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Letter to the Editor: ^{1}H, ^{13}C, and ^{15}N resonance assignments of the N-terminal domain of the SARS CoV nucleocapsid protein

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Biological context

The severe acute respiratory syndrome coronavirus (SARS-CoV) was identified as the pathogenic agent for the worldwide outbreak of SARS in 2003. The virion of SARS encodes four structural proteins: spike (S), envelope (E), matrix (M), and nucleocapsid (N) (Rota et al., 2003). In coronavirus the N protein is involved in the process of virus particle assembly and release, and it is believed to envelope the entire genomic RNA and interact on other important virion structural proteins (Lai and Cavanagh, 1997). The N proteins are representative immunogens in several other coronaviruses, such as murine coronavirus, Turkey coronavirus, and porcine reproductive and respiratory syndrome coronavirus. SARS N protein antibody was demonstrated to be extraordinarily sensitive and specific for reliable SARS diagnosis (Timani et al., 2004). Moreover, as SARS N protein contributes to the RNA assembling process and may serve as a translation enhancer which leads to selective translation of virus genes during infection and replication, it seems to be a potential target for SARS-related drug design. The structure of the SARS N protein RNA-binding domain (residues 49–178) has been determined (Huang et al., 2004). Obviously, the structure of the full length N-domain will give a more precise view of its tertiary structure and biological function. Here we present nearly complete ^{1}H, ^{13}C, and ^{15}N resonance assignments of the backbone and side chain of SARS N protein.

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Methods and results

The gene encoding the N-terminal domain (1–179 residues) of SARS-CoV N protein was cloned into pET-21a Vector (Novagen, Madison, WI). The recombinant plasmid was then transformed into *E. coli* BL21 (DE3)/pLysS cells. The bacteria were cultured overnight at 35 °C in 50 ml Luria–Bertani broth (LB) medium with 100 mg/l ampicillin and 35 mg/l chloramphenicol. Then they were transferred into 1 l LB medium with antibiotics. When the OD_{600} of the culture reached 0.6, the bacteria were harvested by centrifugation at 2000 g for 8 min and then resuspended in 500 ml ^{15}N-labeled or $^{13}C/^{15}N$-labeled or $^{2}H/^{13}C/^{15}N$-labeled M9 medium (Marley et al., 2001). One hour (for ^{15}N and $^{13}C/^{15}N$-labeled medium) or 2 h (for $^{2}H/^{13}C/^{15}N$-labeled medium) later, the bacteria were induced with isopropyl-beta-D-thiogalactopyranoside (IPTG) at a concentration of 100 mg/l. After 6 h induction, the cells were harvested by centrifugation at 8000 g for 15 min and resuspended in 50 mM Tris–HCI buffer (pH = 7.0). The protein was purified with cation exchange chromatography followed by gel filtration. SDS-PAGE showed that the protein purity was over 90%. Uniformly ^{15}N-labeled, $^{15}N/^{13}C$-labeled, and $^{2}H/^{15}N/^{13}C$-labeled NMR samples at about 1 mM concentration were

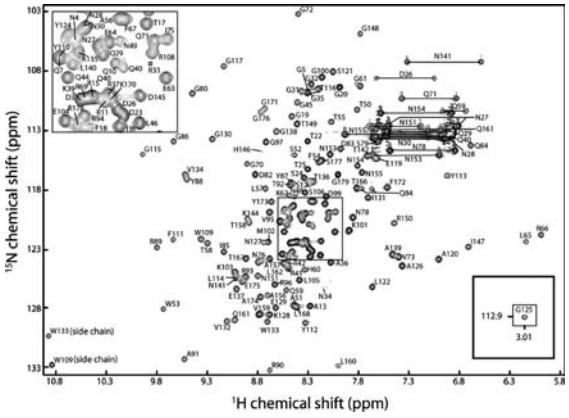

Figure 1. 2D ^{1}H{^{15}N} HSQC (800 MHz) spectrum recorded on [*U*-^{15}N, ^{13}C] N-terminal domain of SARS nucleocapsid protein at pH 7.0 and 298 K. The sidechain NH_2 resonances of Asn and Gln residues are connected by lines. Assignments of signals in the center boxed region are shown in the upper left box. Backbone NH signal from Gly125 is shown separately in the lower right box.

prepared in buffer containing 20 mM potassium phosphate (pH 7.0), 20 mM sodium chloride, 1 mM EDTA, 0.05% NaN_3 in 90% H_2O/10% D_2O, plus Complete, EDTA-free Protease Inhibitor Cocktail (Roche, Germany).

All NMR spectra were recorded at 298 K on Bruker Avance 500 MHz (with cryoprobe), 600, or 800 MHz NMR spectrometers. Backbone assignments were obtained using 2D ^{15}N edited HSQC spectrum, and 3D triple resonance experiments HNCA, HN(CO)CA, HN(CA)CB, HN(COCA)CB, HNCO, and HN(CA)CO with/without deuterium decoupling for triple-labeled and double-labeled samples, respectively. Side chain assignments were obtained from 2D ^{13}C edited HSQC spectrum, and 3D HBHA(CBCA)-CONH, HC(C)H-COSY, HC(C)H-TOCSY, and (H)CCH-TOCSY. All spectra were processed with NMRPipe (Delaglio et al., 1995), and analyzed by NMRView (Johnson and Blevins, 1994). All ^{1}H dimensions were referenced to internal DSS, whereas ^{13}C, ^{15}N dimensions were indirectly referenced to DSS (Wishart et al., 1995).

Extent of assignments and data deposition

Backbone NH resonances were assigned for 159 of the 163 non-proline residues (97%) (Figure 1). The unassigned non-proline residues are: S8, N9, Q164, and G165. 97% of the Cα, 97% of the Cβ, and 95% of the Hα and Hβ resonances in the protein have been unambiguously assigned. In total, more than 90% of aliphatic side-chain ^{1}H and ^{13}C resonances were assigned. The ^{1}H, ^{13}C, and ^{15}N chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 6372.

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References

Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Huang, Q., Yu, L., Petros, A.M., Gunasekera, A., Liu, Z., Xu, N., Hajduk, P., Mack, J., Fesik, S.W. and Olejniczak, E.T. (2004) *Biochemistry*, **43**, 6059–6063.

Johnson, B.A. and Blevins, R.A. (1994) *J. Chem. Phys.*, **29**, 1012–1014.

Lai, M.M. and Cavanagh, D. (1997) *Adv. Virus Res.*, **48**, 1–100.

Marley, J., Lu, M. and Bracken, C. (2001) *J. Biomol. NMR*, **20**, 71–75.

Rota, P.A., Oberste, M.S., Monroe, S.S., Nix, W.A., Campagnoli, R., Icenogle, J.P., Penaranda, S., Bankamp, B., Maher, K., Chen, M.H., Tong, S., Tamin, A., Lowe, L., Frace, M., DeRisi, J.L., Chen, Q., Wang, D., Erdman, D.D., Peret, T.C., Burns, C., Ksiazek, T.G., Rollin, P.E., Sanchez, A., Liffick, S., Holloway, B., Limor, J., McCaustland, K., Olsen-Rasmussen, M., Fouchier, R., Gunther, S., Osterhaus, A.D., Drosten, C., Pallansch, M.A., Anderson, L.J. and Bellini, W.J. (2003) *Science*, **300**, 1394–1399.

Timani, K.A., Ye, L., Ye, L., Zhu, Y., Wu, Z. and Gong Z. (2004) *J. Clin. Virol.*, **30**, 309–312.

Wishart, D.S., Bigam, C.G., Yao, J., Abildgaard, F., Dyson, H.J., Oldfield, E., Markley, J.L. and Sykes, B.D. (1995) *J. Biomol. NMR*, **6**, 135–140.