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Letter to the Editor: Backbone ^{1}H, ^{15}N and ^{13}C assignments for the human rhinovirus 3C protease (serotype 14)

Trent C. Bjorndahl, Hassan Monzavi & David S. Wishart*
Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada, T6G 2N8

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Biological context

The human rhinovirus (HRV) is thought to be responsible for about 30 to 35% of all adult colds. As such, HRV may be considered to be one of the leading causes of human disease and morbidity. This highly variable member of the picornavirus family includes more than 110 known serotypes. These serotypes are broadly grouped into two genera, Rhinovirus A and Rhinovirus B. The Rhinovirus B genus includes serotype 14 (HRV14) which is one of the better studied rhinoviral serotypes. HRV14, as with most picornaviruses, produces a single 250 KDa gene product or polyprotein that contains all the structural and non-structural proteins necessary for viral replication. One of these non-structural proteins is the HRV-3C protease. HRV14-3C is a 182 residue cysteine protease responsible for cleaving itself and a number of other proteins from the HRV polyprotein. Because HRV14-3C plays an important role in viral maturation and because inhibition of this enzyme has shown to be effective at halting viral replication (Matthews et al., 1999), it is thought that 3C protease inhibitors could be effective pharmaceutical agents in halting or curing some colds. X-ray studies of a homologous 3C protease (51% sequence identity) from the Rhinovirus A genus, HRV2-3C (1CQQ.pdb), have shown that this protease exhibits a trypsin-like fold and contains a long shallow grove to accommodate the range of peptide substrates (Wang et al., 1997). To better understand the similarities and differences between HRV14-3C and its HRV2 homologue we have undertaken the task of obtaining the backbone ^{1}H, ^{15}N and ^{13}C chemical shifts for the HRV14-3C protease as a first step in determining its solution structure.

*To whom correspondence should be addressed. E-mail: david.wishart@ualberta.ca

Methods and experiments

HRV14-3C protease was expressed from the plasmid pET-3a in *E. coli* strain BL21(DE3)pLysS. Uniformly labeled (^{15}N and ^{13}C-^{15}N) protein samples were prepared by growing cells on M9 minimal medial containing 1 g l^{-1} $^{15}NH_4Cl$ and/or 4 g l^{-1} ^{13}C glucose. Overexpression of HRV14-3C protein was achieved by growing the host cells to mid-log phase (OD_{600} 1.2) then inducing with 1.6 mM isopropyl-β-D-galactopyranoside at 30 °C for 12 h. Following this induction phase, the cells were harvested, washed with M9 salts, resuspended in lysing buffer (50 mM Tris-HCl at pH 8.8 containing 1 mM EDTA, 5 mM DTT and 6 mM Dnase) and lysed with three cycles of freezing and thawing (−70 °C/4 °C). The supernatant was collected and passed over a 120 mL Q-Sepharose column pre-equilibrated with 50 mM Tris-HCl buffer (pH 8.8 containing 1 mM EDTA and 5 mM DTT) at 4 °C. HRV14-3C eluted following the initial flow through with >95% purity. Yields using this protocol typically approached 60 mg l^{-1}. Fractions containing HRV14-3C were pooled and dialyzed into 20 mM potassium phosphate buffer at pH 6.5 containing 2 mM DTT and 0.5 mM EDTA. Protein concentrations were determined with the Bradford assay. For NMR spectra collection the protein samples were concentrated to ∼1 mM via ultrafiltration in 20 mM potassium phosphate at pH 6.5 containing 10% D_2O, 15 mM DTT and 0.1 mM DSS for internal referencing (Wishart et al., 1995). All experiments were conducted at 25 °C on a Varian 500 MHz INOVA spectrometer fitted with a 5 mm triple-resonance z-

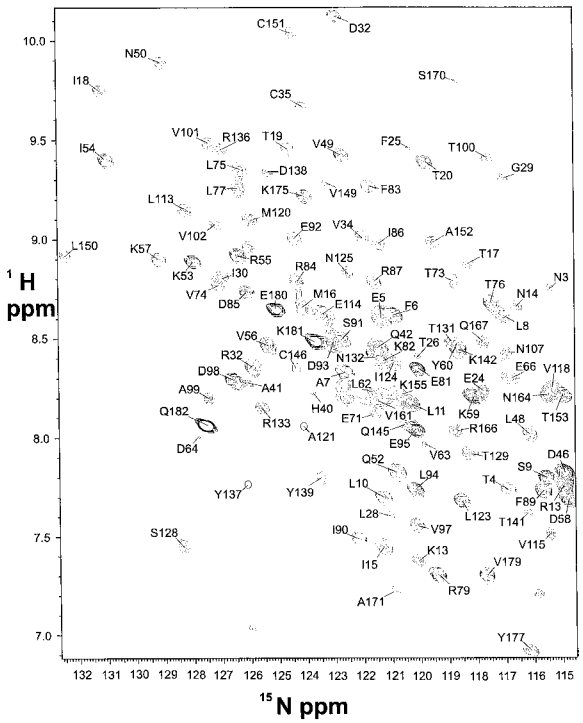

Figure 1. A portion of the ^{15}N HSQC spectrum for HRV14-3C protease. A number of backbone ^{1}H-^{15}N resonances are labeled. Resonances for A121 an Y137 are not visible at this contour level and are represented as open circles.

gradient PFG probe. All experiments were conducted using Varian Protein Pack pulse sequences. A 2D ^{15}N HSQC experiment was collected on a uniformly ^{15}N labeled sample (Figure 1). HNCA, HNCACB, CBCA(CO)NH, HNCO and HNHA spectra were collected on uniformly ^{15}N-^{13}C labeled samples. Spectra were processed with NMRPipe (Delagio et al., 1995) and further analyzed with NMRView (Johnson and Blevins, 1994). Confirmation of assignments was obtained by comparing all observed chemical shifts with calculated chemical shifts using SHIFTX, a program that uses semi-empirical methods to calculate ^{1}H, ^{13}C and ^{15}N chemical shifts from PDB co-ordinate data (Zhang et al., in press). The PDB co-ordinate data was derived from a homology model (SWISS-MODEL) using HRV2-3C (1CQQ.pdb) as a template.

Extent of assignments and data deposition

In total, 92% of all possible backbone shifts were obtained. 85% of $^{1}H^{\alpha}$, 87% of $^{13}C'$, 90% of $^{1}H^{N}$ and ^{15}N, 98% of $^{13}C^{\alpha}$ and 97% of $^{13}C^{\beta}$ chemical shifts were assigned. Backbone assignments for residues S21, H104, S105, F108, L126 and M134 were not attainable under the outlined experimental conditions. However, assignments for 95% of the RNA binding region V74 to R87 and for the active site residues H40, E71 and C146 were made. The backbone shifts have been deposited in the BioMagResBank under accession number BMRB-5659.

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