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Macro Domain from Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Is an Efficient ADP-ribose Binding Module

CRYSTAL STRUCTURE AND BIOCHEMICAL STUDIES*

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Chao-Cheng Cho†, Meng-Hsuan Lin†, Chien-Ying Chuang§*, and Chun-Hua Hsu**†

From the ¤Genome and Systems Biology Degree Program, National Taiwan University and Academia Sinica, Taipei 10617, the ¶Department of Agricultural Chemistry, National Taiwan University, Taipei 10617, and the ©Center for Systems Biology, National Taiwan University, Taipei 10617, Taiwan

The newly emerging Middle East respiratory syndrome coronavirus (MERS-CoV) encodes the conserved macro domain within non-structural protein 3. However, the precise biochemical function and structure of the macro domain is unclear. Using differential scanning fluorimetry and isothermal titration calorimetry, we characterized the MERS-CoV macro domain as a more efficient adenosine diphosphate (ADP)-ribose binding module than macro domains from other CoVs. Furthermore, the crystal structure of the MERS-CoV macro domain was determined at 1.43-Å resolution in complex with ADP-ribose. Comparison of macro domains from MERS-CoV and other human CoVs revealed structural differences in the α1 helix alters how the conserved Asp-20 interacts with ADP-ribose and may explain the efficient binding of the MERS-CoV macro domain to ADP-ribose. This study provides structural and biophysical bases to further evaluate the role of the MERS-CoV macro domain in the host response via ADP-ribose binding but also as a potential target for drug design.

Since the severe acute respiratory syndrome (SARS) outbreak in 2003 (1, 2), a newly discovered disease, Middle East respiratory syndrome (MERS), has been spreading from countries in the Middle East to America (3–5). In the summer of 2015, MERS was reported in North East Asia (6–8). The causative agent of MERS was identified as an unknown coronavirus (CoV) resembling SARS-CoV and referred to as Middle East respiratory syndrome CoV (MERS-CoV) (9–12). MERS-CoV belongs to the genus Betacoronavirus and possesses a positive-strand RNA genome that encodes viral proteins essential to the life cycle of the virus (13, 14). The mortality of MERS is 4-fold higher than SARS (40% compared with 10%) (15). Since the first case report in Saudi Arabia, MERS has been reported in more than 20 countries and has caused more than 400 deaths worldwide (9).

CoVs utilize the RNA genome to encode structural proteins, including spike glycoprotein (S), membrane protein (M), and nucleocapsid protein (N). They encode a large number of non-structural proteins (NSPs) for rapid replication. A single large replicase gene encodes all proteins involved in viral replication. The replicase gene contains two open reading frames (ORFs), ORF1a and ORF1b, which encode two polyproteins, pp1a and pp1ab; production of pp1ab requires a ribosomal frameshift to transcribe the portion encoded by ORF1b (16). ORF1a encodes viral proteases, main protease (Mpro, also called 3CLpro), and papain-like protease (PLpro), which are responsible for cleavage of the ORF1a and ORF1b gene products to produce functional NSPs.

In SARS-CoV, the largest NSP member, NSP3, is a multidomain protein containing the following domains: N-terminal acidic domain, macro domain, SARS-unique domain, PLpro, nucleic acid-binding domain, marker domain (G3M), transmembrane domain, and Y-domain (17). The MERS-CoV genome contains 16 NSPs (Fig. 1); except for 3CLpro and PLpro (18, 19), most of the functional domains within the NSP3 in MERS-CoV remain structurally uncharacterized.

The macro domain is named after the non-histone motif of the histone variant macroH2A, in which it was originally characterized (20–22), a protein module ubiquitous in eukaryotes, bacteria, and archaea. This domain is well known for its affinity to adenosine diphosphate (ADP)-ribose (23–25). Many cellular enzymes bearing macro domains within their structures interact with poly(ADP)-ribose (26–29). Poly(ADP)-ribosylation is a post-translational modification linked with DNA repair, apoptosis, gene regulation, and protein degradation. Thus, macro domain–containing proteins and enzymes may play important roles in regulating various cellular processes (30). Surprisingly, the CoVs studied so far and a few other viruses such as alpha-virus, rubella virus, and hepatitis E virus possess macro domains in their genomes (16). In addition, some viral macro domains were found to have ADP-ribose 1″-phosphate phosphatase (ADRP) activity (31–33), which catalyzes the removal of phosphate from ADP-ribose 1″-phosphate (Appr1p) to produce ADP-ribose. ADRP activity has been reported in a yeast...
protein containing macro domain as well as AF1521 protein in *Archaeoglobus fulgidus* (23, 34, 35). The enzymatic activity of viral macro domains in processing Appr1p is low (33, 36–38) and appears to be dispensable for virus RNA synthesis (31). In addition, the mutant for the CoV mouse hepatitis virus A59 (MHV-A59), encoding a single amino acid substitution of a strictly conserved residue for ADRP activity, replicated to slightly reduced titers in mouse liver but, strikingly, did not induce liver disease (39). The MHV macro domain exacerbates MHV-induced liver pathology, most likely by inducing excessive inflammatory cytokine expression. It was also reported that catalytic residues Asn-809, His-812, Gly-816, and Gly-817 for ADRP activity in hepatitis E virus macro domain are critical for hepatitis E virus replication (40). Accordingly, the development of drugs targeting the viral macro domain may be a strategy for antiviral therapy.

The macro domain of SARS-CoV NSP3 was previously reported to possess ADP-ribose and poly(ADP)-ribose binding ability, which suggests that the macro domain may regulate cellular proteins involved in an apoptotic pathway via poly(ADP)-ribosylation to mediate the host response to infection (36). Structural studies of macro domains from CoVs such as human CoV 229E (HCoV-229E) and feline CoV (FCoV) also revealed interactions with ADP-ribose (41–43) and have offered huge advances in our understanding of viral macro domains. The MERS-CoV genome features a macro domain embedded in NSP3 (Fig. 1). However, we lack structural and functional information regarding the MERS-CoV macro domain.

In the present study, we investigated the MERS-CoV macro domain as an ADP-ribose binding module, with comparison to previously characterized viral macro domains. Furthermore, we determined the crystal structure of the MERS-CoV macro domain in complex with ADP-ribose. Structural comparison of MERS-CoV and other human CoVs revealed divergence in ADP-ribose binding by macro domains. Our study may shed new light on structurally based design of novel antiviral drugs targeting viral macro domains.

### Experimental Procedures

**Protein Expression and Purification**—The DNA sequence containing the MERS-CoV macro domain was synthesized by a local biotechnology company (MDBio, Inc.) and cloned into the pUC57 plasmid. The macro domain fragment was inserted between the NdeI and XhoI sites of the pET28a vector system (Novagen). The forward and reverse PCR primers used for amplification were macro-F (5′-H11032-AATTCATATGCCACTGACATTTTGAACA-3′) and macro-R (5′-H11032-AATTCTCGAGTCTAGATGGTCAGGCTCTTATAC-3′). The resulting plasmid with the inserted sequence was transformed into *Escherichia coli* BL21(DE3) cells, which were grown at 37 °C up to 0.6 A600 with 50 μg/ml of kanamycin. The macro domain fragment was inserted between the NdeI and XhoI sites of the pET28a vector system (Novagen). The forward and reverse PCR primers used for amplification were macro-F (5′-AATTCATATGCCACTGACATTTTGAACA-3′) and macro-R (5′-AATTCGACTGACTGATGGTCAGGCTCTTATAC-3′). The resulting plasmid with the inserted sequence was transformed into *Escherichia coli* BL21(DE3) cells, which were grown at 37 °C up to 0.6 A600 with 50 μg/ml of kanamycin. The expression of the recombinant MERS-CoV macro domain with an His tag at the N terminus was induced in cells with 1 mM isopropyl-β-D-thiogalactoside, followed by growth for 20 h at 16 °C. Cells were collected by centrifugation and resuspended in lysis buffer (25 mM phosphate buffer, pH 7.0, 100 mM NaCl, 0.5 mM dithiothreitol).
Structure of MERS-CoV Macro Domain

His tag was removed by using thrombin, which resulted in four additional residues (GSHM) at the N terminus. The protein was further purified by gel filtration chromatography with a Superdex75 XK 16/60 column (GE Healthcare) in 20 mM Tris-HCl buffer (pH 7.0), 100 mM NaCl.

Circular Dichroism (CD) Spectroscopy—Far-UV CD spectra were measured with 10 μM protein samples in CD buffer (20 mM phosphate buffer, pH 3.5–8.5) placed into a 1-mm path length cuvette and recorded on a JASCO J-810 spectropolarimeter equipped with a Peltier temperature control system (JASCO International Co.). Thermal transition of protein samples with or without preincubation of 1 mM ADP-ribose were monitored at 220 nm from 25 to 95 °C at a scan rate of 1 °C/min. Baseline subtraction, smoothing, and data normalization involved the use of SigmaPlot. The melting temperature (T_m) was calculated with the maximum of the first derivative of the CD signal.

Differential Scanning Fluorimetry (DSF)—Thermal shift assay with DSF involved use of a CFX48 Real-time PCR Detection System (Bio-Rad). In total, a 25–μl mixture containing 2 μl of SYPRO Orange (Sigma), 1.25 μl of dialysis buffer (20 mM Tris-HCl, and 100 mM NaCl, pH 7.0), 10 μl of 1 μM protein sample, and various concentrations of ADP-ribose were mixed on ice in an 8-well PCR tube. Fluorescent signals were measured from 25 to 95 °C in 0.1 °C/30-s steps (excitation, 450–490 nm; detection, 560–580 nm). The main measurements were carried out in triplicate. Data evaluation and T_m determination involved use of the Bio-Rad CFX Manager, and data fitting and dissociation constant (K_d) calculations involved the use of SigmaPlot.

Isothermal Titration Calorimetry (ITC)—Binding of ADP-ribose to the MERS-CoV macro domain was measured by ITC with the Nano Isothermal Titration Calorimeter (TA Instruments). Aliquots of 3 μl of 1.14 mM ADP-ribose were titrated by injection into protein (0.057 mM in 0.98 ml) in 20 mM Tris-HCl (pH 7.0) and 100 mM NaCl. Experiments were carried out at 25 °C with 250 rpm stirring. Background heat from ligand to buffer titrations was subtracted, and the corrected heat from the binding reaction was used to derive values for the stoichiometry of the binding (n), K_d, apparent enthalpy of binding (∆H), and entropy change (∆S). Data were fitted by use of an independent binding model with Launch NanoAnalyze version 2.3.6.

Crystallization and Data Collection—The MERS-CoV macro domain and ADP-ribose were mixed in a molar ratio of 1:15. Initial protein crystallization trials were performed at 283 K by the sitting-drop vapor-diffusion method with commercial crystallization screen kits, 96-well Intelli-plates (Art Robbins Instruments), and a HoneyBee 963 robot (Genomic Solutions). Each crystallization drop was prepared by mixing 0.3 μl of macro domain/ADP-ribose at 10 mg/ml with an equal volume of mother liquor, and the mixture was equilibrated against 100 μl of reservoir solution. The crystals for data collection were grown in 1 week at 283 K with the optimal condition of 100 mM phosphate/citrate (pH 4.2), 2.0 M ammonium sulfate, and 10 mM nicotinamide adenine dinucleotide as the additive. For subsequent anomalous phasing, the crystal was soaked for 8 h in 3 mM mercuric(II) chloride, cryoprotected in mother liquor supplemented with 20% glycerol, and flash-frozen in liquid nitrogen at 100 K. The diffraction images were recorded in a 100-K nitrogen gas stream with use of BL13B1 or BL13C1 beamlines (National Synchrotron Radiation Research Center, Taiwan) and processed by using HKL2000 software (44).

Structure Determination and Refinement—The crystal structure of the MERS-CoV macro domain in complex with ADP-ribose was solved by the mercury(II) derivative single-wavelength anomalous dispersion method by using SHELDX/SHELXE software (45). The initial model was refined by the maximum likelihood method implemented in REFMAC5 (46) as part of the CCP4 suite (47) and rebuilt interactively by inspecting the σ-weighted electron density maps with coefficients 2mF_o – DF_c and mF_o – DF_c in COOT (48). During the later stages, restrained positional and B-factor refinement involved the program phenix.refine (49). Water molecules were manually added at the final stages. The models were evaluated with use of PROCHECK (50) and MOLPROBITY (51). The data collection and structure refinement statistics are in Table 1.

Results and Discussion

ADP-ribose Binding Ability of MERS-CoV Macro Domain—The MERS-CoV macro domain (pp1a residues 1110 to 1273) was expressed and purified from E. coli. The final purified pro-
Structure of MERS-CoV Macro Domain

To understand the affinity of ADP-ribose binding to the MERS-CoV macro domain, both DSF and ITC measurements were used to examine the equilibrium dissociation constant ($K_d$) of ADP-ribose. After fitting DSF data, the $K_d$ was determined to be 3.12 $\mu$M ($r^2 = 0.9628$) (Fig. 3B), which is similar to the calculated $K_d$ of 2.95 $\mu$M based on ITC data (Fig. 3C). In addition, ITC data indicated that ADP-ribose bound to the MERS-CoV macro domain with favorable enthalpy change (exoergic, $\Delta H = -91.04$ KJ/mol). The binding reaction was spontaneous at 25 °C with exergonic Gibbs energy of binding ($\Delta G = -31.56$ KJ/mol). The thermodynamic profile ($\Delta G < 0$, $\Delta H < 0$, and $-T\Delta S > 0$) of ADP-ribose binding to the MERS-CoV macro domain suggests that ADP-ribose is likely stabilized by hydrogen bond formations (52).

We reviewed the results of previously reported binding assays of ADP-ribose binding to CoV macro domains (Table 2). Compared with the $K_d$ of ADP-ribose binding to macro domains of human CoVs such as SARS-CoV (24 $\mu$M) (36) and HCoV-229E (28.9 $\mu$M) (41) and animal coronaviruses such as FCoV ($\approx 400$ $\mu$M) (42), our $K_d$ of 2.95 $\mu$M from biochemical analysis suggests that the MERS-CoV macro domain is a more efficient ADP-ribose binding module. The SARS macro domain possesses poly(ADP)-ribose binding ability and may play a role in the host response to virus (36). We found that the MERS-CoV macro domain interacts with ADP-ribose, which suggests further investigating the role of the macro domain in MERS-CoV infection.

Overall Structure of MERS-CoV Macro Domain in Complex with ADP-ribose—We determined the crystal structure of ADP-ribose-bound MERS-CoV macro domain for further molecular elucidation. The orthorhombic crystals gave good quality x-ray diffraction and belonged to the space group C2221 with the following unit cell dimensions: $a = 41.798$ Å, $b = 120.807$ Å, $c = 67.659$ Å, and $\alpha = \beta = \gamma = 90^\circ$. The structure of the MERS-CoV macro domain was solved by mercury single-wavelength anomalous dispersion (see “Experimental Procedures”). The final protein structure (Fig. 4A) was refined to 1.43-Å resolution with $R$-factor and $R$-free values of 0.1273 and 0.1619, respectively (Table 1). The core of the structure of MERS-CoV macro domain is a seven-stranded $\beta$-sheet in the order of $\beta1-\beta2-\beta7-\beta6-\beta3-\beta5-\beta4$ (Fig. 4B). The central $\beta$-sheet is sandwiched between six $\alpha$-helices, with $\alpha1$, $\alpha2$, and $\alpha3$ packing onto one face and $\alpha4$, $\alpha5$, and $\alpha6$ onto the other. In the initial refinement cycle, a strong bent electron density (continuous at 1 $\sigma$ cutoff) (Fig. 4A) located at the central pocket, was unambiguously identified as an ADP-ribose molecule. This molecule is tightly bound in an uncharged crevice located at the C-terminal end of strands $\beta3$ and $\beta6$ in the loop regions between $\beta3-\alpha2$ and $\beta6-\alpha5$ (Fig. 4A).

A search of the DALI database (53) with the structure of the MERS-CoV macro domain in complex with ADP-ribose used as a model revealed several structural homologs. Top-ranked structures were macro domains of CoVs in complex with ADP-ribose such as those for SARS-CoV (PDB code 2FAV; $Z$ score 27.9; r.m.s. deviation 1.3; sequence identity 45%; sequence similarity 65%) (36), HCoV-229E (PDB code 3EWV; $Z$ score 22.8; r.m.s. deviation 1.8; sequence identity 33%; sequence similarity 56%) (43), FCoV (PDB code 3JZT; $Z$ score 22.6; r.m.s. deviation 1.8; sequence identity 30%; sequence similarity 53%) (42), and infectious bronchitis virus (PDB code 3EWp; $Z$ score 19.5; r.m.s. deviation 1.9; sequence identity 28%; sequence similarity 47%) (43). This finding reflects that the viral macro domains are structurally well conserved. However, variability between all these structures arises from the loops connecting the core secondary structure elements, which display great diversity in sequence, length, and conformation and may correspond to different ADP-ribose binding ability.

Molecular Basis of ADP-ribose Binding in MERS-CoV Macro Domain—To gain insights into the molecular mechanism of ADP-ribose binding, we further investigated the binding pocket for ADP-ribose in the MERS-CoV macro domain. The adenine moiety resides in the hydrophobic cavity containing Gly-19, Ala-21, Ile-47, Pro-123, Leu-124, and Val-152 (Fig. 5A). Coordination of ADP-ribose involves serial hydrogen bond formations and hydrophobic interactions provided by surrounding amino acid residues (Fig. 5B). The side chain of Asp-20 contacts the N-6 atom of the pyrimidine ring in adenine moiety via direct hydrogen bonding. This residue is critical for binding specificity of the macro domain AF1521 in A. fulgidus (54). Structure-based multiple sequence alignment showed that this aspartic acid is conserved among CoV macro domains (Fig. 6A). Oxygen atoms of the pyrophosphate in ADP-ribose contact surrounding residues via hydrogen bonding with nitrogen.
atoms in backbone amides of Ile-47, Ser-126, Gly-128, Ile-129, and Phe-130. The second ribose is stabilized by complex hydrogen bonding with surrounding residues and water molecules (Fig. 5A). The ribose-3′ oxygen atom forms a hydrogen bond with a nitrogen atom in the side chain of Asn-38. The ribose-2′ oxygen atom forms hydrogen bonds with the oxygen and nitrogen atoms in the backbone amides of Lys-42 and Gly-44, respectively. The ribose-1′ oxygen atom forms a hydrogen bond with the nitrogen atom in the backbone amide of Gly-46. A water molecule serves as a bridge between the ribose-1′ oxygen atom, Asn-38, and His-43. This organization of the terminal ribose and surrounding molecules was also observed in the yeast ADRP enzyme (34), which suggests that Asn-38 and His-43 may be critical for the hydrolysis reaction of ADP-ribose-1′-phosphate to ADP-ribose. In addition, equivalent residues critical for ADRP activity in the SARS-CoV macro domain (36) included Asn-35, Asn-38, His-43, Gly-44, Gly-45, and Phe-130, which are conserved in the MERS-CoV macro domain (Fig. 6A). Conservation of catalytically significant residues of ADRP in the MERS-CoV macro domain indicates that the MERS-CoV macro domain may possess ADRP enzymatic activity.

Structural Comparison of Macro Domains in MERS-CoV, SARS-CoV, and HCoV-229E—The structures of the macro domains of other CoVs pathogenic to humans, including SARS-CoV (36) and HCoV-229E (43), have been determined. Superposition of structures of MERS-CoV, SARS-CoV, and HCoV-229E macro domains shows that the major structural divergence lies in the α1 helices, which participates in stabili-
zation of ADP-ribose (Fig. 6B). Of note, in terms of the ADP-ribose binding pockets of the three structures, the structures of ADP-ribose appear at different degrees of curvature at the adenine moieties. In the MERS-CoV macro domain, the side chain position of the equivalent residue, Asp-23, varies significantly from that of Asp-20 in the MERS-CoV macro domain. This variation in side chain positions for Asp residues may result from different compositions of amino acids in the α1 helix. In the MERS-CoV macro domain, Asp-20 forms two hydrogen bonds with the N-6 atom in a pyrimidine ring of
ADP-ribose and nitrogen in the Ile-22 backbone amide in the $\alpha_1$ helix via the same oxygen atom on its side chain, thereby dragging the Asp-20 side chain into the adenine cavity. In contrast, in the SARS-CoV macro domain, Asp-23 forms a hydrogen bond with the N-6 atom of adenine via one of the oxygen atoms in its side chain and with nitrogen atoms in Val-25 and Lys-26 backbone amides via another. Hydrogen bonding with Val-25 and Lys-26 of Asp-23 in the SARS-CoV macro domain causes a variation in side chain orientation from that for Asp-20 in the MERS-CoV macro domain. Furthermore, in the MERS-CoV macro domain, the lengths of hydrogen bonds formed by the Asp-20 side chain with Ile-22 and ADP-ribose are 2.96 and 2.82 Å, respectively. In the SARS-CoV macro domain, the lengths of hydrogen bonds formed by the Asp-23 side chain with Val-25 and ADP-ribose are 3.04 and 2.87 Å, respectively (Fig. 6C). The differential strength of hydrogen bonds formed by Asp with ADP-ribose and residues in the $\alpha_1$ helix of the MERS-CoV and SARS-CoV macro domains may result from the presence of different residues in $\alpha_1$ helices that cause variations in side chain orientation of Asp residues in both struc-

FIGURE 6. Structural comparison of MERS-CoV, SARS-CoV, and HCoV-229E macro domains. A, structure-based sequence alignment of CoV macro domains. Shown are MERS-CoV (PDB code 5DUS); SARS-CoV (PDB code 2FAV); human coronavirus 229E (HCoV-229E; PDB code 3EWR); HCoV-NL63 (PDB code 2VRI); and feline CoV (FCoV; PDB code 3JZT); and infectious bronchitis virus (iBV; PDB code 3EWP). Secondary structures of MERS-CoV macro domain are depicted on the top of the alignment with arrows for $\beta$ strands and cylinders for $\alpha$ helices. Consensus amino acids among macro domains in CoVs with similarity score $>$0.7 are framed in yellow and depicted at the bottom of the alignment. Identical amino acids are in white and framed in red. Blue and green arrowheads on the top indicate amino acids forming hydrogen bonds and providing hydrophobic interactions with ADP-ribose, respectively. Yellow arrowheads at the bottom indicate equivalent amino acids in SARS-CoV macro domain found to abolish or decrease ADRP enzymatic activities when mutated. The number of residues corresponding to the MERS-CoV macro domain indicated by blue, green, and yellow arrowheads is shown on the top of the alignment. B, superposition of macro domains. Structures are shown as a ribbon model with MERS-CoV in blue, SARS-CoV in pink, and HCoV-229E in green. ADP-ribose molecules are shown as a stick model. Structural divergence is circled with a black oval. C, comparison of interactions in the adenine cavity of MERS-CoV and SARS-CoV macro domains. Amino acids and ADP-ribose are shown as a stick model. Hydrogen bonds are shown as dashed lines and bond lengths are indicated in Å units. D, comparison of interactions in adenine cavities of MERS-CoV and HCoV-229E macro domains. Amino acids and ADP-ribose are shown as a stick model. Hydrogen bonds are shown as dashed lines.
tures. As compared with Asp-20 in MERS-CoV and Asp-23 in SARS-CoV, the equivalent residue in HCoV-229E is Asp-19, which does not contact ADP-ribose. Instead of forming a hydrogen bond directly with ADP-ribose, the side chain of Asp-19 in HCoV-229E contacts Thr-22 in the α1 helix via hydrogen bonding with oxygen and nitrogen atoms in the side chain and backbone of Thr-22, respectively. Hydrogen bonding with Thr-22 drags the side chain of Asp-19 in the HCoV-229E macro domain away from the adenine cavity as compared with the position of Asp-20 in the MERS-CoV macro domain (Fig. 6D). Consistent with the previous study, the thermodynamic profile (∆G < 0, ∆H < 0, and −T∆S < 0) of ADP-ribose binding to the HCoV-229E macro domain suggests less contribution of the hydrogen bond to stabilization of ADP-ribose (41) (Table 2). Variations in strength of the hydrogen bond and orientation of the side chain in Asp residues may result in differential binding affinities of ADP-ribose observed in macro domains of MERS-CoV (Kd 2.95 μM), SARS-CoV (Kd 24 μM) (36), and HCoV-229E (Kd 28.9 μM) (41). The relationship between binding affinities of ADP-ribose in macro domains and differential pathogenicity of human CoVs needs further investigation.

Conclusion—Taken together, our biochemical study shows higher binding affinity for ADP-ribose in the MERS-CoV macro domain than macro domains of CoVs characterized to date. Structural analysis revealed that differences in the context of hydrogen bonds formed by the conserved Asp with ADP-ribose and residues in α1 helices in macro domains of MERS-CoV, SARS-CoV, and HCoV-229E may result in differential binding affinities for ADP-ribose. Our studies provide a biochemical basis for further investigating the role of macro domain in MERS-CoV infection and also the precise structural information for the design of novel antiviral drugs.

Author Contributions—C. H. H. conceived the study. C. C. C. and M. H. L. performed purification of the enzyme, biochemical assays, DSF, ITC, and crystallization. C. C. C., M. H. L., and C. Y. C. collected x-ray data. C. C. C. and C. H. H. determined and analyzed the crystal structure. C. C. C. and C. H. H. contributed to the manuscript writing. All authors reviewed the results and approved the final version of the manuscript.

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References
1. Hui, D. S., Chan, M. C., Wu, A. K., and Ng P. C. (2004) Severe acute respiratory syndrome (SARS): epidemiology and clinical features. Postgrad. Med. J. 80, 373–381
2. Shaw, K. (2006) The 2003 SARS outbreak and its impact on infection control practices. Public Health 120, 8–14
3. Kossyvakis, A., Tao, Y., Lu, X., Pokga, V., Tsiodras, S., Emmanouil, M., Mentis, A. F., Tong, S., Erdman, D. D., and Antoniadis, A. (2015) Laboratory investigation and phylogenetic analysis of an imported Middle East respiratory syndrome coronavirus case in Greece. PloS One 10, e0125809
4. Rasmussen, S. A., Gerber, S. I., and Swerdlow, D. L. (2015) Middle East respiratory syndrome coronavirus: update for clinicians. Clin. Infect. Dis. 60, 1686–1689
5. Thabet, F., Chehab, M., Bafaqih, H., and Al Mohaiimeed, S. (2015) Middle East respiratory syndrome coronavirus in children. Saudi Med. J. 36, 484–486
6. Dyer, O. (2015) South Korea scrambles to contain MERS virus. BMJ 350, h3095
7. Hui, D. S., Perlman, S., and Zumla, A. (2015) Spread of MERS to South Korea and China. Lancet Respir. Med. 3, 509–510
8. Park, S. Y., Kim, H. J., Yoo, K. H., Park, Y. B., Kim, S. W., Lee, S. J., Kim, E. K., Kim, J. H., Kim, Y. H., Moon, J. Y., Min, K. H., Park, S. S., Lee, J. J., Lee, C. H., Park, J., Byun, M. K., Lee, S. W., Rhee, C., Jung, J. Y., and Sim, Y. S. (2015) The efficacy and safety of prone positioning in adults patients with acute respiratory distress syndrome: a meta-analysis of randomized controlled trials. J. Thorac. DIS. 7, 356–367
9. Banik, G. R., Khandaker, G., and Rashid, H. (2015) Middle East respiratory syndrome coronavirus “MERS-CoV”: current knowledge gaps. Paeidiatr. Respir. Rev. 16, 197–202
10. Han, H. J., Wen, H. L., Zhou, C. M., Chen, F. F., Luo, L. M., Liu, J. W., and Yu, X. J. (2015) Bats as reservoirs of severe emerging infectious diseases. Virus Res. 205, 1–6
11. Jalal, S. (2015) The emerging threat of MERS. J. Pak. Med. Assoc. 65, 310–311
12. Zaki, A. M., van Boheemen, S., Bestebroer, T. M., Osterhaus, A. D., and Fouchier, R. A. (2012) Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. New Engl. J. Med. 367, 1814–1820
13. Corman, V. M., Eckerle, I., Bleicker, T., Zaki, A., Landt, O., Eschbach-Bldau, M., van Boheemen, S., Gopal, R., Ballhause, M., Bestebroer, T. M., Muth, D., Muller, M. A., Drexler, J. F., Zambon, M., Osterhaus, A. D., Fouchier, R. M., and Drosten, C. (2012) Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill. 17, pii, 20285
14. Geng, H., and Tan, W. (2013) A novel human coronavirus: Middle East respiratory syndrome coronavirus. Sci. China Life Sci. 56, 883–887
15. Zumla, A., Hui, D. S., and Perlman, S. (2015) Middle East respiratory syndrome. Lancet 386, 995–1007
16. Snijder, E. J., Breidenbeek, P. J., Dobbe, J. C., Thiel, V., Ziebuhr, J., Poon, L. L., Guan, Y., Rozanov, M., Spaan, W. J., and Gorbalenya, A. E. (2003) Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. J. Mol. Biol. 331, 991–1004
17. Neuman, B. W., Joseph, J. S., Saikatendu, K. S., Serrano, P., Chatterjee, A., Johnson, M. A., Liao, L., Klaus, J. P., Yates, J. R., 3rd, Wüthrich, K., Stevens, R. C., Buchmeier, M. J., and Kuhn, P. (2008) Proteomics analysis unravels the functional repertoire of coronavirus nonstructural protein 3. J. Virol. 82, 5279–5294
18. Lei, J., Mesters, J. R., Drosten, C., Anemüller, S., Ma, Q., and Hilgenfeld, R. (2014) Crystal structure of the papain-like protease of MERS coronavirus reveals unusual, potentially druggable active-site features. Antiviral Res. 109, 72–82
19. Needle, D., Loutonts, G. T., and Waugh, D. S. (2015) Structures of the Middle East respiratory syndrome coronavirus 3C-like protease reveal insights into substrate specificity. Acta Crystallogr. D Biol. Crystallogr. 71, 1102–1111
20. Chakravarthy, S., Gundimella, S. K., Caron, C., Perche, P. Y., Pehrson, J. R., Karras, G. I., Chatterjee, A., Johnson, M. A., Liao, L., Klaus, J. P., Yates, J. R., 3rd, Wüthrich, K., Stevens, R. C., Buchmeier, M. J., and Kuhn, P. (2008) Proteomics analysis unravels the functional repertoire of coronavirus nonstructural protein 3. J. Virol. 82, 5279–5294
21. Kossyvakis, A., Tao, Y., Lu, X., Pokga, V., Tsiodras, S., Emmanouil, M., Mentis, A. F., Tong, S., Erdman, D. D., and Antoniadis, A. (2015) Laborato-
Structure of MERS-CoV Macro Domain

ADP-ribose binding module. *EMBO J.* **24**, 1911–1920

Martzen, M. R., Mc CRAITH, S. M., Spinieli, S. L., Torres, F. M., Fields, S., Grayhack, E. J., and Phizicky, E. M. (1999) A biochemical genomics approach for identifying genes by the activity of their products. *Science* **286**, 1153–1155

Neuvonen, M., and Ahola, T. (2009) Differential activities of cellular and viral macro domain proteins in binding of ADP-ribose metabolites. *J. Mol. Biol.* **385**, 212–225

Miwa, M., and Sugimura, T. (1971) Splitting of the ribose-ribose linkage of poly(adenosine diphosphate-ribose) by a calf thymus extract. *J. Biol. Chem.* **246**, 6362–6364

Oka, S., Kato, J., and Moss, J. (2006) Identification and characterization of a mammalian 39-kDa poly(ADP-ribose) glycohydrolase. *J. Biol. Chem.* **281**, 705–713

Slade, D., Dunstan, M. S., Barauskaitė, E., Weston, R., Lafite, P., Dixon, N., Ahel, M., Leys, D., and Ahel, I. (2011) The structure and catalytic mechanism of a poly(ADP-ribose) glycohydrolase. *Nature* **477**, 616–620

Ueda, K., Oka, J., Narunyi, S., Miyakawa, N., and Hayashi, O. (1972) Poly ADP-ribose glycohydrolase from rat liver nuclei, a novel enzyme degrading the polymer. *Biochem. Biophys. Res. Commun.* **46**, 516–523

Gibson, B. A., and Kraus, W. L. (2012) New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs. *Nat. Rev. Mol. Cell Biol.* **13**, 411–424

Putics, A., Filipowicz, W., Hall, J., Gorbalenya, A. E., and Ziebuhr, J. (2005) ADP-ribose-1-monophosphatase: a conserved coronavirus enzyme that is dispensable for viral replication in tissue culture. *J. Virol.* **79**, 12721–12731

Putics, A., Gorbalenya, A. E., and Ziebuhr, J. (2006) Identification of protease and ADP-ribose 1-monophosphatase activities associated with transmissible gastroenteritis virus non-structural protein 3. *J. Gen. Virol.* **87**, 651–656

Saikatendran, K. S., Joseph, J. S., Subramanian, V., Clayton, T., Griffith, M., Moy, K., Velasquez, J., Neuman, B. W., Buchmeier, M. J., Stevens, R. C., and Kuhn, P. (2005) Structural basis of severe acute respiratory syndrome coronavirus ADP-ribose-1-phosphate dephosphorylation by a conserved domain of nsP3. *Structure* **13**, 1665–1675

Kumaran, D., Eswaramoorthy, S., Studier, F. W., and Swaminathan, S. (2005) Structure and mechanism of ADP-ribose-1-monophosphatase (Appr-1-pase), a ubiquitous cellular processing enzyme. *Protein Sci.* **14**, 719–726

Shull, N. P., Spinieli, S. L., and Phizicky, E. M. (2005) A highly specific phosphatase that acts on ADP-ribose 1-phosphate, a metabolite of tRNA splicing in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **33**, 650–660

Egloff, M. P., Malet, H., Putics, A., Heinonen, M., Dutartre, H., Frangeul, L., J. Hansen, G., Boomaars-van der Zanden, A. L., Snijder, E. J., Gorbalenya, A. E., and Hillgenfeld, R. (2009) Crystal structures of the X-domains of a Group-1 and a Group-3 coronavirus reveal that ADP-ribose-binding may not be a conserved property. *Protein Sci.* **18**, 6–16

Wojdyla, J. A., Manolaridis, I., Snijder, E. J., Gorbalenya, A. E., Coutard, B., Piotrowski, Y., Hillgenfeld, R., and Tucker, P. A. (2009) Structure of the X (ADRP) domain of nsP3 from feline coronavirus. *Acta Crystallogr. D Biol. Crystallogr.* **65**, 1292–1300

Xu, Y., Cong, L., Chen, C., Wei, L., Zhao, Q., Xu, X., Ma, Y., Bartlam, M., and Rao, Z. (2009) Crystal structures of two coronavirus ADP-ribose-1-monophosphatases and their complexes with ADP-ribose: a systematic structural analysis of the viral ADRP domain. *J. Virol.* **83**, 1085–1092

Otwinowski, Z., and Minor, W. (1997) Processing of x-ray diffraction data collected in oscillation mode. *Method Enzymol.* **276**, 307–326

Sheldrick, G. M. (2010) Experimental phasing with SHELXC/D/E: combining chain tracing with density modification. *Acta Crystallogr. D Biol. Crystallogr.* **66**, 479–485

Mushudov, G. N., Vagin, A. A., and Dodson, E. J. (1997) Refinement of macromolecular structures by the maximum-likelihood method. *Acta Crystallogr. D Biol. Crystallogr.* **53**, 240–255

Winn, M. D., Ballard, C. C., Cowtan, K. D., Dodson, E. J., Emsley, P., Evans, P. R., Keegan, R. M., Kriwinkel, E. B., Leslie, A. G., McCoy, A., McNicholas, S. J., Mushudov, G. N., Pannu, N. S., Potterson, E. A., Powell, H. R., Read, R. J., Vagin, A., and Wilson, K. S. (2011) Overview of the CCP4 suite and current developments. *Acta Crystallogr. D Biol. Crystallogr.* **67**, 235–242

Emsley, P., Lohkamp, B., Scott, W. G., and Cowtan, K. (2010) Features and development of Coot. *Acta Crystallogr. D Biol. Crystallogr.* **66**, 486–501

Adams, P. D., Afonine, P. V., Bunkóczi, G., Chen, V. B., Davis, I. W., Echols, N., Headd, J. I., Hung, L. W., Kapral, G. J., Grosse-Kunstleve, R. W., McCoy, A. J., Moriarty, N. W., Oeffner, R., Read, R. J., Richardson, D. C., Richardson, J. S., Terwilliger, T. C., and Zwart, P. H. (2010) PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr. D Biol. Crystallogr.* **66**, 213–221

Laskowski, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J. M. (1993) Procheck: a program to check the stereochemical quality of protein structures. *J. Appl. Crystallogr.* **26**, 283–291

Chen, V. B., Arendall, W. B., 3rd, Headd, J. J., Keedy, D. A., Immormino, R. M., Kapral, G. J., Murray, L. W., Richardson, J. S., and Richardson, D. C. (2010) MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr. D Biol. Crystallogr.* **66**, 12–21

Haq, I. (2002) Thermodynamics of drug-DNA interactions. *Arch. Biochem. Biophys.* **403**, 1–15

Holm, L., and Rosenström, P. (2010) Dali server: conservation mapping in 3D. *Nucleic Acids Res.* **38**, W545–549

Allen, M. D., Buckle, A. M., Cordell, S. C., Löwe, J., and Bycroft, M. (2003) The crystal structure of AF1521 a protein from Archaeoglobus fulgidus with homology to the non-histone domain of macroH2A. *J. Mol. Biol.* **330**, 503–511

Laskowski, R. A., and Swindells, M. B. (2011) LipidPlot+: multiple ligand-protein interaction diagrams for drug discovery. *J. Chem. Inf. Model* **51**, 2778–2786

Kapplus, P. A., and Diederichs, K. (2012) Linking crystallographic model and data quality. *Science* **336**, 1030–1033