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Multidomain Architecture of Equine Arteritis Virus Helicase Structure Revealing the Conserved Features of Nidovirus Helicase

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1 Introduction

The Nidovirales [1] includes many famous viruses, such as arteriviridae and coronaviruses. Among them, severe acute respiratory syndrome coronavirus (SARS-CoV) caused thousands of deaths and had enormous negative impact on the global economy in 2003. Porcine reproductive and respiratory syndrome virus (PRRSV) has caused huge economic losses in the swine industry in the past 25 years [2]. Viral helicases catalyze the unwinding of DNA/RNA duplex substrates and play important roles in genome replication, DNA/RNA synthesis and virion biogenesis. All positive-stranded RNA viruses with genomes larger than ~7 kilobases encode helicases. Moreover, the domain architecture is conserved among nidovirus. However, no three-dimensional structural information of nidovirus helicases or homologs has been reported to date.

2 Experiment

To understand the regulatory mechanism of the helicase and the interaction with nucleic acids and to help to identify potential drug targets against these viruses, we characterized the crystal structures of Equine Arteritis Virus (EAV) nsp10 helicases. Luckily, we obtained the crystal structures of native EAV nsp10 and its complex with single strand DNA poly(dT). We collected the X-ray diffraction data of EAV nsp10 at beamline NE3A at Photon Factory (KEK) and solved the structure. For detail, please see our publication [3].

3 Results and Discussion

Here, we reported the high resolution structures of the helicase nsp10 from Equine arteritis virus (EAV), alone and in binary complex with ssDNA. Because there was no homologous protein in the PDB database, we used the zinc multiple-wavelength anomalous dispersion (MAD) method to solve the structure of EAV nsp10. The final model is refined to 2 Å with an R_{work} of 19.5% and an R_{free} of 22.4%. The asymmetric unit of the crystal contains one nsp10 molecule. Nsp10 contains four domains: a unique zinc binding domain (ZBD) in the N-terminus and the helicase core consiting of domains 1A and 2A, and an undefined accessory domain 1B (Figure 1).

The N-terminal ZBD has three zinc ions and two RecA-like α/β domains are located in the C-terminus, domain 1A and 2A (Figure 1). The ZBD consists of a specific RING-like module and a novel conserved CHC2/C3H type zinc finger. Domain 1A contains a parallel five-stranded β-sheet sandwiched by three α-helices on one side and two α-helices on the other side. Domain 2A contains a parallel four-stranded β-sheet with five α helices on the side close to the domain 1A. Surprisingly, an unexpected domain, similar to domain 1B in that of SF1B helicases, was found (Figure 1) which is absent in the only solved viral helicase ToMV-Hel structure from SF1. Together with domains 1A and 2A, domain 1B forms the helicase core. The 1B domain has a characteristic beta-barrel fold which consists of five β-strands arranged as two tightly packed anti-parallel β–sheets and is located in front of the domain 1A. A search of the protein data bank showed that the structure of the helicase core is similar to those of Upf1 and Ighmbp2 which both belong to the Upf1-like subfamily [4, 5]. Therefore, structural analysis implies that EAV nsp10 might also belong to the Upf1-like subfamily.

Domain 1B undergoes large conformational changes and binds the 5' single-stranded regions of ssDNA. Structural analysis reveals that the ZBD is essential for EAV nsp10 helicase function. Structural analysis, other biochemical data show that a relay of extensive interactions through the ZBD-helicase core is essential for helicase activity and the binding of nucleic acids is non-specific. Helicases in arteriviruses, maybe together with coronaviruses, represent a unique Upf1-like subfamily with a novel ZBD and have the ability to translocate in a 5'-3' direction.

Our crystal structures of EAV nsp10 show that arterivirus helicases consist of an N-terminal ZBD, which is important to the integrity of nsp10 and required for nucleic acid binding and could serve as interacting surfaces to bind other proteins. A helicase core formed by domains 1A, 1B and 2A, which is similar to Upf1-like subfamily helicase, and forms a substrate binding channel and is greatly different from the recently solved viral SF1 family helicase ToMV-Hel that is only composed of domains 1A and 2A. In addition, the helicase-DNA complex structure is the first solved structure of SF1 family viral helicase in complex with its substrate. The structure shows that viral helicases can have the similar interacting pattern with substrate as that of eukaryotes. Mapping of the mutagenesis study of EAV nsp10 onto the helicase structure provides a molecular basis for understanding the helicase function. Finally, ZBD provides several potential drug targets against nidoviruses, such as SARS-CoV, PRRSV and EAV.

In summary, these results reveal a novel conserved mechanism for ZBD in controlling helicase activity, and provide a framework for elucidating the regulation and function of helicases, and potential drug targets for nidoviruses. Arterivirus nsp10 structurally resembles the cellular nonsense-mediated mRNA decay (Upf1) helicase.

We thus propose that nidoviruses may also use their helicases for posttranscriptional quality control of their large genomes to remove defective genomes from the progeny pool.

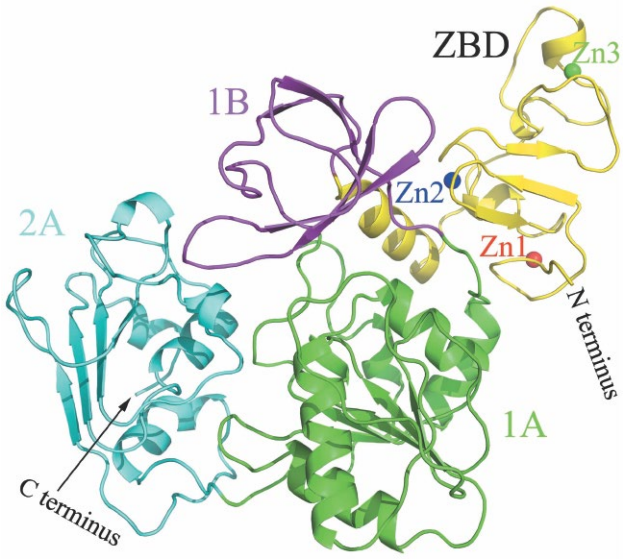

Fig. 1: Overall structures of EAV nsp10. The N-terminal zinc-binding domain (ZBD; yellow), the two RecA-like domains 1A (green) and 2A (cyan) of HEL1 and an additional regulatory domain 1B (magenta).

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References

1. Snijder, E.J. and J.J. Meulenberg, *The molecular biology of arteriviruses.* J Gen Virol, 1998. **79 (Pt 5)**: p. 961-79.
2. Tian, K., et al., *Emergence of fatal PRRSV variants: unparalleled outbreaks of atypical PRRS in China and molecular dissection of the unique hallmark.* PLoS One, 2007. **2**(6): p. e526.
3. Deng, Z., et al., *Structural basis for the regulatory function of a complex zinc-binding domain in a replicative arterivirus helicase resembling a nonsense-mediated mRNA decay helicase.* Nucleic Acids Res, 2014. **42**(5): p. 3464-77.
4. Cheng, Z., et al., *Structural and functional insights into the human Upf1 helicase core.* EMBO J, 2007. **26**(1): p. 253-64.
5. Lim, S.C., et al., *The Ighmbp2 helicase structure reveals the molecular basis for disease-causing mutations in DMSA1.* Nucleic Acids Res, 2012. **40**(21): p. 11009-22.

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