Megabirnavirus structure reveals a putative 120-subunit capsid formed by asymmetrical dimers with distinctive large protrusions

Naoyuki Miyazaki,1,2 Lakha Salaipeth,3 Satoko Kanematsu,4 Kenji Iwasaki1 and Nobuhiro Suzuki3

1Institute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita, Osaka 565-0871, Japan
2National Institute for Physiological Sciences, 38 Nishigonaka, Myodaiji, Okazaki, Aichi 444-8585, Japan
3Institute of Plant Science and Resources, Okayama University, Kurashiki, Okayama 710-0046, Japan
4NARO Institute of Fruit Tree Science, 92 Shimokuruyagawa, Morioka, Iwate 020-0123, Japan

Rosellinia necatrix megabirnavirus 1 (RnMBV1) W779 is a bi-segmented dsRNA virus and a strain of the type species Rosellinia necatrix megabirnavirus 1 of the family Megabirnaviridae. RnMBV1 causes severe reduction of both mycelial growth of Rosellinia necatrix in synthetic medium and fungal virulence to plant hosts, and thus has strong potential for virocontrol (biological control using viruses) of white rot. The structure of RnMBV1 was examined by cryo-electron microscopy and three-dimensional reconstruction at 15.7 Å resolution. The diameter of the RnMBV1 capsid was 520 Å, and the capsid was composed of 60 asymmetrical dimers in the $T=1$ (so-called $T=2$) lattice that is well conserved among dsRNA viruses. However, RnMBV1 has putatively 120 large protrusions with a width of $\sim 45$ Å and a height of $\sim 50$ Å on the virus surface, making it distinguishable from the other dsRNA viruses.

The encapsidated dsRNA viruses infect a broad host range from prokaryotes to eukaryotes, and include medically, veterinary and agriculturally important pathogens (Mertens, 2004; Ahlquist, 2006), but they share numerous functional and structural general principles that reflect common replication and proliferation strategies in their life cycles (Lawton et al., 2000; Mertens, 2004; Nibert et al., 2013, 2014). These viruses are currently classified into 10 families (Totiviridae, Birnaviridae, Megabirnaviridae, Partitiviridae, Picobirnaviridae, Cystoviridae, Chrysoviridae, Quadriviridae, Reoviridae and the proposed family Botybirnaviridae; Table 1). Except for birnaviruses and chrysoviruses (Coulibaly et al., 2005; Luque et al., 2010, 2014; Gómez-Blanco et al., 2012), all of these viruses have single conserved capsid layers that enclose their dsRNA genomes. The conserved capsid layer is a mostly central and contiguous icosahedral shell containing 120 chemically identical copies of a coat protein (CP) that has an $\alpha$-helix-rich $\alpha+\beta$ hold, which has $T=1$ icosahedral symmetry. The $T=1$ lattice contains a CP dimer in an icosahedral asymmetrical unit and is also referred to as a so-called $T=2$ lattice (Cheng et al., 1994; Grimes et al., 1998; Naitow et al., 2002; Nakagawa et al., 2003). This capsid architecture in the $T=2$ lattice appears to be a common attribute of the replication and transcription machinery of almost all dsRNA viruses, which may suggest a common ancestry of dsRNA viruses. In reoviruses and cystoviruses, the conserved capsid layer is covered by additional outer layers in a complete or incomplete $T=13$ lattice, thus having more complex multilayered capsids (Hill et al., 1999; Miyazaki et al., 2008). In the case of cystoviruses, a phospholipid envelope further surrounds the multilayered nucleocapsid encasing a set of three genome segments, each with a polycistronic nature (El Omari et al., 2013; Nemecek et al., 2013). The three-dimensional (3D) structures of viruses in seven of the 10 families have been examined at atomic or near-atomic resolution, whilst those in the families Megabirnaviridae and Quadriviridae remain to be determined.

The family Megabirnaviridae has been recently recognized as one of the seven families of fungal dsRNA viruses by the International Committee of Taxonomy of Viruses (Totiviridae, Partitiviridae, Megabirnaviridae, Chrysoviridae, Quadriviridae, Reoviridae and Botybirnaviridae). In general,
these fungal viruses have no known natural vectors, they lack an extracellular phase in their life cycle, and are spread vertically or horizontally by intracellular means, i.e. via sexual and asexual sporulation, and anastomosis (cell fusion) (Ghabrial, 1998; Ghabrial & Suzuki, 2009). One exception is a fungal dsRNA virus that was recently discovered and shown to be able in infected mycelia (Kanematsu et al., 2014). The ORF3-encoded products were shown to be detectable as a CP fusion product (a 250 kDa protein) probably via a −1 frameshift that is encapsidated into the virion (Salaipeth et al., 2014). ORF3 and ORF4 on dsRNA2 could encode polypeptides with unknown functions (Salaipeth et al., 2014). The ORF3-encoded products were shown to be detectable in infected mycelia (Kanematsu et al., 2014). However, whether ORF4 is expressed remains unknown. RnMBV1 causes a severe reduction of both mycelial growth of \(R.\) necatrix in synthetic media and fungal virulence to plant hosts, and is therefore considered a candidate virocontrol (biological control using viruses) agent with strong potential for controlling white root rot (Chiba et al., 2009; Kondo et al., 2013). In this study, we present the structure of a megabirnavirus (RnMBV1), as determined by cryo-electron microscopy (cryo-EM) and 3D reconstruction, and a comparison of RnMBV1 with other dsRNA viruses. Our observations revealed that RnMBV1 has presumably a 120-subunit capsid in the conserved \(T=2\) lattice, formed by putative asymmetrical dimers with distinctive large protrusions, which represents a feature that is common to, but distinguishable from, other dsRNA viruses.

To determine the structure, RnMBV1 was purified from the fungal strain W779 grown in potato dextrose broth (2 l) for 10 days, as described previously (Chiba et al.,

---

### Table 1. Encapsidated dsRNA viruses

<table>
<thead>
<tr>
<th>Virus family</th>
<th>Hosts</th>
<th>No. of genome segments</th>
<th>Particle size of (T=1) capsid (nm)</th>
<th>Capsid architecture</th>
<th>Size of CP in (T=1) shell [aa (kDa)]*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Megabirnaviridae</strong></td>
<td>Fungi</td>
<td>2(1)(^+)</td>
<td>52</td>
<td>(T=1) (120)</td>
<td>RnMBV1: 1240 (135)</td>
</tr>
<tr>
<td><strong>Totiviridae</strong></td>
<td>Fungi, protozoa</td>
<td>1</td>
<td>40</td>
<td>(T=1) (120)</td>
<td>ScV-L-A: 680 (76)</td>
</tr>
<tr>
<td><strong>Partitiviridae</strong></td>
<td>Fungi, plants, protozoa</td>
<td>2</td>
<td>35</td>
<td>(T=1) (120)</td>
<td>PsV-S: 434 (47)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>37</td>
<td></td>
<td>PsV-E: 420 (46)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>42</td>
<td></td>
<td>FpV1: 637 (70)</td>
</tr>
<tr>
<td><strong>Picobirnaviridae</strong></td>
<td>Vertebrates</td>
<td>2</td>
<td>35</td>
<td>(T=1) (120)</td>
<td>RaPBV: 525 (55)</td>
</tr>
<tr>
<td><strong>Birnaviridae</strong></td>
<td>Vertebrates, molluscs, insects and rotifers</td>
<td>2</td>
<td>–</td>
<td>(T=1) (120) + (T=1) (60)</td>
<td>IBDV: 441 (47)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Phage #6: 769 (85)</td>
</tr>
<tr>
<td><strong>Cystoviridae</strong></td>
<td>Prokaryotes</td>
<td>3</td>
<td>48</td>
<td>(T=1) (120) + (T=1) (60)</td>
<td>PcV: 982 (109)</td>
</tr>
<tr>
<td><strong>Chrysovirusida</strong></td>
<td>Fungi</td>
<td>4</td>
<td>40</td>
<td>(T=1) (60)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Reoviridae</strong></td>
<td>Protists, fungi, invertebrates, vertebrates and plants</td>
<td>9–12</td>
<td>57</td>
<td>(T=1) (120) + (T=1) (60)</td>
<td>RDV: 101 (114)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BTV: 901 (103)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Orthoreovirus: 1275 (142)</td>
</tr>
<tr>
<td><strong>Quadriviridae</strong></td>
<td>Fungi</td>
<td>4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Botybirnaviridae</strong></td>
<td>Fungi</td>
<td>2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*ScV-L-A, *Saccharomyces cerevisiae* virus L-A; PsV-S and -F, *Penicillium stoloniferum* virus S and F; respectively; FpV1, *Fusarium poae* virus 1; RaPBV, rabbit picobirnavirus; IBDV, infectious bursal disease virus; PcV, *Penicillium chrysogenum* virus; RnMV1, *Rosellinia necatrix* quadrivirus 1; RDV, rice dwarf virus; BTV, bluetongue virus.

\(^{+}\)The RnMBV1 mutant with a loss of dsRNA2 is replication competent.

\(^{\dagger}\)This family was proposed to the International Committee of Taxonomy of Viruses in 2014 (code 2014.001a-gF).
Mycelia (~35 g wet weight) were harvested and used for purification of the virus. The total amount of purified RnMBV1 material was rather limited due to the low productivity of the virus, because the growth of the fungal strain W779 is severely reduced by RnMBV1 infection. The purified viral particles of RnMBV1 were embedded in vitreous ice and examined at ~100 K with a cryo-electron microscope (Titan-Krios; FEI) operated at 200 kV and at a nominal magnification of ×47 000. Images were recorded with a 4096 × 4096 pixel charge-coupled device (Gatan UltraScann 4000) at applied defocus ranging from 1.7 to 4.9 μm (Fig. 1a). In addition to the intact RNA-filled full particles (RnMBV1_full), relatively large amounts of empty particles (RnMBV1_empty) lacking RNA were also observed. The empty and full particles were clearly distinguishable by the density at the particle centre (Fig. 1a), and they were selected separately. Approximately 15 particles (including empty and full particles) per electron micrograph were boxed out and the individual images were corrected for the contrast transfer function (only the phase graph were boxed out and the individual images were corrected for the contrast transfer function (only the phase graph). The initial model was calculated using the ‘star-corrected’ module in EMAN (Ludtke et al., 1999). After eight rounds of structural refinement in EMAN, the resultant structure was further refined in the EMAN2 suite (Tang et al., 2007) by the projection matching techniques. The final reconstructions of RnMBV1_full and RnMBV1_empty were computed from 1483 and 1092 particles, respectively. The resolution was assessed with 0.143, 0.3 and 0.5 thresholds (FSC0.143, FSC0.3, and FSC0.5) in the Fourier shell correlation between two reconstructions that was calculated from two halves of each dataset (Grigorieff & Harrison, 2011). The resolution of RnMBV1_full was 16.7 Å with a 0.5 threshold (FSC0.3, 14.0 Å; FSC0.143, 13.0 Å; Fig. S1a, available in the online Supplementary Material) and the resolution of RnMBV1_empty was 17.5 Å with a 0.5 threshold (FSC0.3, 16.7 Å; FSC0.143, 14.0 Å; Fig. S1b). As the capsid structures were indistinguishable between RnMBV1_full and RnMBV1_empty (Fig. 1b, c), the empty and full particles were mixed and further refined to achieve higher resolution. Final 3D reconstruction (RnMBV1_mix) was computed from the mixed particle set (2502 particles). The resolution was slightly improved, but still restricted to 15.7 Å with a 0.5 threshold (FSC0.3, 13.4 Å; FSC0.143, 11.6 Å; Fig. S1c) due to the quality of the sample (the low concentration of the viral particles) that impeded the data collection of the large numbers of viral particle images at higher magnification (smaller pixel size). The three reconstructions (RnMBV1_full, RnMBV1_empty and RnMBV1_mix) were deposited in the Electron Microscopy Database at the European Bioinformatics Institute.

The 3D structures of RnMBV1 are shown in Fig. 1. The diameter of the RnMBV1 capsid was 520 Å, and the RnMBV1 capsid has putatively 120 distinctive large protrusions with a width of ~45 Å and a height of ~50 Å around icosahedral two- and threefold axes, and depressions around icosahedral fivefold axes (Fig. 1d, e). The inner and outer diameters of the contiguous capsid of RnMBV1, excluding the large protrusions, were 190 and 210 Å, respectively. These observations were consistent with the appearance of the particles in electron micrographs of negatively stained RnMBV1, which exhibited spherical particles ~500 Å in diameter with a rough surface (Chiba et al., 2009). The particle size was larger than that of single-layered totiviruses and partitiviruses, whilst it was smaller than those of the inner core capsid layer of multilayered reoviruses (Table 1, Figs. 2 and S2). As seen in the cross-section of RnMBV1, the dsRNA genome was organized in a few concentric layers beneath the capsid shell (Fig. 1b, f), which is the common structural feature among the dsRNA viruses (Prasad et al., 1996; Gouet et al., 1999; Hill et al., 1999; Miyazaki et al., 2008). The cryo-EM structure of RnMBV1 showed two indistinguishable structures in an icosahedral asymmetrical unit, which implied two CP molecules in the icosahedral asymmetrical unit. This spatial arrangement of the CPs was quite similar to those of totiviruses [e.g. Saccharomyces cerevisiae virus L-A (ScV-L-A); Naitow et al., 2002] and reoviruses (e.g. rice dwarf virus; Nakagawa et al., 2003), in which two subunits are side-by-side and approximately parallel to each other around a fivefold axis (Fig. 2). Based on the similarity, the molecular boundaries between subunits were roughly determined (Fig. 1d, e). The two icosahedrally independent CPs were tentatively designated ‘A’ and ‘B’ subunits according to their positions (Fig. 1d), where the A and B subunits surround each icosahedral five- and threefold axis, respectively. The A and B subunits are in non-equivalent environments and interact with the neighbouring subunits in different ways. Due to the limited resolution, the secondary structural elements of the CP of RnMBV1 were not able to be resolved in the cryo-EM density map, but the secondary structure prediction by the PSIPRED server (Bryson et al., 2005) showed that the CP of RnMBV1 contained numerous α-helix and β-strand elements, as in other dsRNA viruses (Fig. 3; McGuffin et al., 2000), and the CP N-terminal half was probably mostly α-helical, whereas the C-terminal half contained many putative β-strands.

The most notable feature in the RnMBV1 structure is the putative 120 distinctive large protrusions from the contiguous capsid shell. Each subunit constituted a single protrusion, and the protrusions of A and B subunits were indistinguishable in terms of size and shape (Fig. 1g). The mass volume of the protruding domain was estimated to be ~5.7 × 10^4 Å^3 after segmentation in the Amira software platform (FEI Visualization Science Group), which corresponded to 46 kDa (34 % of 135 kDa) calculated from a mean partial volume of 0.74 cm^3 g^-1 (1.23 Å Da^-1) for proteins (Matthews, 1968; Kantardjieff & Rupp, 2003). The molecular size of RnMBV1 CP was considerably larger than that of other dsRNA viruses (Table 1). The cryo-EM structure suggested that the large molecular size was partly attributed to the additional large protruding domain of the RnMBV1 CP and that RnMBV1 acquired the additional protruding domain during evolution. The characteristic protrusions in the capsid are reminiscent of viral structures.
Fig. 1. Overall structure of RnMBV1. (a) Electron micrograph of RnMBV1 particles embedded in vitreous ice. The image was recorded at an underfocus value of $\sim 3.0 \, \mu m$, using a cryo-electron microscope (Titan-Krios; FEI) operated at 200 kV and a nominal magnification of $\times 47000$ (1.75 Å pixel$^{-1}$). White arrowheads indicate the empty particle lacking viral genome. Bar, 1000 Å. (b) Surface representation (upper panel) and central cross-section image (lower panel) of a full particle of RnMBV1 (RnMBV1_full), as viewed along an axis with fivefold symmetry. The surface representations are coloured according to the distance from the centre of the viral particle, for which the colour coding used in (b–e) is indicated in (e). (c) Surface representation (upper panel) and central cross-section image (lower panel) of an empty particle of RnMBV1 (RnMBV1_empty), as viewed along an axis with fivefold symmetry. (d) Surface representation of RnMBV1 reconstructed with the mixture of full and empty particles (RnMBV1_mix), as viewed along an axis with fivesfold symmetry. Molecular boundaries of A and B subunits are drawn with continuous orange and red lines, respectively. Bar, 200 Å. (e) Surface representation of RnMBV1_mix, as viewed along an axis of twofold symmetry. A triangle is drawn with a continuous white line to represent an icosahedral face. The representations of the cryo-EM structures in (b–e) were generated using UCSF Chimera (Pettersen et al., 2004). (f) Radial density distribution of RnMBV1_full. The radial positions of structural components are indicated. (g) Structure of the protruding domains. Two protruding domains in each A and B subunit are highlighted in orange and red, respectively.
Fig. 2. Structural comparison between ScV-L-A (a) and RnMBV1 (b): surface representations. To compare the structure of RnMBV1 with ScV-L-A, an electron density map of ScV-L-A was calculated at 10 Å resolution from atomic coordinates (Protein Data Bank ID: 1M1C). The particles are displayed as viewed along an axis of icosahedral twofold symmetry. In the case of ScV-L-A (a), icosahedrally independent two subunits are shown as ribbon models in green (A subunit) and magenta (B subunit). In the case of RnMBV1 (b), the two atomic models of ScV-L-A were manually fitted into the cryo-EM map of RnMBV1 and shown as ribbon models. Bar, 200 Å.

Fig. 3. Secondary structure prediction of the CP of RnMBV1 by the PSIPRED server (Bryson et al., 2005). The secondary structural elements are indicated under the sequences as H (α-helix) or E (β-strand). The amino acid residues predicted as α-helices and β-strands are coloured red and blue, respectively.
in the family Picobirnaviridae and Partitiviridae (Ochoa et al., 2008; Duquerroy et al., 2009; Pan et al., 2009; Tang et al., 2010a, b) (Fig. S2). They have a relatively small single-layered T=2 capsid of 30–42 nm diameter with 60 short surface protuberances rising above the contiguous shell region (Table 1, Fig. S2). Their capsids are composed of 60 quasi-twofold symmetrical dimers of the compact CP, the arrangement of which is somewhat different from those of totiviruses and reoviruses. Two parallel CP dimers (tetramer) form a rhombus, or diamond, tile and the 30 tiles give rise to a spherical particle with a triacontahedral design (Duquerroy et al., 2009). The CPs have an internally inserted ‘protruding’ or ‘arch’ domain in the shell domain forming the contiguous capsid shell and the inserted domains within a quasi-symmetrical dimer form a protuberance on the capsid surface. In the case of the picobirnavirus, the protruding domain is exclusively composed of β-sheets. Interestingly, the secondary structure prediction of the CP of RnMBV1 showed the abundant secondary structural elements of β-strands in the second half of the CP sequence (Fig. 3).

The role that the protruding domain of RnMBV1 CP plays in the RnMBV1 replication cycle is unknown. We assume that it might have some enzymic activities, as with the capsid of ScV-L-A in the family Totiviridae. The ScV-L-A capsids perform enzymatic reactions, decapping of cellular mRNAs and transferring m7G to viral transcripts (cap snatching) at the reaction site on the viral surface (Wickner et al., 2013). The enzymic reactions make viral mRNAs stable and competent as translation templates to avoid the preferred degradation of intrinsic uncapped viral mRNAs. Given the presence of many minicistrons in the extremely long 5′ UTR (~1.6 kb), RnMBV1 probably utilizes a non-canonical translation initiation strategy for 5′-proximal ORFs (ORF1 and ORF3) (A. Jamal, S. Chiba and N. Suzuki, unpublished data). However, if the CP of RnMBV1 has such enzymic activities perturbing host cellular systems, these activities may contribute to the virus-induced severe reduction of both mycelial growth of R. necatrix in synthetic media and fungal virulence to plant hosts, as observed in a previous study (Chiba et al., 2009). Further molecular and structural characterizations at higher resolution would reveal the actual function of the protrusion domain.

In summary, we determined the RnMBV1 capsid structure at a resolution of 15.7 Å by cryo-EM. Our results show conserved dsRNA viral capsid architecture with distinctive large protrusions that are possibly the most important domains in virus replication and proliferation.

Acknowledgements

This work was supported by CREST, Japan Science and Technology Agency, a Ministry of Education, Culture, Sports, Science and Technology of Japan Grant-in-Aid for Scientific Research on Priority Area (Structures of Biological Macromolecular Assemblies) (K.I.) and KAKENHI Grant 25251009 (N.M. and K.I.), and the Program for Promotion of Basic and Applied Research for Innovations in Bio-Oriented Industries (N.S. and S.K.).

References


