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Backbone and side-chain ^{1}H, ^{15}N, and ^{13}C resonance assignments of Norwalk virus protease

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Abstract

Norovirus protease cleaves the virus-encoded polyprotein into six mature nonstructural proteins, presenting itself as an essential enzyme for the viral replication as well as an attractive target for the antiviral drug development. A deeper understanding of the structural mechanism of the protease-substrates/inhibitors interactions by means of solution NMR methods would facilitate a rational design of the virus protease inhibitor. We here report the backbone and side-chain resonance assignment of the protease from Norwalk virus, which is the prototype strain of norovirus. The assignment data has been deposited in the BMRB database under the accession number 17523.

Keywords

Norovirus; Norwalk virus; Viral protease; NMR; Resonance assignments

Biological context

Noroviruses are now recognized as the major cause of acute nonbacterial gastroenteritis outbreaks in all age groups worldwide. It is estimated that there are 23 million annual

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infection cases in the US alone. Despite the serious impact to pubic health and highly contagious nature, no vaccine and antiviral targeting norovirus infection are available to date. Norovirus shows high diversity with five genogroups (GI–GV) with Norwalk virus (NV) being the prototype of GI (GI.1). Norovirus genome is a 7.5–7.7 kb, positive-sense, single stranded RNA containing three open reading frames (ORF1–3) (Lambden et al. 1993). ORF-2 and ORF-3 encode major and minor capsid proteins, VP1 and VP2, respectively. ORF1 encodes a ~200 kDa polyprotein that is co- and post-translationally cleaved by a viral protease to produce at least six nonstructural proteins essential for viral infectious cycle (Sosnovtsev et al. 2006), indicating that the norovirus protease can be a potential target for development of antivirals such as substrate peptide-mimetic drugs.

Norovirus protease, which is alternatively called 3C-like protease to indicate the similarity with the picornavirus 3C protease, consists of 181 amino acids. Norovirus protease belongs to the viral cysteine protease family that adopts a serine-protease (chymotrypsin-like) fold hosting the proteolytic machinery with a cysteine residue acting as a nucleophile instead of serine residue (Someya et al. 2002; Zeitler et al. 2006; Nakamura et al. 2005). Overall structure is comprised of two β barrel-like domains separated by a cleft that harbors the active-site catalytic residues, His30, Glu54, and Cys139, although there exists controversy regarding a role of Glu54 (Zeitler et al. 2006; Nakamura et al. 2005). To date, extensive analysis has been carried out in terms of the substrate specificity of norovirus proteases by using mainly in vitro translational assay; the specificity depends on the primary sequence of the cleavage site, where Gln/Gly (P1/P1′), Glu/Ala and Glu/Gly dipeptides of a polyprotein are cleaved (Blackeney et al. 2003; Hardy et al. 2002; Sosnovtsev et al. 2006). Substrate P4 residue adjoining the cleavage site has also been shown to be critical for the substrate recognition by the protease (Hardy et al. 2002). Crystal structures of Norwalk virus protease (NVpro) and Chiba virus protease (CVpro) and the crystal structure-based substrate docking models have suggested several key interactions for the substrate recognition including S1 and S2 binding pockets that accommodate P1 and P2 residues (Zeitler et al. 2006; Nakamura et al. 2005). However, further studies of the structural basis for the substrate specificity is necessary to understand the protease-substrate interactions and to design substrate-based peptidomimetic inhibitors. We here report the backbone and side-chain ^{1}H, ^{15}N and ^{13}C, resonance assignments of NVpro. The assignments will be valuable information for a detailed structural characterization of the enzyme-substrate interactions and facilitate NMR-based structure activity-relationship studies for the antiviral development.

Materials and experiments

A pET28 (Novagen) plasmid containing NVpro sequence with a stretch of N-terminal six histidine tags was transformed into *Escherichia coli* BL21(Invitrogen) cells. Uniformly ^{15}N- or $^{13}C/^{15}N$-labeled NVpro was expressed in the *E. coli* cells grown in M9 minimal media supplemented with 1 g/L $^{15}NH_4Cl$ and 2 g/L ^{13}C-D-glucose (Cambridge Isotope Laboratories) for ^{15}N-labeling and ^{13}C-labeling, respectively. Briefly, the cells containing the expression plasmid were grown in a starter culture consisting of 50 mL LB media at 37°C for 6–8 h followed by centrifugation. The cells were resuspended in M9 minimal media, grown to an OD_{600} value of 1.0, induced with 1.0 mM isopropyl β-D-thiogalactoside, and further grown at 37°C for 5 h. The cells were harvested by centrifugation, and

subsequently lysed using sonication. After centrifugation of the cell lysate, NVpro was purified using a Ni–NTA affinity column (QIAGEN). Size exclusion chromatography on a Superdex 75 prep grade (GE healthcare) was applied as a final purification step. NVpro was purified as a monomer and the final protein yield was 35–40 mg from 1 L of growing culture. All NMR samples contained 0.5–0.9 mM uniformly ^{15}N- or ^{13}C/^{15}N-labeled NVpro, 50 mM sodium phosphate pH 6.5, 100 mM NaCl, 5 mM DTT, 3 mM NaN_3 in 90% H_2O/10% D_2O or 99% D_2O. In addition, selective amino acid labeling of Val and Ala residues were conducted by growing cells in M9 media containing 150 mg/L of ^{15}N-Ala or ^{15}N-Val, 200 mg/L of the other unlabeled amino acids, and 2 g/L glucose.

NMR measurements were carried out at 25°C on a Varian VNMR 500 MHz, Bruker Avance 700 and 800 MHz spectrometers equipped with cryogenic triple resonance probes. Backbone resonance assignments were achieved using 2D ^{1}H–^{15}N HSQC and 3D HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH, HNCO, HN(CA)CO, ^{15}N-NOESY-HSQC experiments recorded on ^{13}C/^{15}N-labeled NVpro. Following spectra were collected for side chain assignments: HBHA(CO)NH, H(C)(CO)NH, (H)C(CO)NH, HCCH-TOCSY, (H)CCH-TOCSY. Validation of the assignments has also been conducted with ^{13}C-NOESY-HSQC spectrum. All NMR spectra were processed using NMRPipe (Delaglio et al. 1995), and analyzed with Sparky (Goddard and Kneller 2006) and CARA (http://www.nmr.ch) (Keller 2004).

Extent of assignments and data deposition

2D ^{1}H–^{15}N HSQC measurement of NVpro resulted in a well-dispersed spectrum (Fig. 1). Amino acid numbering is made on the authentic NVpro sequence, which excludes N-terminal His tag residues. A total of 94% of backbone $^{1}H^{N}$ and ^{15}N resonances of 177 non-proline residues, 95% of all ^{13}Cα resonances, all ^{13}Cβ resonances, and ^{13}C′ resonances have been unambiguously assigned based on a standard set of triple resonance spectra described above. These assignments were also aided by selective amino acid labeling of Val and Ala residues. The backbone amide resonances that could not be assigned include five N-terminal His tags, T29, Q51, S118, and A170–V171. Based on crystal structure of NVpro, most of the unassigned residues are located on loops connecting β-strands and are due to severe overlap, broadening and missing ^{13}Cα and ^{13}Cβ resonances. It should also be noted that resonance assignments were achieved for a region of L122-G133 for which no electron density was observed in NVpro crystal structure (Zeitler et al. 2006). Side-chain ^{1}H and ^{13}C resonance assignments were ~90% complete with the exceptions of aromatic rings. The secondary structures of NVpro were predicted by TALOS+ program (Shen et al. 2009) using the resonance assignment of ^{13}Cα, ^{13}Cβ, and ^{13}C′ resonances (Fig. 2), which were in good agreement with that of the crystal structure of NVpro (Zeitler et al. 2006). The assignment has been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under the accession number 17523.

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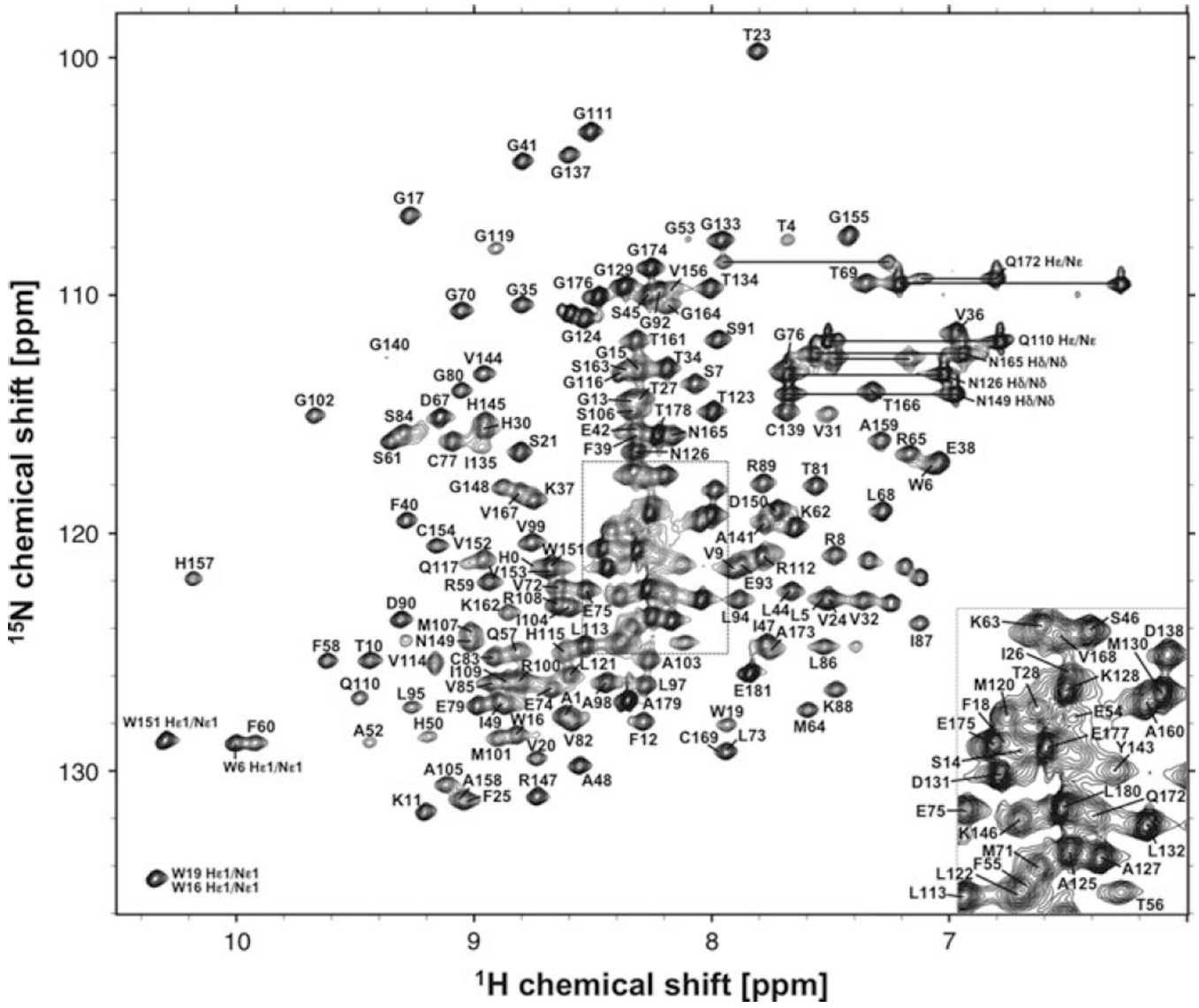

Fig. 1.

2D ^{1}H-^{15}N HSQC spectrum of 0.8 mM $^{13}C/^{15}N$-labeled NVpro recorded at 298 K on a Bruker 800 MHz spectrometer equipped with a cryogenic triple resonance probe. Sequence specific assignments are indicated

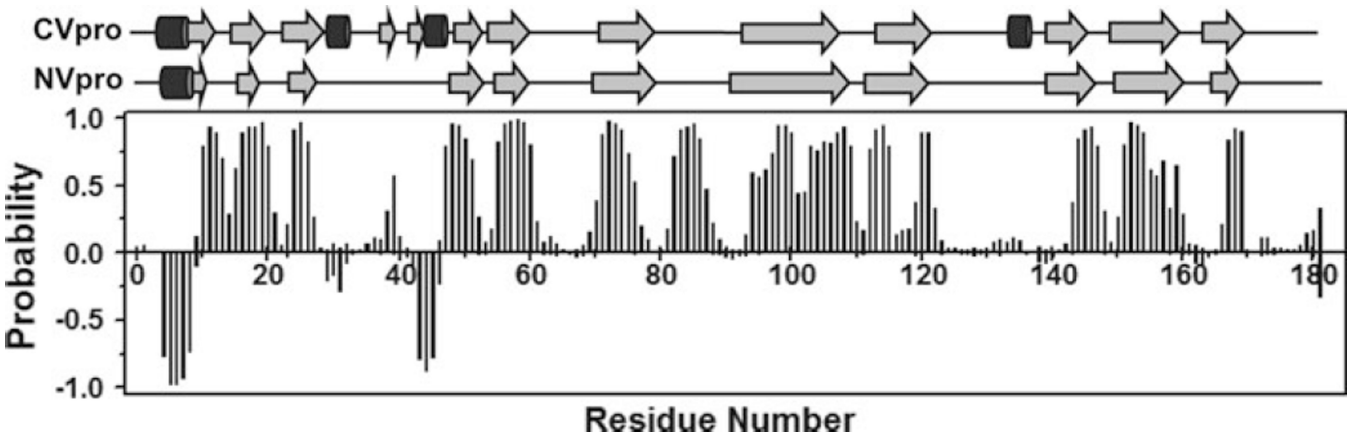

Fig. 2.

Secondary structure prediction for NVpro based on TALOS+ program with obtained chemical shift values. β-strand probabilities are given by positive values and those of α-helix are by negative for clarity. Shown in top of the chart are the secondary structure topology obtained from the crystal structure of NVpro and CVpro (PDB ID: 2FYQ, 1WQS) with α-helix shown as *cylinder* and β-sheet as *arrow*, respectively. The predicted secondary structure corresponds well with that of the crystal structures