Biomol NMR Assign (2011) 5:143–145
DOI 10.1007/s12104-010-9287-9

ARTICLE

^{1}H, ^{13}C and ^{15}N resonance assignments of SARS-CoV main protease N-terminal domain

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Received: 13 October 2010 / Accepted: 13 December 2010 / Published online: 23 December 2010

Abstract The main protease (M^{pro}) of severe acute respiratory syndrome coronavirus (SARS-CoV) plays an essential role in the extensive proteolytic processing of the viral polyproteins (pp1a and pp1ab), and it is an important target for anti-SARS drug development. SARS-CoV M^{pro} is composed of a catalytic N-terminal domain and an α-helical C-terminal domain linked by a long loop. Even though the N-terminal domain of SARS-CoV M^{pro} adopts a similar chymotrypsin-like fold as that of piconavirus 3C protease, the extra C-terminal domain is required for SARS-CoV M^{pro} to be enzymatically active. Here, we reported the NMR assignments of the SARS-CoV M^{pro} N-terminal domain alone, which are essential for its solution structure determination.

Keywords SARS-CoV · Main protease · N-terminal domain · NMR

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Biological context

Severe Acute Respiratory Syndromes Coronavirus (SARS-CoV) was identified as the etiological agent of the pandemic transmissible disease severe acute respiratory syndrome (SARS) (Peiris et al. 2003). SARS-CoV is a positive-sense, single-stranded RNA virus. During infection, two overlapping polyproteins (pp1a and pp1ab) are produced and further cleaved extensively by the main protease (M^{pro}) and the papain-like protease, yielding the components that assemble into the viral replication complex (Ziebuhr 2004). This makes M^{pro} essential for the viral life cycle and to be an attractive target for anti-SARS agent development (Anand et al. 2003).

M^{pro} exists in monomer–dimer equilibrium in solution, and only the dimeric form is the enzymatically active form (Fan et al. 2004). Recently, a super active octamer form of M^{pro} which is formed by 3D domain-swapping of the C-terminal domains is observed (Zhang et al. 2010, Zhong et al. 2009). Crystal structures revealed that each protomer of M^{pro} is composed of an N-terminal domain (residues 1–184) and an α-helical C-terminal domain (residues 201–303) linked by a long loop (Yang et al. 2003). M^{pro} is also known as 3C-like protease ($3CL^{pro}$) for it has similar substrate specificities to that of picornavirus 3C protease ($3C^{pro}$) and its N-terminal domain adopts a similar chymotrypsin-like fold as $3C^{pro}$ does. However, M^{pro} has an extra C-terminal domain which is required for M^{pro} to be active, and its role in activation remains to be elucidated.

In order to characterize the structural difference for the N-terminal domain of SARS-CoV with and without the C-terminal extra domain, we have expressed the N-terminal domain alone (M^{pro}-N, residues 1–199) in *E. coli*, which is well-folded and stable for acquisition of high-

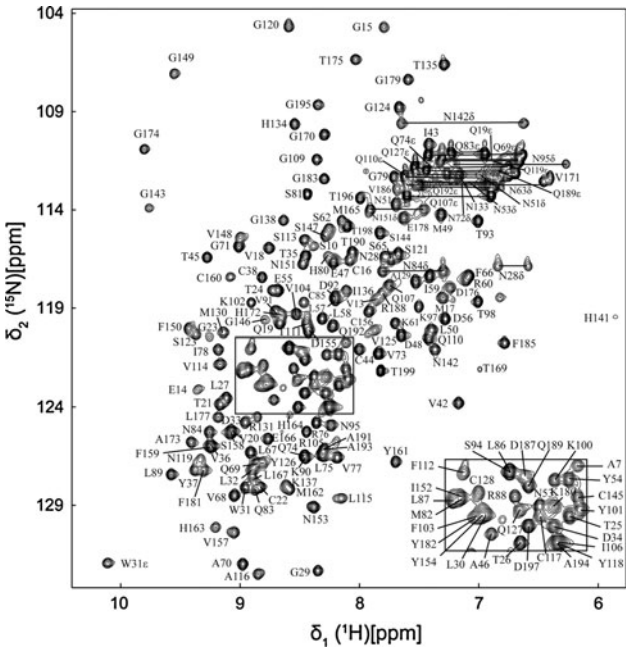

Fig. 1 2D ^{1}H-^{15}N HSQC spectrum of M^{pro}-N. The assignments are annotated by the resonance peaks with one-letter amino acid code and residue number. The side-chain NH_2 peaks of Asn and Gln are connected by *horizontal lines*

quality NMR spectra. Here, we report the nearly complete sequence-specific backbone and side-chain ^{1}H, ^{13}C and ^{15}N resonance assignments of M^{pro}-N.

Materials and methods

The uniformly ^{15}N/^{13}C-labeled or ^{15}N-labeled M^{pro}-N protein was cloned and purified as previously described (Zhong et al. 2008). All NMR samples containing ~0.7 mM M^{pro}-N were in a buffer of 50 mM potassium phosphate (pH 7.0), 30 mM Arginine, 30 mM Glutamine acid, 50 mM NaCl, 1 mM EDTA, 10 mM DTT with 10% D_2O.

All NMR experiments were performed on Bruker Avance 500 MHz, 600 MHz NMR, or 800 MHz spectrometers (all with cryoprobes) at 308 K. The backbone resonance chemical shift assignments were based on 2D ^{1}H-^{15}N HSQC, 3D HNCA, HN(CO)CA, HNCACB, CBCA(CO)HN, HNCO, HN(CA)CO and HBHA(CO)NH NMR spectra. The side chain resonance chemical shift assignments were based on 3D HCCH-COSY, HCCH-TOCSY, (H)CCH-COSY, and (H)CCH-TOCSY NMR experiments data. All NMR spectra were processed with NMRPipe (Delaglio et al. 1995), and analyzed using NMRView (Johnson and Blevins 1994). The chemical shift in the ^{1}H dimension was referenced directly to 2, 2-dimethyl-2-silapentanesulfonic acid (DSS), whereas the chemical shifts in the ^{13}C and ^{15}N dimensions were indirectly referenced to DSS.

Assignments and data deposition

SARS-CoV M^{pro}-N contains 199 residues with a molecular weight of 21.9 kDa. The 2D ^{1}H-^{15}N HSQC spectrum of

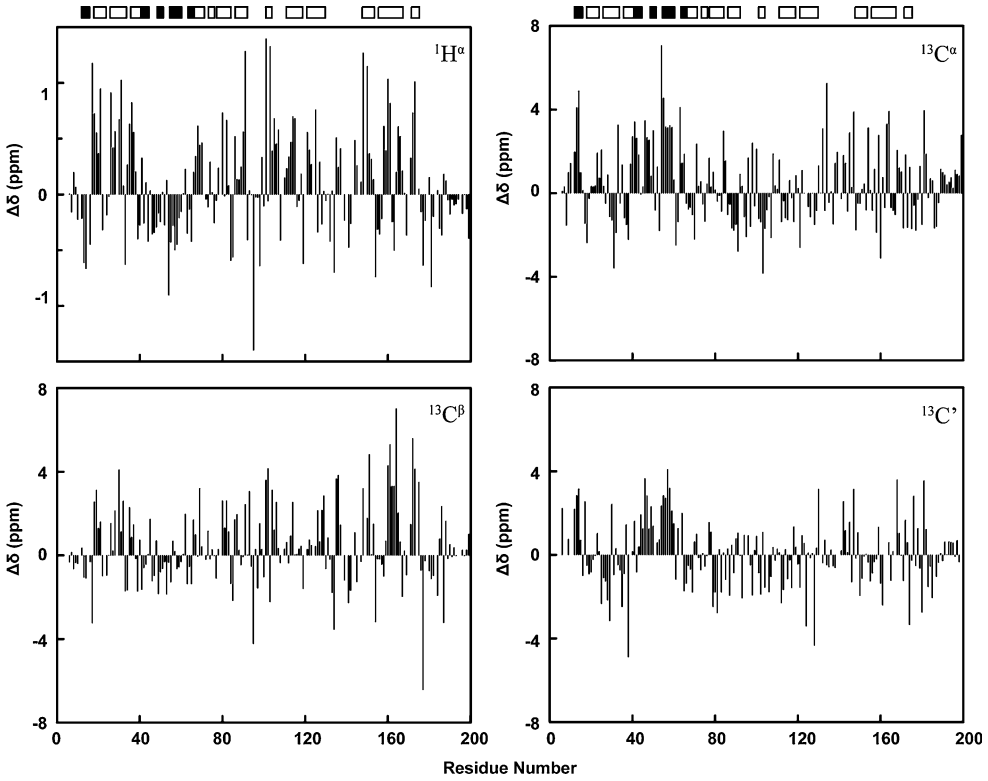

Fig. 2 Secondary chemical shifts for $^{1}H^{\alpha}$, $13C^{\alpha}$, $13C^{\beta}$ and 13C′ resonances versus residue numbers. The random coil chemical shift values were obtained from BMRB database (http://www.bmrb.wisc.edu/published/Ikura_cs_study/index.html). The secondary structural elements of the N-terminal domain in the crystal structure of full length M^{pro} (PDB code: 2H2Z) (Xue et al. 2007) are indicated on the *top of graph*. The *solid* and *hollow boxes* stand for α-helices and *β*-strands, respectively

M^{pro}-N is shown in Fig. 1, with the assignments indicated. More than 90% of backbone and side-chain resonances are assigned. The backbone NH signals for residues M6, F8, K12, N63 and S139 were missing in the 2D ^{1}H-^{15}N HSQC spectrum, while residues S1, G2, F3, R4, K5, G11 and F140 were completely unassigned. The secondary chemical shifts and chemical shift index (CSI) analysis indicate that the secondary structure of SARS-CoV M^{pro}-N is mainly in agreement with that in full length M^{pro}. The assigned ^{1}H, ^{13}C and ^{15}N chemical shifts of the protein have been deposited in BioMagResBank (http://www.bmrb.wisc.edu/) under the accession number 17251 (Fig. 2).

Acknowledgments All NMR experiments were carried out at the Beijing Nuclear Magnetic Resonance Center (BNMRC), Peking University. This work was supported by Grant 2003CB514104 from the 973 Program of China and Grant 30125009 from NSFC to BX.

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