Isolation of a New Member of the Soybean Kunitz-Type Proteinase Inhibitors

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Proteinase inhibitors are present in almost all organisms (Ryan, 1981). Most of these proteins are specific in their interaction with proteinases, inhibiting the proteolytic activity. In plants, this protein family is highly abundant in seeds and tubers of many species. Since high levels of proteinase inhibitor in agricultural crops may result in alterations of the digestive enzymes in human and animals, research has been targeted toward modification of the proteinase inhibitor levels in those plant species (Ryan, 1981).

In soybean (Glycine max), among others, two important families of proteinase inhibitors have been characterized: the Kunitz inhibitor, which shows specificity for trypsin (Ryan, 1981; Kim et al., 1985), and the Bowman-Risk, which inhibits trypsin, chymotrypsin, and elastase (Ryan, 1981, 1988; Tan-Wilson, 1988). These proteins serve an important function in storage protein metabolism by regulating the level of proteinase activity during seed development (Ryan, 1988). It has been shown that they also play an important role against pathogen attack by acting as endogenous insecticides (Hilder et al., 1986).

During screening of a λgt11 cDNA library, we isolated a cDNA clone with an insert size of 802 bp. This insert contains a unique open reading frame of 624 bp, which encoded for a 208-amino-acid polypeptide (Table I). Sequence comparison with other proteins contained in the data base shows a high level of homology to the Kunitz trypsin inhibitor family. Southern blot analysis of the soybean Kunitz trypsin inhibitor indicates that this protein family contains at least 10 members, some of which are organized in tandem pairs (Jofuku and Goldberg, 1989). At present, four of these members have been cloned and characterized (Jofuku and Goldberg, 1989; Song et al., 1993). The KTi3 and KTi-b genes encode for the major Kunitz trypsin inhibitor proteins, TP and TPb, respectively (Kim et al., 1985). Studies of the active site indicated that two amino acids, Arg63 (P1) and Ile64 (P1'), are essential for activity. Thus, trypsin inhibitor activity is detected only when the P1 residue is occupied by Arg or Lys. Loss of specificity or activity occurs when P1 is substituted by other amino acids (Kowalski and Laskowski, 1976; Ryan, 1981). Since the new soybean KTi-S gene contains a Lys residue at position P1, we should expect that the encoded protein has trypsin inhibitor activity. Northern blot analysis of total RNA from soybean cell suspension indicated that the KTi-S transcript is highly abundant in cell suspension under mixotrophic growth condition.

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LITERATURE CITED


Table I. Characteristics of a cDNA coding for a Kunitz trypsin inhibitor protein, KTi-S

| Organism: | Glycine max cv Corsoy. |
| Function: | Trypsin inhibitor (Kunitz type). |
| Source: | cDNA library in λgt11 vector constructed from poly(A)+ RNA from a mixotrophic soybean cell suspension. |
| Sequence Strategy: | Nucleotide sequence was determined by the dideoxy chain termination method, using double-stranded DNA as template. |
| Features of cDNA Structure: | The full-length cDNA clone contains 42 bp of 5' untranslated region, an open reading frame of 624 bp, and 136 bp of 3' untranslated region. |
| Features of Deduced Protein: | Open reading frame of 208 amino acids. The first 25 amino acids at the N terminus correspond to a signal sequence. The active site contains the residues required for trypsin inhibitor activity. |

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