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**BIOCHEMICAL AND STRUCTURAL
STUDIES OF PROTEINS SUPPORTING
THE GENOME REPLICATION OF
ENTEROVIRUSES AND *GIARDIA
INTESTINALIS*.**

Kasturika Shankar

Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av filosofie doktorsexamen framläggs till offentligt försvar i Carl Kempesalen (KBE303), KBC, Tuesday 5th March, 2024, 0900 hrs.

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Fakultetsopponent: Dr. Daniel Hurdiss, Assistant Professor, Veterinary Medicine, Department of Biomolecular Health Sciences, Infectious Diseases & Immunology, Virology, Utrecht University, Netherlands

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Author

Kasturika Shankar

Title

Biochemical and structural studies of proteins supporting the genome replication of enteroviruses and *Giardia intestinalis*.

Abstract

The Enterovirus genus of the Picornaviridae family includes non-enveloped, positive-sense single-stranded RNA (ssRNA) viruses. This genus of viruses causes many diseases such as poliomyelitis by poliovirus (PV), cardiomyopathy by coxsackievirus B3 (CVB3), common cold by rhinoviruses (RVs) and meningitis by Enterovirus 71 (EV 71). The 7.5 kb enterovirus genome encodes a polyprotein, which is subsequently cleaved to yield viral proteins. These viral proteins hijack and modify the membranes of the Golgi and ER to give rise to replication organelles (ROs).

In the first paper of my thesis, we showed that membranes of the ROs act as an assembly line for the assembly of enteroviruses. Using cryo-electron tomography, we studied poliovirus-infected cells. The one feature that we found most interesting was that a protein was tethering viral capsid to membranes as well as two membranes together. We hypothesized that viral protein 2C was a strong candidate. Therefore, I tried to mimic the interaction between 2C and viral capsid using purified Poliovirus and 2C protein. However, after trying several biochemical conditions I could not mimic this interaction. This strongly indicated that I might need to have a membrane in the system and this led me to study the membrane binding activity of 2C in the second paper.

In the second paper, I studied an Enteroviral multi-functional protein: 2C, a highly conserved AAA+ ATPase that plays an important role in the biogenesis of ROs and virus assembly. One of the most interesting features of 2C is its N-terminal membrane-binding domain consisting of 40 amino acids. Using in vitro reconstitution methods and biochemistry, I investigated the association of full-length 2C with lipid vesicles and how this affects the function of the protein. I showed that amino acids 12 to 40 are not only important for membrane binding but also for hexamerization. Moreover, truncation of the first 11 amino acids leads to loss of membrane tethering activity of the protein, which is essential for the formation of ROs. I was also able to demonstrate that the protein is sufficient to recruit RNA to the membrane, as it was not previously known how RNA replication is localized to the membrane and here, we found the possible mechanism. In this realistic reconstituted system, I have shown that 2C is not a helicase but an ATP-independent RNA chaperone. Collectively, these discoveries offer a biochemical foundation for various functions of 2C in enterovirus replication. This sets up a more practical biochemical framework for future research, which could potentially contribute to the development of drugs aimed at thwarting enterovirus infections.

In the third project, I studied deoxyadenosine kinase (dAK) from *Giardia intestinalis*, a protozoan responsible for severe diarrhea spread by the fecal-oral route. *Giardia* is completely dependent on the host for deoxyribonucleosides as it lacks a de novo pathway for their synthesis. dAK uses ATP as a phosphate donor to phosphorylate deoxyadenosine, this activity is essential for genome replication of the protozoan. In this project, we have biochemically and structurally characterized dAK. Here, I used cryo-electron microscopy (cryo-EM) single particle analysis to show that dAK exists as a homo-tetramer in solution. This is an important finding because this is the first example of a non-thymidine kinase1-like deoxy-ribonucleoside kinase with a tetrameric structure and subsequent mutagenesis analysis showed that tetramerization allows the enzyme to achieve a higher affinity for its deoxyadenosine substrate. In summary, in my thesis, I have biochemically and structurally characterized proteins supporting the genome replication of enteroviruses and *Giardia intestinalis*.

Keywords

Biochemistry, Structural Biology, Membrane reconstitution, Infectious diseases, Virus, Protozoa

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