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Letter to the Editor: Sequence-specific ^{1}H, ^{15}N and ^{13}C resonance assignments for an engineered arginine-rich domain of the hepatitis C virus NS3 RNA helicase

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Biological context

The NS3 protein of the hepatitis C virus (HCV) has two essential enzymatic functions for viral maturation and replication (Reed and Rice, 1999), which make it a target for the development of therapeutics. It possesses serine protease activity in the N-terminal 181 residues, whereas RNA-stimulated NTPase and helicase activities reside in the C-terminal 450 residues of the 631 amino acid residue bifunctional enzyme. The 450 amino acid residue HCV RNA helicase is composed of three nearly equal-sized domains (Yao et al., 1997): domain 1 contains the highly conserved NTP-binding motifs I and II which are shared by all helicases and also by a wide variety of other NTP-utilizing enzymes, additional conserved helicase sequence motifs reside in the arginine-rich domain 2 which are believed to be involved in NTP-binding, energy coupling and RNA binding, and the α-helical domain 3 is involved in single-stranded nucleic acid binding (Kwong et al., 1999). An unusual molecular feature of the HCV RNA helicase is a long antiparallel β-loop that extends from the central β-sheet of the second domain to the third domain where the end of the loop becomes an integral part of its structure. We have produced a series of properly folded subdomains of the HCV RNA helicase with reduced molecular sizes which are suitable for novel NMR-based drug discovery methods (Shuker et al., 1996). One of these protein constructs comprises domain 2 of the HCV RNA helicase, where the long β-hairpin that has extensive contacts with domain 3 was replaced by a designed tetra-residue loop to avoid potential protein instability and aggregation. As part of our efforts to use NMR techniques to assist in deciphering the enzyme's structure-function relationships and developing specific small molecule inhibitors, we report here the sequence-specific resonance assignments for this engineered arginine-rich domain 2 of the HCV RNA helicase (residues 327–481 of NS3 with residues 431–451 replaced by the amino acid sequence SDGK), termed d2-HCVh.

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Methods and results

Recombinant d2-HCVh was expressed in *E. coli* strain BL21(DE3) as a C-terminal fusion to an N-terminal 6-histidine tag and a thrombin cleavage site using the pET28b(+) expression vector (Novagen) which contained plasmid pNS3(327–430,SDGK,452–481). The latter was derived from plasmid pJC84 (Grakoui et al., 1993), which encodes the entire NS3 region of the 1a strain of HCV. The cell cultures were grown at 37 °C in M9 minimal medium with $^{15}NH_4Cl$ as the sole nitrogen source and/or ^{13}C-glucose as the sole carbon source. Recombinant protein expression was induced with IPTG (1 mM final concentration) at an optical density (600 nm) of 0.7–1.0. The protein was purified by a combination of Ni-NTA and gel filtration chromatography. The protein yield was typically around 10 mg L^{-1}. Samples containing 0.2–1.0 mM protein were prepared in 75 mM K_iPO_4, 5 mM perdeuterated dithiothreitol (d_{10}-DTT), 0.015% NaN_3, 0.4 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF), 5% or 99.99% D_2O at pH 6.5.

NMR experiments were performed at 25 °C on a Varian *INOVA* 500 MHz spectrometer. Spectra

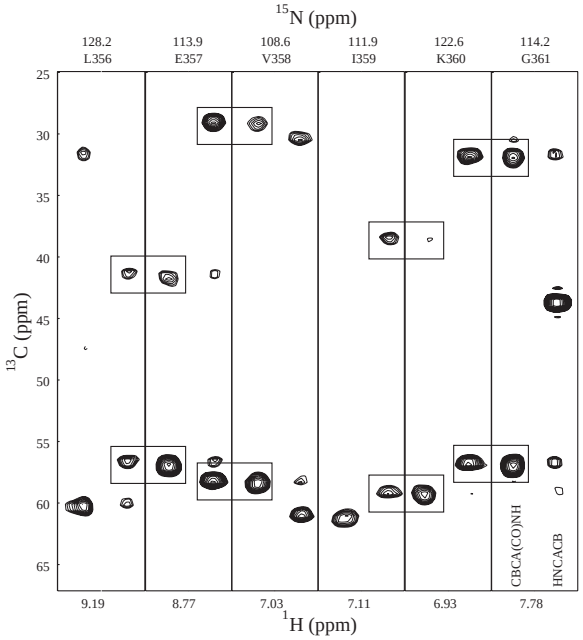

Figure 1. Representative strips from the CBCA(CO)NH and HNCACB spectra showing sequential connectivities for residues L356 to G361 in the first helix of d2-HCVh. Backbone amide ^{15}N and ^{1}H chemical shifts are shown for the indicated residues at the top and bottom, respectively, of each panel.

were processed with FELIX980 (Molecular Simulations Inc.) and analyzed with NMRView (Johnson and Blevins, 1994) on a Silicon Graphics workstation. The CBCA(CO)NH, HNCACB, (H)CC(CO)NH-TOCSY (Montelione et al., 1992) and HNCO (Muhandiram et al., 1994) experiments were used to obtain ^{1}HN, ^{15}N, ^{13}Cα, ^{13}Cβ and ^{13}C$'$ resonance assignments (Figure 1). Side chain proton and ^{13}C carbon resonances were assigned using HCCH-TOCSY (Kay et al., 1993) and ^{15}N-edited TOCSY-HSQC experiments. Aromatic side chain resonance assignments were obtained using a combination of ^{13}C-edited NOESY-HSQC and 2D experiments which provide correlations of ^{13}Cβ with ^{1}Hδ and ^{1}Hε chemical shifts of aromatic side chains (Yamazaki et al., 1993).

Extent of assignments and data deposition

The current construct includes a G-S-H-M sequence at the N-terminus of d2-HCVh which is not included in the assignment statistics. The ^{1}HN and ^{15}N resonances for all (129) possible (non-Pro residues) backbone amides were assigned. The ^{13}Cα and ^{13}Cβ resonance assignments are complete, while 90% ^{13}C$'$ resonance assignments were determined. Complete side chain resonance assignments were achieved for 132 residues, while for residues I347, F349, I359, F367, L414 and the lysine within the truncated β-hairpin S-D-G-K on average one resonance could not be assigned due to spectral overlap. The δ methyl groups for all Leu residues and the γ methyl groups for all Val residues, except V329 and V331, have been stereospecifically assigned. The ^{1}Hδ_2, ^{1}Hε_1, ^{13}Cδ_2, and ^{13}Cε_1 resonances for H333, H364 and H369 were assigned. The ^{1}H, ^{15}N, and ^{13}C chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4791.

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