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Kunitz Soybean Trypsin Inhibitor is Modified at its C-terminus by Novel Soybean Thiol Protease (Protease T1)

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Abstract: Kunitz soybean trypsin inhibitor (KSTI) is hydrolyzed during seed germination to yield amino acids needed to support initial seedling growth. The type of KSTI from *Glycine max* (L.) Merrill cv. Toyokomachi is KSTI-T*i*^{b}. The KSTI-T*i*^{b} from 4-day-old post-germination cotyledons (KSTI-T*i*$^{b'}$) has 3 or 4 amino acid residues cleaved off at the C-terminus. This KSTI modification is important to understand the mechanism of degradation in seed reserve proteins by proteases. Protease K1 also cleaves amino acid residues at the C-terminus of KSTI but it removes 5 amino acid residues. Therefore, we presumed the KSTI-T*i*$^{b'}$ was produced by a protease other than protease K1. In this study, the protease T1 responsible for cleavage of KSTI-T*i*^{b} at the C-terminus was purified. The enzyme was estimated to have a molecular mass of 33 kDa from its mobility on SDS-PAGE gels. The N-terminal amino acid sequence of the purified protease T1 corresponded to amino acids Phe-73 to Phe-92 of both thiol protease isoforms A and B from the soybean leaf, and shared 83% identity with the partial amino acid sequence of the membrane-associated cysteine protease from mung bean seedlings, a protease known to perform post-translational cleavage of C-terminal peptides of target proteins. Finally, this enzyme was shown to convert KSTI-T*i*^{b} to KSTI-T*i*$^{b'}$.

Keywords: C-terminus degradation, Inhibitory activity, Kunitz soybean trypsin inhibitor, Protease T1

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