

**BIOPHYSICAL AND STRUCTURAL CHARACTERIZATION
OF HEPATITIS A VIRUS (HAV) CAPSID PROTEIN VP1
GENERATED IN A HETEROLOGOUS EXPRESSION
SYSTEM**

ANSHU NAIN



KUSUMA SCHOOL OF BIOLOGICAL SCIENCES

INDIAN INSTITUTE OF TECHNOLOGY DELHI

APRIL 2024

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HEPATITIS A VIRUS (HAV) CAPSID PROTEIN VP1
GENERATED IN A HETEROLOGOUS EXPRESSION SYSTEM**

by

ANSHU NAIN

Kusuma School of Biological Sciences

Submitted

in fulfilment of the requirements of the degree of

Doctor of Philosophy

to the



Indian Institute of Technology Delhi

APRIL 2024

Dedicated To my

Beloved Parents

&

Loving Husband

For their unconditional love, endless support &
encouragement

CERTIFICATE

This is to certify that the thesis entitled “**Biophysical and Structural characterization of Hepatitis A Virus (HAV) capsid protein VP1 generated in a heterologous expression system**” being submitted by **Ms. Anshu Nain** to the **Kusuma School of Biological Sciences, Indian Institute of Technology Delhi** for the award of the degree of “**Doctor of Philosophy**” is a record of the bonafide research carried out by her, which has been prepared under my supervision and guidance in conformity with rules and regulation of the Indian Institute of Technology Delhi, India. The results prescribed in it have not been submitted in part or full to any other University or Institute for the award of any Degree/Diploma.

Date:

Prof. Manidipa Banerjee

Place:

Kusuma School of Biological Sciences
Indian Institute of Technology Delhi
New Delhi-110016, India

ACKNOWLEDGEMENTS

*First and foremost, I would like to express my deepest gratitude to my PhD supervisor, **Prof. Manidipa Banerjee** who made this venture possible. She is an excellent scientist, a great mentor, and moreover an exceptional supervisor. I always enjoyed our scientific discussions as she simplifies complex theories in small sentences with good scientific English that made me to understand and excel my research. I am very thankful for her professional and personal support during the entire journey of my PhD tenure. Her constant support and believe gave me the confidence and a chance to develop my scientific skills which helped me to become an independent researcher. She has always been an inspiration to me for her expertise, enthusiasm, and creativity. Just because of her, today I am certain that wherever I will go next, I would be able to manage the unknown challenges coming my way both professionally and personally. I'm confident and ready because of the training and knowledge I gained under her supervision. Words are not sufficient to express my feelings and gratitude for her.*

*I am also grateful to my SRC members, **Prof. James Gomes, Prof. Bishwajit Kundu and Prof. Ritu Kulshreshtha** (Department of Biochemical Engineering and Biotechnology, IIT Delhi) for their valuable inputs, discussions and encouragement over the years. I am also thankful to other KSBS faculties who always inspired and motivated me.*

*I also acknowledge the cooperation of the non-academic staff who helped me in doing my administrated work smoothly. A special thanks to **Mini Sharma** who in spite of her busy schedule helped me in various administrative work.*

*PhD journey become amazing if you get a chance to work with the fantastic people in a friendly lab environment and I'm very fortunate to get that chance in KSBS. I am very thankful to my lab members **Dr. Saumya Verma Bajaj, Dr. Ashutosh Shukla, Dr. Aditya Padhi, Dr Priyanka Nair, Dr Kamalika Banerjee, Dr. Debajit Dey, Dr. Kimi Azad, Dr. Sukanya Ghosh, Chandra Shekhar Kumar, Ramesh Kumar, Akanksha Saini, Maryam Khursheed, Inamur Rahman, Debashish Panda and Manthan Raj** who made the lab environment friendly, helped and respect each and every member in their respective work. A special heartfelt thanks goes to **Chandra Shekhar Kumar** who always helped me in the lab. I really enjoyed both scientific and non-scientific discussions and my tea breaks with him.*

*I would also like to thank my batchmates **Harsha, Prashant and Vivek** for their discussions over coffee and parties which we had during my PhD tenure. I would like to give special thanks to all my amazing and irreplaceable friends, **Dr. Mayuri Khandelwal, Dr. Kapil Manglani, Dr. Manju Narwal, Dr. Ashutosh Shukla, Riti Rawat and Arun Dhaiya**, who were emotional support for me throughout my PhD journey. They were always stood by my side in every circumstance and made me realize the worth of true friends. Spending time together, sharing experiences and making memories with them was just an amazing part of my PhD life in IIT. Whenever I got upset or stressed of work, they made me smile and never allow me to get down. They were very supportive and have been there with me through all tough times.*

*I am also thankful to **Dr. Subhomoi Borkotoky, Dr. Parvez Alam, Dr Kamal Gupta, Dr. Jeetu and Anjali** for being there with me as close friends and colleagues. I am thankful that I earned them as friends for life.*

*I would like to thank **IIT Delhi** and **KSBS** for providing me the infrastructure and resources to carry out my experiments. I would also like to thank **IIT Delhi** for providing me the financial support to attend National and International conferences. I'm thankful to the **Indian Council of Medical Research** for providing me the fellowship during my PhD tenure.*

At last, I am thankful and grateful to my family. I am indebted to my parents for their unconditional love, care and endless support. They are my pillar of strength. They always stood by my side in each and every step I took. They always motivated and encouraged me throughout my life. I am blessed to have them in my life. Today, whatever I am, it just because of them. They gave me wings to fly and freedom to live.

*This acknowledgement is incomplete without mentioning the most amazing and wonderful person in my life, **Mohit**, my loving husband and best friend. Without him I would never be able to achieve my goals. Throughout my PhD journey, he was so supportive and caring which I cannot express in words. He was always with me whenever I needed him. He always supported me in my decisions.*

Last but not the least, I would like to thank Almighty who blessed me with all the wonderful people in my life and gave me strength to accomplish my goals.

*I owe my thesis to my family- My parents **Mummy, Papa** and **Maa**, My husband **Mohit**, My sister **Riya** and My brother **Mayank** for their love, care and support that makes me truly blessed with such a wonderful, supporting and loving family.*

Anshu Nain

ABSTRACT

Hepatitis A Virus (HAV) is a quasi-enveloped picornavirus that causes acute hepatitis in humans and infects approximately 1.4 million individuals annually, not including the asymptotically infected population. HAV, from being endemic in the Indian subcontinent and the far east, is slowly becoming a threat to public health. This is because improved socioeconomic conditions in specific pockets have drastically reduced the population of resistant individuals and increased the probability of outbreaks, with increased possibility of liver failure. The available vaccines against HAV are based on generation of infectious particles in culture and are therefore too expensive for universal vaccination efforts. HAV is very slow growing in culture and procuring large quantities of purified virus drives up the cost of generating inactivated or live attenuated vaccines. Under the given circumstances, generating cheaper subunit vaccines against HAV is a priority. Recombinantly generated HAV structural proteins may also be utilized for other usages, like development of effective diagnostic tests.

We attempted several strategies for recombinant production of one of the major capsid proteins, VP1, from HAV in *E. coli*. While several efforts resulted in the formation of soluble aggregates or co-elution of VP1 with the bacterial chaperone GroEL, correctly folded VP1 was eventually generated in a discrete oligomeric form upon purification of the protein from inclusion bodies and refolding. The oligomers resemble pentamers of VP1 from other picornaviruses and appear to have correct secondary and antigenic surface structure. These complexes can be utilized for understanding the molecular pathway of HAV capsid assembly and can also have potential biomedical usages in prevention and diagnostics of HAV infections.

सारांश

हेपेटाइटिस ए वायरस (एचएवी) एक अर्ध-आच्छादित पिकोर्नावायरस है जो मनुष्यों में तीव्र हेपेटाइटिस का कारण बनता है और सालाना लगभग 1.4 मिलियन व्यक्तियों को संक्रमित करता है, जिसमें बिना लक्षण वाली संक्रमित आबादी शामिल नहीं है। एचएवी, भारतीय उपमहाद्वीप और सुदूर पूर्व में स्थानिक होने से धीरे-धीरे सार्वजनिक स्वास्थ्य के लिए खतरा बनता जा रहा है। ऐसा इसलिए है क्योंकि विशिष्ट इलाकों में बेहतर सामाजिक आर्थिक स्थितियों ने प्रतिरोधी व्यक्तियों की आबादी में भारी कमी ला दी है और लिवर फेलियर की संभावना के साथ प्रकोप की संभावना बढ़ गई है। एचएवी के खिलाफ उपलब्ध टीके संस्कृति में संक्रामक कणों की उत्पत्ति पर आधारित हैं और इसलिए सार्वभौमिक टीकाकरण प्रयासों के लिए बहुत महंगे हैं। एचएवी संस्कृति में बहुत धीमी गति से बढ़ रहा है और बड़ी मात्रा में शुद्ध वायरस की खरीद से निष्क्रिय या जीवित क्षीण टीके बनाने की लागत बढ़ जाती है। दी गई परिस्थितियों में, एचएवी के खिलाफ सस्ते सबयूनिट टीके तैयार करना प्राथमिकता है। पुनः संयोजक रूप से उत्पन्न एचएवी संरचनात्मक प्रोटीन का उपयोग प्रभावी नैदानिक परीक्षणों के विकास जैसे अन्य उपयोगों के लिए भी किया जा सकता है।

हमने ई. कोली में एचएवी से प्रमुख कैप्सिड प्रोटीनों में से एक, वीपी1 के पुनः संयोजक उत्पादन के लिए कई रणनीतियों का प्रयास किया। जबकि कई प्रयासों के परिणामस्वरूप घुलनशील समुच्चय का निर्माण हुआ या बैक्टीरियल चैपरोन ग्लोब के साथ वीपी1 का सह-संयोजन हुआ, सही ढंग से मुड़ा हुआ वीपी1 अंततः समावेशन निकायों से प्रोटीन के शुद्धिकरण और रीफोल्डिंग पर एक अलग ऑलिगोमेरिक रूप में उत्पन्न हुआ था। ऑलिगोमर्स अन्य पिकोर्नावायरस के वीपी1 के पेंटामर्स से मिलते जुलते हैं और उनमें सही माध्यमिक और एंटीजेनिक सतह संरचना होती है। इन परिसरों का उपयोग एचएवी कैप्सिड असेंबली के आणविक मार्ग को समझने के लिए किया जा सकता है और एचएवी संक्रमण की रोकथाम और निदान में संभावित जैव चिकित्सा उपयोग भी हो सकता है।

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LIST OF ABBREVIATIONS

The following list gives account of various abbreviations and acronyms used throughout the thesis

HAV	Hepatitis A Virus
VP	Viral protein
E.coli	Escherichia coli
UIP	Universal Immunization Programme
HPV	Human Papilloma Virus
CCMV	Cowpea Chlorotic Mottle Virus
FMDV	Foot and mouth disease virus
eHAV	enveloped Hepatitis A Virus
HAVcr-1	Hepatitis A virus cellular receptor 1
TIM-1	T-cell immunoglobulin and mucin domain 1
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
cDNA	Complementary DNA
GST	Glutathione-S-Transferase
H ₆	Hexahistidine
LB	Luria-Bertani
IPTG	Isopropyl β -D-1-thiogalactopyranoside
PMSF	Phenyl methyl sulfonyl fluoride
SDS-PAGE	Sodium Dodecyl Sulfate-PolyAcrylamide Gel Electrophoresis
Ni-NTA	Nickel-Nitrilotriacetic acid
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
HRP	Horseradish peroxidase
ddH ₂ O	double distilled water
TEM	Transmission electron microscopy
FPLC	Fast protein liquid chromatography

LC/MS-MS	Liquid chromatography–mass spectrometry
Cryo-EM	Cryogenic electron microscopy
SEC	Size exclusion chromatography
ATP	Adenosine triphosphate
CHAPS	3-cholamidopropyl) dimethylammonio)-1-propane sulfate
DLS	Dynamic light scattering
PBS	Phosphate-Buffered Saline
RT	Room Temperature
PVDF	Polyvinylidene fluoride
TBST	Tris-Buffered Saline with Tween
PBST	Phosphate-Buffered Saline with Tween
CD	Circular dichroism
ELISA	Enzyme-linked immunosorbent assay
SD	Standard Deviation
QELS	Quasi-elastic light scattering
TMB	Tetramethylbenzidine
CTF	Contrast transfer function