RNA-Binding Capabilities of the C-Terminal Domain in the Nucleocapsid Protein in Mouse Hepatitis Virus

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1. Abstract

The nucleocapsid protein is essential for packaging the viral genome of Mouse Hepatitis Virus (MHV), a type of coronavirus similar to the SARS-CoV viruses. The C-terminal domain of this nucleocapsid protein (N-CTD) is thought to play a role in specific packaging of RNA. However, the RNA binding capacity of N-CTD remains unknown. Our research juxtaposes the RNA binding capacity of N-CTD with a version of the nucleocapsid protein with an additional tail on the end (N-CTD-Bd3). After purifying both proteins, we ran electrophoretic mobility shift assays (EMSAs) comparing protein binding to different genetic materials. We hypothesized that the N-CTD would have greater binding to specific RNA than N-CTD-Bd3. However, our results indicate that the additional Bd3 tail does not have a great impact upon the binding ability of the protein, as both the N-CTD and the N-CTD-Bd3 were binding to non-specific and specific RNA in a similar manner. Future research is needed to determine the effect of repetition within the specific RNA marker and how it impacts the binding abilities of the proteins. We anticipate that this work is useful in revealing more about specific RNA binding in MHV, which could also be applicable to other closely related coronaviruses.

2. Introduction

Mouse Hepatitis Virus (MHV), otherwise known as Murine Hepatitis Virus, is in the family Coronaviridae and genus Betacoronavirus. It is the largest type of positive-sense, single-stranded RNA viruses, with a 30 kilobase genome and a diameter of around 100 nm. The nucleocapsid protein within the virus is responsible for binding to the viral genome (see Figure 1).

Mouse Hepatitis Virus is a coronavirus very closely linked to contagious human coronaviruses like SARS-CoV-1 (the viral agent that causes Severe Acute Respiratory Syndrome, or SARS) and SARS-CoV-2 (the viral agent that causes COVID-19). These coronaviruses cause respiratory and gastrointestinal diseases in humans and animals, leading to several recent health crises. From 2002 to 2003, SARS had 8,098 cases and 774 deaths across 30 countries. As of August 2022, the official death toll of COVID-19 is greater than 6 million people, and this number is likely to be an underestimate.

Because of this human health threat, Mouse Hepatitis Virus is a very important virus to be studying. Since MHV is similar to the SARS coronaviruses, knowing more about it will help us better understand the SARS coronaviruses. Additionally, MHV is not transmittable to humans, which makes the virus easier and safer to study in a laboratory environment.

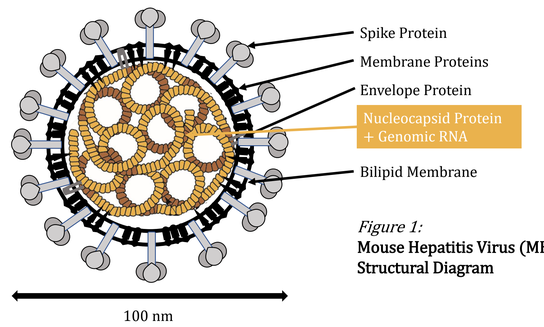


Figure 1:
Mouse Hepatitis Virus (MHV) Structural Diagram

The ability of MHV to bind solely to its own RNA remains a mystery. A specific RNA marker was discovered by Kuo and Masters in 2013 to be critical to specific binding of RNA in the virus. Beyond identifying the specific marker, studies into the N-terminal Domain of the nucleocapsid protein have found that the region is not responsible for specific RNA binding. Currently, it is theorized that another region in the nucleocapsid protein, the C-Terminal Domain (CTD), plays a crucial role in solely binding to viral RNA.

3. Materials and Methods

Plasmids with Kanamycin resistance for both MHV-N-CTD and MHV-N-CTD-Bd3 were obtained from the Masters lab and transformed into Rosetta (De3) pLysS *E.Coli* cells. Colonies were transferred into an overnight culture growth, then grown at 37 degrees Celsius at 250 rpm until the OD reading was 0.6 at 600 nm. The growth was induced with IPTG and left in the same conditions for an additional 3 hours. Afterwards, the cells were spun down, lysed via sonication, and spun down once more to obtain the supernatant.

N-CTD was purified using a wash buffer of buffer A (25 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$, 10% glycerol, and 5 mM beta-mercaptoethanol) plus 20 mM imidazole pH 8.0 and 0.3 M NaCl, lysis buffer of buffer A plus 5 mM imidazole pH 8.0 and 1.0 M NaCl, and elution buffer (buffer A plus 400 mM imidazole pH 8.0 and 0.3 M NaCl). The purification was performed by hand over a His-Trap column (Cytiva). N-CTD-Bd3 was purified the same way as N-CTD, then purified once more with the same lysis buffer, two wash buffers of buffer A plus 20 mM imidazole pH 8.0 with 1.0 M NaCl and 0.3 M NaCl respectively, and an elution buffer of buffer A plus 800 mM imidazole pH 8.0 and 0.3 M NaCl. The purification for MHV-N-CTD-Bd3 was performed by an Amersham Biotech AKTA prime chromatography machine. Both were purified at room temperature (~22 degrees Celsius).

Genetic materials:

PS 25 (5′ UAC CGG AGC CCA CAA GGU AAU CCG G 3′)

silPS 25 (5′ UAC AGA AGC CCA CAA GGA AAC CCA G 3′)

PS 25 is the tip of the specific MHV RNA marker discovered by Kuo and Masters in 2013, and the silPS25 contained 5 silent mutations from the PS 25 (both highlighted in Figure 2 to the right). All genetic materials were fluorescently labeled at the 5′ end.

EMSAs were conducted with a solution containing either N-CTD or N-CTD-Bd3, 1x reaction buffer (20 mM Tris-HCl pH 7.5, 50 mM NaCl, 5 mM $MgCl_2$, and 50% glycerol) and 10 nM concentration of genetic material were used for complexes. Complexes were allowed to rest in ice baths for 30 minutes to an hour before 6x Loading Dye No SDS was added, and they were loaded into 25% Acrylamide gels and run at 150 V for 35-45 minutes.

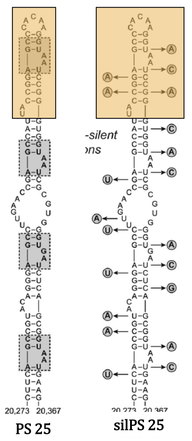


Figure 2:
Specific RNA markers in MHV binding

4. Results

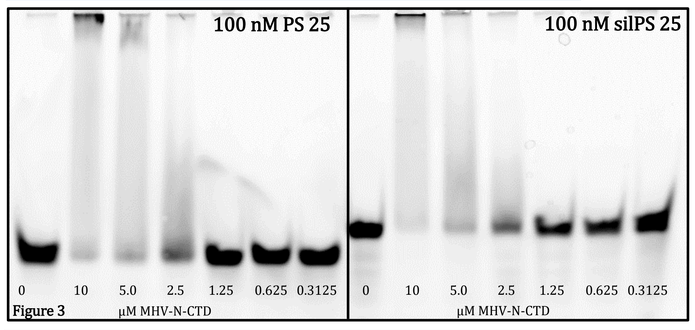


Figure 3

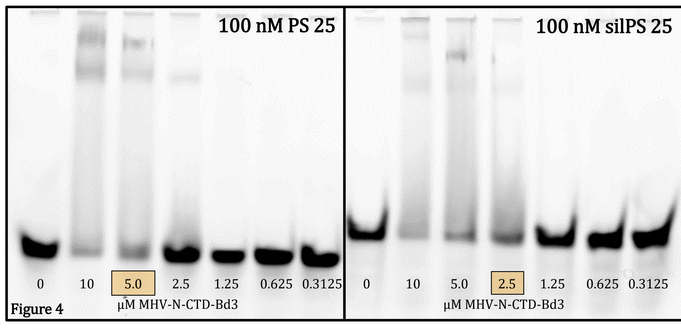


Figure 4

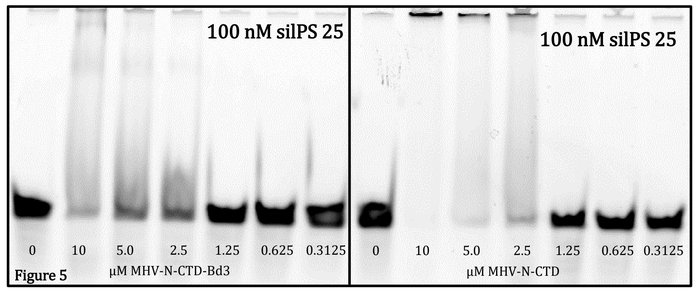


Figure 5

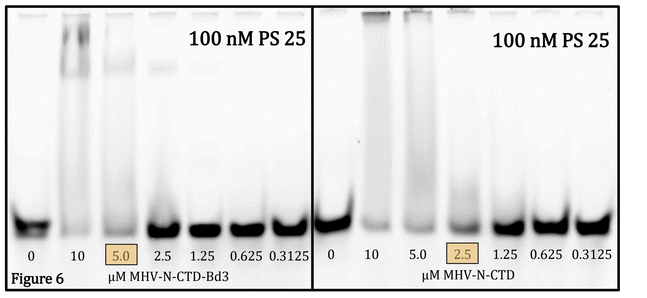
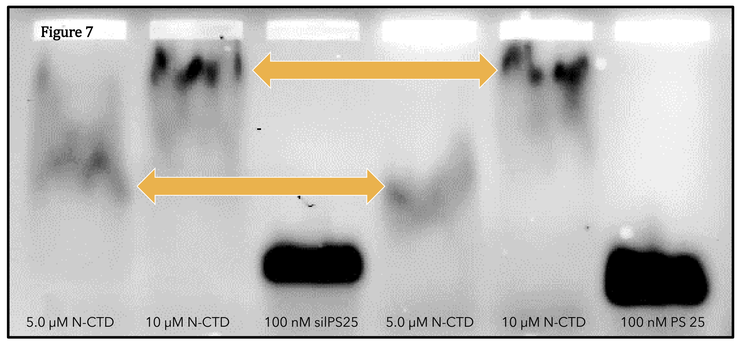


Figure 6



5. Conclusion

- MHV-N-CTD does not specifically bind with the packaging signal (Figure 3)
- MHV-N-CTD-Bd3 binds more tightly to silPS 25 than it does to PS 25 (Figure 4)
- N-CTD-Bd3 and N-CTD bind similarly to silPS 25 (Figure 5)
- PS 25 binds more tightly to MHV-N-CTD than to MHV-N-CTD-Bd3 (Figure 6)
- More than one band indicates that there may be multiple MHV-N-CTD binding to one RNA (Figures 3-7)

Contrary to our expectations, MHV-N-CTD does not bind specifically to the PS 25 marker in comparison to the silPS 25 marker (Figure 3). This may indicate that the C-Terminal Domain is not involved with the specificity of RNA binding in MHV. Additionally, the MHV-N-CTD-Bd3 protein is more likely to bind to the silently mutated RNA than the specific marker (Figure 6), indicating that the additional Bd3 tail may inhibit specific binding of the protein. However, both of these findings could be because the complex only contained a small portion of the total RNA structure. Future studies may benefit from including a larger or full structure of the wild type RNA molecule that Kuo and Masters discovered to be critical for specific RNA binding. In addition to the acrylamide gels (Figures 3-6) we ran an agarose gel at a higher voltage for a shorter amount of time, specifically 240 V for 15 minutes, to clarify faint double bands that were visible in previous gels (Figures 3-6). Two bands were clearly identified (Figure 7), indicating that there is likely to be more than one complex forming with higher amounts of protein. Because the RNA strands used are relatively short, we presume that once one protein dimer forms a complex with the RNA, it is favorable for another protein dimer to physically connect to the initial protein.

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