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ARTICLE

NMR assignments of the macro domain from Middle East respiratory syndrome coronavirus (MERS-CoV)

Yi-Ping Huang[1] · Chao-Cheng Cho[2,3] · Chi-Fon Chang[1] · Chun-Hua Hsu[2,3,4]

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Abstract The newly emerging human pathogen, Middle East respiratory syndrome coronavirus (MERS-CoV), contains a macro domain in the highly conserved N-terminal region of non-structural protein 3. Intense research has shown that macro domains bind ADP-ribose and other derivatives, but it still remains intangible about their exact function. In this study we report the preliminary structural analysis through solution NMR spectroscopy of the MERS-CoV macro domain. The near complete NMR assignments of MERS-CoV macro domain provide the basis for subsequent structural and biochemical investigation in the context of protein function.

Keywords Viral macro domain · MERS-CoV · ADP-ribose binding module · NMR spectroscopy

Yi-Ping Huang and Chao-Cheng Cho have contributed equally to this work.

✉ Chi-Fon Chang
chifon@gate.sinica.edu.tw

✉ Chun-Hua Hsu
andyhsu@ntu.edu.tw

1 Genomics Research Center, Academia Sinica, Nankang, Taipei 11529, Taiwan

2 Genome and Systems Biology Degree Program, National Taiwan University and Academia Sinica, Taipei 10617, Taiwan

3 Department of Agricultural Chemistry, National Taiwan University, Taipei 10617, Taiwan

4 Center for Systems Biology, National Taiwan University, Taipei 10617, Taiwan

Biological context

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is a kind of RNA virus belongs to genus *Betacoronavirus* in *Coronavaridae* of enveloped, which is a positive-stranded RNA virus and becomes a novel zoonotic virus in recent years (de Groot et al. 2013). MERS-CoV transmits respiratory tract infections from original host, the bat, to human by intermediate host, the camel, although the detail viral life cycle is still unknown (Han et al. 2015). The first human case was reported in 2012 at Saudi Arabia about a man with severe acute pneumonia and secondary renal failure (Zaki et al. 2012). Through close contact to infected patients, the human-to-human transmissions of the MERS-CoV virus spread very quickly. Since the first report, MERS-CoV has caused over 400 people died among 1100 cases in 23 countries. There is no efficient specific drug to use for this newly human pathogen yet, thus drug development is the emergency to conquer for the researchers.

The macro domain, a protein module specifically recognizing ADP ribose (Karras et al. 2005), could be found in many bacteria, archaea, plants and vertebrates, suggesting their evolutionary conservation (Han et al. 2011). Some macro domains possess phosphatase activity towards ADP-ribose-1″-phosphate, which is a by-product of tRNA splicing (Putic et al. 2005). Furthermore, macro domains are found in nonstructural proteins (nsps) of several positive-strand RNA viruses, including alphaviruses, rubella virus, hepatitis E virus (HEV), and coronaviruses [including the severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV)] (Neuvonen and Ahola 2009; Fehr and Perlman 2015; Hurst-Hess et al. 2015; Parvez 2015). The enzymatic activity of viral macro domain is

dispensable for coronavirus replication (Putic et al. 2005), however it seems to play a role in the pathogenesis of mouse hepatitis virus infection (Eriksson et al. 2008). In addition, viral macro proteins may act via ADP-ribose binding to influence the cellular macro domains-regulated pathways either to promote virus replication or to inhibit host responses directed against the virus (Neuvonen and Ahola 2009). Since the biochemical, enzymatic and structural analysis of MERS-CoV macro domain is not available, here we present the expression, purification, and chemical shift assignments of MERS-CoV macro domain. The NMR chemical shift assignments serve as the basis for further structural characterization and ligand screening, which provides insight into the conformational properties of this domain in solution and contributes to understanding its function.

Materials and methods

Cloning, overexpression and purification of MERS-CoV macro domain

The DNA sequence containing MERS-CoV macro domain (aa. 1110–1274) was chemical synthesized and cloned into NdeI/XhoI site of pET-28a(+) vector system (Novagen). Bacteria of the *E. coli* strain BL21(DE3) transformed with the pET-28a (+)-macro domain plasmid were grown in 4 liters of LB medium at 37 °C until the absorbance at 600 nm reach 1.0. Cells were then harvested by centrifugation and re-suspended in one liter of M9 minimal medium supplemented with 1 g/L of $^{15}NH_4Cl$ and 2 g/L of $^{13}C_6$-glucose (Cambridge Isotope Laboratories). Isopropyl-β-D-thiogalactoside (IPTG, 1 mM) was added to induce His-tagged protein for 20 h at 16 °C. The cell culture was harvested by centrifugation at 6000 rpm.

For purification, cell pellets were re-suspended in 50 mL buffer containing 25 mM sodium phosphate and 100 mM NaCl at pH 7.0 and then disrupted by sonication for 20 min. The cell extract was clarified by centrifugation at 12,500 rpm for 30 min at 4 °C to remove debris. The supernatant was then applied to Ni–NTA column (GE, Healthcare) equilibrated with the same re-suspension buffer, and His-tagged protein was eluted with 200 mM imidazole. The purified His-tagged macro domain was then digested with thrombin for 6 h at 16 °C to remove the His-tag. Finally, macro domain protein (20 kDa, with extra Gly, Ser, His and Met at N-terminus) was purified using size-exclusion Superdex75 XK 16/60 column (GE, Healthcare). The purified protein was concentrated to 0.1–0.5 mM in 20 mM sodium phosphate (pH 6.5) and 100 mM or 150 mM NaCl for NMR structural studies.

NMR spectroscopy

All NMR experiments were carried out at 293 K on Bruker Avance 600 MHz NMR or 800 MHz spectrometers equipped with 5 mm triple resonance cryoprobe and Z-gradient. The data was acquired and processed using the software Topspin2.1 (Bruker, Germany) and further analyzed using Sparky, version 3.114 (T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco), following the procedures as described previously (Yang et al. 2010; Chen et al. 2014). ^{1}H chemical shifts were externally referenced to 0 ppm of 2,2-dimethyl-2-silapentane-5-sulfonate, whereas ^{13}C and ^{15}N chemical shifts were indirectly referenced according to IUPAC recommendations (Markley et al. 1998). Protein backbone resonance assignments were based on standard triple resonance experiments (Sattler et al. 1999): HNCACB, CBCA(CO)NH, HNCO and HN(CA)CO. Aliphatic side-chain assignments were primarily done by HCCH-TOCSY and HCCH-COSY with the help of HCC(CO)NH and HBHA(CO)NH experiments.

Assignments extension and data deposition

The backbone resonance assignments were nearly complete. Figure 1 illustrates the 2D (^{1}H–^{15}N) HSQC spectrum and assignments of the amide resonances. Except for the first four amino acids on N-terminal (Gly^{-2}, Ser^{-1}, His^{0} and Met^{1}) and six Proline residues (Pro^{1}, Pro^{72}, Pro^{96}, Pro^{118}, Pro^{123}, and Pro^{134}), amides of all other residues (158 out of 168) have been assigned under the experimental conditions (pH 6.5 at 293 K). Among these 158 residues, all other backbone resonances ($^{1}H\alpha$, $^{13}C\alpha$, $^{13}C\beta$ and ^{13}C) are 100 % completed. Completeness of ^{1}H resonances assignment, including side-chain, calculated by CYANA3.9 (Güntert 2004) is 86.7 %. Secondary structure elements of MERS-CoV macro domain were identified by calculating the chemical shift deviations of the $C\alpha(\Delta\delta C\alpha)$ and $C\beta(\Delta\delta C\beta)$ from the random coil values and was corroborated by analysis of the chemical shift data using the program TALOS+ (Shen et al. 2009). Positive and negative values of the difference between $\Delta\delta C\alpha$ and $\Delta\delta C\beta$ correspond to α-helix and β-sheet secondary structure, respectively and correlated well with TALOS+ index (Fig. 2). Six helices and seven β-strands could be deduced for MERS-CoV moacro domain protein based on the secondary chemical shift analysis, which results in residues 21–27, 47–54. 58–69, 104–115, 134–144 and 156–162 in α-helices and residues 5–10, 14–19, 32–25, 78–81, 89–94, 119–124 and 148–153 forming β-sheets. The resonance assignments have been deposited to the BioMagResBank (http://www.bmrb.wisc.edu/) under the accession number 26657.

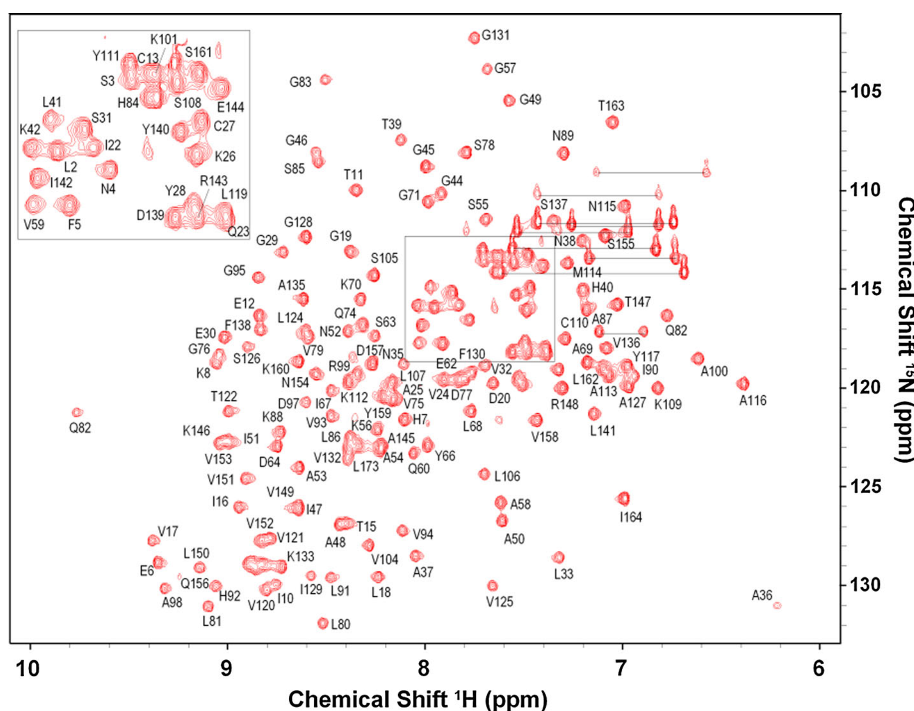

Fig. 1 ^{1}H–^{15}N HSQC spectrum of MERS-CoV macro domain recorded at 600 MHz cryogenic-probe with pH 6.5 at 293 K. Backbone amide ^{1}H and ^{15}N cross peaks are shown. The *horizontal lines* connect pairs of the side chain NH_2 protons from Asn and Gln residues

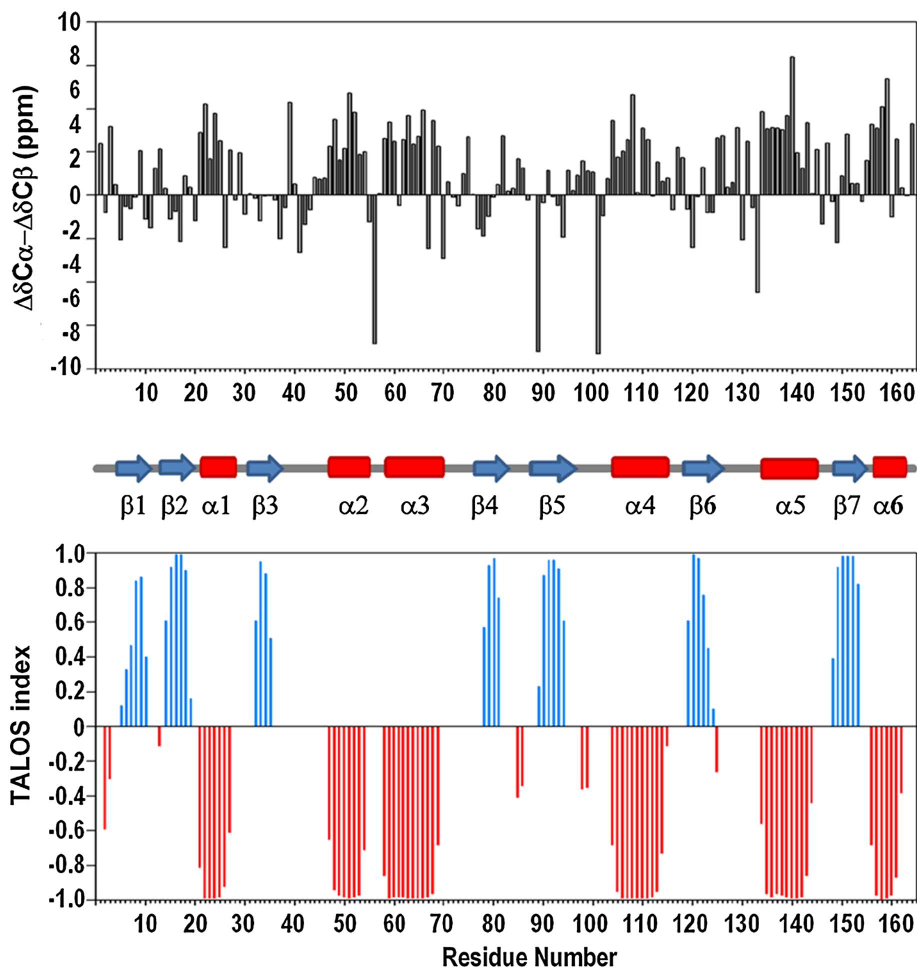

Fig. 2 The secondary structure assessment of MERS-CoV macro domain. *Upper* The parameter $\Delta\delta C\alpha - \Delta\delta C\beta$ shows the deviation of Cα and Cβ experimental values from the corresponding random coil values. Positive and negative values suggest α-helix and β-strand structure, respectively. *Middle* Chemical shift analysis resulting secondary structure elements of macro domain are represented. *Lower* TALOS+ index shows the prediction of secondary structure distribution based on backbone N, H, Cα, Hα, C and side chain Cβ chemical shift values. Negative and positive values suggest α-helix (in *red*) and β-strand (in *blue*) structure, respectively

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