Plant Gene Register PGR97-015


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Nucleotide Sequence of a cDNA (Accession No. U76004) Encoding Rice Bowman-Birk Proteinase Inhibitor

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Abbreviations: Ose, Oryza sativa embryogenesis

Bowman-Birk proteinase inhibitors belong to the serine proteinase inhibitor family. They are small and cysteine-rich protein with a molecular mass of 8-20 kD. These proteinase inhibitors are widespread in both monocotyledons and dicotyledons and appear to function as part of the defense system against insect attack. Bowman-Birk proteinase inhibitor has double-headed homologous regions within a single peptide chain. Each region contains a reactive site for a serine proteinase. The amino acid sequence of a rice bran trypsin inhibitor (RBTI, Tashiro et al., 1987) showed a high degree of sequence homology to wheat germ trypsin inhibitor (Odani et al., 1986) and soybean Bowman-Birk inhibitor (Odani and Ikenaka, 1972). This RBTI consist of 133 amino acid residues including 18 cysteine residues which are involved in 9 disulfide bridges in the molecule. This sequence showed that RBTI is composed of 4 domains, domains I and III, and domains II and IV being homologous to the first and the second domains of soybean Bowman-Birk inhibitor, respectively (Odani and...
Ikenaka, 1972). This domain comparison demonstrated that RBTI is a duplicated structure of the Bowman-Birk type inhibitor (Tashiro et al., 1987). Although the entire amino acid sequence of RBTI has been determined (Tashiro et al., 1987), its complete cDNA sequence has not been reported.

In this paper, we report the nucleotide sequence of a rice embryo-specific cDNA clone Ose727A encoding amino acid sequences almost identical to that of RBTI (Tashiro et al., 1987). The cDNA fragment of Ose727A is 1050 nucleotides in length, consisting of a 159-nucleotide 5' untranslated region, an open reading frame of 588 bp, and a 303-nucleotide 3' untranslated region including three putative polyadenylation signals (AATAAA). The deduced translation product consist of 195 amino acid residues and the amino acid sequence of 60 to 192 shows 100% identity to the 133 amino acids of RBTI (Tashiro et al., 1987). The additional 59 amino acid residues on the N-terminal of cDNA Ose727A may serve as a signal peptide of the RBTI. In addition to the 59 amino acid residues on the N-terminus, three extra amino acids Ala-Glu-Asp on the C-terminus of the deduced protein product are not found in RBTI.

The signal peptides of proteinase inhibitor for soybean (Hammond et al., 1984), cowpea (Hilder et al., 1989), pea (Domoney et al., 1995), maize (Rohrmeier and Lehle, 1993) and alfalfa (McGurl et al., 1995) have been reported and they may serve to guide the mature proteinase inhibitor to various locations in the cell. For example, a signal peptide of 44 or 55 amino acid residues in alfalfa trypsin inhibitor (ATI) may target the ATI to the ER and then eventually deposit it in the vacuole of the cell (McGurl et al., 1995). Whether the signal peptide reported in the Ose727A is responsible for targeting the mature RBTI to its appropriate location requires further investigation. We are currently using in situ localization to identify the location of RBTI in the cell.

Acknowledgments:

This study was supported by grants from the National Science Council, Republic of China. We thank Dr. Tim Setter for the valuable comments and polishing on the manuscript.

Table 1. Characteristics of a rice cDNA clone Ose727A - a Bowman-Birk proteinase inhibitor
Organism:

Rice (Oryza sativa L. var. Lomello)
Gene location:
Multiple copy nuclear gene revealed from Southern hybridization

Gene Product:
A full-length cDNA clone encoding a Bowman-Birk type proteinase inhibitor

Source:
cDNA library in Lamda UniZAP XR vector (Stratagene, USA) constructed using poly(A) mRNA isolated from rice embryos 10-days after pollination (DAP).

Techniques:
Differential screening; in vivo pBluescript plasmid excision; restriction fragment subcloning; dideoxy sequencing of both strands; Northern and Southern blot hybridization.

Methods of identification:
Nucleotide sequences of Ose727A and the deduced translation product were compared with the sequence data in GenBank through the BLAST algorithm. The Ose727A was identified as Bowman-Birk type trypsin inhibitor based on its amino acid sequence homology with a known rice Bowman-Birk type trypsin inhibitor (Tashiro et al., 1987).

Features of the deduced proteins:
The deduced protein has a calculated molecular mass of 21.6 kD including a 59 amino acid residues of putative signal peptide and a 136 residues of mature proteinase inhibitor. The mature protein is almost identical to RBTI (Tashiro et al., 1987) including 18 cysteine residues which make 9 disulfide bonds and this protein contains 4 reactive sites for proteinase binding. The estimated PI value is 5.18.

Expression characteristics:
Embryo-specific expression. The size of the transcript for Ose727A is about 1.1 kb, measured by RNA gel hybridization assay.

Literature Cited:


