496(1aJ05)

VACUOLAR PROCESSING ENZYME IS SELF-CATALYTICALLY ACTIVATED BY SEQUENTIAL REMOVAL OF THE C-TERMINAL AND N-TERMINAL PROPEPTIDES

Nagako HIRAIWA[1, 2], Takeshi MIZUTANI[3], Yumiko MAKINO[3], Akio MURAKAMI[3, 4], Mikio NISHIMURA[1], Ikuko HARA-NISHIMURA[1, 5], ([1] Dept. Cell Biol., Nat. Inst. Basic Biol., Okazaki 444-8585, [2]JT Biohistory Research Hall, Osaka 569-1125, [3]Analytical Instruments, Nat. Inst. Basic Biol., Okazaki 444-8585, [4]Research Center for Inland Seas, Kobe Univ., Kobe 656-2401, [5] Dept. Bot., Grad.Sch.Sci, Kyoto Univ., Kyoto 606-8502)

A vacuolar processing enzyme (VPE) responsible for maturation of various vacuolar proteins are synthesized as an inactive precursor. To clarify how to convert the VPE precursor into the active enzyme, we expressed point mutated VPE precursors of castor bean in Saccharomyces cerevisiae. A VPE with a substitution of the active site showed no ability to convert itself into the mature form. The mutated VPE was converted by the action of the VPE that had been purified from castor bean. Substitution of the conserved Asp-Asp at the putative cleavage site of the C-terminal propeptide with Gly-Gly abolished both the conversion into the mature form. In vitro assay with synthetic peptides demonstrated that a VPE exhibited an activity towards Asp residue to remove the N-terminal propeptide. Taken together, the results indicate that the VPE is self-catalytically maturated to be converted into the active enzyme by removal of the C-terminal propeptide and the N-terminal one.[1)]

1)Hiraiwa et al. (1999) FEBS Lett., 447, 213-216

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PROTEOLYTIC MOBILIZATION OF RIBULOSE 1,5-BISPHOSPHATE CARBOXYLASE/OXYGENASE (Rubisco) IN SENESCING *Phaseolus vulgaris* LEAVES

Takao MINAMIKAWA, Kiminori TOYOOKA, Takashi OKAMOTO, Ikuko HARA-NISHIMURA[1], Maki KONDO[2], Mikio NISHIMURA[2] (Dept. Biol. Sci., Tokyo Metropolitan Univ., Hachioji, Tokyo 192-0897, [1]Dept. Bot., Kyoto Univ., Kyoto 606-8502, [2]Dept. Cell Biol., Natl. Inst. Basic Biol., Okazaki 444-8585)

Mainly using protein immunoblot and immunoelectron microscopic techniques, the mobilization of Rubisco was observed in detached primary leaves of *P. vulgaris* plants during senescence (4 to 8 days at 25^{0}C in darkness). Immunoblotting indicated that the amounts of both Rubisco LSU and SSU during senescence decreased, while polypeptides of smaller mol wt that were immunoreactive to LSU were detected on the blots. The amount of EP-C1 (one of the major cysteine proteinases in *P. vulgaris* plants and immunoreactive with antibody to *Vigna mungo* SH-EP) increased but that of VmPE-1 (a putative processing enzyme of EP-C1) decreased. During the senescence, number of chloroplasts in a leaf cell decreased (day 4), and their shapes turned round and disordered (day 8). Gold particles from the anti-SH-EP antiboday were observed only in the vacuole in fresh leaves, but, in senescing leaves, they were also localized in chloroplasts that came into contact with the vacuole or were enclosed by it. The results indicate the involvement of vacuolar proteinases in the Rubisco degradation in senescing leaves.

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Regulation of Endopeptidase in Cucumber Leaves -Regulation by endogenous specific inhibitors-

Yasuo YAMAUCHI, Yukinori EJIRI, Kiyoshi TANAKA, Lab. Plant Biotech., Fac. Agr., Tottori Univ., Tottori 680-0945

Cucumber (*Cucumis sativum* L.) leaves contain two major proteases, one is an endopeptidase of pI 4.5 cleaving the C site of Glu residue, and another is an endopeptidase of pI 5.0 cleaving the C sites of Arg and Lys. Previously, we reported that CEP 5.0 (Cucumber Endopeptidase of pI 5.0) is a chloroplast serine endopeptidase regulated by Arg, ATP and Mg^{2+}. Here, we report that activity of CEP 4.5 is regulated by endogenous specific inhibitors.

We searched inhibitory activity of CEP 4.5 in cucumber leaves, in result, at least 3 biochemically different inhibitors were present. We purified a heat stable, non-competitive inhibitor, with molecular weight of 25 kDa, and found that it was specific inhibitor against CEP 4.5 because of showing no inhibition against other animal or bacterial endopeptidases.

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ANALYSIS OF THE REGULATORY SUBUNITS OF Clp PROTEASE IN CHLOROPLAST

Kazumi NAKABAYASHI, Masaki ITO, Akira WATANABE, Dept. Biol. Sci., Grad. Sch. Sci., Univ. Tokyo, Tokyo 113-0033

Plant Clp protease is localized in chloroplasts and consists of two types of subunits. One is the regulatory subunit, ClpC, which possesses ATPase activity, and the other is the catalytic subunit, ClpP. *Arabidopsis* contains ClpC as regulatory subunits besides ERD1 that is isolated earlier as one of the dehydration responsive clones. We isolated cDNA from *Arabidopsis clpC* and examined its gene expression. Accumulation pattern of transcripts from *AtclpC* was apparently distinct from that from *Erd1*, whose level increased in such processes as dehydration, dark treatment and natural senescence. The results suggested functional difference between these two regulatory subunits. In order to characterize these two regulatory proteins, we prepared the mature proteins by overexpression of corresponding cDNA sequences in *E. coli* and determined their ATPase activities. Results of the *in vitro* experiments will be reported.