Molecular cloning and characterization of a cDNA encoding asparaginyl endopeptidase from sweet potato (Ipomoea batatas L. Lam) senescent leaves

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Abstract

Asparaginyl endopeptidase is a cysteine endopeptidase that has strict substrate specificity toward the carboxyl side of asparagine residues, and is possibly involved in the post-translational processing of proproteins. In this report one full-length cDNA, SPAE, was isolated from senescent leaves of sweet potato (Ipomoea batatas (L.) Lam). SPAE contained 1479 nucleotides (492 amino acids) in the open reading frame, and exhibited high amino acid sequence homologies (c. 61–68%) with asparaginyl endopeptidases of Vicia sativa, Phaseolus vulgaris, Canavalia ensiformis, and Vigna mungo. SPAE probably encoded a putative precursor protein. Via cleavage of the N- and C-termini, it produced a mature protein containing 325 amino acids (from the 51st to the 375th amino acid residues), the conserved catalytic residues (the 173rd His and 215th Cys amino acid residues), and the putative N-glycosylation site (the 332nd Asn amino acid residue). Semi-quantitative RT-PCR and western blot hybridization showed that SPAE gene expression was enhanced significantly in natural senescent leaves and in dark- and ethephon-induced senescent leaves, but was much less in mature green leaves, stems, and roots. Phylogenic analysis showed that SPAE displayed close association with vacuolar processing enzymes (legumains/asparaginyl endopeptidases), which function via cleavage for proprotein maturation in the protein bodies during seed maturation and germination. In conclusion, sweet potato SPAE is probably a functional, senescence-associated gene and its mRNA and protein levels were significantly enhanced in natural and induced senescent leaves. The possible role and function of SPAE associated with bulk protein degradation and mobilization during leaf senescence were also discussed.

Key words: Asparaginyl endopeptidase, dark, ethephon, leaf senescence, sweet potato.