High-pressure X-ray Crystallographic Studies of Complex Viral Assemblies

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Introduction

The field of macromolecular crystallography has been expanding rapidly as a result of the availability of beamlines at third-generation sources, the maturation of analysis techniques (helped by both improved methodologies and improved computer hardware), and fast, large area, charge-coupled device (CCD) detectors. The expansion is also aided by the use of crystallization robots, recombinant gene products, and new crystallization strategies. One great challenge is to improve the diffraction from crystals of biological interest.

We demonstrated that diffraction from crystals of an icosahedral virus, Cowpea mosaic virus (CPMV), could be dramatically improved under high pressure [1]. This method, if proved generally applicable, would be of high value in x-ray crystallography. With the availability of HP-CAT (APS Sector 16), we have reproduced the results that previously obtained at the European Synchrotron Radiation Facility (ESRF) in Grenoble, France, and have collected sufficient data for the structural study of the pressure effects on the CPMV structure. This opens the way for in-depth studies for application of this method to virus and protein crystallography.

Methods and Materials

Cubic CPMV crystals were grown under conditions previously reported [2]. The pressure medium was composed of 100 mM ammonium sulfate, 3% PEG6k, 55 mM potassium phosphate, and 23% MPD and had a neutral pH.

Two types of pressure cells were employed, the Merrill-Bassett and compact diamond anvil cells (DACs), with stainless steel and inconel gaskets. Both types of DAC were found suitable for the data acquisition. A schematic presentation of a DAC is shown in Fig. 1. Ruby fluorescence was used for the pressure calibration.

The wavelength of the x-ray was 0.4241 Å. The crystal-to-detector distance was 1000 mm. The oscillation angle was 0.3°, and the exposure time was from 10 to 30 seconds. The size of the beam was 100 × 100 μm, which was focused on the detector. The detector was a MAR345 imaging plate system.

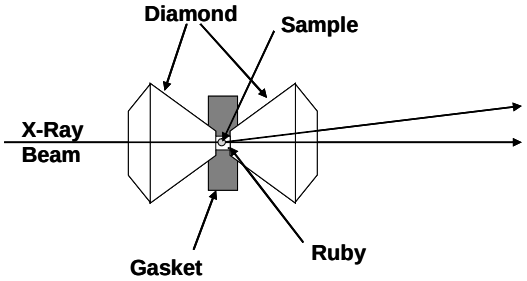

FIG. 1. Schematic of DAC used in this experiment. The sample chamber was a 500-μm-diameter hole drilled in the gasket.

Results

For comparison and calibration of experimental setting, a data set was acquired at the same beamline station (16-ID-B) at atmospheric pressure. About 60 images were recorded from 7 crystals. The diffraction limits were about 3.5-3 Å. With few exceptions, the preliminary data processing showed that the crystals were of primitive cells, indicating a deviation from the ideal I23 space group.

For the data collection at high pressure, the bottom portion of the DAC was first assembled with the gasket. The chamber made in the middle of the gasket at a size of about 0.5 mm in diameter was filled with the pressure medium. Virus crystals about 0.3 × 0.3 × 0.2 mm in size were transferred into the chamber with a capillary. Ruby chips were also loaded into the chamber for pressure calibration before the DAC was fully assembled. The pressure was increased at steps of about 0.5 kbar. Diffraction data were acquired at the desired pressure.

When the pressure was below 3 kbar, the crystals diffracted similarly to those at atmospheric pressure. When the pressure was increased to between 3.2 and 3.5 kbar, a drastic improvement in diffraction was observed (Fig. 2). Because of the poor accuracy of ruby methods of calibration of pressure for this application, the precise point at which the disorder-to-order transition occurred was not determined.

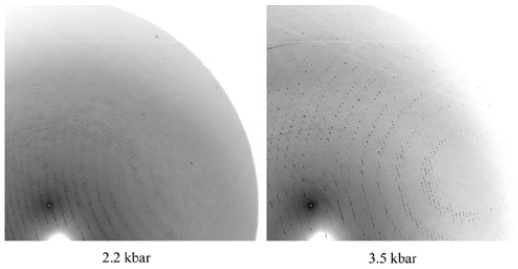

FIG. 2. The diffractions of a CPMV crystal in DAC cell. Left panel shows the diffraction to about 3 Å at 2.2 kbar pressure, which is similar to that at atmospheric pressure. Right panel shows the diffraction of the same crystal at 3.5 kbar. The edge of the diffraction is about 2.1 Å.

With a further increase of pressure to about 4 kbar, the crystals lost diffraction completely. The crystals remained intact, however, with only a slight change to the crystal morphology, as the edges of the crystals became smooth, which could only be discerned on close inspection. The physical property of the crystals, in contrast, underwent a significant alteration with the treatment of pressure at 4 kbar. The pressure-treated crystals could no longer be dissolved in aqueous solutions and became resistant to physical force. The packing of the pressure-treated crystals will be investigated.

Attempts were also made to reverse the effect of pressure on the crystals by lowering the pressure. Probably as a result of friction, graduate loosening of screws on the DAC did not lead to the linear lowering of pressure. Instead, a sudden drop in pressure was observed when the screws were entirely loosened. The diffraction from the crystals, first pressurized to 3.5 kbar and then depressurized to atmospheric pressure, was poor. However, it could not be determined whether it was due to the release of pressure or due to the shock of the pressure jump.

Five crystals were employed in the data collection at a pressure of 3.5 kbar for a total of 30 images. Preliminary data processing showed that all of these images were of perfect I23 space groups. A manuscript on the mechanistic studies of this disorder-to-order transition is being prepared.

Discussion

It is significant that the pressure can be employed to improve the quality of macromolecular crystals. However, its generality has yet to be demonstrated. We are preparing a new round of experiments with a selection of other macromolecular crystals for high-pressure studies.

Acknowledgments

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