Molecular characterization of a wound-inducible inhibitor I gene from potato and the processing of its mRNA and protein

Thomas E. Cleveland,1 Robert W. Thornburg2 & Clarence A. Ryan*
Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340, USA *author for offprints); 1present address: USDA/ARS, Southern Regional Research Laboratory, P.O. Box 19687, New Orleans, LA 70448, USA; 2present address: Department of Biochemistry and Biophysics, Iowa State University, Ames, IA 50011, USA

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Abstract

Genomic blotting of restriction fragments of Russet Burbank DNA indicated that at least 6 copies of Inhibitor I are present in the tetraploid potato genome. A library of potato genes in bacteriophage λ was screened for the presence of Inhibitor I genes using a wound-inducible tomato Inhibitor I cDNA as a hybridization probe. One phage with an insert of 13.1 kb was isolated that hybridized most strongly with the probe. A 4.2 kb Eco RI fragment containing the gene was isolated from the clone and 2.2 kb region was sequenced that included about 800 bp of both the 5' and 3' regions. The gene contained two introns of 479 and 417 bp respectively, and the splice junctions were typical of other eukaryotic genes. Putative TATAA and CAAT boxes were identified. The nucleotide sequence, when compared with a wound-inducible tomato Inhibitor I cDNA, exhibited over 90% identity. The gene codes for a prepro-Inhibitor I protein of 96 amino acids. The putative pre-sequence of 19 amino acids, differs in only one residue from that of tomato Inhibitor I. The potato pro-sequence, however, is lacking a tetrapeptide that is found in the tomato pro-sequence in the region of pro-peptide processing. This deletion, together with a substitution of a Gln for a Leu (4 residues toward the N terminus) provides an explanation for the differences at the N-termini between tomato and potato Inhibitor I natural proteins by providing different processing sites in the two pro-inhibitors. Thus, amino acid sequence differences between the N termini of tomato and potato Inhibitor I are easily explained by the mutational events. The different proposed pro-processing sites of the tomato and potato inhibitors suggest that a processing protease may be present in the vacuole with a specificity for Asn-X and Gln-X bonds.

Introduction

In leaves of tomato and potato plants, wounding by chewing insects, or other severe mechanical damage, initiates the synthesis of two inhibitors of serine proteinases [9, 10], called Inhibitor I (Mr 8100) and Inhibitor II (Mr 12300) [6, 18] throughout the plants. The systemic response is caused by the release and transport of a putative wound hormone, whose activity has been associated with fragments of plant cell walls that are released from the damaged cells [4, 22, 25].
The two proteinase inhibitors are also synthesized in potato tubers where they increase in concentration throughout tuber growth, eventually comprising several percentages of the soluble proteins. The levels of inhibitors found in tubers can vary with variety [24], and in Russet Burbank potatoes, Inhibitors I and II represent about 2% and 5% respectively of the soluble proteins of the apical cortical tissues of the tubers. It is not known whether the tuber inhibitors and wound-inducible inhibitors in leaves are coded by the same genes.

In this communication we report the isolation and characterization of a complete gene coding for Inhibitor I from the Russet Burbank potato genome. The gene contains over 90% base identity with a wound-induced Inhibitor I gene from tomato [12], including both the 5' and 3' regions flanking the open reading frame. This gene is therefore considered to be a wound-inducible gene. The structure and organization of the potato Inhibitor I gene and the deduced amino acid sequence of the potato Inhibitor I protein provide new understanding of the regulation and expression of the Inhibitor I mRNA and protein processing during wound-induced gene expression in potato leaves.

Materials and methods

Materials

Restriction endonucleases, T4 ligase, DNA polymerase I (large fragment), agarose for electrophoresis and isopropylthiogalactoside were purchased from Bethesda Research Laboratories, Inc. Gathersburg, MD). Conditions for enzyme reactions were according to instructions by the supplier. Nitrocellulose paper was from Schleicher and Schuell, Inc. (Keene, NH). Reagent grade chemicals for buffers and salt solutions, 5-bromo-4-chloro-3-indolyl-B-D-galactoside (X-Gal) and ampicillin were obtained from Sigma (St. Louis, MO). Deoxy- and dideoxyribonucleotides and all M13 sequencing reagents were from P-L Biochemicals, Inc. (Milwaukee, WI). All radioisotopes ([α-32P] dATP and [α-35S] dATP) were purchased from New England Nuclear (Boston, MA). Reagents utilized for oligonucleotide primer synthesis were from Applied Biosystems (Foster City, CA). X-Omat AR film for autoradiography was from Eastman Kodak Company (Rochester, NY).

Estimation of gene copy number in genomic DNA from potato

DNA, isolated from Russet Burbank potatoes three weeks after sprouting, was digested (5–10 µg/digest) with restriction enzymes and the fragments were separated by electrophoresis on agarose gels. Hybridization of the fragments with nick-translated tomato Inhibitor I cDNA was carried out in the gels after denaturing the DNA and drying the gels. Hybridization was carried out at 42 °C in 10% dextran sulfate, 50% deionized formamide and 0.01 M NaCl for 16 h. The gels were washed twice for 30 min in 2 x SSC buffer at room temperature, then for 30 min at 55 °C in the same buffer containing 0.1% SDS. Relative size (in kb) of all restriction fragments, after electrophoresis, was determined by comparison with Hind III digested bacteriophage λ DNA as standards.

Screening of a potato genomic library

The potato genomic library utilized in this study was provided by D. M. Anderson, Director of Research, Phytogen Corporation, 101 Waverly Drive, Pasadena, CA 91105. It was constructed by ligating partial Eco RI digests (approximately 10 to 20 kb fragments) of Russet Burbank potato nuclear DNA into bacteriophage lambda (Charon 4) DNA. About 1.2 x 10^6 clones were obtained after packaging. The plate-amplified library contained approximately 5.0 x 10^9 pfu ml^-1 when titersed in this laboratory using E. coli K802 as the host strain, and a background of 7% blue plaques when plated on X-Gal.

About 3.5 x 10^5 plaques (5.0 x 10^4 per 15 cm petri plate) were screened using nick-translated tomato Inhibitor I cDNA [7] as a probe. Slight modifications of previously published methods were used for amplifying and blotting the library,