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Nucleotide Sequence Analyses of p10 Gene and its Promoter of *Hyphantria cunea* Nuclear Polyhedrosis Virus

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=국문초록=

Hyphantria cunea Nuclear Polyhedrosis Virus p10유전자와 프로모터의 염기서열 결정

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Hyphantria cunea nuclear polyhedrosis virus p10유전자와 프로모터의 염기서열을 결정하였고, p10단백질의 아미노산 서열을 유도했다. pBP10재조합클론 (Cha *et al.*, 1995)에 삽입이 되어 있는 p10유전자의 염기서열을 결정한 결과 p10유전자의 ORF는 285 bp였고, p10단백질은 95개의 아미노산으로 구성 되었으며, 분자량은 10.26 kDa이었다. 프로모터내에는 TATA box 와 전사개시부위인 TAAG 염기가 발견되었다. poly (A) signal부위인 AATAAA염기서열은 3'-말단상류의 65염기부위에 위치했다. p10단백질의 N-말단은 소수성이었으며, C-말단은 고도로 친수성이었다. p10단백질에는 cysteine, histidine, tryptophane, tyrosine, glutamine, asparagine잔기가 없었다.

Key Words: *Hyphantria cunea* NPV, p10 gene sequence, p10 protein.

INTRODUCTION

Nuclear polyhedrosis viruses (NPV) are member of Baculoviridae [1]. NPVs are double-stranded DNA viruses which replicate in the nucleus of a variety of arthropods, primarily insects of the orders *Lepidoptera, Hymenoptera,* and *Diptera*[2]. Baculoviruses are studied extensively both for their potential as insecticidal agents [2] and as expression vectors [3,4]. NPV genes are expressed in a temporally regulated program consisting of at least three phases of gene expression; (i) an early phase prior to DNA replication, (ii) a late phase viral DNA replication and the formation of budded extracellular virus, and (iii) a very late phase dominated by high level expression of p10 and polyhedrin genes [5].

At 48 h post-infection these two polypeptieds constitute about half of the protein mass in insect cells [6]. Polyhedrin is the major constituent of the viral occlusion bodies that are found exclusively in the nuclei of baculovirus infected insect cells [7]. The p10 protein is associated with large fibrillar structures in both nuclei and cytoplasm of baculovirus infected insect cells [8]. This p10 protein has been sugested to be involved in the release of polyhedra from infected cells [9].

Hyphantria cunea nuclear polyhedrosis virus (HcNPV) was isolated from dead larvae of

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Fall Webworm, *H. cunea* by Lee [10]. Genetic analysis of *H. cunea* NPV was reported by Lee and Lee [10], and the polyhedrin gene of *H. cunea* NPV was cloned and sequenced [11]. The p10 gene of *H. cunea* NPV was cloned and expressed in *Escherichia coli*[12]. The DNA sequence of the p10 genes of six different NPVs has been reported [12-16,18]. However the nucleotide sequence of the p10 gene of the *H. cunea* NPV is not reported yet.

In this article, we report the cloning and sequencing of *H. cunea* NPV p10 gene, and also deduced amino acid sequences of the gene.

MATERIALS AND METHODS

Plasmid vector and host cell

For cloning genes, pBluescript SK (+) and pBP10 [12] were used and the plasmids were propagated in *E. coli* XL-1.

Plasmid DNA elements were isolated by Maniatis *et al.* [19], and Lee *et al.* [20].

Subcloning of p10 gene of *H. cunea* NPV

For the sequencing, the pBP10 plasmid [12] was subcloned. The pBP10 plasmid was digested with *Hind*III and electrophoresed in 0.8% Seakem GTG agarose gel. The *Hind*III-digested fragment was recovered from the agarose gel by the electroelution method, extracted with phenol, precipitated with ethanol, and suspended in distilled water at 1㎎/㎖. The fragment was inserted into the *Hind*III-digested and CIP-treated pBluescript SK (+) vector by T4 DNA ligase in 10X ligation buffer containing 10mM ATP and the reaction was incubated at 18℃ for 4 h and then transformed in *E. coli* XL1 [11]. In addition, *Hind*III-digested pBP10 vector was treated with 0.01 unit of calf intestinal alkaline phosphatase for 15 min at 37℃, extracted with phenol, precipitated with ethanol, and suspended in distilled water at 1㎎/㎖. The *Hind*III-digested and CIP-treated pBP10 vector was self-ligated and transformed *E.coli* XL-1 Blue by described previously. And then, the clones were isolated and identified. The strategy of subcloing is illustrated in Fig. 1.

DNA sequencing

DNA fragments to be sequenced were cloned into pBluescript plasmid (Stratagene). Sequence determinations were made by the dideoxy chain termination method [21] with pBluescript T3, T7 and M13 reverse primers, using Sequenase version 2.0 system kit (United States Biochemical).

Deducing amino acid sequence and Hydrophilicity plot

After the p10 gene sequencing, the amino acid sequences of p10 protein of HcNPV were duduced from the data. Hydrophilicity plot of the amino acid sequence of the p10 protein of HcNPV was constructed using the algorithms of Kyte & Doolittle [22], calculated with the Peptide structure program of the GCG sequence analysis software package, and plotted with the Lotus and Freelance programs (Lotus Development Corporation). The computer program plots the sum of hydrophilicity values for seven contiguous amino acids over the position of the middle amino acid in each sector. Above the zero line the plot indicates sectors with an overall hydrophilic character, whereas below the zero line it indicates sectors with an overall hydrophobic character (Fig. 4).

RESULTS AND DISCUSSION

Nucleotide sequence analyses of p10 gene and its promoter of *H. cunea* NPV

The *Eco*RI-Q fragment of *H. cunea* NPV genome was cloned into the *Eco*RI site of pUC8 plasmid by Lee *et al.* [23], and this clone was named pHE-Q. *Eco*RI fragment was recloned into the *Eco*RI site of pBluescript KS (+) and then transformed into *E. coli* XL-I. This clone was named pBP10 [4]. The clone

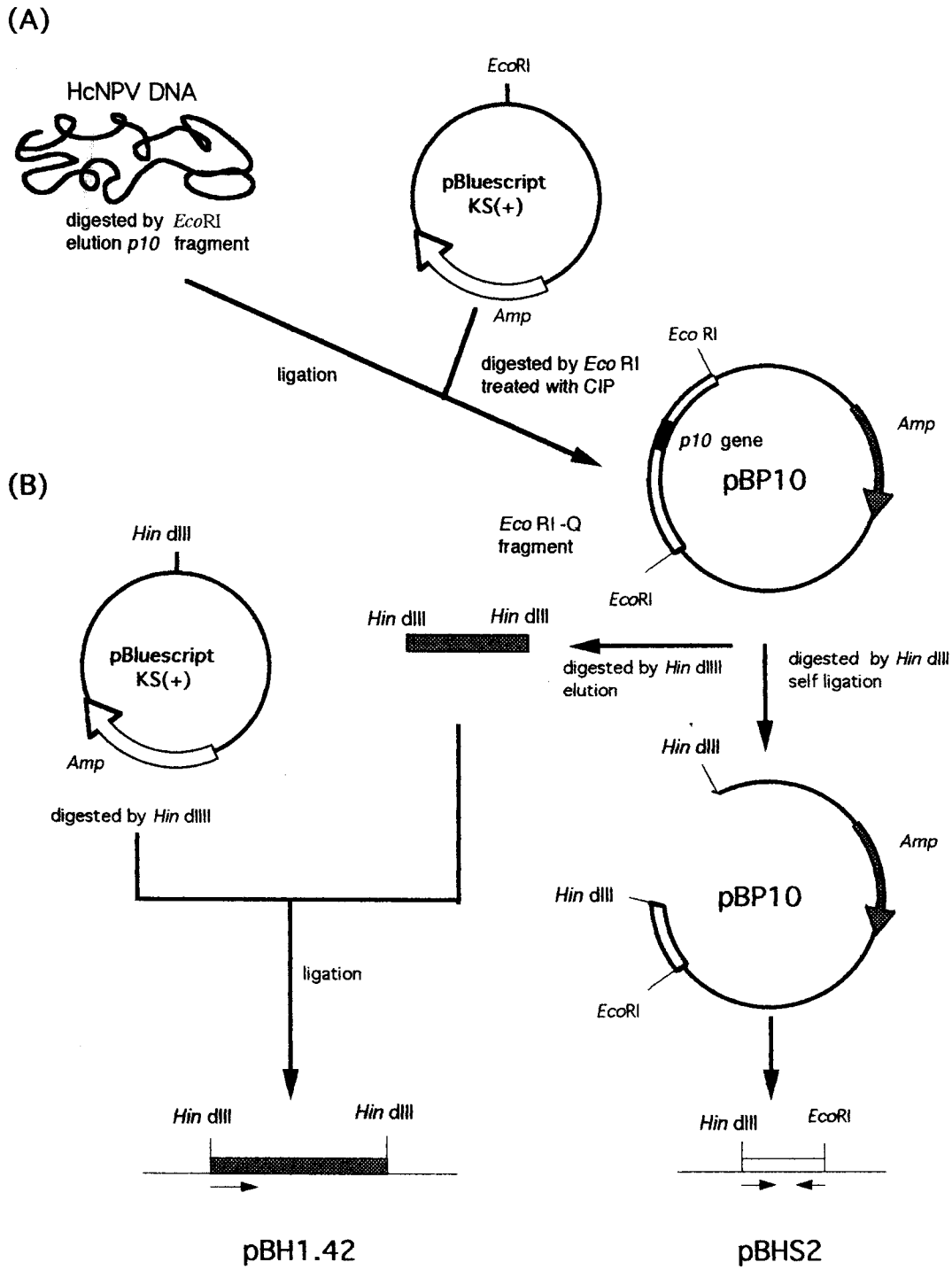

Fig. 1. Scheme for the cloning and sequencing of *H. cunea* NPV p10 gene fragment. (A): Cloning of *Eco*RI-Q fragment containing the *H. cunea* NPV p10 gene into pBluescript KS(+), which was named pBP10. (B): *Hind*III fragment in the pBP10 was inserted into *Hind*III site of pBluescript KS(+), which was named pBH1.42 and the rest of the *Hind*III-digested pBP10 vector was self-ligated, which was named pBHS2. The two recombinants were sequenced by the direction.

pBP10 was reisolated, then redigested with *Eco*RI and rehybridized with the probe to the *Eco*RI-Q fragments. The pBP10 plasmid was digested with *Hind*III and then the resulting 1.42 kb fragment was ligated into the *Hind*III site of pBluescript SK (+) plasmid vector and transformed into *E. coli* XL-I, and the rest of the *Hind*III-digested pBP10 clone was self-ligated and transformed into *E. coli* XL-I. These recombinant plasmids were named pBH1.42 and pBHS2, respectively (Fig. 1).

Sequence determination of pBHS2 was made with pBluescript T3 and T7 primers, and that of pBH1.42 with pBluescript M13 and T7 primers by the dideoxy chain termination method [21]. The p10 gene sequence and deduced amino acid sequence are shown Fig. 2. The putative *H. cunea* NPV p10 gene ORF (open reading frame) consisted of 285 bp, and therefore p10 protein consisted of 95 amino acid in its sequence with a predicted molecular weight of 10.26 kDa (Fig. 2). The p10 gene sequences of *A. californica* NPV, *Orgyia pseudotsugata* NPV, *Spodoptera exigya* NPV and *Bombyx mori* NPV were reported by Kuzio *et al.*, [13], Leisy *et al.*, [14], Zuidema *et al.*, [18] and Hu *et al.*, [26], respectively. We found that the p10 gene sequence of the HcNPV with 285 bp was the same to that of *A. californica* NPV. However the sequences of other five p10 genes were different in sizes; 276 bp in *O. pseudotsugata* NPV, 264 bp in *S. exigya* NPV and 210bp in B. mori NPV. *H. cunea* NPV was most distantly related to *S. exigya* NPV [18] which was different with respect to 81 amino acids.

The leader of p10 gene transcript was AT-rich (85.7%) within -70 nucleotides (Fig. 2). The 5'-noncoding leader sequence of p10 gene promoter contained the TATA box (Hogness box) and the transcription start site TAAG (Fig. 2). The putative TATA box (the start site of gene transcribed by RNA polymerase II) was located at sites -85 to -79 and the TAAG sequence was located at positions -70 to -67

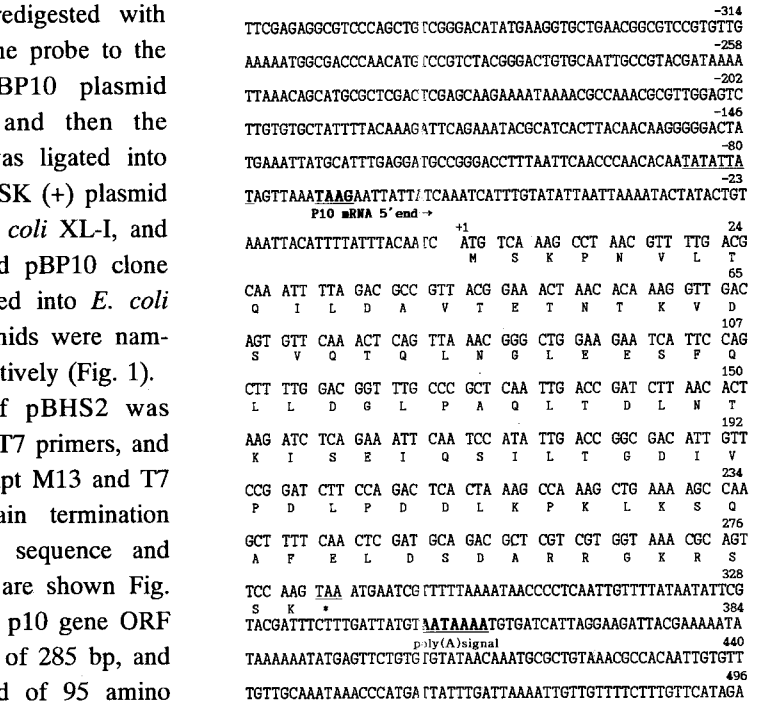

Fig. 2. Nucleotide sequence and deduced amino acid seqeunce of *H. cunea* NPV p10 gene and its promoter. TATA box and TAAG regions are in the promoter region. AUG(translational starting codon) and TAA(termination codon) are in ORF. Poly(A) signal is located at 3'-downstream of the termination codon. p10 gene ORF contained 285 bp long.

with respect to the translational start codon of HcNPV p10 ORF (Fig. 2). Poly (A) signal sequence in the 3' noncoding region of the putative p10 gene was located at 66 to 73 nucleotides downstream of the translation stop codon (Fig. 2). The *H. cunea* NPV p10 gene ORF was identical to that of the *A. californica* p10 gene.

The *H. cunea* NPV p10 gene transcript without a poly(A) tail has a leader of 68 nucleotides, a coding sequence of 285 nucleotides and a 3' non-coding sequence of above 70 base-pair (Fig. 2). Transcription initiation motif is conserved in all baculovirus late gene [24,25]. This heterogeneity (AUAAG) at the 5'-end of the p10 gene mRNA also exists in p10 transcript from other

(A) TAAATAAGAATTATTATCAAATCATTTGTATATTAATTAAATACTATACTGTAATTACATTTTATTTACAATCATG

(B) TAATAAGTATTTTACTGTTTTCGTAACAGTTTTGTAATAAAAAAACCTATAAATATG

Fig. 3. Comparison of the promoter sequences of p10 gene (A) and polyhedrin gene (B) of *H. cunea* NPV. The two genes are functional at the same time in the infection cycle. They have a substantially different nucleotide sequences in their promoters. However both the transcription intiation sites of the two genes were a common core sequence, TAAG.

NPVs as determined for *A. californica* NPV [13] and *B. mori* NPV [26], for *O. pseudotsugata* MNPV [14] by S1 nuclease analysis and for *S. exigya* MNPV [18] by primer extension analysis. AATAAA sequence was at the site 65 base upstream from 3' terminus (Fig. 2). This sequence is normally found a short distance upstream from the 3' terminus of eukaryotic mRNAs and serves as part of a signal for the processing of longer primary transcript and for poly (A) addition.

The promoter of the p10 gene, although functional at the same time in the infection cycle as the polyhedrin promoter, has a substantially different nucleotide sequence (Fig. 2 and 3). Also the two genes contained common transcriptional initiation sites, TAAG. Mutation of this core sequence abolished the activity of both the polyhedrin and the p10 promoters [6,24,27,28], suggesting that the TAAG motif plays an important role in the regulation of the p10 gene and polyhedrin gene expressions.

Deducing of amino acid sequence and hydrophilicity

HcNPV p10 protein consisted of 95 amino acid in its sequence with a predicted molecular weight of 10.26 kDa (Fig. 2). In the p10 protein sequence, a hydrophobic region was present at the N-terminus of the protein, whereas the C-terminus was highly hydrophilic (Fig. 4). The p10 protein of *H. cunea* NPV did not contain cysteine, histidine, tryptophan, tyrosine, glutamine and asparagine residues. The molecular weight of the purified p10 protein produced by the *E. coli* was different from that of the p10 protein produced by *S. frugiperda* cell [12].

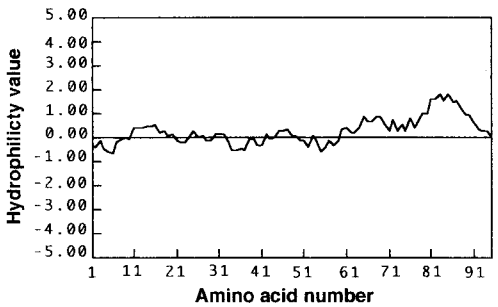

Fig. 4. Hydrophilicity plot assay of the p10 protein of *H. cunea* NPV. The plot was constructed using the algorithms of Kye & Doolittle (8) calculated with the Peptide structure program of the GCG sequence analysis software package, and plotted with the Lotus and Freelance programs (Lotus Development Corporation). The computer program plots the sum of hydrophilicity values for seven contiguous amino acids over the position of the middle amino acid in each sector. Above the zero line the plot indicates sectors with an overall hydrophilic character, whereas below the zero line it indicates sectors with an overall hydrophobic character. The N-terminus of the protein appeared hydrophobic region, whereas the C-terminus was higly hydrophilic.

SUMMARY

The sequences of p10 gene and its promoter of *Hyphantira cunea* NPV were determined. According to the sequence analysis, the putative p10 gene ORF has 285 bp. The 5'-noncoding leader sequence of the p10 gene promoter contained the TATA box and the putative transcription initiation site TAAG motif. Poly (A) tail signals, AATAAA sequence was at site 65 base upstream from the 3' terminus.

The deduced amino acid sequence of p10 protein was 95 with a predicted molecular weight of 10.26 kDa. In the p10 protein se-

quence, a hydrophobic region was present at the N-terminus of the protein, whereas the C-terminus was highly hydrophilic. The p10 protein of *H. cunea* NPV did not contain cysteine, histidine, tryptophan, tyrosine, glutamine and asparagine residues.

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