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Crystallization of the membrane-containing bacteriophage PRD1 in quartz capillaries by vapour diffusion

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Crystals of bacteriophage PRD1, a virus containing an internal lipid bilayer, have been grown in thin-walled quartz capillary tubes by vapour diffusion as a means of eliminating mechanical handling of the crystals during data collection. It has been found that the addition of polyethylene glycol 20 000 (PEG 20K) to the mother liquor that bathes the crystals allows far higher resolution diffraction intensities to be observed. Growing and treating the crystals in this way has produced a small number of crystals which are particularly amenable to X-ray diffraction analysis.

1. Introduction

The dsDNA bacteriophage PRD1 is the type organism of the Tectiviridae family and infects a broad range of Gram-negative hosts such as *Escherichia coli* and *Salmonella enterica* (Olsen *et al.*, 1974; Bamford *et al.*, 1995). It is chiefly characterized by the presence of a host-derived lipid bilayer lying beneath the icosahedral protein capsid, which encapsulates the phage DNA (Butcher *et al.*, 1995). The 15 kbp genome encodes some 20 structural proteins, around ten of which are thought to be embedded in the membrane (Bamford, Cockburn *et al.*, 2002). The virus uses the promiscuous Inc P, N or W encoded DNA-transfer complex as a receptor on its host (Olsen *et al.*, 1974). Binding to this receptor initiates a sequence of events that lead to DNA translocation into the host cytosol, during which the membrane is thought to transform into a tube-like structure possibly penetrating the host cell envelope (Grahn *et al.*, 2002*a*,*b*).

Solving the crystal structure of PRD1 offers the opportunity to study an intact membrane in atomic detail. In addition, PRD1 shares a number of striking structural similarities with adenovirus. The major capsid proteins of the viruses have the same fold (Benson *et al.*, 1999) and are the only known viruses to be constructed from a pseudo $T = 25$ capsid lattice (Butcher *et al.*, 1995; San Martin *et al.*, 2001). The capsids of both viruses are stabilized by minor 'cementing' proteins and have similar receptor-binding spike complexes at the capsid vertices (Burnett, 1997; Rydman *et al.*, 1999, 2001; San Martin *et al.*, 2002). This had led to the proposal that PRD1 and adenovirus share a common evolutionary ancestor (Benson *et al.*, 1999; Hendrix, 1999; Belnap & Steven, 2000). Indeed, it has been proposed that structural comparisons can be used in a systematic way to reveal viral lineages (Bamford, Burnett *et al.*, 2002).

Recently, we reported that crystals of PRD1 could be grown in sitting drops (Bamford, Cockburn *et al.*, 2002). At 66 MDa, this represents the largest virus for which diffraction-quality crystals have been reported. Although a small number of these crystals diffracted to high resolution, the virus particles are unstable; PRD1 crystals are very delicate and the mechanical handling involved in preparing them for data collection rendered them useless in most cases. In addition, extensive attempts to develop a protocol for cryocrystallography of PRD1 crystals failed (Bamford, Cockburn *et al.*, 2002). There are several cases in the literature where fragile crystals have been grown in quartz capillaries for *in situ* data collection (Gavira *et al.*, 2002; Wikoff *et al.*, 1998; Thunissen *et al.*, 1995; Besseman *et al.*, 1991; Glotz *et al.*, 1987). This paper describes the development of a method for growing PRD1 crystals in thin-walled quartz capillary tubes by vapour diffusion which has obviated the need for crystal handling and rendered X-ray data collection practical.

2. Experimental

Bacterial cells were grown in Luria–Bertani (LB) medium (Sambrook & Russell, 2000). PRD1 *sus*539 amber mutant (Grahn *et al.*, 1999) was propagated on *S. enterica* sv. Typhimurium suppressor strain DB7156 (pLM2) (Mindich *et al.*, 1976). The mutant virus particles were produced on *S. enterica* wild-type strain DS88 (Bamford & Bamford, 1990) in liquid cultures at 310 K followed by purification in sucrose gradients and by a MemSep anion-exchange chromatography cartridge (Millipore; Bamford & Bamford, 1991; Walin *et al.*, 1994). Protein concentration was determined according to Bradford (1976) using bovine serum albumin as a standard. The

virus preparation was used undiluted, with concentrations ranging from 7 to 20 mg ml^{-1}. The virus was fixed using 0.025%(*w*/*v*) gluteraldehyde immediately after the final anion-exchange chromatography step and crystallizations were set up 15 min after glutaraldehyde addition.

The basic setup for the capillary crystallizations is shown in Fig. 1. A 3 µl volume of the virus and precipitant solution was injected into a thin-walled quartz capillary 15 mm in length and 0.5 mm in diameter (Pantak Ltd). The capillary was then fixed to a sitting-drop microbridge (Harlos, 1992) using Plasticine and the assembly placed into the well of a sitting-drop tray (VDX, Hampton Research, USA). The mother liquor was equilibrated against 1 ml of reservoir solution by vapour diffusion *via* the open ends of the capillary. The conditions for crystallization were derived from those used for sitting drops (Bamford, Cockburn *et al.*, 2002). The solution inside the capillary tube was a 1:1 mix of the virus solution described above with a solution of 3.7–4.3%(*w*/*v*) PEG 8000, 400 m*M* NaCl and 100 m*M* potassium phosphate buffer pH 7.2 as precipitant. The reservoir solution consisted of 16–20% PEG 8000 with salt and buffer as above.

Crystals grown in capillaries were further treated by the addition of small amounts (~1 µl) of 20%(*w*/*v*) PEG 20K solution, injected into the capillary using a glass Pasteur pipette drawn out to a fine point in a butane flame. The PEG 20K was added to the capillaries at least one week prior to data collection, since it was found that high-molecular-weight dye (Blue Dextran, molecular weight 2 000 000; Sigma–Aldrich, Poole, England) took around one week to diffuse through a capillary section containing the mother liquor.

Diffraction data were collected on beamlines ID13 and ID14 at the European Synchrotron Radiation Facility (ESRF), Grenoble, France. During data collection, the capillaries containing the crystals were removed from the microbridges and sealed with vacuum grease (Dow Corning, USA). The mother liquor was not drained from the capillaries in order to avoid disturbing the crystals unnecessarily. They were then attached to goniometer heads (Huber, Germany) using Plasticine and pre-aligned on an optical Huber goniometer (using protocols established previously for other virus-structure determinations) before being exposed to the beam. Crystals frequently detached from the capillary wall during this preparation and they were centred using the strategy described by Bamford, Cockburn *et al.* (2002). Typical values for the beam size, oscillation range, crystal-to-plate distance and wavelength were 80 × 80 µm, 0.25°, 440 mm and 0.933 Å, respectively. The data were collected at room temperature using ADSC Quantum 4, Quantum 210 and 165 mm MAR CCD detectors (ADSC, California, USA; MAR Research, Hamburg, Germany). The data were processed using special versions of *DENZO* (Otwinowski & Minor, 1997) and *SCALEPACK* (Otwinowski & Minor, 1997) compiled to handle the large numbers of data collected from many non-consecutive images.

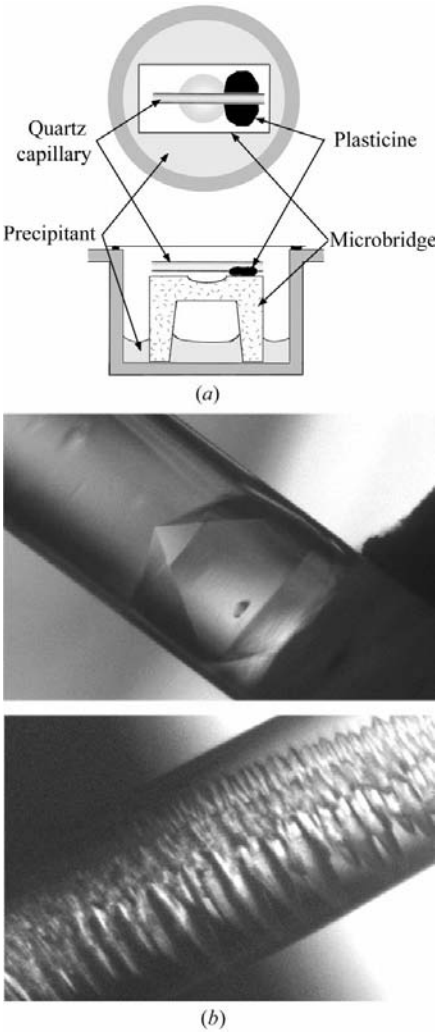

Figure 1
The crystallization of PRD1 in quartz capillaries. (*a*) Basic setup for PRD1 vapour-diffusion experiments in capillaries. The virus and precipitant solution are injected into a quartz capillary, which is then supported on a sitting-drop microbridge with Plasticine. This assembly is then sealed within a VDX plate well containing 1 ml of precipitant mother liquor to allow equilibration to take place. (*b*) Differing crystal morphologies of PRD1 grown in quartz capillaries. The crystals with more conventional morphologies (above) gave diffraction to approximately 20 Å resolution, whereas crystals with an irregular fern-like appearance (below) gave diffraction to Bragg spacings of up to 4 Å.

3. Results and discussion

3.1. Crystallization

Crystals with a variety of morphologies grew to fill the capillaries within a fortnight (see Fig. 1), but gave the same pattern of very poor diffraction as seen with crystals grown in sitting drops (Bamford, Cockburn *et al.*, 2002). However, the crystals could be made to diffract to higher than 4 Å resolution by the addition of a solution of 20%(*w*/*v*) PEG 20 000 to the mother liquor. The amount of PEG 20K added was hard to control and was limited by the varying amount of empty volume inside the capillary caused by dehydration of the mother liquor. The approach used was to add as much PEG 20K as possible in every case, which typically gave a final concentration of 5–10%(*w*/*v*). The rate of diffusion of PEG 20K within the capillaries was estimated by the diffusion of dye (see §2) to be sufficiently slow to necessitate the doping of crystals with PEG 20 K at least one week before data collection. Using this pre-doping protocol enabled the success rate of improvement of the resolution of diffraction to be significantly enhanced.

3.2. Data collection

Despite their irregular appearance, crystals with a long fern-like morphology such as that shown in Fig. 1(*b*) gave the most reliable high-resolution diffraction, in contrast to those having more conventionally attractive morphologies, which invariably gave very poor diffraction (a high-resolution limit of approximately 20 Å). The experimental procedure was to direct the X-ray beam at the spine of the crystal and take successive exposures at 100 µm intervals along its length. The capillary was rotated by 1° about the φ axis between exposures. Using the procedure, up to 20 diffraction images could be collected from a single crystal, despite the data collection taking place at room temperature. This method formed the basis of our data-collection strategy.

The majority of the crystals exposed to the beam were isomorphous with the crystals grown in sitting drops. These had the space group $P2_12_12_1$, with unit-cell parameters around $a = 895$, $b = 905$ and $c = 925$ Å (there was some variation from crystal to crystal; Bamford, Cockburn *et al.*, 2002). However, a minority of the crystals grown in capillaries belonged to a different crystal form. These had the same space group as the others, but a slightly larger unit cell: $a = 903.0$, $b = 920.6$, $c = 926.2$ Å. Treating the virus as a solid icosahedron with planar facets and a

Table 1
Data-processing statistics for the two cells of PRD1 crystals, showing that the quality of the data collected from cell 2 is substantially better than for cell 1.

	Cell 1			Cell 2		
	Overall (100–4.2 Å)	5.3–5.0 Å	4.4–4.2 Å	Overall (100–4.2 Å)	5.3–5.0 Å	4.4–4.2 Å
Completeness (%)	43.3	43.1	7.6	14.6	6.9	0.3
$I/\sigma(I)$†	1.7	1.0	0.8	2.4	1.1	0.9
χ^2	1.50	1.01	0.97	1.30	1.13	1.95
$R_{\rm merge}$‡ (%)	43.3	67.8	67.2	22.4	49.7	72.2

† $I/\sigma(I)$, mean signal-to-noise ratio for the diffraction data. ‡ $R_{\rm merge} = \sum_j \sum_h (|I_{j,h} - \langle I_h \rangle|)/\sum_j \sum_h (\langle I_h \rangle)$, where h is the unique reflection index, $I_{j,h}$ is the intensity of the symmetry-related reflection and $\langle I_h \rangle$ is the mean intensity.

vertex-to-vertex diameter of 740 Å gives solvent contents of 31 and 33% for the first and second cell types, respectively. In both forms, the crystallographic asymmetric unit contains one whole virus particle, thus giving 60-fold non-crystallographic symmetry. Around 180 capillaries have been exposed to the beam, yielding 194 useful diffraction images. The data for each crystal form are summarized in Table 1, showing that the second crystal form gives substantially higher quality diffraction data. There was no discernible correlation between the crystal form and the morphology.

3.3. Discussion

The growth of the crystals in capillaries, in conjunction with pre-selection of crystals with the fern-like morphology and crystal annealing using high-molecular-weight PEG, has considerably enhanced the success rate of PRD1 data collection. It is hoped that some of the techniques described here will be transferable to other enveloped viruses.

The annealing effect of the PEG 20K on the crystals is as yet uncharacterized. Presumably, it effects a more ordered crystal packing of the virions through dehydration (Esnouf *et al.*, 1994). Whether or not the second crystal form arises from a different pathway during the annealing or whether it reflects differences already established during the growth of the crystals is unclear, as is how this is reconciled with an increase in solvent content. All crystals of the second form were obtained from one particular virus preparation but have been impossible to reproduce so far. Data collected from these crystals is being used for structural analysis.

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