Three-dimensional structure of the Chinese Sacbrood bee virus

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Abstract The RNA of Chinese Sacbrood Bee Virus (CSBV) was purified and used as template to obtain a 1096 bp cDNA fragment by RT-PCR amplification. This DNA fragment was cloned into pGEM-T Easy Vector for sequencing. Analyses of the sequenced CSBV RNA fragment revealed a nucleotide sequence homology of 87.6% and a deduced amino-acid sequence homology of 94.6% with that of the Sacbrood Virus (SBV), indicating that CSBV is a different but highly homologous virus of SBV. The three-dimensional (3D) structure of CSBV was determined at 2.5 nm resolution by using electron cryo-microscopy (cryoEM) and computer reconstruction methods. The 3-D structure showed that the capsid has a \( T = 1 \) (or \( P = 3 \)) icosahedral capsid shell with a smooth surface. There were 12 pentons at its icosahedral vertices (5-fold axes) and 132 holes penetrating the shell. The 3-D structure also revealed densities corresponding to the CSBV genome, suggesting icosahedrally-ordered RNA organization, a novel feature not previously reported for any picornaviruses.

Keywords: Chinese Sacbrood virus (CSBV), sequence, three-dimensional structure, electron cryo-microscopy.

Chinese Sacbrood disease is a widespread viral disease seriously impacting Chinese bee farming. This disease is the most harmful to bee larvae but can also affect the health and behavior of adult Chinese honey bees. It was first observed in Guangdong Province in 1972 and quickly spread to the entire China and the countries of Southeast Asia, leading to devastating bloom to the honey industry in this region. Yang\(^1\), Yuan\(^2\) and Dong\(^3\) had shown that the disease was caused by a virus, subsequently named Chinese Sacbrood bee virus (CSBV), which belongs to the small RNA virus family, the Picornaviridae. The genome of the virus was +ssRNA and there were four structural proteins at its capsid. CSBV was similar to the Sacbrood Bee Virus (SBV) in physiological and biochemical features, while their antigenicities were different and had no cross infection. In this work, we used molecular biology techniques to clone and sequence the CSBV genome. Sequence analyses indicated that CSBV was different but highly homologous to SBV. We have also determined the 3-D structure of CSBV at 2.5 nm resolution by cryoEM and computer reconstruction.
1 Materials and methods

1.1 The purification of CSBV

The infected larvae were kindly provided by the Entomology Institute of Guangdong Province. In our procedure, the infected larvae were mixed with 0.1 mol/L PBS (pH 7.6) buffer and then ground, followed by adding chloroform to resolve the adipose tissue. Highly purified and concentrated CSBV sample was obtained by several steps of differential speed centrifugation as previously described[4]. To examine the purity and concentration of the virus preparation, about 5μl aliquot of the purified virus sample was applied onto the glow-discharged support film of a copper EM grid and negatively stained with PTA 2% (pH 7.4) and observed in a JEOL 100CX transmission electron microscope.

1.2 Clone and sequence of the virus genome

The RNA of CSBV was obtained and purified with mRNA Capture Kit. Two primers for cDNA synthesis were designed according to the sequence of the structural protein gene of poliovirus which also belongs to the Picornaviridae[5,6], including fragments: L: 5′GCCGGTACCATAGGTTATCAAGCC3′ and R: 5′GCACTGCAGCCAAGAGTTATGCCACAAG3′. Using the purified RNA as template for cDNA synthesis and the two fragments L and R as primers, a DNA fragment was obtained and amplified through RT-PCR. Then the fragment was cloned into pGEM-T Easy Vector, and finally sequenced by Genda Technology Corp., Canada. The software packages DNASIS and PROSIS were used to calculate sequence homology between CSBV and other picornaviruses[7].

1.3 Electron cryo-microscopy

About 5 μL aliquot of the purified virus was applied to a copper EM grid coated with a holey carbon support film. The grid was blotted nearly dry by pressing a piece of Whatman 1# filter paper directly against it and then rapidly plunged into a liquid ethane (cooled by liquid nitrogen). Because the sample was cooled rapidly enough (about −103 degrees centigrade per second), the virus particles were kept in a lay of vitrified ice. The grid was then transferred from the ethane into liquid nitrogen, and mounted into a liquid nitrogen cooled Gatan 626 cryo-specimen holder. The cryo-specimen holder was then rapidly and carefully inserted into the JEM-4000 TEM[8]. Images were recorded at 60000× magnification, 400 keV with an electron dose of ~1200 e/nm2. All these steps were carried out with the sample kept at −168°C. Images of each area were recorded twice as follows. The first image was close-to-focus and the second one was far-from-focus which was 2 μm further away from the defocus value of the first one. Both images were recorded on Kodak-SO-163 electron image films, which were developed in full length D-19 for 12 min and fixed for 20 min at 20 centigrade.

1.4 Image analysis and 3D reconstruction

1.4.1 Selection and digitization of micrographs. Prior to computer analysis, the micrographs