17A/2010G576

Crimean– Congo hemorrhagic fever virus nucleoprotein reveals endonuclease activity in bunyaviruses

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1 Introduction

Crimean-Congo hemorrhagic fever virus (CCHFV), a virus with high mortality in humans, is a member of the genus *Nairovirus* in the family *Bunyaviridae*, and is a causative agent of severe hemorrhagic fever (HF). It is classified as a biosafety level 4 pathogen and a potential bioterrorism agent due to its aerosol infectivity and its ability to cause HF outbreaks with high case fatality (~30%) (1). However, little is known about the structural features and function of nucleoproteins (NPs) in *Bunyaviridae*, especially in CCHFV. Here we report a 2.3-Å resolution crystal structure of the CCHFV NP. The protein has a racket-shaped overall structure with distinct "head" and "stalk" domains and differ significantly with NPs reported so far from other negative-sense single-stranded RNA viruses. Furthermore, CCHFV NP shows a distinct metal-dependent DNA-specific endonuclease activity. Single residue mutations in the predicted active site resulted in a significant reduction in the observed endonuclease activity. Our results represent a new folding mechanism and function for a nucleoprotein of negative strand RNA virus NP, extend our structural insight into bunyavirus NPs, and provide a potential target for antiviral drug development to treat CCHFV infection.

2 Experiment

Diffraction data of the native crystal of CCHFV NP was first collected to 3.0 Å at 100 K using a MAResearch M165 CCD detector on beamline 1W2A at the Beijing Synchrotron Radiation Facility (BSRF). Anomalous diffraction data for selenomethionine derivatives were collected to 2.3 Å at 100 K using an ADSC Q315 CCD detector on beamline BL17 at the Photon Factory (Japan). All data sets were indexed, integrated and scaled using the HKL2000 package. The orthorhombic crystal form belongs to space group $P2_12_12_1$ with cell parameters a= 58.3 Å, b= 67.9 Å, and c= 131.5 Å.

Heavy atom searching, initial phase calculation and density modification were performed with PHENIX. The resulting electron density map was displayed with COOT and an initial model was built in manually. Several rounds of simulated annealing, restrained individual atomic displacement parameter refinement, energy minimization and individual B-factor refinement were carried out with PHENIX. Solvent molecules were located from stereochemically reasonable peaks in the σA-weighted 2*Fo−Fc* difference *Fourier* electron density map (1.2 σ). Model geometry was verified using the program PROCHECK. All structure figures were drawn with the program PyMOL. The coordinates and structure factors have been deposited with the RCSB under accession codes: 3U3I. The authors declare no competing financial interest.

3 Results and Discussion

The correct assembly of viral genomic RNA by nucleocapsid protein is crucial for single stranded virus assembly and maturation, and is also a significant process to protect viral RNA from host cell degradation. Additionally, recent structural work on LASV NP has extended our knowledge of virally encoded NP proteins beyond the packaging of viral genomic RNA (15, 16, 19), while the exact function of the N-terminal domain is still under discussion. Our finding that, despite the structural similarity between the head domain of CCHFV NP and the N-terminal domain of LASV NP, they may not function equivalently. CCHFV NP shows weak binding with RNA, and did not show any cap-snatching affinity *in vitro*. However, CCHFV reveals a novel endonuclease function which is not previously described for any member of this virus family.

The crystal structure of CCHFV NP reported in this work extends our knowledge of nucleocapsid protein of Bunyaviruses. The structure of CCHFV NP features a

racket-shaped overall structure, composed by a head domain which resembles the topology found in LASV NP but has a distinct nuclease function instead of cap binding. CCHFV NP potential surface displays several positive regions while unexpectedly show very weak binding activity with RNA *in vitro*. These data provide the new insight into the biological role of NP in Bunyavirus replication and provide valuable information for the development of intervention strategies designed to lessen the pathogenic burden of this important group of viral infections.

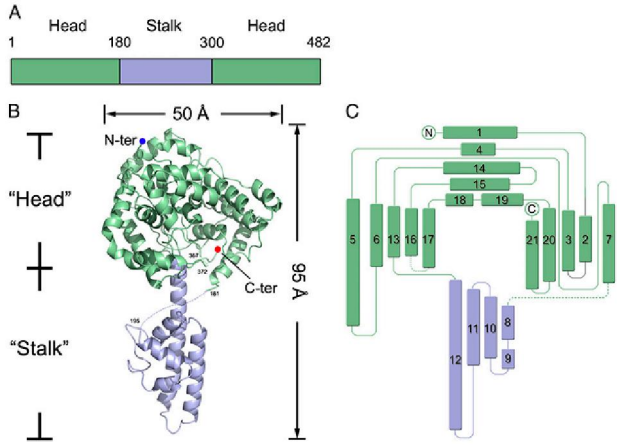

Fig. 1: Structure of CCHFV NP.

(A) Schematic diagram of domain organization of CCHFV NP in primary sequence. Stalk and head domain are colored as light blue and green, respectively.

(B) Overall structure in cartoon representation, missing residues are linked by dotted lines, and (C) in topology diagram. Head and stalk domains are colored in green and light blue, respectively.

Acknowledgement

We thank the staff at Beijing Synchrotron Radiation Facility and Photon Factory for their help with data collection; we also thank Prof. Hong-bing Shu from Wuhan University who supplied the plasmids for INF induction reporter assays. This work was supported by the National Natural Science Foundation of China, the Ministry of Science and Technology 973, the Special Program for Key Basic Research of the Ministry of the Science and Technology, and the Knowledge Innovation Program of the Chinese Academy of Sciences.

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