Title
Characterization of crystals of satellite panicum mosaic virus

Permalink
https://escholarship.org/uc/item/1n93z5tz

Journal
Journal of Molecular Biology, 238(5)

ISSN
0022-2836

Authors
Day, J
Ban, N
Patel, S
et al.

Publication Date
1994-05-19

DOI
10.1006/jmbi.1994.1339

License
CC BY 4.0

Peer reviewed
Characterization of Crystals of Satellite Panicum Mosaic Virus

John Day, Nenad Ban, Samit Patel, Steven B. Larson and Alexander McPherson

Department of Biochemistry
University of California, Riverside
Riverside, CA 92521, U.S.A.

Satellite panicum mosaic virus (SPMV) has been purified from pearl millet and obtained in a variety of different crystal forms, at least two of which appear suitable for high resolution X-ray diffraction analysis. The first is of cubic space group $P4_32$ with $a=b=c=183.1$ Å and two virus particles in the unit cell. The second is of a primitive orthorhombic space group with $a=166.1$ Å, $b=266.7$ Å and $c=269.1$ Å, with four virus particles in the unit cell. While the cubic crystal has as its asymmetric unit one twelfth of the icosahedron, or five capsid protein subunits, the asymmetric unit of the orthorhombic crystals is an entire particle. The cubic crystals diffract to at least 2.8 Å resolution. We have also succeeded in crystallizing, but not yet characterizing the master virus, PMV.

Keywords: satellite virus; X-ray diffraction; crystallization; panicum

There are three well characterized, protein encapsidated, satellite viruses of plants, each of $T=1$ icosahedral symmetry (reviewed by Francki, 1985). These are satellite tobacco necrosis virus (STNV$^+$; Kassanis, 1962) satellite tobacco mosaic virus (STMV; Valverde & Dodds, 1986; Dodds, 1991), and satellite panicum mosaic virus (SPMV; Masuta et al., 1987). These have as their master viruses tobacco necrosis virus (TNV), tobacco mosaic virus (TMV), and panicum mosaic virus (PMV; Niblett & Paulsen, 1975), respectively. The host plants for the first two master-satellite pairs are chiefly tobacco, while that for the last pair is millet. In every case, the satellite virus can replicate in the host cell only when it is co-injected with its master virus upon which it relies for enzymatic assistance (Francki, 1985).

The structures of both STNV (Liljas et al., 1982), which has a genome size of 1239 nucleotides and a capsid protein molecular mass of 21,000 Da, and STMV (Larson et al., 1993a), of genome size 1059 bases and capsid protein molecular mass of 17,500 Da, have been determined by X-ray crystallography to high resolution (Jones & Liljas, 1984; Larson et al., 1993b). In the latter case, segments of double helical genomic RNA were observed associated on the interior of the capsid with each pair of dyad related proteins. Each RNA segment contained seven base-pairs plus unpaired 3' terminal nucleotides at either end (Larson et al., 1993a).

SPMV (Niblett & Paulsen, 1975) is the smallest of all of the encapsidated satellite viruses (Buzen et al., 1984; Masuta et al., 1987). It has a genome length of 850 nucleotides and a capsid protein of 157 amino acid residues, two less than for STMV. It shows no amino acid or nucleotide sequence homology with either STMV, STNV, or its own master virus, PMV. Furthermore, it shows no immunological cross-reactivity with any of these viruses. Nonetheless, experience would suggest that it may be structurally similar to the other satellite viruses and that the organization of its RNA within the capsid might be similar as well.

Pearl millet was grown from seed in a temperature controlled greenhouse and infected mechanically with a mixed inoculum of PMV and SPMV approximately three weeks after planting. After three additional weeks, the leaves exhibited strong symptoms of infection and were harvested. From about one kilogram of millet stems and leaves, nearly one and a half grams of the crystalline satellite virus could be obtained using a modified procedure of Masuta et al. (1987). The major alteration to the purification procedure was the replacement of the sucrose gradient centrifugation step by PEG-NaCl fractionation. Precipitate was resuspended in 0.1 M phosphate buffer (pH 7.4; 1 ml per mg of tissue). At this point the preparation contained a significant excess of SPMV over PMV as shown by SDS-PAGE. The concentration of the solution, that contained both viruses as well as 0.1 M phosphate buffer (pH 7.2), was slowly raised to 6% (w/v) PEG 8000 by adding 0.25 volume of 30% PEG 8000 and
allowing it to stand at 4°C for 24 hours. This resulted in the precipitation, in amorphous form, of virtually all of the PMV. Following centrifugation of the PMV, the supernatant concentration of PEG 8000 was increased to 10% (w/v) and NaCl was added to a final concentration of 0.2 M. After 4 hours at 4°C the microcrystalline precipitate of SPMV was recovered by centrifugation. Both the PMV and the SPMV fractions were judged to be better than 95% pure, based on silver stained SDS-PAGE gels.

Crystals were mounted in quartz capillaries by conventional techniques and diffraction photographs recorded on Buerger precession cameras of crystal to film distance 90 mm. The X-ray source was an Enraf-Nonius Diffractis generator with conventional sealed tube operated at 40 kV and 32 mA. Crystals were subsequently examined on a San Diego Multiwire Systems multiwire area detector system with a Rigaku RU-200 generator fitted with a Supper graphite crystal monochromator as X-ray source. Using the multiwire detector system the unit cell dimensions were confirmed and refined.

Crystals of SPMV were grown under a wide variety of conditions ranging from pH 4.5 to 8.5 and using both ammonium sulfate and PEG as precipitating agents. Two crystal forms, however, demonstrated particular promise as subjects for a high resolution structure analysis based on their size, quality and diffraction properties. These crystals were grown by vapor diffusion methods using glass depression plates in plastic sandwich boxes (see McPherson, 1982) at 23°C over a period of about ten days. The reservoir solution was 30% saturated ammonium sulfate in water. The sample droplets were composed of 8 µl of an 8 mg/ml virus solution buffered with 0.1 M cacodylate (pH 6.8) plus 8 µl of the reservoir.

The diffraction pattern from these crystals could be indexed on a primitive orthorhombic lattice consistent with real space dimensions of a = 166.1 Å, b = 266.7 Å and c = 229.1 Å. At this point we could not assign a space group unequivocally due to the large cell dimensions. If it is assumed that there are four virus particles in each unit cell of volume 12.58 × 10^6 Å^3, then the volume per particle would be almost exactly the same as for the cubic crystals above. This means that the four virus particles occupy general positions within the unit cell and that the asymmetric unit of the crystals must be an entire particle or 60 capsid protein subunits plus associated nucleic acid. The diameter of the virus, as judged from electron micrographs (Franci, 1985; Masuta et al., 1987), is 170 Å; hence, the unit cell can be only one virus particle thick along the c direction. These orthorhombic crystals do not appear to exhibit the diffraction quality of the cubic crystals.

Crystals of PMV were obtained after resuspending the virus pellet in water and centrifuging at low speed to clarify. Vapor diffusion yielded small octahedral crystals of about 0.1 mm maximum dimension in both Crychem plates and glass depression plates contained in plastic boxes (McPherson, 1982). The microdrops were formed by combining 8 µl of PMV (15 mg/ml) and 2 µl of 0.1 M phosphate buffer (pH 7.4) with 10 µl of the precipitant solution. The precipitant solutions producing the best results to this point are 3.5% (w/v) PEG 8000, 4% (w/v) PEG 20,000, or 30% saturated ammonium sulfate. All crystals were grown at room temperature over 2 to 10 days.

Presently, the PMV crystals have not been characterized because of their small size. We are optimistic, however, that this problem will be overcome and that we can proceed with the three-dimensional structure analysis of the master virus as well as its satellite.

We are in the process of undertaking a full three-dimensional structure analysis of both forms of the SPMV crystals we have described here using molecular replacement approaches combined with phase averaging and extension. Given the likelihood
that SPMV and STMV will bear a reasonable resemblance to one another, we anticipate that these methods will prove successful. We are further hopeful that the structure of SPMV, combined with the results obtained in the STMV analysis, will allow us to define in more detail the conformation and distribution of the genomic RNA within the virus particle.

This research was sponsored by grants from NASA and the NIH. The authors wish to thank Professor P. J. Desjardins for his advice and assistance, and Professor A. Jackson for providing us with both the pearl millet seed and the virus inoculum.

References


Edited by A. Klug

(Received 17 February 1994; accepted 17 February 1994)