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Nucleotide Sequence of a Truncated Proteinase Inhibitor I Gene of Potato

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감자에서 분리된 절단형 단백질분해효소 억제제 I 유전자의 염기서열

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ABSTRACT

A genomic clone carrying a proteinase inhibitor I sequence was isolated and characterized. The clone contained a 0.7 kb *Eco*RI fragment hybridized with tomato inhibitor I cDNA. The nucleotide sequence of the *Eco*RI fragment revealed presence of a truncated form of a proteinase inhibitor I gene of potato. The truncated gene contained the 5' flanking region and the first exon of a functional proteinase inhibitor I gene. Although the 5' flanking region contained the regulatory sequences TATAAA and CCACT, a deletion of 40 bp occurred between them.

INTRODUCTION

Proteinase inhibitors are usually present in storage organs such as seeds and tubers, of plants in the Leguminosae, Gramineae and Solanaceae families (Laskowski and Kato, 1980). In potato, two nonhomologous inhibitors I and II are major inhibitors in tubers, comprising about 2.5% and 4.0% of soluble proteins of tubers, respectively (Belitz *et al.*, 1971; Ryan *et al.*, 1976). They usually inhibit the activity of serine proteinases such as chymotrypsin and trypsin (Ryan, 1981).

Potato inhibitor I proteins specific toward chymotrypsin are a mixture of heterogenous pentamers of 40,000 daltons composed of subunits of 8,000 daltons. At least four different protomers of inhibitor I proteins are present in potato tubers (Melville and Ryan, 1972), suggesting that they might be encoded by different genes in the potato genome. Potato inhibitor I proteins are synthesized and stored along with inhibitor II proteins as tubers develop. Furthermore, they are induced in leaves of potato upon wounding mechanically or by insect attacks (Ryan and Huisman, 1967; Green and Ryan, 1972).

For the understanding of regulation of inhibitor I gene expresssion in potato, we have carried out various experiments concerning their structure and expression. We determined the copy number of inhibitor I genes in the genome of Russet Burbank potato, which revealed presence of about 10 inhibitor I genes in the genome (Lee and Park, 1989). We also isolated and characterized an inhibitor I gene from a genomic library of Russet Burbank potato. The inhibitor I gene seemed to be functional. In this report, we characterized an inhibitor I truncated gene which contained part of a functional inhibitor I gene as compared with other inhibitor I genes (Cleveland *et al.*, 1987; Lee and Park, 1989).

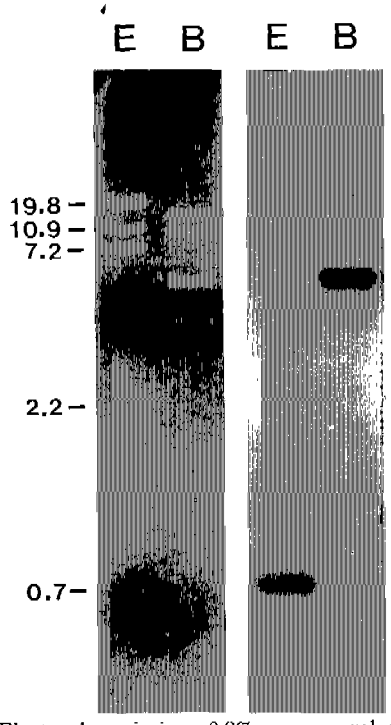

Fig. 1. Electrophoresis in a 0.8% agarose gel of λ DNA of clone 28 isolated from a potato genomic library after digestion with restriction enzymes (left) and Southern hybridization with radioactively labeled tomato inhibitor I cDNA after transfer onto GeneScreen *Plus* membrane (right). Size of restriction fragments is shown in kb determined with *Hind*III-digested lambda DNA as size markers. E, *Eco*RI; B, *Bam*HI.

MATERIALS AND METHODS

Enzymes and chemicals. Restriction and DNA modifying enzymes were purchased from Promega. The DNA sequencing kit was purchased from New England Biolabs. [α-^{32}P]dATP was from New England Nuclear. Other chemicals were purchased from Sigma Chemical Co.

Screening of a potato genomic library. About 5×10^4 bacteriophage were screened by the method of Woo (1979) from a *Eco*RI-partial genomic library of potato (*Solanum tuberosum* cv Russet Burbank). *E. coli* strain K802 was used as the host for infection of bacteriophage. Inserts of tomato inhibitor I cDNA clone, pT_1-24 (Graham *et al.*, 1985) was used as the probe after labeling radioactively by nick-translation.

Molecular cloning. Standard procedures were used for recombinant DNA works as described by Maniatis *et al.* (1982). Localization of inhibitor I sequences in the genomic clone was carried out by hybridization with tomato inhibitor I cDNA as a probe (Southern, 1975; Wahl *et al.*, 1979) after digestion of λ DNAs with *Eco*RI or *Bam*HI. DNA fragments from the λ clone were subcloned into pU19 (Yanisch-Perron *et al.*, 1985). *E. coli* strain JM 101 was used for multiplication of plasmid DNAs.

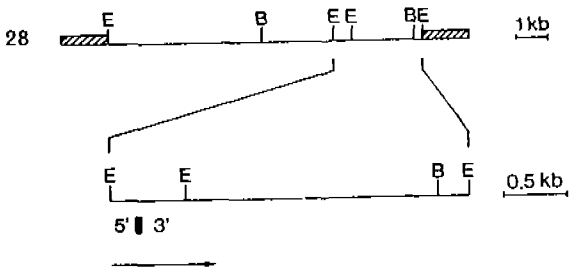

Fig. 2. Restriction map of the clone 28 carrying a truncated inhibitor I gene. The region of cloned genomic DNA only is shown. Horizontal arrow indicates the extend and direction of sequences. Symbols: E, *Eco*RI; B, *Bam*HI.

Nucleotide sequencing. The nucleotide sequence of DNA fragments was determined by the dideoxynucleotide chain termination method (Sanger *et al.*, 1977) with denatured plasmid DNAs and the universal primers. Plasmid DNAs of subclones were subjected to unidirectional deletions with exonuclease III and S1 nuclease as described by Henikoff (1984). Plasmid DNAs with serial deletions of about 200 bp in size were selected and used as template for DNA synthesis after denaturation with alkali (Mierendorf and Pfeffer, 1987). The reaction products were separated in 6% and 8% polyacrylamide-urea gels. Ambiguity at the nucleotide sequences was corrected by repeated sequencings.

RESULTS AND DISCUSSION

Isolation of a clone carrying an inhibitor I gene. We have isolated six genomic clones containing inhibitor I genes from a *Eco*RI-partial genomic library of potato by screening it with tomato inhibitor I cDNA as a probe (Lee and Park, 1989). One of the clones, clone 28 was found to carrying three *Eco*RI fragments of 0.7, 2.2 and 7.2 kb in size, respectively. As shown in Fig. 1, the 0.7 kb *Eco*RI fragment was identified to hybridize with tomato inhibitor I cDNA. Restriction map of and location and direction of the inhibitor I gene on the clone 28 determined on the bases of the gel electrophoresis and hybridization are shown in Fig. 2.

```
                                                    50                                                  100
GAATTCAATTTTCACTTTCCCTCCGTATTTCTCGTATGTAATTAAAAATATATCAAAACATAATATTTTTTTCCTTTATCTTCAAACTGTTCAACATGTG

                                                   150
GTTGTTACACCACTAGTATGCCTATAAATTTATGTGATGCACTCATACAAATTCACTCAATTCCTTCTACTCTTTACAACTAAAAGAAA ATG GAG
                                                                                              Met Glu

  200                                                 250
TCA AAG TTT GCT CAC ATC ATT GTG TTC TTT CTT CTG AAAAAATATTCTTTTAAAATGTAAGTTAAAGTTTTCATAATTTGACTTTCGA
Ser Lys Phe Ala His Ile Ile Val Phe Phe Leu Leu

            300                                 350
ATAGCTAAATTTCACTTGAGACGGGAAAGCGTATTTGATATCTTATAACAATTATTTTATCTAATGACAAATTATGATAAGAAATATTTATTATGAGCTA

            400                                 450
TTTAATGACAAATTAATAATAATTTCATAGCTAAATTTTTAAATTTTTATTATGATGAAATGATGAAAGAAAACATAAAGAAAAAACTAAAAAAGAGATG

            500                                 550
ATACCAAAAGTAAAATTGCTATGGTCCCCTAATGACATGTTCTTATTCATGCCCAATTTTTGTCAGTATATAAAACACATAATAAATGTGTTATAATTGT

            600                                 650
AAGTATTTTTTATTTGCTCCTAATTATTCTGCCTTTCAATTTATTTAGTTGCGTGTGAATTCAATATACCAACCATGCATTAACTCTTTAGACATTTCCC

            700                                 750
ATATTTTATTATGTAATTGATAAAATTGTTTCTTCTTTTGTATTTTTATAATAAATTAATAAGAGGAATTTCTTTTAAACCAAAAAAAGAAGAAGAATTT

            800
GGGTAAAAAAAGGTATATTTTTCAATATACAAGGACTAGATAGAGCACCTAACTAATGATC
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Fig. 3. Nucleotide sequence of the truncated inhibitor I gene. DNA sequences which resemble the consensus sequences of promoter elements (TATAAA and CCACT) of other eukaryotic genes are boxed.

```
          -200                -180                -160                -140                -120
PI-I-3.4: GAATTAAATTTTCACTGTCCCTCCTCACTTCTCGTATGTAATTAAAAATATATCAAAACCTAATAATTTTTTCCTTTATCTTCAAATTGT
          ****** ********** *******    ********************************** ************************** ***
PI-I-0.7: GAATTCAATTTTCACTTTCCCTCCGTATTTCTCGTATGTAATTAAAAATATATCAAAACATAATATTTTTTTCCTTTATCTTCAAACTGT

                    -100                -80                 -60                 -40
PI-I-3.4: TCAACATGTGGTTGTTAGACACCACTAGTAAATAGTACATCACTAGTCACTACAATGAAGGCAACCTGTGCCTATAAATTTATGTGATGC
          ****************  **********                                          ********************
PI-I-0.7: TCAACATGTGGTTGTTA--CACCACTAGT----------------------------------------GCCTATAAATTTATGTGATGC

          -20                 +1                  +20                 +40
PI-I-3.4: ACTCATACAAATTCACTCAATTCCTTCTACTCTTTACAACTAAAAGAAA ATG GAG TTA AAG TTT GCT CAC ATC ATT GTT
          ************************************************ *** *** * * *** *** *** *** *** *** **
PI-I-0.7: ACTCATACAAATTCACTCAATTCCTTCTACTCTTTACAACTAAAAGAAA ATG GAG TCA AAG TTT GCT CAC ATC ATT GTG

                    +60                 +80
PI-I-3.4: TTC TTT CTT CTT GCA ACT T/GTTAGTACCC
          *** *** *** **    * *   *  **  *
PI-I-0.7: TTC TTT CTT CTG AAA AAA T ATTCTTTTTA
```

Fig. 4. Comparison of the nucleotide sequences at the 5' flanking regions of the inhibitor I functional (PI-I-3.4) and truncated (PI-I-0.7) genes. The nucleotide sequence of the functional gene is from Lee and Park (1989). DNA sequences which resemble the consensus sequences of promoter elements (TATAAA and CCACT) of other eukaryotic genes and the translation initiation codon (ATG) are boxed. A right-handed slash positions the 5' border of the first intron.

Structure of the inhibitor I gene. The *Eco*RI fragments present in clone 28 were subcloned into the *Eco*RI site of pUC19. A subclone containing the 0.7 kb *Eco*RI fragment was subjected to DNA sequencing after unidirectional deletions with exonuclease III and S1 nuclease. The nucleotide sequence of part of the 2.2 kb *Eco*RI loca-

ted 3' to the 0.7 kb fragment was also determined. Fig. 3 shows the nucleotide sequence determined. It revealed that the 0.7 kb *Eco*RI fragment contained the 5' flanking region and the first exon only of a functional inhibitor I gene (Cleveland *et al.*, 1987; Lee and Park, 1989). This result indicates that the inhibitor I gene is a truncated gene of an inhibitor I gene. When the 5' flanking region of the truncated inhibitor I gene was compared with that of a functional inhibitor I gene, high level of homology was obseved. However, a deletion found beween the TATAAA and CCACT sequences, possible promoter elements, may prevent the 5' flanking region from acting as a promoter region (Joshi, 1987). The 5' border sequence of the first intron was changed from GT to AT (Brown, 1986).

Since recombinant DNA techniques were applied to plant genes, several nonfunctional truncated and pseudo genes were found to be present in the genome of plants, especially those with multiple genomes such as soybean. Leghemoglobin gene family in soybean with the tetraploid genome is composed of functional, truncated and pseudo genes (Brisson and Verma, 1982; Lee and Verma, 1984). Potato is also known to be tetraploid generated by genome duplication. It was found that the inhibitor I gene family in tetraploid potato is a multigene family composed of about 10 different genes (Lee and Park, 1989). Therefore, it is not surprising that the inhibitor I gene family contains truncated gene(s). The inhibitor II family in potato also contains pseudo and truncated genes (Choi *et al.*, 1990). It is not yet clear what roles truncated and pseudo genes play in the plants. They might be evolutionary vestiges or play an important role as origins of new genes (Little, 1982).

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적 요

감자의 유전자은행에서 단백질분해효소 억제제 I 유전자를 가지고 있는 하나의 clone을 분리하고 그 특성을 분석하였다. 분리된 clone 28에는 각각 크기가 0.7 kb, 2.2 kb 그리고 7.2 kb인 3개의 *Eco*RI 절편이 존재하고 있었는데, 이를 토마토의 억제제 I cDNA와 hybridization을 수행한 결과 0.7 kb *Eco*RI 절편에 억제제 I 유전자가 존재하고 있음을 확인하였다. 억제제 I 유전자를 간직하고 있는 0.7 kb *Eco*RI 절편을 pUC19에 subcloning하고 염기서열을 결정하였다. 그 결과 이 억제제 I 유전자는 기능을 간직한 유전자의 5' 근접부위와 첫번째 exon 만을 간직하고 있는 절단형 억제제 I 유전자임이 판명되었다. 5' 근접부위에는 조절 염기서열로서 TATAAA와 CCACT가 존재하고 있었으나 이들 사이에 40 bp의 제기가 일어나 있었다.

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