Structure Determination of a *T*=4 Virus, *Helicoverpa armigera stunt virus*

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Introduction

Helicoverpa armigera stunt virus (HaSV) is a nonenveloped, single-stranded *T*=4 RNA virus whose capsid undergoes a large-scale, pH-induced conformational change. Previously, a similar virus, *Nudaurelia capensis ω virus* (NωV), was the main focus of the investigation on this pH-induced phenomenon [1]. There were at least two types of NωV capsids, a precursor form of about 480 Å and a mature form of about 410 Å [1]. The structure of the NωV mature form was analyzed by x-ray crystallography [2]. The precursor form and the structural transition were studied by electron cryo-microscopy and image reconstruction [1].

To better understand the pH-induced structural rearrangement of virus capsids, HaSV was expressed, and the mature form was crystallized for x-ray crystallographic studies.

Methods and Materials

No suitable cryo conditions were found, and 68 crystals were used in the data collection at room temperature for 226 images. DENZO was used in the data processing [3]. A summary of diffraction statistics is in Table 1. GLRF was employed for the calculation of rotation functions [4]. Molecular replacement in real space was performed with RAVE [5].

TABLE 1. Statistics in data processing.

Space group	P1
Unit cell (a)	403.84 Å
Unit cell (b)	405.31 Å
Unit cell (c)	405.80 Å
α	119.2°
β	114.5°
γ	94.7°
D_{max}/D_{min}	40.0/2.5 Å
Completeness	25.8 (%)
Total reflections	1,994,232
Unique reflections	1,702,088
Ave<I/σ(I)>	4.2
Overall R_{merg}	16.2 (%)
V_m	2.4 $Å^3$/Da

Results

Crystals of the mature form of HaSV diffracted x-rays to at least 2.5 Å resolution, and the structure was determined by molecular replacement. The initial phases were calculated from the NωV atomic model [1] to 4-Å resolution and improved by iterative cycles of averaging over the 60-fold noncrystallographic symmetry and phase extension to 3.2-Å resolution at steps of single reciprocal units. A model was built into the averaged electron map, and a new set of phases was calculated to 2.5 Å. The final structure was determined by averaging to 2.5 Å. A portion of the electron density to 2.5 Å resolution is shown (Fig. 1).

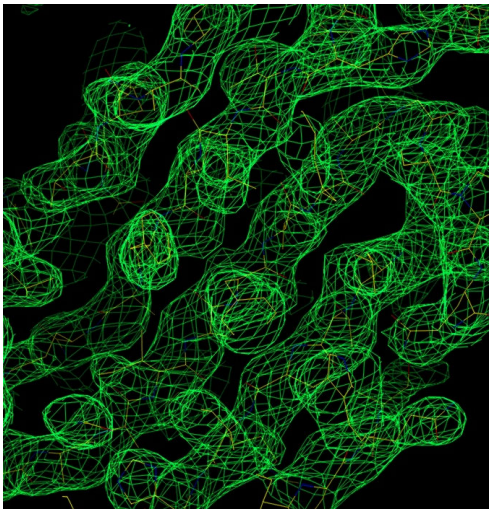

FIG. 1. A portion of the electron density map of HaSV.

Discussion

Despite the low percentage of data completeness, the electron density is well defined, and a high-quality, refined model of HaSV atomic structure can be expected in the near future. With the structure determination of two similar viruses, NωV and HaSV, a comparative study will be conducted to gain better insight into the pH-induced maturation of virus capsids. These investigations will provide useful paradigms for studies of other viral capsids and macromolecular machineries that undergo large-scale structural rearrangements. Examples of such viruses are poxvirus, herpesvirus, and bacteriophages.

Acknowledgments

We thank V. Reddy, L. Gan and T. Lin for helping with data collection. Useful discussions with V. Reddy and J. Speir on structure determination are also gratefully acknowledged. Use of the APS was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-ENG-38.

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