 (gift from Dr de la Torre and Althea A. Capul, *Scripps Research Institute*, La Jolla, California) and was expressed in *Escherichia coli* BL21 (DE3). LFV-Z was expressed at 37°C and induced by addition of 0.4 mM IPTG (supplemented with 100 μ M ZnSO₄), at 20°C for 20 h. Cells were harvested by centrifugation and resuspended into lysis buffer (PBS supplemented with 200 mM NaCl, 0.5 mM PMSF, protease inhibitor tablet, 0.1 mg/ml lysosyme, 50 μ M TCEP). After cells were disrupted by sonication, the lysate was cleared by centrifugation and the supernatant was loaded onto glutathione sepharose 4B beads (GE Healthcare) for 4 hours at 4°C. After the resin was washed 4 times with 20 ml of cold TBS (pH 7.0), the GST-fusion protein was eluted with 50 mM reduced glutathione in TBS, cleaved with 40U PreScission protease overnight at 4°C, and extensively dialyzed against phosphate buffer (20 mM NaH₂PO₄ pH 7.2, 200 mM NaCl, 50 μ M TCEP). The cleaved protein was subsequently purified by gel filtration (HiLoad 16/60 Superdex 75 pg, Amersham Biosciences) using the same buffer. Final yield of pure Z protein was 5 mg per liter of culture. The molecular weight (11,458.4 Da) was confirmed by mass spectrometry.

Isotopically enriched LFV-Z was prepared from cells grown on minimal M9 media containing [¹⁵N]ammonium chloride with or without [¹³C₆]glucose (Cambridge Isotopes Laboratory, Andover, MA). Here, the yield was 3.2 mg per liter of culture.

The solution conditions used for NMR assignment of LFV-Z were 0.2 mM protein in 93% H₂O/7% D₂O containing 20 mM phosphate buffer (pH 7.2), 200 mM NaCl, 50 μ M TCEP, 50 μ M ZnSO₄, 0.02% NaN₃. NMR experiments were acquired at 600 MHz on a Varian INOVA spectrometer equipped with an HCN cold probe at 20°C. Assignment of the main-chain atoms was achieved using the following triple resonance experiments in Biopack: HNCO, HNCA, HNCACB and CBCA(CO)NH; while HCCH-TOCSY, C(CO)NH-TOCSY, HBHA(CO)NH, 2D (HB)CB(CGCD)HD and 2D (HB)CB(CGCDCE)HE experiments were carried out for assignment of the ¹H and ¹³C side chain resonances. Due to the low concentration of LFV-Z and time-dependent stability, the HNCO, HNCA, HNCACB, CBCA(CO)NH and C(CO)NH-TOCSY experiments were acquired using non-uniform sampling acquisition schemes readily available from the Biopack software under the “Digital Filter/NLS” page in VNMRJ. Specifically, modifications to the pulse sequence for non-linear acquisition were made according to Vladislav Orekhov (Swedish NMR Centre, see Biopack manual). For the non-TOCSY experiments, 415 complex fids were acquired using a sampling scheme generated using the COAST software (Rovnyak et al. 2004), corresponding to a maximum of 60 and 32 points in the ¹³C and ¹⁵N dimensions respectively, and a reduction in acquisition time of ~ 4.5 fold. For the C(CO)NH-TOCSY experiment 1000 complex fids were collected, using a sampling schedule generated from the non-uniform sampling scheduler from Mark Maciejewski (<http://sbtools.uchc.edu/nmr/sample%5Fscheduler/>) corresponding to a maximum of 100 points in ¹³C and 32 points in ¹⁵N, leading to a reduction in acquisition time of ~ 2-fold. All non-linear experiments were processed using the Rowland NMR toolkit (RNMRTK, <http://webmac.rowland.org/rnmrtk/>). Heteronuclear ¹H- ¹⁵N NOE spectra were recorded in an interleaved manner in which each sequential fid was recorded with and without 3.5 seconds of saturation using a 120° pulse (Farrow et al. 1994). All linearly sampled spectra were processed using the NMRPipe software package (Delaglio et al. 1995) and analyzed using Sparky 3.114 (Goddard and Kneller 2003).

Assignment and data deposition

All the backbone ^1H , ^{13}C and ^{15}N resonances were fully assigned, except for residues Gly2, Lys4-Ala6, Glu10-Lys12, His25, Ser78 and Ala79 (Fig. 1A). The scarcity of peaks in the N-terminus indicates a highly mobile region. This was supported by the heteronuclear NOE experiment (Fig. 1B). Nearly all resonances were assigned in the well-ordered RING domain (Leu26-Leu70). Indeed, 97% of the protons and 99% of the carbons were unambiguously assigned for this region. In total, 74% of the proton and 85% of the carbon side chains were obtained for the entire protein. $\text{C}\alpha$, $\text{C}\beta$ and CO chemical shifts were analyzed for secondary structure propensities. The results clearly indicated the presence of an α -helix between residues Leu51 and Leu57 (Fig. 1C). Many other RING domains also contain this α -helix when the loop between the third and the fourth pair of cysteines is sufficiently long (~ 10 residues). For the rest of the protein, no regular secondary structure elements could be assigned, which is consistent, for example, with the RING finger structure of NOT4 (Hanzawa et al. 2001). However, other RING finger domains exhibit very small β -strands or extended regions throughout the structure and in particular between the first and the second pair of cysteines. This is the case for Cbl (Zheng et al. 2000) or BARD1 (Brzovic et al. 2001) for example. These latter were not clearly identified using this method. The chemical shifts have been deposited in the BMRB (accession number 15660).

Acknowledgments

We particularly thank Dr. Juan Carlos de la Torre and Althea A. Capul for providing the LFV-Z plasmid and technical advice for publication. We also thank Nadeem Siddiqui for mass spectrometry analysis. Research was supported by NIH R01 80728.

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Fig. 1. (A) Two-dimensional ^{15}N HSQC spectrum of LFV-Z at 293K and pH 7.2. The backbone amide and Trp side-chain resonances are assigned. The side-chain amide groups of Asn and Gln are connected by horizontal lines. (B) Backbone $\{^1\text{H}\}$ - ^{15}N heteronuclear NOE values for Z at 20° C. The schematic of zinc binding is shown at the bottom (solid line, site I; dotted line, site II). (C) Chemical Shift Index (CSI) analysis for the Ca resonances. The predicted helix structure is indicated as a cylinder.