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Plant-specific insertions in the soybean aspartic proteinases, soyAP1 and soyAP2, perform different functions of vacuolar targeting

Kaede Terauchi[a], Tomiko Asakura[a,b,*], Haruko Ueda[c], Tomoko Tamura[a], Kentaro Tamura[c], Ichiro Matsumoto[a], Takumi Misaka[a], Ikuko Hara-Nishimura[c], Keiko Abe[a]

[a]*Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan*
[b]*Laboratory of Food Science, Atomi Junior College, Bunkyo-ku, Tokyo 112-8687, Japan*
[c]*Department of Botany, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan*

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Summary

Most aspartic proteinases (APs) of plant origin are characterized by the presence of plant-specific insertion (PSI) in their primary structure. PSI has been reported to function as signals for both transport of AP molecules from the endoplasmic reticulum (ER) and for their targeting to the vacuole. To determine the functions of the PSIs in soyAP1 and soyAP2 identified in our previous study, we examined their subcellular localization by transient expression of a green fluorescent protein (GFP) fusion protein in the protoplasts of *Arabidopsis* suspension-cultured cells. Both soyAP1-GFP and soyAP2-GFP were targeted to the vacuole. To confirm the role of the PSI, we prepared PSI-deleted soyAP1 and soyAP2, and investigated their vacuolar targeting by the same method. While the former deletion mutant was always transported to the vacuole, the latter sometimes remained in the ER and was only sometimes transported to the vacuole. These observations indicated that, in the case of soyAP1, the PSI is not involved in vacuolar targeting, also suggesting that the function of the PSI differs depending on its origin.

Abbreviations: AP, aspartic proteinase; ER, endoplasmic reticulum; GFP, green fluorescent protein; PSI, plant-specific insertion.

*Corresponding author. Laboratory of Food Science, Atomi Junior College, 1-5-2 Otsuka, Bunkyo-ku, Tokyo 112-8687, Japan. Tel.: +81 3 3941 5483; fax: +81 3 3941 8333.
E-mail address: asakura@mail.ecc.u-tokyo.ac.jp (T. Asakura).

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Introduction

Aspartic proteinases (APs) belong to EC 3.4.23, a class of proteolytic enzymes that have two particular aspartic acid residues in the active center. Many typical plant APs are comprised of both pre- and pro-regions as well as a C-terminal region containing a plant-specific insertion (PSI)(Mutlu and Gal, 1999; Simões and Faro, 2004). The pro-form of each AP is processed into the mature enzyme by removal of both the pro-sequence and the PSI by autolysis (Ramalho-Santos et al., 1998; White et al., 1999; Park et al., 2001; Castanheira et al., 2005). A previous study indicated that the mature enzyme is formed by removal of the PSI after transport to the vacuole (Glathe et al., 1998). In other studies, recombinant PSI-deleted mutants were shown to retain the original AP activities (Asakura et al., 2000; Törmäkangas et al., 2001).

APs exist in vacuoles, which are weakly acidic, and are probably involved in the degradation of used proteins. Many digestive enzymes as well as APs have vacuole targeting signals in their N-terminal pro-sequences and C-terminal sequences. NPIR in barley aleurain is an example of an N-terminal vacuole targeting signal (Holwerda et al., 1992), while the sequence VFAEAIAANSTLVAE in barley lectin is an example of a C-terminal targeting signal (Bednarek and Raikhel, 1991). The PSI has thus been investigated as a vacuolar targeting signal in AP molecules (Gal and Raikhel, 1993; Neuhaus and Rogers, 1998; Marty, 1999; Vitale and Raikhel, 1999; Törmäkangas et al., 2001). The PSI has been reported to be involved in export from the endoplasmic reticulum (ER) for vacuolar targeting in the barley AP, phytepsin (Törmäkangas et al., 2001). On the other hand, cardoon AP (cardosin B), which has a PSI, is not accumulated in the vacuole but is transported out of the cell (Vieira et al., 2001). Thus, the role of the PSI in vacuolar targeting is not unequivocal.

Previously, we reported the two soybean APs, soyAP1 expressed in dry seed and soyAP2 expressed in a variety of vegetative tissues (Terauchi et al., 2004). As *soyAP1* mRNA differs from *soyAP2* mRNA in terms of period- and tissue-specific expression, these two enzymes are expected to have different patterns of intracellular localization. The present study was performed to investigate the subcellular localization of soyAP1 and soyAP2. Since PSI is a candidate of the vacuolar sorting signal, we prepared a PSI deletion mutant and investigated its effect on the sorting.

Materials and methods

Plasmid construction

Green fluorescent protein (GFP) fused soyAPs were expressed under the control of the 35S promoter. GFP was fused to each of the C-termini of the full-length structures, soyAP1 and soyAP2, and the PSI-deleted mutants, soyAP1ΔPSI and soyAP2ΔPSI, with a four-glycine residues insertion as a linker (Figs. 1A–D). To prepare soyAP1ΔPSI, the 104 amino acids from residue 323Q to 426M were removed, while the 103 amino acids from residue 318V to 420S were removed to prepare soyAP2ΔPSI (Figs. 1C and D). The plasmid containing synthetic GFP (S65T), pTH-2 (CaMV35S promoter-GFP(S65T)-NOS terminator with pUC18 backbone)(Chiu et al., 1996), was provided by Dr. Yasuo Niwa, University of Shizuoka. The sense primer for soyAP1-GFP fusion protein and soyAP1ΔPSI-

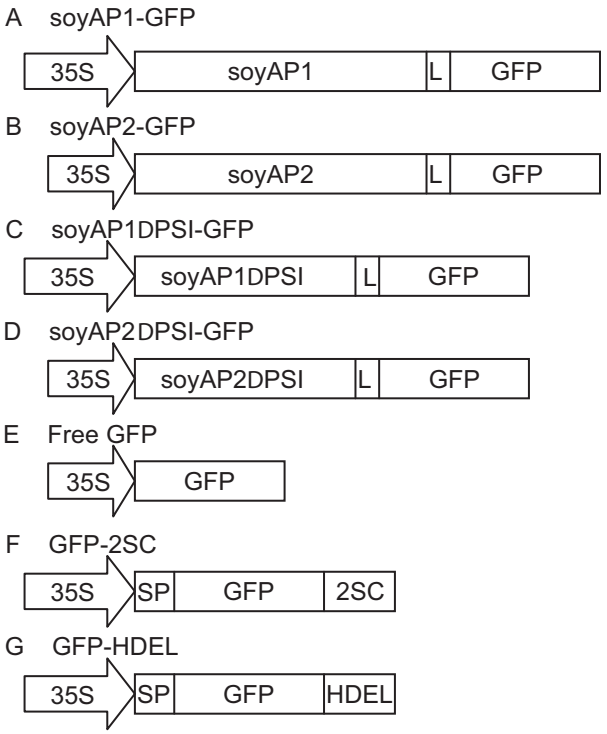

Figure 1. Constructs of GFP fusion protein expression vectors under control of the 35S promoter. (A) Full-length soyAP1 (soyAP1-GFP). (B) Full-length soyAP2 (soyAP2-GFP). (C) SoyAP1-deleted PSI (soyAP1ΔPSI-GFP). (D) SoyAP2-deleted PSI (soyAP2ΔPSI-GFP). (E) Free GFP. (F) GFP-2SC. (G) GFP-HDEL. 35S, cauliflower mosaic virus 35S promoter; L, linkers comprised of four glycine residues combined with the C-termini of soyAPs; GFP, synthetic GFP (S65T); SP, signal peptide of pumpkin 2S albumin; 2SC, C-terminal 18-amino acid sequence of pumpkin 2S albumin including a putative vacuolar-targeting signal; HDEL, ER retention signal.