Acta Cryst. (1995). D**51**, 850–852

Crystallization and preliminary X-ray analysis of two density populations of feline calicivirus particles.

By LAN ZHOU and MING LUO,* *Center for Macromolecular Crystallography, Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL* 35294, *USA*

(*Received* 5 *October* 1994; *accepted* 14 *February* 1995)

Abstract

Two density populations of full-size feline calicivirus (FCV), the intact infectious particles (P_H) and the empty capsids (P_L), have been crystallized using the hanging-drop method. Exposed to high-intensity synchrotron radiation, P_H and P_L crystals were shown to diffract X-rays to about 3.0 and 5.5 Å resolution, respectively. The P_H crystal belongs to an orthorhombic crystal system with unit-cell dimensions $a = 889.0$, $b = 995.0$, $c = 436.6$ Å. Based on the V_M value (3.4 Å^3 Da^{-1}), it was estimated that one crystallographic asymmetric unit of P_H crystals contains the unique content of an entire virus particle, not necessarily from the same particle. This implies the presence of 60-fold non-crystallographic redundancy. The particle orientation was obtained from a locked rotation function.

Introduction

Feline calicivirus (FCV) (Fastier, 1957) is a small single-stranded positive-sense RNA virus. FCV belongs to the calicivirus family, which include the prototype vesicular exanthema of swine virus (VESV) (Tan, 1970; Smith & Akers, 1976), the San Migeul sea lion virus (SMSV) (Smith & Akers, 1976), the primate calicivirus (Smith, Skilling, Ensley, Benirschke & Lester, 1983), the rabbit hemorrhagic disease virus (RHDV) (Liu, Xue, Pu & Qian, 1984) and the Norwalk virus and Norwalk-related viruses (Kapikian & Chanock, 1990). There is increasing evidence that the caliciviruses are more widespread and may cause infection more often than was previously believed.

The appearance of the calicivirus particles is highly characteristic and distinct from other animal viruses. The electron cryomicroscopic study of a primate calicivirus (Prasad, Matson & Smith, 1994) showed that the calicivirus has a diameter of 405 Å and exhibits $T = 3$ icosahedral symmetry. The main features on the surface of calicivirus are the 32 large surface hollows, 50 Å deep and 90 Å wide, located at the icosahedral fivefold and threefold axes. There are 90 arch-like dimeric spikes surrounding these hollows. Amino-acid sequence alignment indicates that the capsid protein of calicivirus may share structural homology to some of the spherical viruses such as picornaviruses, of which atomic structures have been determined by X-ray crystallography [Rossmann & Johnson, 1989 (review)]. The major capside proteins of icosahedral RNA viruses share a common structure motif, an eight-stranded antiparallel β-barrel. In the $T = 1$ picornaviruses, the three major types of capsid proteins, VP1, VP2 and VP3, are situated at essentially the same positions as the *A*, *C* and *B* sununits of $T = 3$ plant viruses These animal viruses, therefore, are named pseudo $T = 3$ viruses. However, not all icosahedral viruses have the eight-stranded antiparallel β-barrel motif in the capside protein. Two of the exceptions are bacteriophage MS2 (single-stranded DNA virus) (Colmohammadi, Valegard, Fridborg & Liljas, 1993) and blue tongue virus (double-stranded RNA virus) (Stuart, 1994). In bacteriophage MS2, the capsid protein contains a five-stranded β-sheet facing the interior of the virion, and a hairpin and a two helices on the exterior (Colmohammadi *et al.*, 1993). In blue tongue virus, the major capsid protein VP7 consists of an 'outer' and an 'inner' domain with respect to the virion center. The outer domain is an antiparallel β-sandwich and bears strong similarity to the 'jelly roll' of hemagglutinin. The structure of the inner domain is unusually composed entirely to α-helices and extended loops (Stuart, 1994). No crystal structure is currently available for caliciviruses. We here present the first report of the growth and preliminary X-ray diffraction analysis of crystals of two density populations of feline calicivirus (FCV strain F9) particles.

Materials and methods

The FCV F9 strain virus was propagated in Crandell–Reese feline kidney (CRFK) cells and purified by CsCl equilibrium centrifugation. (Zhou & Luo, 1994). Two density populations of viral particles were observed after centrifugation. The buoyant density of the heavy particle (P_H) was 1.33 g ml^{-1} while the light particle (P_L) had a buoyant density of 1.22 g ml^{-1}. Western blot analysis, infectivity assay and viral RNA isolation (Zhou & Luo, 1994) of these two forms of viral particles indicated that both populations contain the same

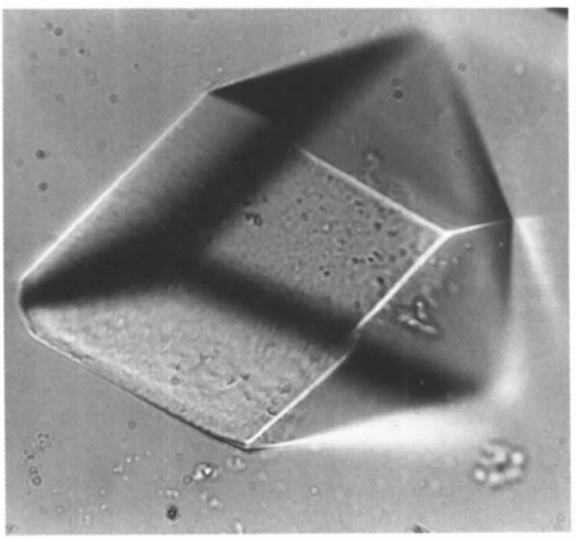


Fig. 1. Crystal of heavy particles (P_H) of feline calicivirus.

* Author for correspondence.

capsid protein and that P_H is the intact infectious FCV particle and P_L appeared to be empty capsid.

Both purified P_H and P_L were concentrated by ultracentrifugation. Five different buffers, phosphate buffer (pH 7.4), Tris–acetate (pH 7.4 and pH 8.4), boric acid buffer (pH 7.4 and pH 8.6) and PEG 3350 were used in initial crystallization trials for FCV. All experiments were set up using the hanging-drop vapor-diffusion method at 295 K. On the basis that the solubility of most proteins will be increased by adding salt and that a sodium salt partially stabilized a feline calicivirus against heating (Lee & Gillespie, 1973), sodium chloride was included during FCV purification and crystallization. Crystallization set up in all the five buffers produced small crystals within 1 d. The crystals grown at pH 8.4 or pH 8.6 appeared to be very unstable and to aggregate with some precipitates a few days later. Finally, crystals of P_H (Fig. 1) with a maximum size of 0.8–1.0 mm were grown from drops containing 5–6% PEG 3350, 20 m*M* boric acid buffer (pH 7.4), 0.5 *M* NaCl and 0.2% β-mercaptoethanol within 4 d. Crystals of P_L with a maximum size of 0.1–0.15 mm were also obtained when PEG 3350 was used as precipitant and 20 m*M* boric acid (pH 7.4) with 0.5 *M* NaCl as the buffer.

Results and discussion

FCV P_H and P_L crystals were examined for their diffraction using the beamline 7-1 at the Standford Synchrotron Radiation Laboratory (SSRL) and an MAR Research image-plate system. The P_H and P_L crystals diffracted X-rays to at least 3.0 and 5.5 Å resolution, respectively, when a wavelength 1.08 Å and 300 s exposure per frame were used. An oscillation image of P_H crystal is shown in Fig. 2. X-ray diffraction data of P_H crystals were collected from randomly oriented crystals by oscillation of 0.15°. The crystal-to-detector distance was set to 390 mm. The program *OSC*-123 (Kim, 1989) was used to index the oscillation data. The P_H crystals belong to an orthorhombic crystal system with lattice parameters $a = 889.0$, $b = 996.0$ and $c = 436.6$ Å. Integration of the diffraction data was carried out using the profile-fitting program package *OSC* developed by Rossmann and co-workers (Rossmann, 1979; Rossman, Leslie, Abel-Meguid & Tsukihara, 1979), in space group $C222$, though we could not eliminate $C222_1$ at this time. A total of 226 image plates were included in the data set. There were 1 764 795 total observations ($I/\sigma > 3$), of which 1 396 078 were unique reflections (Table 1). The overall R factor for the data beween 15 and 3 Å resolution is 17.58%.

The volume of the unit cell is large enough to accommodate eight virus particles. We estimate one virus particle per crystallogaphic asymmetric unit with a crystal-packing parameter (Matthews, 1968) V_m of 3.4 Å^3 Da^{-1}, which is comparable to values obtained for crystals of other relatively large spherical viruses, such as SV40 virus (Liddington *et al.*, 1991). The orientation of the FCV full particles in the unit cell was determined with a locked rotation function (Tong & Rossman, 1990), using data between 15 and 7 Å resolution with 156 607 observed reflections (~ 58% completeness). Among these reflections, 128 terms were selected that are larger than four times the mean intensity of all the observed reflections in the resolution shell. The initial search was conducted in Eulerian space (defined as in, Rossmann & Blow, 1962) between 0 and 88° in all three angles, using 8° intervals. The highest peak in the map was located at 0,0,0° with a height of

Table 1. *Data set of FCV P_H crystals*

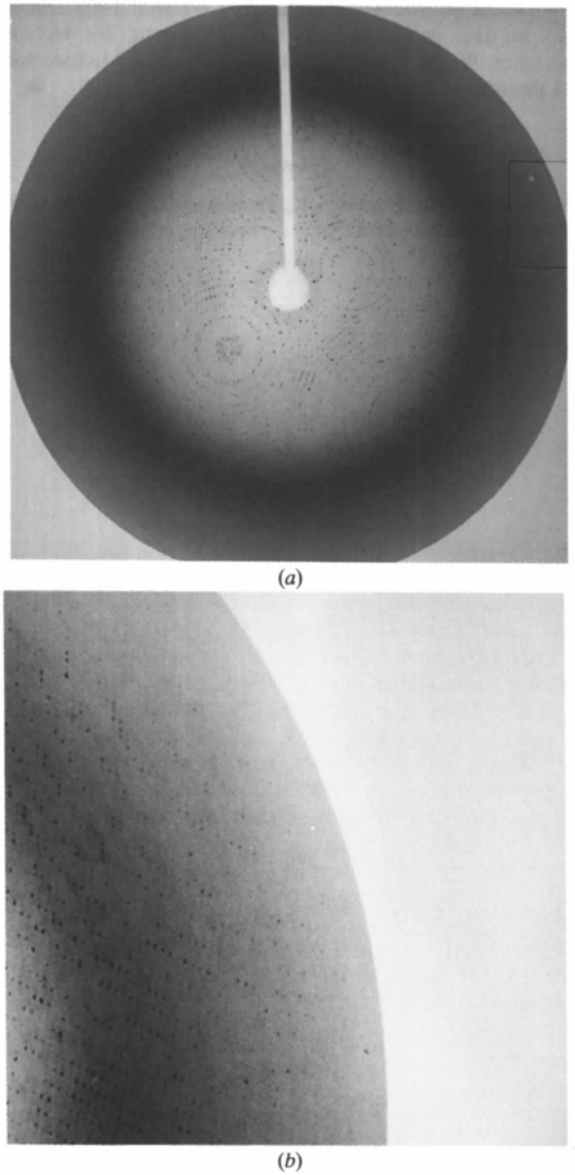
(*a*) Data processed

Total No. of image plates	226
No. of observed reflections	1764795
No. of unique reflections	1396078
Internal consistency R_I* (%)	17.58
$I > \sigma(I)$	3.0
Partiality	> 0.5

(*b*) Completeness of data set

Resolution (Å)	15.0	10.0	7.5	5.0	3.5	3.0
Completeness (%)	50.2	58.8	58.6	51.2	40.2	37.0

* $R_I = [(\sum_h \sum_i |I_h - I_{hi}|)/(\sum_h \sum_i I_{hi})] \times 100$, where I_h is the mean intensity of the i observations of reflection h.



(*a*)

(*b*)

Fig. 2. (*a*) A 0.15° oscillation diffraction pattern obtained from a P_H crystal, taken at the Stanford Synchrotron Radiation Laboratory. (*b*) The magnification of the squared area in (*a*). The wavelength was 1.08 Å and the crystal-to-image-plate distance was 390 mm.

17.38 and was 8.36σ above the background. The next lower peak had a height of 11.31 and was 5.08σ above background. Successive search intervals of 2.0 and 0.5° with 11 826 large terms were used around the position 0,0,0°. The orientation of the virus particle appeared to be coincident with that of the standard icosahedron defined by Tong & Rossmann (Tong & Rossmann, 1990), with three orthogonal twofold axes parallel to crystallographic unit-cell axes. It is, therefore, possible that several virus particles sit on crystallographic symmetry axes while maintaining the unique content of one virion in the asymmetric unit.

Indexing of image data from P_{I_L} crystals indicated that P_L crystals belong to same crystal system with same unit cell as the P_H crystals.

We thank Dr Marek Jedrzejas, Clint White and Jun Zhao for help in data collection and Carl X. Zhang for technical assistance. We truly appreciate the help from Dr Michael Soltis and the staff at SSRL.

References

COLMOHAMMADI, R., VALEGARD, K., FRIDBORG, K. & LILJAS, L. (1993). *J. Mol. Biol.* **234**, 620–639.

FASTIER, L. B. (1957). *Am. J. Vet. Res.* **18**, 382–389.

KAPIKIAN, A. Z. & CHANOCK, R. M. (1990). *Viology*, edited by B. N. FIELDS, Vol. 1, pp. 507–548. New York: Raven Press.

KIM, S. (1989). *J. Appl. Cryst.* **22**, 53–60.

LEE, K. M. & GILLESPIE, J. H. (1973). *Infect. Immun.* **7**, 678–679.

LIDDINGTON, R. C., YAN, Y., MOULAI, J, SAHLI, R., BENJAMIN, T. L. & HARRISON, S. C. (1991). *Nature (London)*, **354**, 278–284.

LIU, S. J., XUE, H. P., PU, B. Q. & QIAN, N. H. (1984). *Vet Med.* **16**, 253–255.

MATTHEWS, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

PRASAD, B. V. V., MATSON, D. O. & SMITH, A. W. (1994). *J. Mol. Biol.* **204**, 256–264.

ROSSMANN, M. G. (1979). *J. Appl. Cryst.* **12**, 225–238.

ROSSMANN, M. G. & BLOW, D. M. (1962). *Acta Cryst.* **15**, 24–31.

ROSSMANN, M. G. & JOHNSON, J. (1989). *Annu. Rev. Biochem.* **58**, 533–573.

ROSSMANN, M. G., LESLIE, A. G. W., ABDEL-MEGUID, S. S. & TSUKIHARA, T. (1979). *J. Appl. Cryst.* **12**, 570–581.

SMITH, A. W. & AKERS, T. G. (1976). *J. Am. Vet. Med. Assoc.* **169**, 700–703.

SMITH, A. W., SKILLING, D. E., ENSLEY, P. K., BENIRSCHKE, K & LESTER, T. L. (1983). *Science*, **221**, 79–81.

STUART, D. I. (1994). Univ. of Oxford, Laboratory of Molecular Biophysics Progress Report 1993–1994, pp. 38–39.

TAN, R. J. S. (1970). *Jpn. J. Med. Sci. Biol.* **23**, 419–424.

TONG, L. & ROSSMANN, M. G. (1990) *Acta Cryst.* A**46**, 783–792.

ZHOU, L. & LUO, M. (1994). *Virology*, **305**, 530–533.