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Authors

Koszelak, S
Dodds, JA
McPherson, A

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Preliminary Analysis of Crystals of Satellite Tobacco Mosaic Virus

Satellite tobacco mosaic virus (STMV), a small $T=1$ icosahedral plant virus, has been crystallized in a form suitable for high-resolution X-ray diffraction analysis. The crystals, which diffract to better than 2.5 Å resolution, are of space group $I222$ and have unit cell dimensions of $a=176$ Å, $b=192$ Å and $c=205$ Å. The centers of the virus particles occupy 222 symmetry points in the unit cell and one quarter of the virus particle constitutes the asymmetric unit, which is therefore comprised of 15 capsid protein molecules. From packing considerations, the maximum diameter of the STMV particles cannot exceed 165 Å, and it is probably 5 to 10 Å less than this value.

Numerous plant virus groups support the replication of another virus (Kassanis, 1981; Buzen *et al.*, 1984; Gingery & Louie, 1985) and the dependent viruses are known as satellite viruses. Several of these small icosahedral viruses of about 170 Å diameter (1 Å = 0.1 nm) have been characterized (Francki, 1985). They have sedimentation values of 42 to 50 S, single-stranded RNA genomes ranging in size from 826 to 1239 bases and coat proteins of molecular weight 17,000 to 25,000.

Satellite tobacco mosaic virus (STMV†) is one of the more recently characterized of the satellite viruses and is found in association with tobacco mosaic virus (TMV) strain U5 (Valverde & Dodds, 1986, 1987). Electron microscopy shows STMV to have the morphology typical of icosahedral virus particles (Valverde & Dodds, 1987). This is interesting since STMV is therefore the first reported case of a spherical satellite virus having a helper that is rod-shaped, in this case TMV. Its single-stranded RNA genome of 1060 nucleotides is encapsidated in a protein that is antigenically unrelated to TMV and has a molecular weight of 17,500. STMV shows little sequence similarity to its helper virus in hybridization experiments. It is similarly distinct from another satellite virus which it physically resembles, satellite tobacco necrosis virus (STNV). This latter virus (STNV) has been crystallized (Klug, 1971; Åkervall *et al.*, 1971) and the entire three-dimensional structure of its capsid elucidated by X-ray diffraction analysis (Liljas & Strandberg, 1984).

Although there are superficial resemblances between STMV and STNV, owing principally to the $T=1$ icosahedral symmetry of their capsids, the two viruses are otherwise quite different. The coat protein of STNV is 23,000 M_r and the genome size is 1239 bases. STMV has a coat protein of 17,500 M_r and a genome of 1060 bases. While both viruses are

reported to have diameters of 170 Å, the approximately 20% less weight of STMV would suggest that it may be of somewhat smaller diameter.

Because of its unique features and its clear differences from the only other satellite virus whose three-dimensional structure is known, we undertook attempts to crystallize STMV in a form suitable for X-ray diffraction analysis. We report here our success in these attempts and a preliminary diffraction analysis.

STMV was purified from TMV U5-infected tissue by rate zonal density-gradient centrifugation as described by Valverde & Dodds (1987). Prior to crystallization, it was dialyzed against water containing a trace of chlorobutanol and concentrated in an Amicon B15 absorption concentrator to 20 mg/ml. It was stored as a stock solution at 4°C.

Crystallization trials were carried out at 4°C and 23°C. Ultimately, it was judged that the higher temperature was more favorable for crystal growth. A broad screen of conditions was carried out initially using ammonium sulfate and polyethylene glycol 4000 (PEG 4000) as the precipitating agent. Later, other salts such as NaCl and $\text{NaC}_2\text{H}_4\text{O}_2$ were also included. The technique employed in the initial screen and all subsequent crystallization attempts was the vapor diffusion method using sitting drops on nine-well glass depression plates as described by McPherson (1982).

Although crystals were obtained, generally in as little as 24 hours, from all of the salt solutions as well as the PEG 4000 trials, the best crystals were grown according to the following formula. The drop was initially composed of 5 μl of 20 mg STMV/ml, 5 μl of 40 mM-cacodylate, phosphate or Tris buffer and 10 μl of the reservoir. The pH values of the buffers were either 6.0, 6.5 or 7.0, although crystals could also be grown, less reproducibly, at both higher and lower pH values. The reservoirs were 25 ml of 10% to 18% saturated ammonium sulfate solutions buffered at 40 mM concentration with the same buffer as that used in the microdroplet.

Crystals were photographed under a low-power

† Abbreviations used: STMV, satellite tobacco mosaic virus; TMV, tobacco mosaic virus; STNV, satellite tobacco necrosis virus.

light microscope and mounted in quartz capillaries along with a small amount of mother liquor by conventional techniques. X-ray diffraction photographs were obtained from the crystals using Buerger precession cameras with a crystal-to-film distance of 100 mm. The X-rays were generated by an Enraf-Nonius generator fitted with a standard focus tube operated at 1400 W. Exposure times were generally of the order of 12 to 24 hours for precession angles of 7° to 10°.

Crystals of STMV having typical polyhedral habits were grown by equilibration against 15% saturated ammonium sulfate. Extensive investigation of the reciprocal lattice using a series of low-angle precession photographs proved there to be three orthogonal major zones. Each of the zones exhibited reflections falling on rectangular nets, all having mirror-mirror symmetry. Alternate reflections along all reciprocal lattice lines on all three zones were systematically absent and this was true of upper layers of reciprocal space as well. The reciprocal lattice, therefore, had mmm symmetry with $h+k+l \neq 2n$ reflections systematically absent. The space group of the crystals was therefore, $I222$ or $I2_12_12_1$. The unit cell dimensions were measured to be $a=176$ Å, $b=192$ Å, $c=205$ Å, and the volume to be $V=6.93 \times 10^6$ Å³.

The unit cell clearly must contain at least two virus particles to account for the body centering, but assumption of more than two would result in a cell having a prohibitively high density. The unit cell of the crystals, therefore, must contain exactly two virus particles. This has the consequence that each particle must provide four asymmetric units of the crystal. To accomplish this, the space group must be $I222$, the center of each virus particle must lie at a 222 symmetry point in the unit cell, and dyad axes of the virus must coincide with 2-fold crystallographic symmetry axes. This is possible if the virus has icosahedral symmetry, as all evidence suggests, since an icosahedron contains 222 point group symmetry as a subset of its own symmetry. The nucleic acid contained within the capsid clearly cannot demonstrate such symmetry and, therefore, it must be assumed that it does not contribute in a systematic way to the discrete diffraction pattern of the crystals.

A further consequence of the necessity that a quarter of the virus particle comprise the asymmetric unit of the crystals is that the asymmetric unit must be composed of 15 identical capsid proteins. Thus, the asymmetric unit is 15-fold redundant and the subunits must be arrayed in the asymmetric unit according to the $T=1$ icosahedral symmetry of the virus.

The volume in the crystals per STMV particle is $V=3.46 \times 10^6$ Å³ while that for STNV is $V=4.5 \times 10^6$ Å³ per virus particle. Unless the viruses in the $I222$ crystals of STMV are packed substantially closer than in the $C2$ crystals of STNV, it would be necessary to conclude that the STMV particles are about 20% less in volume than the STNV particle. This is consistent with the roughly 20% shorter

genome and 20% less mass of the protein coat of STMV with respect to STNV. In the $I222$ unit cell of the STMV crystals reported here, we can calculate the center-to-center distance for the particles in the crystallographic unit cells. This is simply the distance between the point 0,0,0 and point $\frac{1}{2}, \frac{1}{2}, \frac{1}{2}$, and from the unit cell dimensions is 165 Å. This center-to-center distance is also the maximum possible diameter of the virus particle since a larger diameter would result in interpenetration of virus particles. Thus, STMV cannot have a diameter greater than 165 Å, and probably, it is somewhat less than this. Thus, STMV must have a diameter at least 5 to 10 Å less than the 170 Å previously estimated (Valverde & Dodds, 1987). It must, furthermore, be the smallest virus, in terms of both size and weight, to be crystallized and analyzed by X-ray diffraction analysis.

We were gratified to observe that in still photographs, even with a standard X-ray tube with conventional collimation, strong reflections were observed to well beyond 2.5 Å resolution. In addition, no inordinate decay was noted with X-ray exposure up to 40 hours. Thus, we feel that the crystals will prove to be entirely adequate for a complete high-resolution analysis of the virus structure.

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Stanley Koszelak¹
James Allan Dodds²
Alexander McPherson^{1†}

¹Department of Biochemistry
University of California at Riverside
Riverside, CA 92521, U.S.A.

²Department of Plant Pathology
University of California at Riverside
Riverside, CA 92521, U.S.A.

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† Author to whom all correspondence should be addressed.

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