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Crystalline Cowpea Chlorotic Mottle Virus

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A new crystal form of cowpea chlorotic mottle virus has been characterized. The crystals exhibit needle-like morphology and diffract to 8-Å resolution. The crystal has orthorhombic symmetry of space group $P2_12_12_1$; the spatial distribution of the virus particles is distorted cubic close packing.

Cowpea chlorotic mottle virus (CCMV) is a small spherical RNA plant virus. The RNA consists of four distinct species with respective molecular weights of 1.2, 1.1, 0.7, and 0.3×10^6 daltons (5). These are combined in three nucleoprotein particles: two virions containing, respectively, RNA components one and two and a third virion containing components three and four. All three particles are required for complete infective efficiency.

Each virion appears to have an identical protein capsid composed of 180 protein subunits which places the isometric particles in the icosahedral surface lattice class $T = 3$ (8). Amino acid composition studies indicate the molecular weight of each subunit to be 19 600 daltons (6) while polyacrylamide gel electrophoresis shows 19 200 daltons (1). The average virion molecular weight is thus approximately 4.6×10^6 daltons.

Crystals of CCMV showing cubic, needle-like, and tetragonal bipyramidal morphology have been reported previously (12). Only the cubic crystals gave useful X-ray diffraction patterns. Crystalline arrays of CCMV have also been studied with the electron microscope (3, 4, 10). The optical diffraction patterns and X-ray intensity distribution both indicated the same particle-packing arrangement and an average virion diameter of approximately 260 Å at a pH below 6.5. Above this pH, the virus diameter increases to greater than 300 Å. The growth of large needle-shaped crystals of CCMV which diffract to 8-Å resolution is reported here.

The virus was grown in cowpea plants, *Vigna unquiculata* (L.), and purified according to Bancroft *et al.* (7). The crystals were obtained at room temperature from a buffered aqueous solution of 1.8 *M* sodium phosphate at a virus concentration between 10 and 20 mg/ml. They were grown over a period of 1 month in depression slides covered by glass coverslips and sealed with grease. This procedure facilitated the placing of the extremely soft crystals into capillary tubes. The morphology of the crystals was very sensitive to pH. The thickest needle-like crystals ($0.4 \times 0.3 \times 1.5$ mm) were grown at pH 6.0; below this the crystals were very thin, while above pH 6.2 a predominance of small (0.01 mm) cubic crystals was observed. The needles showed weak birefringence along their longest dimension.

The crystals were examined with CuK_α radiation produced by an Elliott rotating anode X-ray generator and focused by two perpendicular mirrors (9). Reflections were observed to 8-Å resolution on still photographs after 3 hr of exposure. Precession photographs (Fig. 1) showed the space group to be $P2_12_12_1$ with $a = 522$, $b = 383$, and $c = 308$ Å. The crystals exhibited prominent {101} faces.

If the unit cell is assumed to contain four virus particles, that is, one per asymmetric unit, then the value of V_m (11) is 3.3 Å^3/dalton, which is close to that of 3.6 Å^3/

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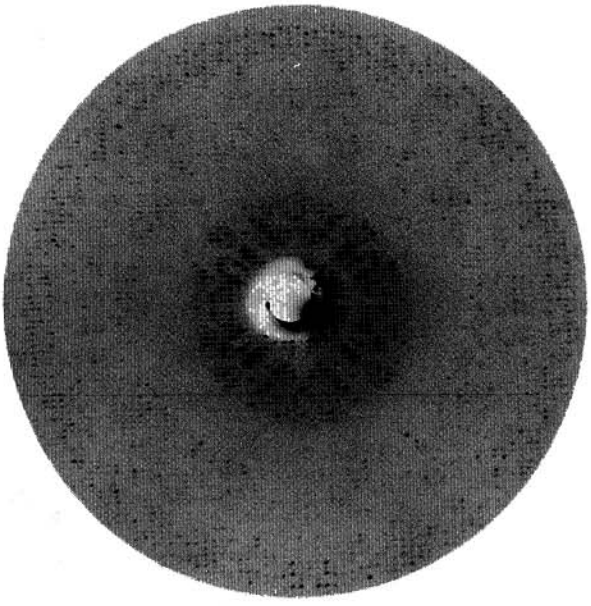

FIG. 1. A $\mu = 1.5°$ unscreened precession photograph of the (hk0) zone of the CCMV needle-like crystals taken using CuK_α radiation produced by a 30-mA tube current and a 40-kV potential difference between electrodes. Exposure time was 12 hr. The photograph shows the (hk0) zone and one upper level (hk1) zone.

dalton found for the various crystal forms of southern bean mosaic virus (2). Any other assumption gives a value for V_m which lies outside acceptable limits (11). From the cell dimensions, space group, and virus diameter it was possible to limit the position of the particle center in the asymmetric unit. A systematic search allowing interparticle distances to be greater than 265 Å gave a particle center with fractional coordinates $x = 0.13 \pm 0.03$, $y = 0.22 \pm 0.04$, and $z = 0.05 \pm 0.05$. Furthermore, a particle positioned at this site makes six close contacts of 266 Å. The distribution thus generated by the crystallographic symmetry is a distorted cubic close packed arrangement, with the hexagonal close packed planes lying parallel to {110}. In contrast, the packing arrangement found by Rossmann *et al.* (12) for the F432 crystals is hexagonal close packed. No information is available, however, for the orientation of the icosahedral symmetry axes in either case.

It is improbable that there is separation of the three different virus particles during crystallization in view of their identical protein coats, which may account for the low resolution X-ray diffraction patterns observed. Separation of the different particle types might result in crystals which diffract to higher resolution.

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