Nucleotide sequence of the Buzura suppressaria single nucleocapsid nuclear polyhedrosis virus polyhedrin gene


1 Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071 and 2 Shanghai Institute of Biochemistry, Chinese Academy of Sciences, Shanghai 200031, People’s Republic of China

A portion of the genome of the Buzura suppressaria (Lepidoptera) single nucleocapsid nuclear polyhedrosis virus (BsSNPV) containing the polyhedrin gene was sequenced. An open reading frame of 738 nucleotides encoded a protein of 246 amino acids and represented the polyhedrin gene. A conserved TAAG motif, associated with transcriptional start sites in other polyhedrin genes, was identified 51 nucleotides upstream of the polyhedrin gene. A putative polyadenylation signal, AATAAA, was found immediately downstream of the polypeptide termination codon. Comparison of the amino acid sequence of BsSNPV polyhedrin with other NPV polyhedrins and granulosis virus granulins showed that the BsSNPV polyhedrin was most closely related to the polyhedrin of Orgyia pseudotsugata (Lepidoptera) SNPV and most distantly related to the polyhedrin of Neodiprion sertifer (Hymenoptera) SNPV.

Buzura suppressaria (of the Geometridae, Lepidoptera) is the major pest of tea in China. It is a polyphagous pest which also causes severe damage to tung oil, citrus and metasequoia plants. The single nucleocapsid nuclear polyhedrosis virus (SNPV) of B. suppressaria (BsSNPV) (Xie et al., 1979; Gan, 1981; Chu et al., 1979) has been used for biological control of this insect (Xie & Peng, 1980; Peng et al., 1989). A physical map of the BsSNPV DNA has been determined and the genome was found to be in the order of 129 kbp (Liu et al., 1993).

The polyhedrin gene is the most extensively studied baculovirus gene but most of the information has been derived from studies on multiple nucleocapsid NPVs (MNPVs). Only two SNPV polyhedrins have been sequenced, those from Orgyia pseudotsugata (Op) of the Lepidoptera (Leisy et al., 1986a) and Neodiprion sertifer (Ns) of the Hymenoptera (see Rohrmann, 1992). Because OpSNPV polyhedrin and NsSNPV polyhedrin have only a low degree of amino acid sequence identity, sequencing of a third SNPV polyhedrin gene will be useful in understanding the relationship of SNPV polyhedrins. We therefore compared the BsSNPV polyhedrin gene and flanking regions with those of other baculoviruses.

As previously reported (Liu et al., 1992), a 20 kb KpnI-I fragment of BsSNPV DNA hybridized with the Autographa californica (Ac) MNPV EcoRI-I fragment, which contains the polyhedrin gene. The KpnI-I fragment was cloned in both orientations in a M13mp18 vector according to Maniatis et al. (1982). The vector containing the KpnI-I fragment was digested with PstI and BamHI to produce 3' and 5' overhangs, respectively. A series of overlapping fragments were then generated by digestion with exonuclease III and nuclease S1 (Erase-a-Base System, Promega). DNA sequencing was carried out using the dideoxynucleotide chain terminating method of Sanger et al. (1977), employing the T7 DNA polymerase sequencing system (Promega). Since the polyhedrin gene sequence did not reside totally within the KpnI-I fragment, the adjacent KpnI-HindIII fragment was cloned and the portion containing the 3' end of the polyhedrin gene was sequenced.

A total of 2340 nucleotides of BsSNPV were sequenced, which included the polyhedrin gene and its flanking regions (Fig. 1). The polyhedrin gene is 738 nucleotides long and has the potential to encode a polypeptide of 246 amino acids. In the 5' non-coding region, a putative transcriptional start site is present with the canonical core sequence TAAG (Vlak & Rohrmann, 1985) at position -51. In the 3' non-coding region, a poly(A) signal sequence, AATAAA, (Birnstiel et al., 1985) was found five nucleotides downstream from the translational stop codon TAA. Our sequence is different from the partial, N-terminal polyhedrin sequence reported previously for the BsSNPV polyhedrin gene (Zhang et al., 1991), but this could be due to the use of two different virus isolates.

The promoter region of the BsSNPV polyhedrin gene was found to be similar to those of other baculovirus
Fig. 1. The nucleotide sequence of BsSNPV polyhedrin gene and flanking regions. Predicted amino acids are indicated with one-letter code designations. The TAAG consensus sequence for late baculovirus transcription initiation and a potential polyadenylation signal are underlined. The KpnI recognition sites are shown in bold.

<table>
<thead>
<tr>
<th>Nucleotide Position</th>
<th>Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Y</td>
</tr>
<tr>
<td>2</td>
<td>Q</td>
</tr>
<tr>
<td>3</td>
<td>E</td>
</tr>
<tr>
<td>4</td>
<td>V</td>
</tr>
<tr>
<td>5</td>
<td>L</td>
</tr>
<tr>
<td>6</td>
<td>E</td>
</tr>
<tr>
<td>7</td>
<td>L</td>
</tr>
<tr>
<td>8</td>
<td>V</td>
</tr>
<tr>
<td>9</td>
<td>Y</td>
</tr>
<tr>
<td>10</td>
<td>D</td>
</tr>
<tr>
<td>11</td>
<td>N</td>
</tr>
<tr>
<td>12</td>
<td>K</td>
</tr>
<tr>
<td>13</td>
<td>R</td>
</tr>
<tr>
<td>14</td>
<td>E</td>
</tr>
<tr>
<td>15</td>
<td>Y</td>
</tr>
</tbody>
</table>

polyhedrin genes (Fig. 2). These similarities include the A at position -3 (Kozak, 1983) and the TAAG motif involved in transcription at position -51 (Rohrmann, 1986). The promoter region of the BsSNPV polyhedrin gene is most closely related to OpSNPV (Leisy et al., 1986) with identical length (51 nucleotides) and only a five-nucleotide difference in the sequence. We found no homology between the sequences upstream of the BsSNPV polyhedrin gene promoter or downstream of the gene and those of AcMNPV (Possee et al., 1992). No major open reading frames were observed in the upstream region.

The amino acid sequence of the BsSNPV polyhedrin was compared to those of 12 other NPV polyhedrins (Fig. 3). The BsSNPV polyhedrin (246 amino acids) is similar in size to most other NPV polyhedrins, including NsSNPV (also 246 amino acids), but not to granulosis viruses (GVs) (size variation at N terminus, data not shown). At amino acid residues 33 to 36 a KRKK sequence is present, which in the AcMNPV polyhedrin is reported to be a nuclear localization signal (Jarvis et al., 1991).

In contrast to MNPs, which appear to be confined to
polypeptide is most closely related to that of OpSNPV, with 80.7% nucleotide and 95.5% amino acid identity. Many insect orders (Vlak & Rohrmann, 1985). Analysis of lepidopteran insects, SNPVs have been isolated from nucleotide and amino acid sequence identity ranging from 70-6 to 80-6% and 82.9 to 94.3%, respectively. It is worth mentioning that a closer relationship was found between BsSNPV polyhedrin and granulins (Pieris brassicae GV, Chakerian et al., 1985; Trichoplusia ni GV, Akiyoshi et al., 1985), with nucleotide identity of 57.2 to

Fig. 3. Comparison of the amino acid sequences of 12 NPV polyhedrins. Dots indicate identity with BsSNPV polyhedrin. The sources of the sequences are described in Fig. 2, except for and NsSNPV (see Rohrmann, 1992).
58.4% and amino acid identity of 56.2 to 57.1%, than between the Bs- and NsSNPV polyhedrins (see Rohrmann, 1992). These data give further credence to the hypothesis that NsSNPV evolved from the common ancestor of the lepidopteran GVs and NPVs before GVs evolved from the lepidopteran NPVs (Rohrmann, 1992). Although the BsSNPV polyhedrin was most closely related to that of OpSNPV, distinct lineages of MNPVs and SNPVs within the lepidoptera are not evident.

Future research will focus on transcriptional mapping of the 3' and 5' termini of the BsSNPV polyhedrin gene, and further sequencing of its flanking region. The generated data will give additional information on the organization of SNPV genomes in comparison to those of MNPVs.

We are grateful to Dr B. M. Arif for kindly providing the unpublished amino acid sequence of CMNNPV polyhedrin and helpful reviewing of the manuscript, and to Dr Lu Zhaohai for excellent research assistance. This work was supported in part by the National Foundation for Natural Sciences of China and the Director Foundation of the Wuhan Institute of Virology.

References


(Received 22 October 1992; Accepted 4 March 1993)