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Protease Activity in Plant Tissues (VIII)

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Abstract

Caseinolytic and 4-nitroanilide hydrolytic activities of various plant tissues were examined. Favorable 4-nitroanilide hydrolytic activity was found in the extracts of rhizome of Freesia, *Freesia refracta* (Jacq.) Klatt. No attractive caseinolytic activity was found in a series of the present plant tissues.

Key words: plant protease; aminopeptidase; endopeptidase.

Introduction

The plant tissues of some species have a remarkable protease activity. The physiological significance of the protease have little known. Typical plant proteases so far isolated have belonged mainly to the cysteine protease group (1). In the plant proteases, enzymatic properties of papain [EC 3. 4. 22. 2] (2) has been considerably investigated. We recently isolated a cysteine protease: phytolacain (formerly called phytolacin) from sarcocarp of pokeweed, *Phytolacca americana* (3). The enzyme was different from papain in the substrate specificity for some synthetic substrates (3). The results show that a new type of cysteine proteases are present in other plants. As the continuation of our previous papers (4-10), we attempted the screening test to find a new type plant protease.

Experimental

Foliage plants were obtained from flower shops, fruits and cereals were purchased from greengrocers and other plants were collected locally in Kagoshima prefecture. Casein was a product of E. Merck, Darmstadt, West Germany. Ala-pNA, Leu-pNA were obtained from Peptide Institute, Inc.; Osaka. Other reagents were purchased from Wako Pure Chemical Industries Ltd.

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Preparation of Sample Solution for Proteolytic Activity Assay-All samples were added to equal weight of 0.067 M phosphate buffer, pH 7.3, and homogenated with mixer. The homogenates were filtered through a cotton cloth and centrifuged for 10 min at $3000 \times g$. The proteins of the supernatant were precipitated with adding sodium sulfate to 20-25% (w/v). The resultant precipitate was collected with centrifugation, and diluted to the point of appropriate concentration for assay with 0.067 M phosphate buffer, pH 7.3.

Assay of Protease-Proteolytic activity was measured by the method of Kunitz (11) with casein as a substrate. One ml of sample solution was preincubated for 10 min at 30° , and then added to 1 ml of a solution of 0.067 M phosphate buffer containing 1% (w/v) casein, pH 7.3, at 30° . After incubation for 30 min the reaction was terminated by the addition of 3 ml of 5% trichloroacetic acid. After standing for 30 min at room temperature, the precipitate was removed by filtration through Toyo filter paper No. 5C and the absorbancy at 280 nm of the trichloroacetic acid-soluble peptides formed was determined with Hitachi spectrophotometer U-1100.

The rates of enzymatic hydrolysis of 4-nitroanilide substrates were followed spectrophotometrically in 0.067 M phosphate buffer, pH 7.3, at 410 nm with the spectrophotometer.

A unit of activity was defined as that amount which yielded 0.001 $A_{280\text{nm}}$ (0.001 $A_{410\text{nm}}$) unit of change per min in a 1-cm cell under the conditions mentioned above. The specific activity is expressed as the number of enzyme units per 1 ml of juice.

Results and Discussion

The results of the screening test are shown in Table 1 and Table 2.

Caseinolytic activity was observed in two plants (Table 1). The extracts of rhizome of Common Hyacinth, *Hyacinthus orientalis* L. and leaf of Hyssop, *Hyssopus officinalis* L. had caseinolytic activity. But this activity units were very small compared to the previous data (4-10). Other plant tissues had not any proteolytic activity.

The extracts of four plants hydrolyzed 4-nitroanilide (Table 2). The bulb of Freesia, *Freesia refracta* (Jacq.) Klatt showed for Ala-pNA and Leu-pNA. The activities for both substrates were the largest compared with the previous data (8). Bulb of Freesia also has a high caseinolytic activity (9). Subsequently the extracts of bead free fruit (drupes), *Melia azedarach* L. var. japonica Makino and leaf of parsley, *Petroselinum crispum* (Mill.) Nyman ex A.W. Hill strongly hydrolyzed Leu-pNA. The results suggested that the plant tissues contained relatively stable aminopetidases. The *Melia azedarach* L. var. japonica Makino, was found in the west area of Japan. The fruit of *Melia azedarach* have been used for the treatment of chaps and chilbains in the skin in Japan. The fruits containing large amount of protease were interested, but no enzyme have been so far isolated from *Melia azedarach* fruit. Therefore, we are proceeding the purification of the protease from the fruits of *Melia azedarach*.

Table 1. Caseinolytic Activity of Extracts from Plant Tissues

Plant	Plant parts	Activity (Units)	
		Leu-pNA	Ala-pNA
Akou (<i>Ficus superba</i> Miq. var. <i>japonica</i> Miq.)	Sarcocarp	0	
Berugamottominto, Bargamot Mint (<i>Mentha × piperita</i> L. var. <i>citrata</i> (J. F. Ehrh.) Briq.)	Leaf	0	
Gama, Common Cat-Tail (<i>Typha latifolia</i> L.)	Ear	0	
Hisoppu, Hyssop (<i>Hyssopus officinalis</i> L.)	Leaf	10	
Hiyashinsu, Common Hyacinth (<i>Hyacinthus orientalis</i> L.)	Rhizome	8	
Itarianpaseri, Italian Parsley (<i>Petroselinum crispum</i> "Neapolitanum")	Leaf	0	
Jinjyaminto, Red Mint (<i>Mentha × gentilis</i> L.)	Leaf	0	
Komonseiji, Common Sage (<i>Salvia officinalis</i> L.)	Leaf	0	
Komontaimu, Common Thyme (<i>Thymus vulgaris</i> L.)	Leaf	0	
Kurariseiji, Fetid Clary Sage (<i>Salvia sclarea</i> L.)	Leaf	0	
Monaruda, Bee-balm (<i>Monarda didyma</i> L.)	Leaf	0	
Otomeyuri, Rosy Lily (<i>Lilium rubellum</i> Baker)	Rhizome	0	
Oregano, Common Marjoram (<i>Origanum vulgare</i> L.)	Leaf	0	
Painappuruseiji, Pineapple Sage (<i>Salvia elegans</i> Vahl)	Leaf	0	
Rubabu, Rhubarb (<i>Rheum rhabarbarum</i> L.)	Leaf	0	
Soreru, Suiba, Common Sorrel (<i>Rumex acetosa</i> L.)	Leaf	0	
Tsubaki, Common Camellia (<i>Camellia japonica</i> L.)	Immature fruit	0	

Table 2. 4-Nitroanilide Hydrolytic Activity of Extracts from Plant Tissues

Plant	Plant parts	Activity (Units)	
		Leu-pNA	Ala-pNA
Furijjia, Freesia (<i>Freesia refracta</i> (Jacq.) Klatt)	Bulb	605	1,470
Paseri, Parsley (<i>Petroselinum crispum</i> (Mill.) Nyman ex A.W. Hill)	Leaf	190	18
Sendan, Bead Tree (<i>Melia azedarach</i> L. var. <i>japonica</i> Makino)	Sarcocarp	40	14
Tingensai (<i>Brassica rape</i> var. <i>chinensis</i>)	Leaf	24	24

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