Crystallographic Studies of the Bacteriophage HK97 Head I

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Introduction

The bacteriophage HK97 offers an accessible system for mechanistic studies of viral maturation and large-scale conformational changes of macromolecular machineries. Its capsid, upon formation, undergoes a maturation process with large-scale structural rearrangements from a precursor Prohead to the final mature capsid Head II. HK97 differs from all other known viruses in that almost all 420 subunits form autocatalytic, intermolecular cross-links in maturation to Head II, resulting in molecular chainmail. There is a crucial intermediate in the late stage of the maturation, called Head I. Head I is morphologically similar to Head II but without the chainmail [1]. To better understand the mechanism of HK97 capsid maturation, a stable Head II mutant was produced and crystallized for x-ray crystallographic studies.

Methods and Materials

Head I crystals were flash-frozen for the data acquisition. The crystals diffracted x-rays to 5 Å resolution. The unit cell dimensions were in excess of 1000 Å in two axes. In order to collect a high-quality data set, a helium cone 650 mm in length was used, and the beam was focused at the detector 700 mm from the crystals. A data set to 6.4 Å resolution was acquired for the structural studies. The data were processed with DENZO [2], and the rotation function searches were conducted with a GLRF package [3]. The molecular replacement in real space was carried out with RAVE [4].

Results

An image of the diffraction is shown in Fig 1. The results of data processing and space group analysis are listed in Table 1. There are four particles per unit cell, and the reference particle is centered at fractional coordinates of (0.156, 0.156, 0.000). One icosahedral two-fold axis coincides with the crystallographic two-fold axis, and there is a 30-fold noncrystallographic redundancy. The initial phases were calculated from the atomic model of Head II to 12 Å and improved by iterative rounds of real-space averaging and phase extension at single steps of reciprocal lattice intervals.

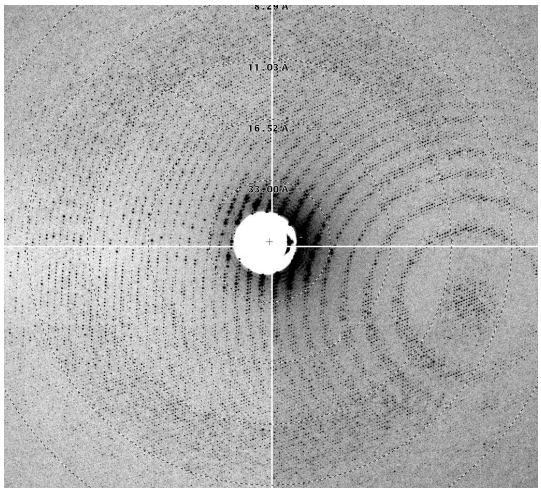

FIG. 1. A diffraction image acquired at APS beamline station 14-BM-C.

TABLE 1. Statistics in data processing.

Space group	$P4_32_12$
Unit cell (a), (b)	1009.03 Å
Unit cell (c)	729.47 Å
α, β, γ	90^o
D_{max}/D_{min}	50.0/6.4 Å
Measured reflections	5,200,447
Unique reflections	454,633
Completeness	60.0% [26.3%][a]
Overall R_{merg}	7.8% [33.4%][a]
$\langle I/\sigma(I)\rangle$	9.6 [1.65][a]
V_m	14.3/4.8[b]
Particles/cell	4

[a] Values in brackets indicate highest-resolution shell between 6.63 and 6.4 Å.

[b] With/without dsDNA genome.

Preliminary analysis of the averaged map shows that the orientation and possibly the fold of the pentons of Head I are dramatically different from those of Head II, while little difference in hexons can be detected between Head I and II (Fig. 2), which suggests that covalent cross-linking induces structural rearrangement in the pentons during maturation, in addition to the fortification of the capsid.

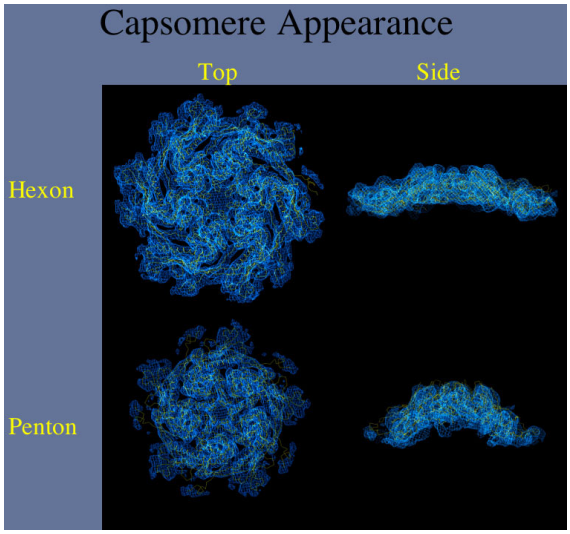

FIG. 2. The electron density map of Head I, shown in blue, superposed on the atomic model of Head II, shown in yellow.

Discussion

The crystal structure of a maturation intermediate of an HK97 capsid has been determined with data acquired from beamline station 14-BM-C. A detailed analysis of the structure is underway, which will shed light on the large-scale mechanistic change of a complex macromolecular machinery.

Acknowledgments

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