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Barley stripe mosaic virus: Structure and relationship to the tobamoviruses

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ABSTRACT

Barley stripe mosaic virus (BSMV) is the type member of the genus *Hordeivirus*, rigid, rod-shaped viruses in the family *Virgaviridae*. We have used fiber diffraction and cryo-electron microscopy to determine the helical symmetry of BSMV to be 23.2 subunits per turn of the viral helix, and to obtain a low-resolution model of the virus by helical reconstruction methods. Features in the model support a structural relationship between the coat proteins of the hordeiviruses and the tobamoviruses.

Introduction

Barley stripe mosaic virus (BSMV; Fig. 1) is the type member of the genus *Hordeivirus*, a group of rigid, rod-shaped filamentous viruses in the family *Virgaviridae* (Adams et al., 2009). The virus is tripartite; virions are composed of a single type of coat protein that helically encapsidates one of three different *g*RNAs, and particle lengths vary from 110 to 150 Å (Harrison et al., 1965; Chiko, 1975). Several reviews of the *Hordeivirus* genus (Jackson and Lane, 1981; Jackson et al., 1989, 2009) and a number of publications describing hordeivirus transport within plants (for example, Verchot-Lubicz et al., 2010) have been published; additionally, there has been much recent interest in BSMV as a vector for virus-induced gene silencing and related applications (Jackson et al., 2009; Lee et al., 2012). However, little detailed structural information is available for this group of viruses.

Structural studies from almost 50 years ago provide some information about the morphology of BSMV. X-ray fiber diffraction experiments (Finch, 1965) showed that the virus has a central hole of radius 15 to 20 Å and a maximum radius between 105 and 115 Å; the pitch of the primary helix was determined to be 26.1 ± 0.2 Å. The possibility of a strong structural feature at a radius of 65 Å in the virus particle was discussed, and it was proposed that the RNA might be located at a radius of 55 Å. The experiments showed that there were $5q+1$ or $5q-1$ coat protein subunits in five turns of the viral helix, with q an integer, but the value of q was not known. Ultracentrifugation and electron microscopy (EM) experiments (Kiselev et al., 1966) indicated the presence of three main types of aggregates in repolymerized coat protein preparations: disks of approximately 24 coat protein subunits per turn, rod-like aggregates, and longer helical aggregates. Negative stain electron micrographs (Atabekov et al., 1968) suggested that there were between 20 and 22 coat protein subunits per turn of the viral helix, and that the coat protein subunit of BSMV could be approximated by a prolate ellipsoid of length 75–80 Å and diameter 25–26 Å. Stoichiometric calculations (Veerisetty, 1978) predicted the viral RNA to be at a radius of 63 Å. For those calculations, the number of coat protein subunits per turn was estimated to be 26, with three nucleotides associated with each coat protein subunit.

We have recently combined fiber diffraction analysis with EM to produce low-resolution models of a number of flexible filamentous plant viruses (Kendall et al., 2008, 2013). We have now applied this approach to determine the symmetry and obtain a model of BSMV.

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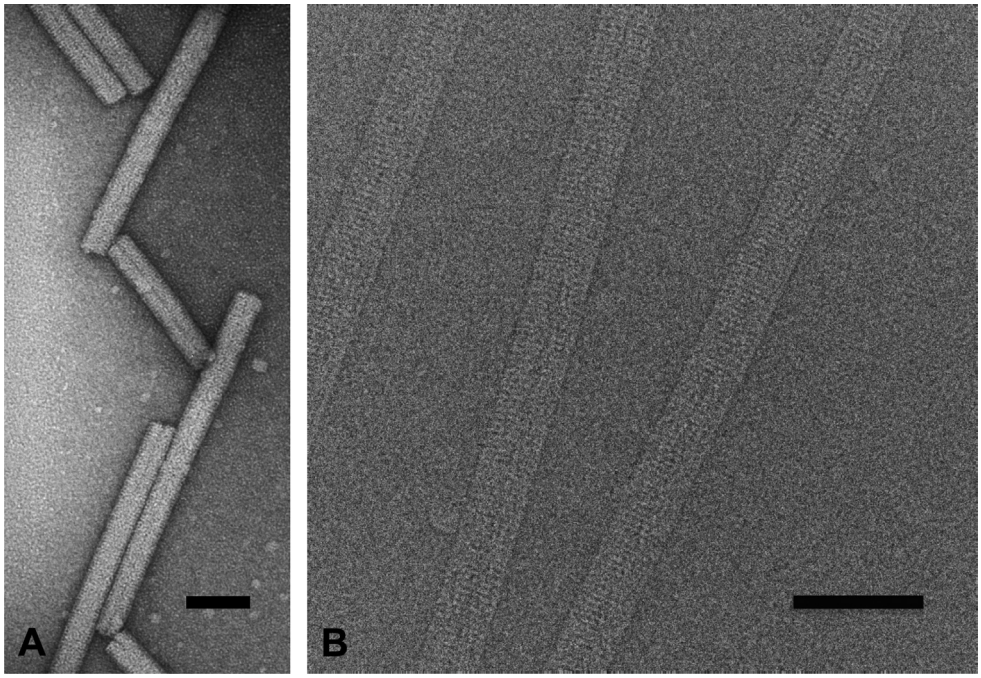

Fig. 1. (A) Negative stain electron micrograph of barley stripe mosaic virus (Materials and methods). (B) Cryo-electron micrograph of barley stripe mosaic virus (Materials and methods) with density inverted. Scale bars 500 Å.

Results

Data collected from fiber diffraction experiments were used to obtain initial parameters for model refinement from cryo-EM data.

Fiber diffraction

The X-ray fiber diffraction pattern from BSMV (Fig. 2) shows that the fibers are noncrystalline, with continuous diffraction along layer lines. The pattern is dominated by a series of near-meridional layer lines whose spacing corresponds to a virus helical pitch of 25.8 ± 0.2 Å, close to the pitch determined by Finch (1965). The meridian is the line through the origin and parallel to the fiber axis, approximately vertical in this figure. Between the near-meridional layer lines, off-meridional layer lines are located at spacings one-fifth those of the near-meridional layer lines, that is, corresponding to a 129 Å repeat or five turns of the viral helix. Finch (1965) pointed out that these spacings and the distribution of intensities on the off-meridional layer lines suggest that there are $5q+1$ coat protein subunits in five turns of the viral helix, where q is an integer. Although the possibility of $5q-1$ could not be completely excluded, the pair of layer lines 20 and 21 near the meridian (Fig. 2) supports $5q+1$; the pair would be 19 and 20 if the number of subunits in the repeating unit were $5q-1$ (Kendall et al., 2008).

The first intensity maximum on layer line 1 (Fig. 2) is at reciprocal space radius $R=0.34$ Å^{-1}. The intensity at a point R on a layer line depends on $J_n(2\pi Rr)$, where J_n is the Bessel function of order n, R is the reciprocal space radius (the distance along the layer line from the meridian), and r is the radius of the structural feature giving rise to the diffracted intensity. On the first layer line, n is the integer nearest to the number of subunits in each turn of the diffracting helix, corresponding to q as defined above (Cochran et al., 1952; Chandrasekaran and Stubbs, 2012). At low resolution, r is typically a little less than the maximum particle radius, that is, the principal diffracting features are at the boundary between the particle and the surrounding solution. Our estimate of that radius from electron micrographs agrees with the value of about 110 Å derived from diffraction data by Finch (1965); $r=100$ Å and $R=0.034$ Å^{-1} would correspond to the first maximum of the Bessel function of order $n=23$. While this value for n is a very rough estimate, it is consistent with the literature estimates discussed

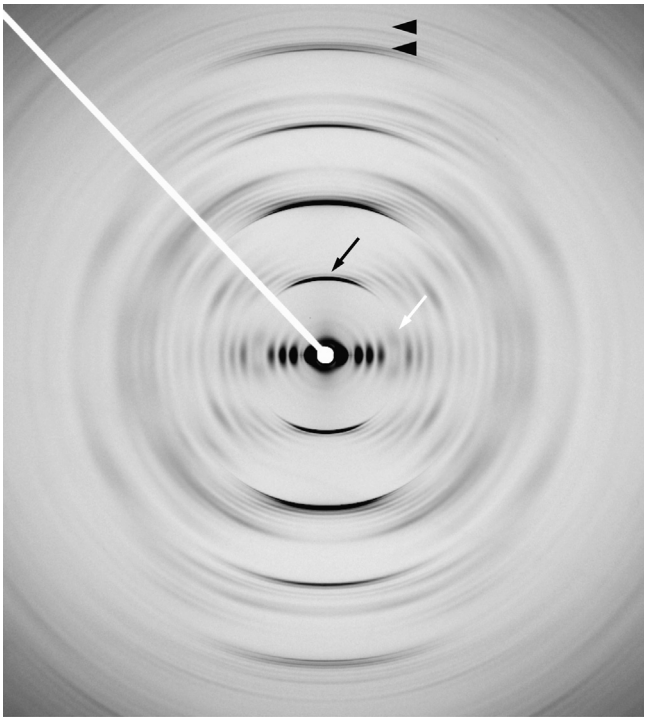

Fig. 2. X-ray fiber diffraction pattern from an oriented sol of BSMV. White arrow: first intensity maximum on layer line 1, at reciprocal space radius $R=0.34$ Å^{-1}. Black arrow: first near-meridional layer line, corresponding to a helical pitch of 25.8 Å. Arrowheads: diffracted intensities on layer lines 20 and 21.