Data collection and structure determination of Pariacoto virus

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Introduction

Pariacoto virus (PaV) is a single-stranded RNA virus recently isolated in Peru from the Southern army worm (Spodoptera eridania). It is a nonenveloped T = 3 icosahedral insect virus of the family nodaviridae. The viral genome consists of two messenger-sense RNA molecules: RNA1 (3011 nucleotides) and RNA2 (1311 nucleotides), which encode proteins A, the viral contribution to the RNA replicase, and protein alpha, the precursor of the capsid coat protein, respectively. As in other nodaviruses [1], the coat protein alpha undergoes a post-assembly autocleavage to yield coat proteins beta and gamma. This maturation process is required for infectivity.

Methods and Materials

The virus was purified as described [2] and crystallized by the hanging-drop vapor-diffusion method at room temperature. The reservoir consisted of 1 ml of 75 mM Li2 SO4, 5 mM CaCl2, and 4% PEG 8000 in pH = 7.5 50 mM tris-HCl buffer. The droplet was a mixture of 1 µl reservoir solution and 1 µl virus sample at a concentration of ~20 mg/ml. Crystals appeared within 4–5 days. The diffraction data were collected under cryo conditions using 35% (v/v) ethylene glycol as the cryoprotectant on a CCD detector in station 14-ID-B at the Advanced Photon Source (APS) operating at a wavelength of 1.00 Å. A total of 360 images was collected with an oscillation angle of 0.5° and a crystal-to-detector distance of 275 mm. The data were processed with the program DENZO/SCALEPACK [3] to 3.5 Å resolution. A total of 3,486,085 observations were merged into 1,150,365 unique reflections, which were 98.3% complete with a merged R-factor of 0.12 and overall I/σ of 8.0. The crystal belonged to the P21 space group with cell parameters a =3 28.6 Å, b = 348.2 Å, c = 424.1 Å, and β = 91.0°.

Results

The crystal structure was determined by molecular replacement and real-space averaging. The cell dimension indicated that there was one virus particle in the asymmetric unit of the crystal. The rough orientation of the particle in the unit cell was determined by a locked rotation function calculated with the program GLRF [4], and the particle center was near (a/4, 0, c/4) based on the packing geometry. The accurate parameters of the particle position and orientation were determined by an iterative R-search using a poly-alanine model from the crystal structure of flock house virus (FHV) [5]. The particle was found to shrink by ~2.5% compared with the size of FHV. The poly-alanine model was then correctly oriented and positioned in the unit cell to calculate an initial electron density map at 3.5 Å resolution. This map was subjected to cycles of 60-fold real-space averaging with the program RAVE [6]. The initial mask was created from the model with the program MAMA [7] and was edited during averaging to avoid truncation of density by the mask. The averaged map at the final cycle gave rise to an R-factor of 0.21 and a correlation coefficient of 0.85 and was readily interpretable. The atomic model was built using the averaged map with the program O [8]. An extended N-terminal segment of subunit A was clearly defined in the electron density map. A 25-nucleotide-long RNA duplex was modelled.

Discussion

A dodecahedral RNA scaffold was formed inside the virus capsid by 30 copies of the RNA duplex which account for ~1/3 of the total viral genomic RNA. The basic-residue-rich extended N-terminus of subunit A interacts extensively with the RNA duplex, which neutralizes the abundant negative charge of the RNA phosphate groups. Its very amino end inserts into the major groove of the RNA duplex.

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References