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Review

A novel delivery platform based on *Bacteriophage MS2* virus-like particles

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ABSTRACT

Our objective here is to review the novel delivery platform based on *Bacteriophage MS2* virus-like particles (VLPs), including introduction to their structure, their potential as a delivery platform, and their expected use in medicine and other fields. *Bacteriophage MS2* VLPs are nanoparticles devoid of viral genetic material and can self-assemble from the coat protein into an icosahedral capsid. As a novel delivery platform, they possess numerous features that make them suitable and attractive for targeted delivery of RNAs or DNAs, epitope peptides, and drugs within the protein capsid. In short, as a novel delivery platform, MS2 VLPs are suitable for delivery of targeted agents and hold promise for use in diagnostics, vaccines, and therapeutic modalities.

Contents

1. Introduction

Virus-like particles (VLPs)—nanoparticles devoid of viral genetic material that are usually formed by one or several structural proteins—have external structure and antigenicity similar to those of native viruses. Recently, considerable efforts have been devoted to construction of VLPs, making them attractive as possible

Abbreviations: CCPD, covalent coat protein dimer; CP, coat protein; DC, dendritic cell; FMDV, foot-and-mouth disease virus; GM-CSF, granulocyte-macrophage colony-stimulating factor; HPV, human papilloma virus; miR, microRNA; PAP, prostatic acid phosphatase; VLP, virus-like particle.

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nanocarriers (Carrico et al., 2008; Kovacs et al., 2007; Wu et al., 2005). Combining a good safety profile with strong immunogenicity, VLPs are expected to gain widespread use in numerous fields, such as *in vitro* diagnostics, vaccines, and therapeutic modalities.

Before 2013, more than 110 VLPs had been constructed from viruses belonging to 35 families (Zeltins, 2013), among which MS2 VLPs—an icosahedral capsid self-assembled from 180 copies of a single coat protein (CP) and measuring 22–29 nm in diameter—represent a novel delivery platform and became a hot research area within several years because of their attractive features. First, MS2 VLPs can offer effective, convenient ways to package and deliver RNAs or DNAs, epitope peptides, and drugs within bacteriophage capsids (Sun et al., 2011; Wei et al., 2009; Wu et al., 2005; Zhang et al., 2015a,b). Second, they can have excellent adjuvant properties and thus induce innate and cognate immune responses. Additionally, they are safer and more effective than traditional vaccines derived from attenuated or inactivated infectious viral strains. Furthermore, they are capable of tissue-specific targeting after modification with a ligand, and this feature can ensure that the targeted agents carried by MS2 VLPs are more effective.

Clinical applications of MS2 VLPs are perhaps their most exciting feature. Their main applications belong to the field of vaccine development. A series of research findings showed that an MS2 VLP-based vaccine can effectively induce innate and cognate immune responses and can be used as a specific preventive intervention in some diseases, such as foot-and-mouth disease (Bittle et al., 1982; Dong et al., 2015; Van Lierop et al., 1992; Wong et al., 2000), prostate cancer (Li et al., 2014), and illnesses caused by human papilloma virus (HPV) (Tumban et al., 2012). Another important application of MS2 VLPs is therapeutic modalities because these particles can deliver drugs or biologics to a specific tissue (Ashley et al., 2011; Pan et al., 2012a; Yao et al., 2015). Moreover, by packaging specific RNA molecules into the recombinant MS2VLPs, researchers can construct so-called armored RNAs. Because their structure is similar to that of the native virus and their concentration is already calibrated, the armored RNAs can be used as "standards" or "calibrators" for detection of the corresponding native viruses and for validation of an experiment with the help of a "control" (Das et al., 2006; Zhan et al., 2009). Our objective here is to review the novel delivery platform based on *Bacteriophage MS2* VLPs, including introduction to their structure, their potential as a delivery platform, and their expected use in medicine and other fields.

2. Characteristics of MS2VLPs

2.1. Structure of MS2VLPs

MS2 is an icosahedral RNA bacteriophage with the triangulation number $T = 3$, and its crystallographic structure was solved at 2.8 Å resolution (Fig. 1A) (Borodavka et al., 2012; Golmohammadi et al., 1993; Valegård et al., 1990, 1991). The genome of *Bacteriophage MS2* is a positive-sense single-stranded RNA molecule of 3569 nucleotides and encodes four proteins: the major coat protein (CP), the maturation protein (A-protein), the replicase (an RNA polymerase necessary for genome multiplication), and the lysis protein (Fig. 1B).

CP makes up the bulk of the bacteriophage, assembling into an icosahedral structure ~26 nm in diameter. CP molecules first form a dimer. The dimer can bind to an RNA hairpin and then undergoes an allosteric conformational change in the FG loop region (Bleckley and Schroeder, 2012), forming an asymmetric A/B dimer rather than a symmetric C/C dimer (Borodavka et al., 2012; Dykeman et al., 2010; Dykeman and Twarock, 2010; Rolfsson et al., 2010; Stockley et al., 2007). The FG loops of the B conformation of CP surround the fivefold vertices of the icosahedron, and the A and C conformations interdigitate at the threefold vertices of the icosahedron, thus forming an icosahedron of 60 A/B dimers and 30 C/C dimers (Golmohammadi et al., 1993; Valegård et al., 1990). Eventually, *Bacteriophage MS2* can form a simple ribonucleoprotein structure composed of 180 CP molecules.

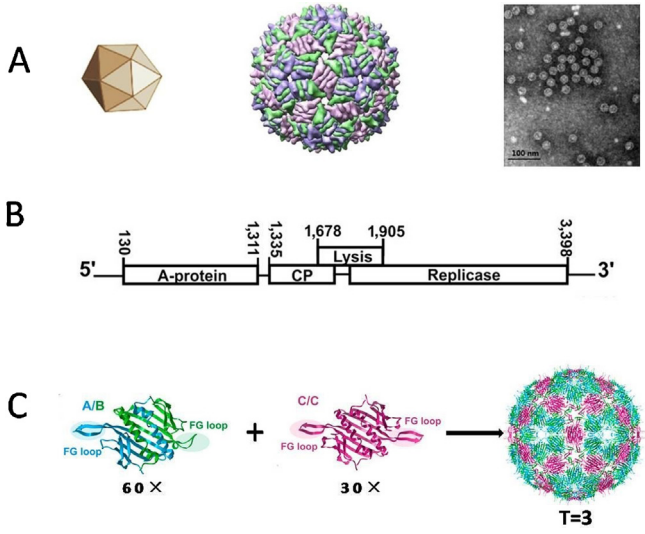

Fig. 1. Structure of *Bacteriophage MS2*. (A) External morphological characteristics of *Bacteriophage MS2* with the triangulation number $T = 3$. The left panel depicts a geometric model of MS2. The middle panel depicts surface representation of *Bacteriophage MS2*: a crystal structure of *in vitro* assembled MS2 coat protein (CP) with synthetic RNA hairpins. A/B dimers are blue and green. C/C dimers are purple. The right panel depicts an electron-microscopy image of wild-type MS2. The scale bar is 100 nm. (B) Structures of the A/B and C/C dimers. The FG loop (highlighted) exists in an extended conformation in the A and C subunits but is bent back toward the core of the protein in B subunits. Sixty A/B dimers and 30C/C dimers assemble into the icosahedron of MS2. (C) Genetic map of the MS2 genome. The genome of *Bacteriophage MS2*encodes four proteins: CP (the major protein), the maturation protein (A-protein), the replicase (an RNA polymerase necessary for genome multiplication), and the lysis protein. Adapted from Bleckley and Schroeder (2012), Borodavka et al. (2012), Li et al. (2014), and Pan et al. (2012a,b). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The three quasiequivalent conformers of CP differ primarily in the conformation of the FG loop. The loops of the A subunit are similar to those of subunit C because they have extended conformations and interact with each other at the capsid's threefold axes, but in the B subunit, the loop is bent back toward the globular core of the subunit, interacting with the other B loops at the particle's fivefold axes (Fig. 1C) (Valegård et al., 1990).

Both *in vitro* (Mastico et al., 1993; Stockley et al., 1993) and *in vivo* (Knolle and Hohn, 1975), CP molecules are capable of self-assembling into capsids at $T = 3$. In very rare cases, aberrant forms, such as capsids at $T = 1$, have also been documented (Knolle and Hohn, 1975).

2.2. Self-assembly of Bacteriophage MS2

As discussed above, we know that *Bacteriophage MS2* can assemble from 180 copies of CP into a monodisperse, icosahedral capsid with a diameter of 22–29 nm (Mastico et al., 1993; Stockley et al., 1993). Generally, the final fully formed capsids represent the state of lowest free energy of the component proteins and nucleic acids (Stonehouse and Stockley, 1993). Therefore, after its assembly, the final fully formed MS2 VLP has a stable structure, and this feature forms the basis for delivery of all kinds of targeted agents by MS2VLPs.