PREPARATION AND PHYSIOCHEMICAL CHARACTERIZATION OF ENGINEERED VIRUS LIKE PARTICLE, DEMONSTRATION OF ITS STABILITY AND EFFICACY AS TARGETED DRUG DELIVERY VEHICLE

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Preparation and physiochemical characterization of engineered virus like particle, demonstration of its stability and efficacy as targeted drug delivery vehicle

by

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Dedicated to my mother and father for their unconditional support, everlasting love, affection and care

"ईश्वर: अस्ति मम शक्ति:

CERTIFICATE

This is to certify that the thesis entitled **"Preparation and physiochemical characterization of engineered virus like particle, demonstration of its stability and efficacy as targeted drug delivery vehicle"** being submitted by **Ms. Pragati Vishwakarma** to the **Kusuma School of Biological Sciences,** Indian Institute of Technology Delhi, New Delhi for the award of the degree of **"Doctor of Philosophy"** is a record of the bonafide research work carried out by her, prepared under my supervision, in conformity with the rules and regulations of the 'Indian Institute of Technology Delhi'. The research report and the results present in the thesis have not been submitted to any other University or Institute for the award of any other degree or diploma.

Date: 23rd January 2025
Place: New Delhi

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"**ईश्वर**: **अस्ति** मम **शक्ति**:"

-Pragati Vishwakarma

ABSTRACT

Virus like particles are multimeric, macromolecular protein assemblies. Capsid protein of virus self-assembles to form VLPs, devoid of any genetic material. Structural features of viral capsid makes VLPs amenable for surface modification, cargo encapsulation and protein engineering for biomedical applications. Capsid protein of bacteriophage MS2 forms a 27nm icosahedral VLPs of T=3 symmetry. Change in the subunit interaction causes the change in symmetry, size and nature of particle, often affecting the applications. A point mutation of S37P in MS2 VLPs shifts the size of particle to 17nm with T=1 symmetry. However, does the change in size and symmetry influences the stability and application of mutant MS2 VLPs remain unexplored.

In this present work, we have prepared and characterized the MS2 VLPs together with its engineered variant for its stability, cargo loading efficiency and drug delivery efficacy. Recombinant production of WT and mutant MS2 VLPs have been achieved by expression in *Escherichia coli* (*E.coli*) host. The *E.coli* purified MS2 VLPs were subjected to a series of biochemical and biophysical characterization using plethora of analytical techniques. Interestingly, our results indicated the higher melting temperature of mutant MS2 VLPs as compared to WT, attributed to the different sets of interaction such as hydrogen bonding or hydrophobic interaction at interdimer angle. Furthermore, our findings

highlighted that the thermal unfolding of MS2 VLPs follows a sequential process involving particle destabilization, nucleic acid exposure/melting, and disassembly of VLP. This observation underscores the disruption of cooperative intersubunit interactions and protein–nucleic acid interactions, shedding light on the mechanism of heat-induced VLP disassembly.

VLPs offer an advantage for encapsulation of poorly soluble drugs, due to hydrophobic nature of interior core of capsid. MS2 VLPs have been utilized for encapsulation of diverse cargoes via conjugation or passive uptake. In the next part of this work, curcumin, a highly hydrophobic flavonoid molecule has been encapsulated in interior volume of WT and mutant MS2 VLPs. The results depicted the large interior volume of WT, encapsulating more number of curcumin molecules per particle as compared to small size mutant MS2 VLPs. Peptide attachment on the surface of mutant MS2 VLPs suggested for the enhanced surface accessibility of amino acid for conjugation and higher binding affinity to target cells. Together, our work established that mutant MS2 VLPs has potential to be fabricated or modified likewise WT MS2 VLPs. Furthermore, in vitro delivery efficiency of WT and mutant MS2 VLPs were characterized by targeting triple-negative breast cancer (MDA-MB-231) cells. Fabricated and drug formulated WT and mutant MS2 VLPs showed comparable results on cell targeting, biocompatibility, cytotoxicity and apoptosis assessment. Moreover,

mutant-tlyP1 MS2 VLPs depicts higher binding and apoptosis induction, given to higher uptake by cells due to small size of particles. In conclusion, present work characterized and established the engineered variant of MS2 VLP as a potential nanocarrier, in agreement with versatile application of WT MS2 VLPs.

सार

वायरस का कैप्सिड प्रोटीन किसी भी आनुवंशिक सामग्री से रहित, वायरस जैसे कण या वीएलपी (VLP) बनाने के लिए स्वयं एकत्रित होता है। वायरस जैसे कण (VLP) मल्टीमेरिक मैक्रोमॉलिक्यूलर प्रोटीन असेंबलियां हैं। वायरल कैप्सिड की संरचनात्मक विशेषताएं वीएलपी को सतह संशोधन, कार्गो एनकैप्सुलेशन और बायोमेडिकल अनुप्रयोगों के लिए प्रोटीन इंजीनियरिंग के लिए उपयुक्त बनाती हैं। बैक्टीरियोफेज MS2 का कैप्सिड प्रोटीन T=3 समरूपता का 27nm इकोसाहेड्रल VLP बनाता है। सबयूनिट इंटरैक्शन में परिवर्तन से कण की समरूपता, आकार और प्रकृति में परिवर्तन होता है, जो अक्सर अनुप्रयोगों को प्रभावित करता है। MS2 VLP में S37P का एक बिंदु उत्परिवर्तन कण के आकार को T=1 समरूपता के साथ 17nm पर स्थानांतरित करता है। हालांकि, क्या आकार और समरूपता में परिवर्तन उत्परिवर्ती MS2 VLP की स्थिरता और अनुप्रयोग को प्रभावित करता है ? WT और उत्परिवर्ती MS2 VLPs के पुनः संयोजक उत्पादन को एस्चेरिचिया कोली (E.coli) मेज़बान में निर्मित किया गया है। E.coli शुद्ध MS2 VLPs को विश्लेषणात्मक तकनीकों की श्रृंखला का उपयोग करके इसके जैव रासायनिक और जैवभौतिकीय लक्षण का वर्णन किया गया था। दिलचस्प बात यह है कि हमारे परिणामों ने WT की तुलना में उत्परिवर्ती MS2 VLPs के उच्च तापीय गलन के तापमान को इंगित किया, जो कि इंटरडिमर कोण पर हाइड्रोजन बॉन्डिंग या हाइड्रोफोबिक इंटरैक्शन जैसे विभिन्न प्रकार के इंटरैक्शन के लिए जिम्मेदार है। इसके अलावा, हमारे निष्कर्षों ने इस बात पर प्रकाश डाला कि MS2 VLPs का थर्मल अनफ़ोल्डिंग एक अनुक्रमिक प्रक्रिया का अनुसरण करता है जिसमें कण अस्थिरता, न्यूक्लिक एसिड एक्सपोजर/पिघलना और VLP का विघटन शामिल है। यह अवलोकन सहकारी इंटरसबयूनिट इंटरैक्शन और प्रोटीन-न्यूक्लिक एसिड इंटरैक्शन के विघटन को रेखांकित करता है MS2 VLPs का उपयोग

संयुग्मन या निष्क्रिय अपटेक के माध्यम से विविध कार्गो के एनकैप्सुलेशन के लिए किया गया है। इस कार्य के अगले भाग में, कर्क्यूमिन, एक अत्यधिक हाइड्रोफोबिक फ्लेवोनोइड अणु को WT और उत्परिवर्ती MS2 VLPs की आंतरिक मात्रा में एनकैप्सुलेट किया गया है। परिणामों ने WT की बड़ी आंतरिक मात्रा को दर्शाया, जो छोटे आकार के उत्परिवर्ती MS2 VLPs की तुलना में प्रति कण अधिक संख्या में कर्क्यूमिन अणुओं को एनकैप्सुलेट करता है। उत्परिवर्ती MS2 VLPs की सतह पर पेप्टाइड लगाव ने संयुग्मन के लिए अमीनो एसिड की बढ़ी हुई सतह पहुंच और लक्ष्य कोशिकाओं के लिए उच्च बंधन आत्मीयता का सुझाव दिया। साथ में, हमारा काम यह स्थापित किया कि उत्परिवर्ती MS2 VLPs को WT MS2 VLPs के समान ही निर्मित या संशोधित किया जा सकता है। इसके अलावा, WT और उत्परिवर्ती MS2 VLPs की इन विट्रो डिलीवरी दक्षता को ट्रिपल-नेगेटिव ब्रेस्ट कैंसर (MDA-MB-231) कोशिकाओं को लक्षित करके चिह्नित किया गया था। निर्मित और दवा से तैयार WT और उत्परिवर्ती MS2 VLPs ने कोशिका लक्ष्यीकरण, जैव-संगतता, साइटोटॉक्सिसिटी और एपोप्टोसिस आकलन पर तुलनीय परिणाम दिखाए। इसके अलावा, उत्परिवर्ती-tlyP1 MS2 VLPs उच्च बंधन और एपोप्टोसिस प्रेरण को दर्शाता है, जो कणों के छोटे आकार के कारण कोशिकाओं द्वारा उच्च अवशोषण को दिया जाता है। निष्कर्ष में, वर्तमान कार्य ने WT MS2 VLPs के बहुमुखी अनुप्रयोग के साथ सहमति में, एक संभावित नैनोकैरियर के रूप में MS2 VLP के इंजीनियर्ड संस्करण की विशेषता और स्थापना की।

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ABBREVIATIONS

%	Percentage
°C	Degree Celsius
A280	Absorbance at 280 nm
A260	Absorbance at 260 nm
ATP	Adenosine triphosphate
APS	Ammonium persulphate
BSA	Bovine serum albumin
bp	Base pair
CD	Circular dichroism
CV	Column volumes
Cm	Chloramphenicol resistance marker
DTT	1, 4-Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
g	Gram
GdnHCl	Guanidium Hydrochloride
h	Hour/hours
IPTG	Isopropyl- β- D-1-thiogalactopyranoside
kb	Kilobase pair
kDa	Kilodalton
LA	Luria Agar
LB	Luria Bertani broth

M	Molar
mg	Milligram
min	Minute
ml	Milliliter
mM	Millimolar
MRE	Molar residue ellipticity
MW	Molecular weight
Ni-NTA	Nickel-nitrilotriacetic acid
nm	Nanometer
OD600	Optical density measured at 600 nm
O/N	Overnight
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase Chain Reaction
PMSF	Phenylmethylsulfonyl fluoride
rpm	Revolution per minute
RT	Room temperature
WT	Wild type