# Letter to the Editor: Backbone $^1$H, $^{15}$N and $^{13}$C resonance assignments of the NTPase subdomain of the hepatitis C virus NS3 RNA helicase

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## Biological context

The RNA helicase of the hepatitis C virus (HCV) is essential for viral maturation and replication (Reed and Rice, 1999). RNA helicases represent a family of enzymes that unwind double-stranded RNA in an NTP-dependent fashion, and in most cases, this activity is stimulated in vitro by the addition of RNA or DNA. However, the mechanism of how NTP binding and hydrolysis are coupled to the unwinding process is largely unknown for the HCV RNA helicase. RNA-stimulated NTPase and helicase activities reside in the C-terminal 450 residues of the 631 amino acid residue nonstructural protein 3 (NS3) of HCV which, in addition, contains serine protease activity in its N-terminal 181 residues. Crystal structures of the HCV NS3 helicase domain, both by itself (Yao et al., 1997) and in complex with ssDNA (Kim et al., 1998), showed that it is composed of three nearly equal-sized subdomains. Subdomains 1 and 2 have little sequence identity, but share the same structure composed of a large central β-sheet flanked by α-helices, and are homologous in structure to the central region of the RecA protein (Korolev et al., 1998). Subdomain 3 (residues 482–631) is mostly α-helical and contains part of the ssDNA binding site (Kim et al., 1998). All conserved motifs, which are used to classify helicases into superfamilies and are implicated in helicase function (Gorbalenya and Koonin, 1993), are located within the two N-terminal RecA-like subdomains. For NMR-based drug discovery techniques (Shuker et al., 1996) and to better understand the structure–function relationships using NMR and crystallography together with mech-

anistic studies, we have engineered the HCV NS3 helicase domain and several subdomains of this enzyme that are correctly folded (Gesell et al., personal communication). Previously, we reported NMR resonance assignments for subdomain 2 (Liu and Wyss, 2000). Here, we report sequence-specific backbone resonance assignments for the NTPase subdomain 1 (termed dl-HCVh). dl-HCVh 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. In addition, it contains motif Ia and the TxGx motif which are involved in single-stranded nucleic acid binding (Kim et al., 1998), and motif III which, based on structural comparisons, is involved in coupling NTP hydrolysis to nucleic acid unwinding and translocation (Korolev et al., 1998).

## Methods and experiments

Recombinant dl-HCVh was expressed in *E. coli* strain BL21(DE3) as a C-terminal fusion to an N-terminal six-histidine tag and a thrombin cleavage site using plasmid pNS3(181–324) that encodes for residues 181–324 of HCV NS3 (1a strain) inserted into the NdeI and HindIII sites of the pET28b(+) expression vector (Novagen). Following thrombin cleavage, the final purified product contains an additional GSHM sequence at its N-terminus due to a cloning artifact. The cell cultures were grown at 37 °C in M9 minimal medium with $^{15}$NH$_4$Cl as the sole nitrogen source and/or $^{13}$C-glucose as the sole carbon source. D$_2$O (99%) was used in M9 medium to produce perdeuterated protein samples. Recombinant protein expression was induced with IPTG (1 mM final concentration) at an optical density (600 nm) of 0.7–1.5. The pro-
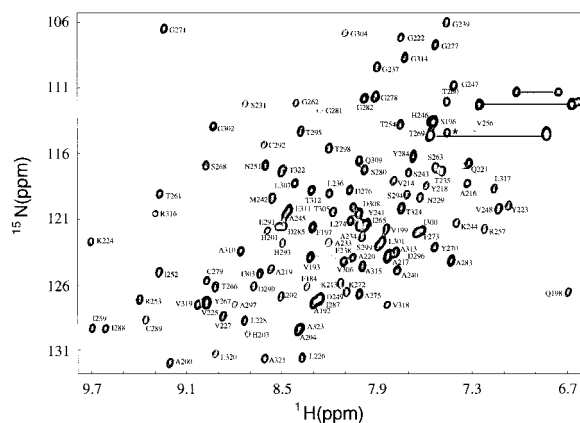
*Figure 1.* 2D $^1$H-$^{15}$N HSQC of 0.2 mM dl-HCVh. Pairs of peaks connected by a horizontal line are from NH$_2$ resonances of Asn and Gln side chains. The peak with an asterisk is from an arginine side chain.

tein was purified by a combination of Ni-NTA and size-exclusion chromatography. The protein yield was typically around 25 mg L$^{-1}$. Samples containing 0.1–0.3 mM protein were prepared in 20 mM Tris-HCl, pH 7.4, 50 mM NaCl, 5% glycerol, 5 mM perdeuterated dithiothreitol (d$_{10}$-DTT), 0.015% NaN$_3$, 0.4 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF), 5% D$_2$O.

NMR experiments were performed at 20 °C on a Varian UNITY *plus* 600 MHz spectrometer. Spectra were processed with FELIX980 (Molecular Simulations, Inc.) and analyzed with NMRView (Johnson and Blevins, 1994) on a Silicon Graphics workstation. The HNCOCACB, HNCACB, HNCA, HNCOCA, and HNCO (Muhandiram et al., 1994) experiments were used to obtain $^1$HN, $^{15}$N, $^{13}$C$\alpha$, $^{13}$C$\beta$ and $^{13}$C$'$ resonance assignments (Figure 1).

## Extent of assignments and data deposition

We noticed that dl-HCVh is more stable at higher pH ($\geq$ 7.4) due to its high pI (6.54) and it starts to aggregate at concentrations greater than about 300–500 $\mu$M (Gesell et al., personal communication). Aggregation and slow conformational exchange contributions to the line widths present a challenge for obtaining good quality triple resonance data sets. To increase

the sensitivity of the NMR experiments a perdeuterated $^{15}$N/$^{13}$C-labeled NMR sample of dl-HCVh at a protein concentration of 300 $\mu$M was used. Of the possible 134 non-proline backbone amide resonances, 119 were observed in a 2D $^1$H-$^{15}$N HSQC spectrum, all of which could be assigned (Figure 1). Missing peaks are presumed to be from residues in slow conformational exchange and consequently broadened beyond detection. Unassigned residues are clustered at the N-terminal region (residues 185–189) and in one of the conserved sequence motifs (residues 205–211 of motif I). $^{13}$C$\alpha$, $^{13}$C$\beta$, and $^{13}$C$'$ resonance assignments were obtained for 129, 112, and 115 residues, respectively. Work toward completion of side-chain assignments was hindered by the poor quality of $^{13}$C-edited spectra such as HCCH-TOCSY and $^{13}$C-edited NOESY-HSQC, due to line broadening caused by conformational exchange and/or aggregation. The $^1$H, $^{15}$N, and $^{13}$C chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4885.

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