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Increased resolution data from a large unit cell crystal collected at a third-generation synchrotron X-ray source

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A third-generation synchrotron source was used to collect data from crystals with a very large unit cell. There was an increase in the effective resolution of the data from 5 to 3.5 Å. Data were collected on crystals of HK97 mature empty capsids, space group $P2_1$, with unit-cell parameters a = 580, b = 625, c = 790 Å, β = 90.0°. Like other crystals with very large unit-cell dimensions, the intensity falls off rapidly as a function of resolution, with a precipitous drop beginning at 9 Å resolution. Synchrotron data from these crystals were previously observed at the CHESS F1 beamline to about 3.5 Å resolution, but the intensities could not be accurately measured beyond 5 Å. In experiments conducted at the Advanced Photon Source (APS) beamline 14-BM-C, data from identical crystals could be processed to a resolution of 3.5 Å. The lifetime of the crystals in the beam was increased from one exposure per crystal volume to between four and eight exposures per crystal volume. More than 500 images were collected in two trips, allowing the extension of the resolution of the data set and the structure determination to 3.5 Å resolution. Factors in the increased resolution may include X-ray flux, beamline geometry and low background scatter. These results suggest that other crystals with large unit cells and pronounced intensity falloff with resolution may benefit from the use of this or similar beamlines.

1. Introduction

The maximum resolution obtained in a single-crystal diffraction experiment depends on many factors, including the inherent order of the crystal, the flux and brilliance of the X-ray source and the properties of the beamline optics and detector. The resolution-dependent intensity falloff from macromolecular crystals can be severe, creating a need to collect data at synchrotron sources. Large macromolecular assemblies and viruses often have very large unit cells and present additional difficulties in data collection, including the spatial resolution of closely spaced Bragg reflections and a very high percentage of partially recorded reflections. This places stringent requirements on the X-ray flux, beam optics and detector. Collecting high-resolution data from crystals with very large unit-cell dimensions requires a small focal projection of the source onto the detector plane, a long crystal-to-detector distance to provide reasonable spot separation and to reduce the background scattering from the crystal, a non-scattering beam path and a large detector with high quantum efficiency and large dynamic range to record data at high diffraction angles. Cryo-data collection, routine for proteins, has proven intractable for many large unit cell crystals, creating additional constraints on data collection.

Here, we report the results of data collection at the Advanced Photon Source (APS) beamline 14-BM-C from crystals of HK97 mature empty capsids. HK97 is a dsDNA bacteriophage in the lambdoid family that infects *Escherichia coli*. Although the bacteriophages have been used as model systems in biology for almost the entire past century and have been investigated using many biophysical methods, it has not been possible to obtain a crystal structure of a dsDNA phage capsid. The large size and high complexity of these phage present an obstacle to obtaining crystals that diffract well. The bacteriophage tail and associated appendages break the external icosahedral symmetry of the particle and prevent crystallization unless they are removed. HK97 has the advantage of not requiring the tail attachment assembly (connector), separate scaffolding proteins or capsid decoration proteins for correct particle assembly in an expression system (Duda, Hempel, *et al.* 1995; Duda, Martincic *et al.*, 1995). The large size of the dsDNA bacteriophage capsids, most of which are about 600 Å in diameter, generates a correspondingly large unit cell, which makes data collection and analysis challenging.

We have previously reported the crystallization of HK97 capsids (Wikoff *et al.*, 1998) and preliminary crystallographic results to a resolution of 7 Å (Wikoff *et al.*, 1999). The space group was determined to be $P2_1$, with unit-cell parameters a = 580, b = 625, c = 790 Å, β = 90.0°. For that work, a synchrotron data set was collected to 5 Å resolution and initially processed to 7 Å resolution. Diffraction to higher resolution was observed, but it was not possible to measure accurately this very weak data. Here, we report that when data from essentially identical crystals were collected at APS beamline 14-BM-C, it was possible to extend the resolution of the data to 3.5 Å. This result allowed the extension of the data set and the structure from 5 to 3.5 Å resolution. These results suggest that additional controlled experiments are warranted to determine if similar problems involving very large unit cells and weakly diffracting crystals could benefit from data collection at this or similar beamlines.

2. Experimental methods

HK97 mature empty capsids (Head II) were produced (Duda, Hempel *et al.*, 1995; Duda, Matrincic *et al.*, 1995) and crystallized as described previously (Wikoff *et al.*, 1998). Briefly, crystals were grown directly in quartz X-ray capillaries using a batch method. 2–4 µl of mature empty Head II (in 20 m*M* Tris–HCl pH 7.5, 100 m*M* KCl, 1 m*M* 2-mercaptoethanol) at a concentration of 40–70 mg ml^{-1} was mixed with an equal volume of precipitant. The precipitant was 50 m*M* citrate pH 5.0, 0.85 *M* ammonium sulfate, 1.5% PEG 8000. Mineral oil was drawn into a 1–2 mm quartz capillary, followed by the sample/precipitant mixture and then more mineral oil; the ends were then sealed with wax. Capillaries in which large single crystals grew were used for data collection.

Oscillation data were previously collected on Fuji image plates at the CHESS F1 beamline using an oscillation angle of 0.3°, a wavelength of 0.908 Å and a crystal-to-detector distance of 680 mm (Wikoff *et al.*, 1998). The crystal was translated to a new position after each exposure. Most data were collected without draining the mother liquor from the crystals, because of their extreme fragility. Occasionally, crystals with slippage problems were partially drained.

In general, the approach to data collection at APS was identical to that described previously. Many of the crystals from which data were collected at APS were from the same batches of virus that were used for previous data collection at CHESS and some were grown at the same time. Data were collected on APS beamline 14-BM-C in two trips, in June and August 1998. Most of the data were collected at 273–277 K; a range of temperatures were screened up to 298 K. A MAR345 detector was used with a helium path between the sample and the detector to reduce air scatter. Oscillation angles were sampled between 0.12–0.2°, with most images either 0.15 or 0.18°, with exposure times between 30 and 60 s. The crystal-to-detector distance was 555 or 600 mm and the wavelength was 1.04 Å. Nine images were collected at a distance of 455 mm to determine the maximum measurable resolution. Crystals were translated to an unexposed region after four to eight exposures. The images were examined using *MARVIEW*; the *DENZO* program (Otwinowski & Minor, 1997) was used to index and process the images.

3. Results

The diffraction and data collection from HK97 Head II crystals to 5 Å resolution have been described previously (Wikoff *et al.*, 1998, 1999). Diffraction from the CHESS F1 beamline could be observed on some images to 3.5 Å resolution, but the data were very weak and could not be accurately measured. The data collection was therefore optimized to collect lower resolution data, which was processed to an upper limit of 5 Å resolution. The diffraction quality of each image was judged by eye; many crystals diffracted to less than 5 Å, with a range on the outer resolution between 9 and 5 Å. Only one image could be collected at each position on the crystal. After each exposure, the crystal decayed severely and was then translated to a new position. The decay was visible not only in the diffraction pattern, but in the crystal itself, which had a round hole of about 0.2–0.4 mm deep.

There was a substantial improvement in data collected at APS. A diffraction image of an HK97 head II crystal collected at APS beamline 14-BM-C is shown in Fig. 1. The reflections are well separated and can be observed to the edge of the detector at 3.45 Å resolution. This data could be processed to 3.45 Å using the program *DENZO*. Data were collected in one location of the crystal for four to eight images and, depending on size, the crystal was translated to a new position. The measureable data varied in resolution limit from about 5 to 3.45 Å. The X-ray beam did not produce holes in the crystals even after several exposures, but small cracks were occasionally observed. No significant differences could be detected between data collected at 277 K and room temperature. Nine images were collected at a crystal-to-detector distance of 455 mm to determine the maximum resolution that could be

obtained. These images indicated that the crystals diffract to a maximum of 3.3–3.4 Å resolution.

A total of 328 images were collected from 31 crystals in June 1998 and 223 images were collected from 30 crystals in August 1998. They were inspected visually and the maximum resolution was estimated. Each image was autoindexed separately using *DENZO* and processed to the geometric limit of the image. The signal-to-noise ratio for each image was then evaluated by plotting $I/\sigma(I)$ *versus* resolution for the whole reflections. Based upon this plot, the resolution bin in which the mean $I/\sigma(I) = 2.0$ was determined and the image was then reprocessed to this resolution limit. The resolution determined by this method correlated in general with the resolution determined by visual inspection. It was found that the images varied widely in terms of maximum resolution, but that many of the images could be processed to >3.8 Å resolution based upon the criteria of $I/\sigma(I) > 2.0$. One such plot is shown in Fig. 2, comparing an image from CHESS F1 and an image from APS; the values are consistently higher for the APS data.

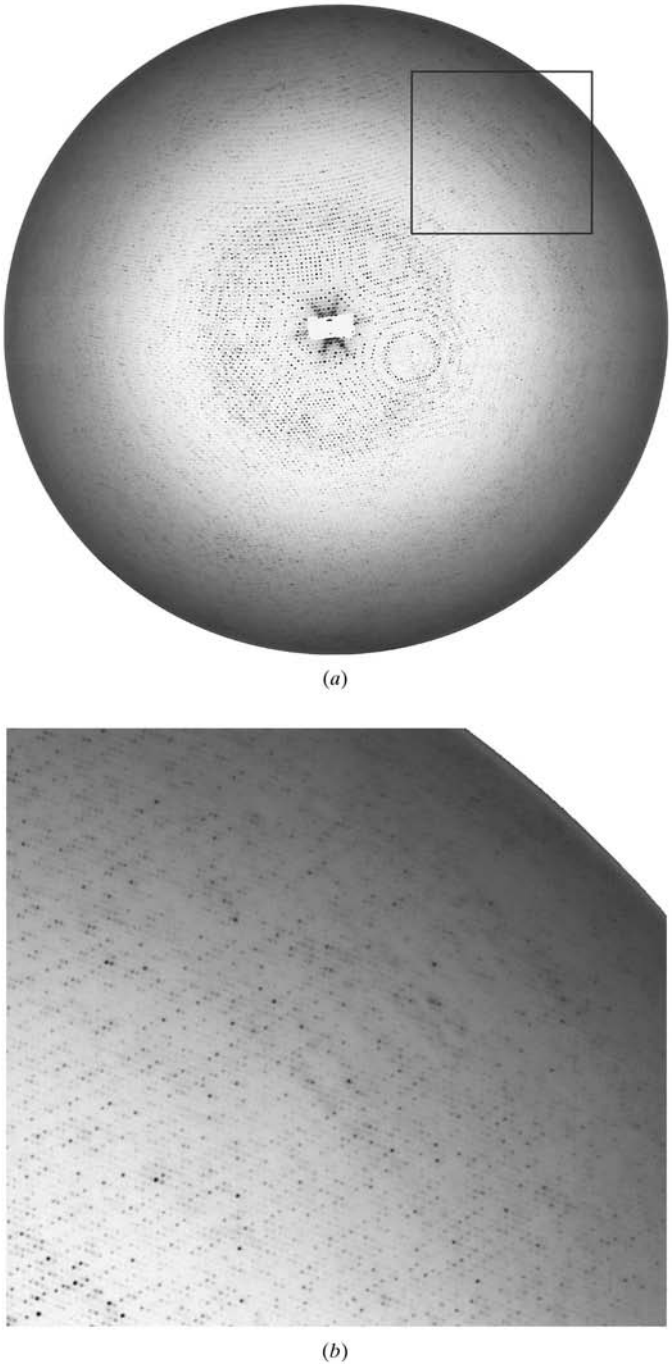

Figure 1
(*a*) An HK97 Head II diffraction pattern collected on the MAR345 detector at the Advanced Photon Source beamline 14-BM-C at a wavelength of 1.04 Å. The oscillation angle was 0.15°, with an exposure time of 45 s. The crystal-to-detector distance was 555 mm. The boxed area in (*a*) is magnified in (*b*) showing a detail of the diffraction image. The image was processed to a resolution of 3.45 Å. A total of 1935 whole reflections and 49 001 partial reflections were measured on the image.

4. Discussion

The resolution of the measureable HK97 data was increased from 5 to 3.5 Å using beamline 14BM-C. The crystals were not a significant factor in the resolution gain, as many were grown at the same time and from identical batches of material. Three factors contributed to the quality of the high-resolution data collected at APS: high X-ray flux, beam optics and low background scatter.

The 14-BM-C beam uses convergent beam optics, maintaining the low emmittance from the bending magnet source of X-rays. The source is essentially round in shape with a

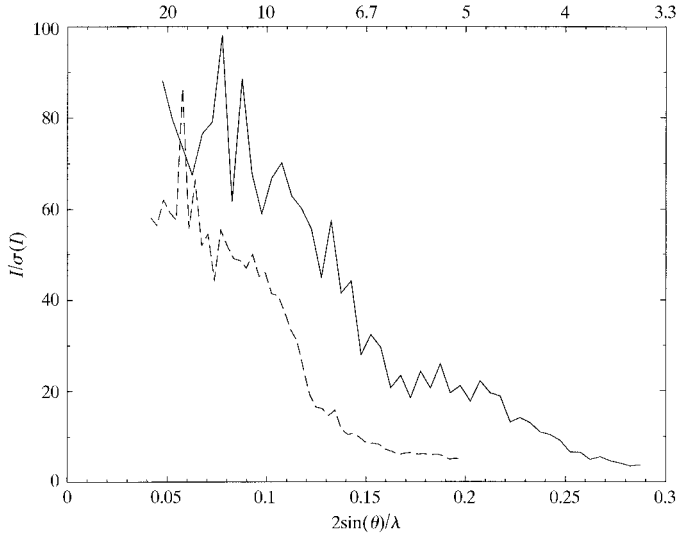

Figure 2
Comparison of the integrated intensities for whole reflections of an image collected at CHESS and an image collected at APS (shown in Fig. 1). The CHESS image was processed to a maximum of 5 Å resolution and the APS image was processed to 3.45 Å resolution. $I/\sigma(I)$ is plotted as a function of resolution for whole reflections. For the CHESS image there were a total of 8168 whole reflections and 11 422 partial reflections measured and a mean of 224 reflections per bin between 9 and 5 Å resolution. The input mosaicity for the data processing was 0.09. For the APS image there were a total of 1935 whole reflections and 49 001 partial reflections measured and the mosaicity was 0.12, which resulted in the large fraction of partial reflections in the data. There were a mean of 48 reflections per bin in the plot. Note the higher signal at identical resolutions of the APS images compared with the CHESS images.

diameter of 0.3 mm and the beam convergence is 1.95 mrad in the horizontal direction and 0.12 mrad in the vertical direction at 12 keV. This source size combined with partially demagnifying optics can be imaged onto the detector surface with a spot size of 0.16 mm horizontally and 0.3 mm vertically. This produces an intensity gain factor of approximately 4000, making the bending magnet combined with a reasonable bandpass monochromator comparable in flux (1×10^{12} photons s^{-1}) to a beamline with an insertion device, though at a narrower bandpass.

The amount of background X-ray scattering falling on the detector was a significant factor in the resolution gain. The long exposure times, as high as 60 s, in combination with a very weak signal make the amount of background scatter an important factor in determining the measurable signal. The small focal spot size is achieved with focusing optics and not with passive apertures, which typically tend to scatter and hence produce background on the diffraction pattern. The apertures in station 14-BM-C are all used as guards, not as beam-defining delimiters. A helium path was used at APS to reduce air scatter; the amount of beamline shielding may also be a factor.

Surprisingly, it was possible to collect between four and eight images at each crystal location. In the previous data set, crystal decay allowed only one exposure to be collected in a given crystal location, after which the crystal was translated. The exposures were typically 45–60 s at APS, whereas previously we had used 90 s exposures to collect 5 Å data. The shorter exposure time to collect higher resolution data suggests higher flux; paradoxically, there was less crystal damage per exposure. If factors other than flux were involved, the shorter exposure time may have reduced the crystal damage. The beam at APS is convergent, producing a significantly larger beam profile at the crystal than at the detector. This spreads the heat load over a wider area for a given amount of total flux on the crystal, resulting in less physical damage to the crystal and slower decay. The convergent beam geometry is an advantage for relatively large samples, such as HK97, reducing the power density deposited in the crystal while increasing the diffracting volume. The beam dimensions at the sample were approximately 0.35 mm vertically and 0.8 mm horizontally.

Image-plate detectors were used to collect the HK97 data. A MAR 345 detector was used at APS, whereas off-line Fuji image plates were used at CHESS. While the properties of these image plates and scanners should be very similar, as both use Fuji image plates, the area of the MAR345 is somewhat larger and symmetric; there is no variability between image plates or cassette covers as with off-line image plates. Although the 180 s read-out time of the MAR detector was not favorable for time-dependent radiation decay, the large detector size allowed a wider angular range of data to be measured symmetrically in a single pass.

In conclusion, data to as high as 3.45 Å resolution were accurately measured from HK97 crystals at APS beamline 14-BM-C. This was a substantial improvement over the previous 5 Å resolution data set. More than 500 images were collected in two visits to APS, from which it should be possible to extend the resolution of the HK97 Head II data set and the structure to 3.5 Å resolution. The increase in resolution was not produced by differences in the crystals, but may be a consequence of a combination of high flux, the beamline optics and decreased background scatter producing an increased signal-to-noise ratio at higher resolution. These experiments were designed for optimum data collection rather than to evaluate the beamline and there are many variables that may have contributed to the higher resolution data; additional controlled experiments would be required to assess the beamline. Nevertheless, other large unit cell crystals with very weak intensity diffraction at high resolution may benefit from data collection at the APS 14-BM-C beamline.

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