Structural and functional elucidations of SARS-CoV-2 macro domain, a potential drug target for combating COVID-19

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Abstract

The pandemic outbreak of a novel coronavirus, SARS-CoV-2, has threatened the global public health and economy since late December 2019. SARS-CoV-2 encodes the conserved macro domain within non-structural protein 3, which may reverse cellular ADP-ribosylation and potentially cut the signal of a viral infection in the cell. Herein, we report that the SARS-CoV-2 macro domain was examined as a poly-ADPR binding module and possessing mono-ADPR cleavage enzyme activity. After confirming the ADPR binding ability via a biophysical approach, the X-ray crystal structure of the SARS-CoV-2 macro domain was determined and structurally compared with those of other viruses. Fragment screening by saturation transfer difference nuclear magnetic resonance (STD-NMR) is a method with the advantages of high-throughput to identify small molecules binding with biological targets, especially the promised drug target. Moreover, the fragment library screening using STD-NMR was established. We had identified small molecules that bind to the SARS-CoV-2 macro domain. To verify the screening-hits, the ^{1}H, ^{13}C, and ^{15}N resonance assignments of the SARS-CoV-2 macro domain were also completed. This study provides structural, biophysical, and biochemical bases to further evaluate the role of the SARS-CoV-2 macro domain in the host response via ADP-ribose binding but also as a potential target for drug design against COVID-19.

Keywords **- SARS-CoV-2 macro domain; ADP-ribose; COVID-19; crystal structure; STD-NMR screening.**

Introduction

A novel virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was identified as the causal pathogen of the newly emerged disease COVID-19. The macro domain is a protein module ubiquitous in eukaryotes, bacteria, and archaea. SARS-CoV-2 harbors a macro domain in its nonstructural protein 3 (NSP3) located in the open reading frame 1ab (ORF1ab). Accumulated evidence for virus macro domains pointed out a key relevance to host cellular ADP-ribosylation [1], which is a post-translational modification correlated with a wide spectrum of biological phenomena, such as the innate immune response. In the present study, we characterized the basic protein properties and confirmed the biochemical function of PAR binding and deMARylation of the SARS-CoV-2 macro domain. Furthermore, we describe the crystal structure of the SARS-CoV-2 macro domain in complex with ADPR by X-ray crystallography. Fragment screening by STD-NMR is a method with the advantages of high-throughput to identify small molecules binding with biological targets, especially the promised drug target. Hence, our study may be the essential groundwork for revealing the SARS-CoV-2 macro domain's function to beat against COVID-19.

Experiments

Protein expression and purification

Production of the recombinant SARS-CoV-2 macro domain was similar to that for the MERS-CoV macro domain[1].

X-ray crystallization, data collection and structural determination

The SARS-CoV-2 macro domain and ADPR were mixed in a molar ratio of 1:16. Protein crystallization trials were performed at 283 K by the sitting-drop vapor-diffusion method with commercial screening kits. Each crystallization drop was prepared with 1 μL macro domain/ADPR at 14 mg/mL mixing with an equal volume of mother liquor, and the mixture was equilibrated against a 100 μL reservoir solution. The crystal was cryoprotected in 4M lithium formate and flash-cooled in liquid nitrogen. The diffraction images were recorded in a 100-K nitrogen gas stream with the use of the BL05A beamline (National Synchrotron Radiation Research Center, Taiwan) and processed by using HKL2000 software. The crystal structure was determined by the molecular replacement method by using Phaser in the PHENIX package. The initial structure was refined with iterative cycles of simulated annealing, energy minimization, and manual rebuilding by using PHENIX refinement and COOT. Molecular visualizations were generated with PyMOL.

STD-NMR fragment screening

Fragment binding was investigated by saturation transfer difference NMR (STD-NMR) experiments with cocktails containing fragments and the SARS-CoV-2 macro domain protein. STD-NMR experiments were conducted at 600 MHz on a Bruker Avance spectrometer with cryoprobe. The potential binders were verified by NMR chemical shift perturbation.

Results and Discussion

To examine the ADPR binding ability of the SARS-CoV-2 macro domain, we used isothermal titration calorimetry (ITC). The ITC measurements confirmed ADPR binds to SARS-CoV-2 macro domain at 1:1 stoichiometry with a dissociation constant (Kd) of 17.18 ± 6.03 μM (Figure 1A-B). Differential scanning fluorimetry (DSF) was used to examine the binding ability. After fitting DSF data, the Kd was determined at 20.60 ± 7.7 μM (Figure 1C), which is similar to the calculated Kd based on ITC data. Furthermore, nanoDSF analysis revealed that the

addition of ADPR in the micromolar range significantly increased the melting point of the SARS-CoV-2 macro domain (Figure 1D).

De-mono-ADP-ribosylation (de-MAR) activity of viral macro domains was found critical for replication of pathogenic chikungunya virus, hepatitis E virus, and SARS-CoV-1. Hence, the deMAR enzyme activity of the SARS-CoV-2 macro domain was examined. The mono-ADP-ribosylation signals were decreased with increasing macro domain concentration, which indicates the de-MAR activity of the SARS-CoV-2 macro domain (Figure 2A-B). When triggered by a viral infection, Poly-ADP-ribosylation (PARylation) acts as a stress signal to induce apoptosis and/or necrosis in the cell. There is much evidence for the PAR binding ability of various viral macro domains. Therefore, we confirmed the ability of the SARS-CoV-2 macro domain to interact with PAR by immuno-dot-blotting (Figure 2C).

For a comprehensive analysis of the viral macro domain, we determined the crystal structure of the SARS-CoV-2 macro domain in complex with ADPR (Figure 3A). The overall structure of the SARS-CoV-2 macro domain consisted of six α-helices and one seven-stranded β-sheet, the classical construction of macro domains (Figure 3B). The β-sheet is oriented at an order of β1-β2-β7-β6-β3-β5-β4 and sandwiched between α-helices (α1, α2, and α3 located at one side and α4, α5 and α6 at the other side), so the SARS-CoV-2 macro domain showed a baseball glove-like structure and a groove between α-helices formed the ligand-binding site.

Macro domains of three beta-CoVs (MERS-CoV, SARS-CoV-1, and SARS-CoV-2), causing severe public health problems, bound to ADPR with different binding abilities. The macro domain from MERS-CoV presented the strongest binding affinity to ADPR. The ADPR binding ability was better for the SARS-CoV-2 than the SARS-CoV-1 macro domain. This order was associated with the ADPR adenine group orientation (Figure 4). In the SARS-CoV-2 macro domain, the N-6 nitrogen of the ADPR adenine group and D22 side chain formed a 2.9-Å hydrogen bond. The distance between the backbones of V24 and D22 was 3.0 Å. Moreover, the F156 residue located near the pyrimidine group of ADPR in the SARS-CoV-2 macro domain. The ring-stacking effect was suspected to contribute to the orientation of ADPR. It was likely a correlation between ADPR-binding ability and the N-6 nitrogen orientation at the adenine group of ADPR inside the ligand-binding site of the viral macro domain.

Furthermore, fragments bound to the SARS-CoV-2 macro domain were screened by high throughput STD-NMR (Figure 5). Twenty-one small molecules containing ring structures were listed to be the hits. The identified fragments were verified by NMR chemical shift perturbations. These fragments may be potential pre-drugs against COVID-19 by inhibiting the function of the SARS-CoV-2 macro domain.

References

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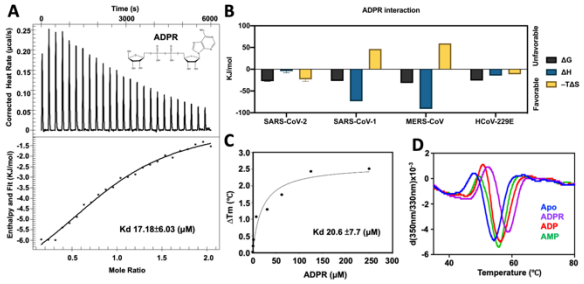

Figure 1. ADPR binding ability of SARS-CoV-2 macro domain.

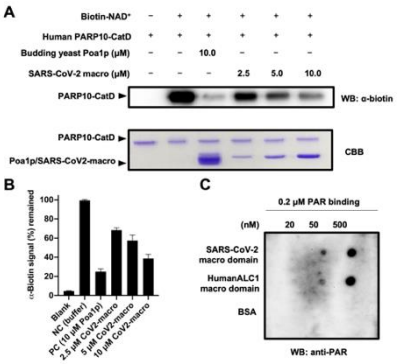

Figure 2. Functions of SARS-CoV-2 macro domain.

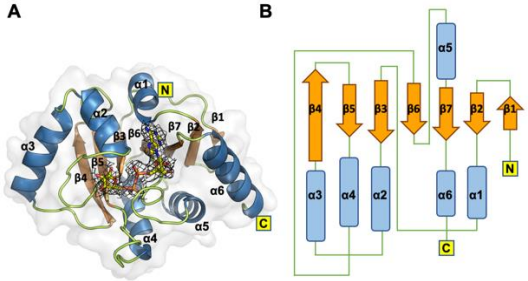

Figure 3. Structure of SARS-CoV-2 macro domain.

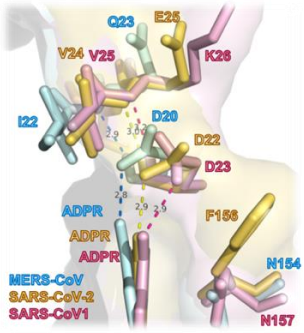

Figure 4. Ligand binding site of virus macro domains.

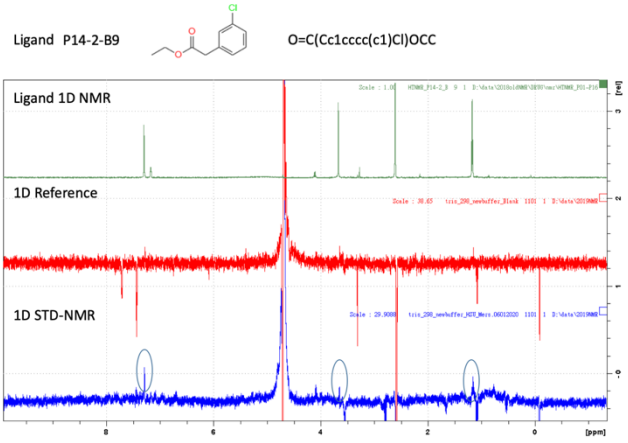

Figure 5. Fragment hits identified by STD-NMR