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SHORT COMMUNICATION

Crystallization and Preliminary X-ray Analysis of the Core Particle of Bluetongue Virus

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Core particles of bluetongue virus serotype 1 (South Africa) have been crystallized. The crystals, which grow up to 0.8 mm in diameter, belong to a primitive orthorhombic space group and have point group symmetry 222. The unit cell dimensions are 754 × 796 × 823 Å^3 and the crystallographic asymmetric unit contains one-half of a core particle. The best crystals diffract strongly to 4.8 Å Bragg spacings, which is the maximum resolution to which we can measure data with the detectors available, suggesting that useful diffraction extends well beyond this. Core particles of serotype 10 have also been crystallized but the crystals have yet to be analyzed by X-ray diffraction.

Bluetongue virus (BTV) is a member of the *Orbivirus* genus in the family Reoviridae. It causes an acute disease in sheep and wild ruminants, but a milder disease in cattle and goats, and is endemic in many parts of the world. In common with other members of the Reoviridae BTV has a double-capsid structure. The icosahedral core, which contains a genome of ten segments of double-stranded (ds) RNA, consists of two major proteins, VP3 (M_r = 103,000) and VP7 (M_r = 38,000), and four minor proteins (VP1, VP4, VP6, and VP6a) (*1–5*). The outer shell, which has been shown by electron cryomicroscopy to be well-ordered (*6*), consists of two major proteins, VP2 and VP5, and may be associated with a minor protein component (NS2) (*5*). It can be removed by treatment with high concentrations of magnesium chloride or in some cases, depending on the virus strain, by caesium chloride (*5*).

The structure of the core, which has a diameter of 690 Å (69 nm), has been determined to a resolution of 30 Å by electron cryomicroscopy (*7*). The surface of the core is built up from 260 trimers of VP7 (i.e., 780 copies per virion) which form a T = 13, *l* lattice. The inner layer or subcore is made up of the other major core protein VP3, which forms a scaffold on which the trimers of VP7 sit. The ratio of VP7 to VP3 has been estimated to be 13:2 (J. N. Burroughs, unpublished observations), which would put the number of copies of VP3 at 120 per virus particle. Recent evidence from electron cryomicroscopy suggests that the triangulation number of the subcore lattice is T = 1 (*8*), consistent with it being assembled from 60 dimers of VP3.

Preliminary X-ray studies have been carried out on

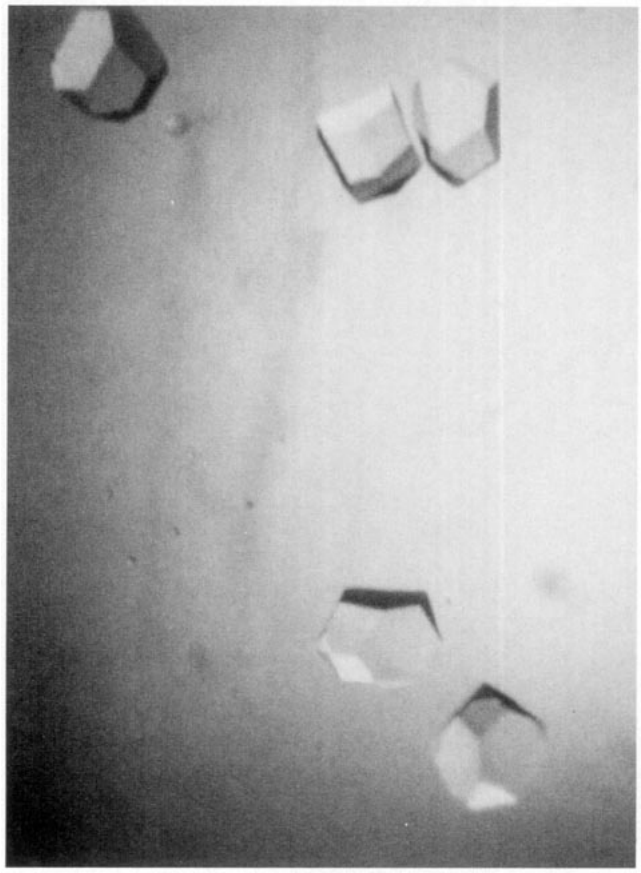

FIG. 1. Crystals of the core particle of BTV type 1 (South Africa). The maximum dimensions are approximately 0.45 mm.

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0042-6822/95 $6.00

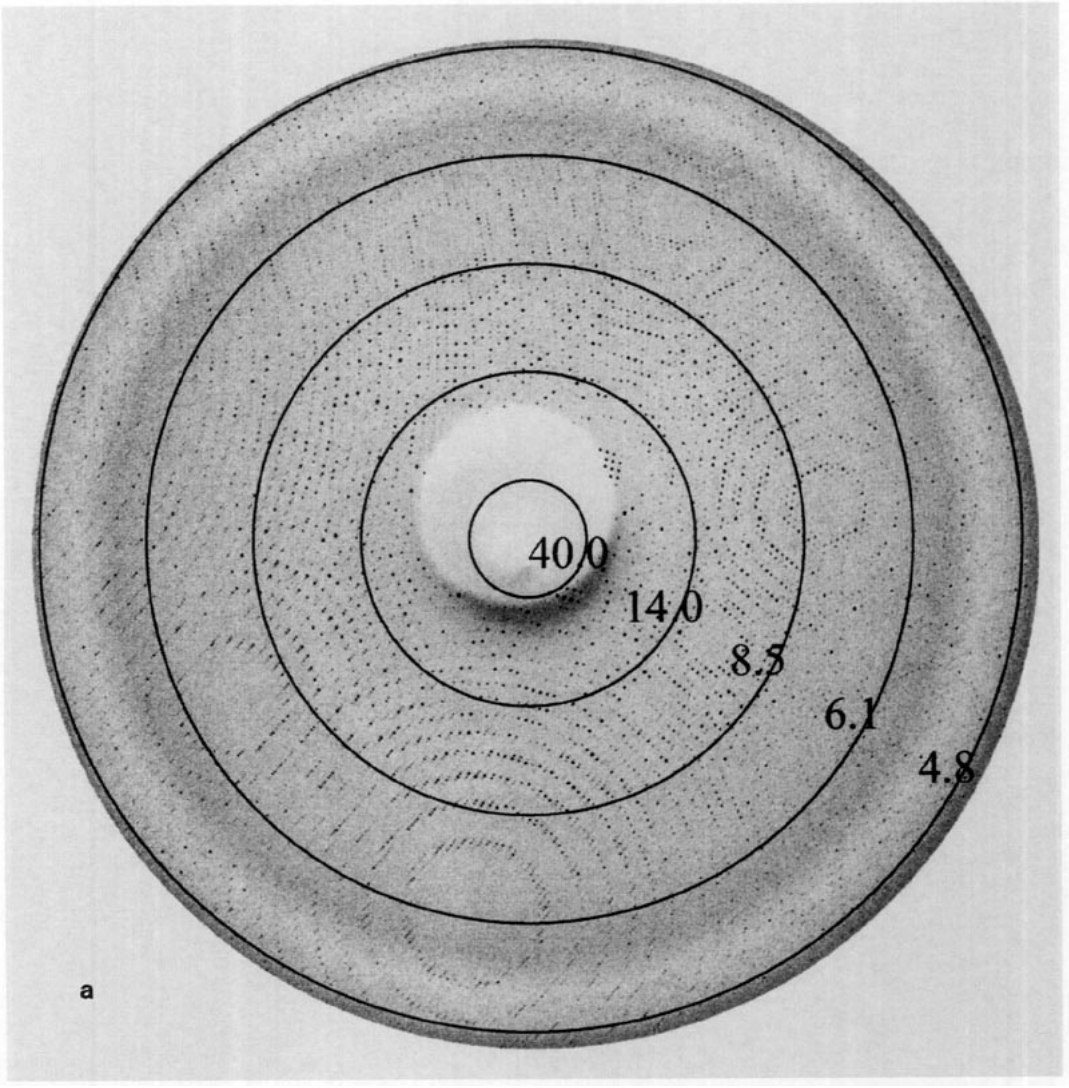

FIG. 2. (a) X-ray diffraction pattern from a crystal of BTV type 1 core particles, obtained using a 30-cm diameter Marresearch imaging plate, with an oscillation range of 0.2°, wavelength 0.88 Å, and crystal to film distance of 777 mm. The images were recorded at station 9.6 of the SRS Daresbury with an exposure time in the order of 4 min. They were produced by PSIMAGE (Robert Esnouf, unpublished program). (b) Enlargement of an area of the image shown in (a). The Bragg spacing (in Å) is indicated by circles of increasing radius from the position of the direct beam.

two related viruses, reovirus type 3 cores (*9*) and the Phytoreovirus, rice dwarf virus (*10*), with crystals diffracting to a resolution of 8 and 6.5 Å, respectively. The structure of baculovirus-expressed BTV type 10 (USA) VP7 has recently been solved at 2.6 Å resolution (*11*). It crystallizes as a trimer and has a lower α-helical domain and an upper jelly roll β-sandwich domain. It is our intention to use this structure as a starting point to obtain initially a medium resolution structure for the BTV core particle and we report here its crystallization and a preliminary X-ray analysis.

The strain of BTV type 1 (South Africa) (BTV 1SA), identical to that previously described (*5*), was chosen for the first trials because it was the most studied strain at the Pirbright laboratory and could be purified in the highest yield. It was grown in BHK-21 cell monolayers and core particles purified by established methods (*5*). They were purified finally by two cycles of isopycnic caesium chloride centrifugation, using solutions which had been filtered through 0.22-μm filters (Millipore), followed by exhaustive dialysis against 0.1 *M* Tris–HCl buffer, pH 8.0. Purified cores of BTV 1SA in this buffer were insoluble at 4° and pelleted at 1 *g*, but were fully soluble at 29°, the temperature at which all crystallization experiments with BTV 1SA cores were performed. This property facilitated concentration of the cores, although they were not concentrated beyond 3–4 mg/ml because of their tendency to aggregate. Any particulate matter was removed from the redissolved cores (typically at a concentration of 3 mg/ml) by microcentrifugation (10,000 *g* for 2 min at room temperature). They were then adjusted to 25% (v/v) ethylene glycol and 3.3% (w/v) 1,2,3-heptanetriol.

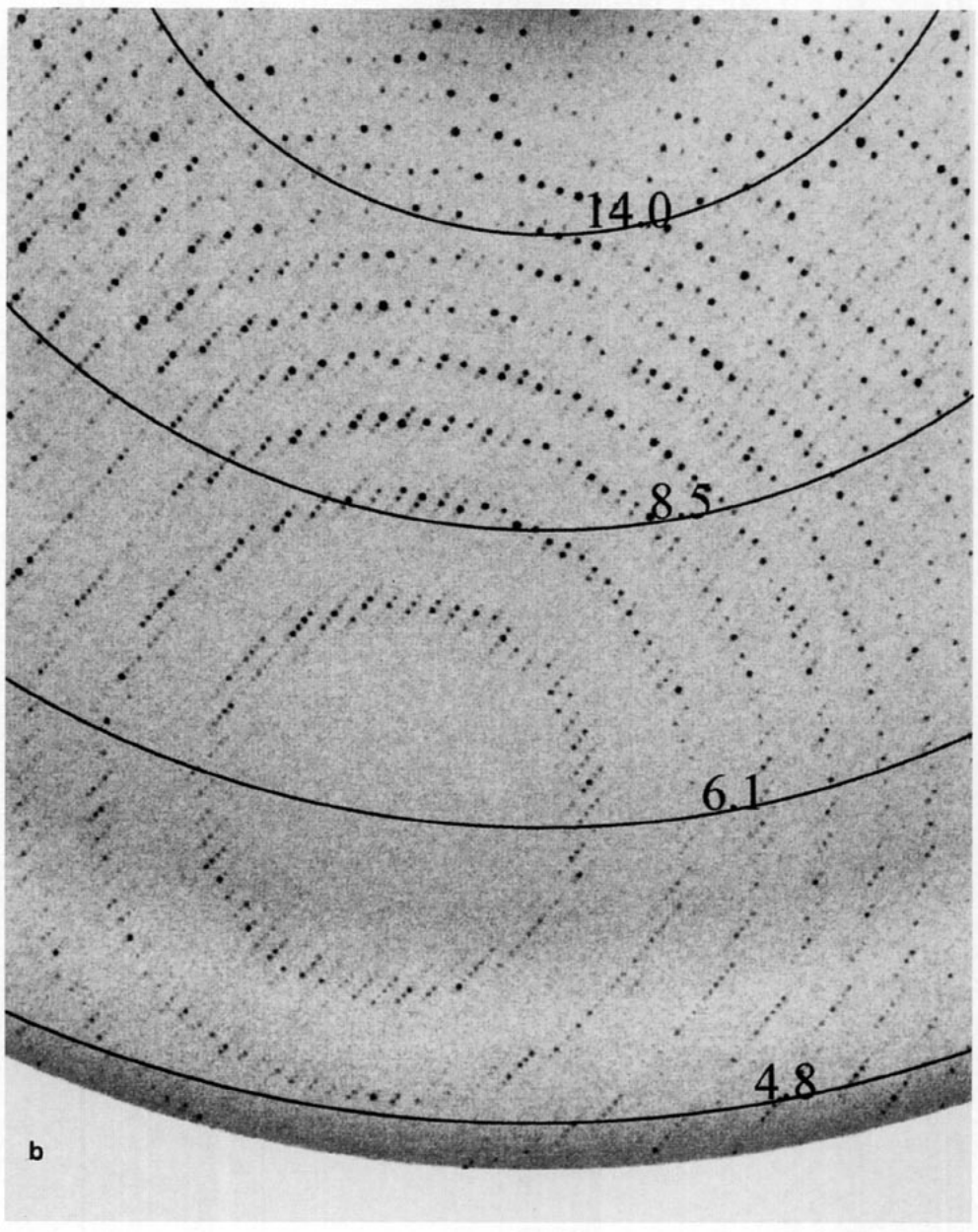

FIG. 2—*Continued*

Crystallization trials were carried out by vapor diffusion (sitting drop) using microbridges supplied by Crystal Microsystems (*12*). The precipitant solution in the reservoir ranged from 11 to 16% saturated ammonium sulfate in 0.1 *M* Tris–HCl buffer, pH 8.0. In some trials 15% ethylene glycol was also included in the reservoir solution. Usually 10 μl of treated cores were mixed with 5 μl of reservoir solution. Regular crystals grow with the morphology of half rhombic dodecahedra, to a diameter of 0.3 mm in approximately 4 weeks and then more slowly to a maximum diameter of 0.8 mm (Fig. 1). The largest crystals, though fewer in number, were obtained together with noncrystalline aggregates, when ethylene glycol was incorporated in the reservoir solution.

Recently core particles of BTV type 10 (USA) have also been crystallized. They too precipitate at 4° in 0.1 *M* Tris–HCl buffer, pH 8.0, but can only be fully redissolved at room temperature when the concentration of this buffer is reduced to 0.01 *M*. The conditions used for crystallization were similar to those for BTV 1SA except that cores, at 22°, were adjusted to 16.6% ethylene glycol only and the precipitant buffer was 0.01 *M* Tris–HCl, pH 8.0. The rate of growth, final size, and morphology were similar to BTV 1SA crystals.

Core particles of BTV 1SA are stable in crystals for at least 1 year at 29°, as demonstrated by diffraction to medium resolution (4.8 Å) from these same crystals (Fig. 2a). The crystals were mounted in quartz capillary tubes

at the Institute for Animal Health (Pirbright) and transported to the SERC Synchrotron Radiation Source (Daresbury, UK) for X-ray diffraction experiments, in accordance with the disease protocols agreed with the Ministry of Agriculture, Fisheries and Food (MAFF). Experiments were performed with radiation of approximate wavelength 0.88 Å, at station 9.6 with the machine operating at 2 Gev and 50 to 220 mA. Data were collected on a Marresearch 30-cm imaging plate as a series of contiguous 0.2° oscillations (Fig. 2a). To resolve the reflections the beam crossfire in the horizontal direction was reduced by limiting the width of the beam incident on the monochromator. Slits, immediately before the crystal, reduced the size of the beam incident on the crystals to 0.15 × 0.15 mm^2 (the crystal to detector distance was set to 777 mm). The higher Bragg angle data became weak after one or two images had been collected; however, up to five images containing useful data to 8 Å could be collected from a single position of a crystal. These data could be processed with DENZO (*13*) using standard protocols. The better crystals diffracted to 4.8 Å with little fall off of the intensity of the data at the edge of the detector (Fig. 2b). This suggests that useful diffraction extends well beyond this resolution.

The crystals have unit cell dimensions of 754 × 796 × 823 $Å^3$ and have point group symmetry 222. There is still an ambiguity in assigning the space group. Packing considerations indicate that half a core particle is present in each crystallographic asymmetric unit, suggesting that the space group is $P2_12_12$; however, we are not yet able to assign the twofold rotation axis to a particular principle axis. The current data are a marked improvement on those reported for other members of the Reoviridae. It is envisaged that the data could further be improved by use of the high energy synchrotron source at Grenoble, France, where the beam characteristics are intrinsically better suited to collecting data off large unit cells. A set of diffraction data is currently being assembled and together with our knowledge of the structure of the major outer core protein VP7, such data should enable the structure of the BTV type 1 core particle to be solved.

Recombinant baculovirus expression systems are available for BTV 10 (USA) which enable core-like particles (CLPs) to be synthesized in insect cells (*14–16*). Similar conditions to those for BTV10 cores are being used in crystallization trials with purified CLPs. In initial experiments preparations containing only VP3 and VP7 are being used, but subsequent trials will be carried out with CLPs containing VP3 + VP7 and VP1, VP4, or VP6. By X-ray analysis of crystals of these structures, the position within the core of the minor structural proteins may be determined if they possess icosahedral symmetry. Using such expression systems it will also be possible to modify particular sites on CLPs by genetic manipulation and then to study the effect of such changes on protein and particle structure by X-ray analysis of CLP crystals.

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