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PROTEASE ACTIVITY IN PLANT TISSUES (VI)

By

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Abstract

Caseinolytic activity of various plant tissues were examined. Favorable protease activity was found in the extracts such as bulbs of Freesia, *Fresia refracta* Klatt, sarcocarp of Andes melon, *Cucumis melo* ssp. *melo* 'Andes', sarcocarp of Papaya melon, *Cucumis melo* L. var. Papaya and seeds of Black gram, *Vigna mungo* (L.) Hepper.

Introduction

Typical plant proteases so far isolated have belonged mainly to the cysteine protease group. In the plant proteases, enzymatic properties of papain [EC. 3.4.22.2] (1) has been considerably investigated. We recently isolated a cysteine protease from sarcocarp of pokeweed, *Phytolacca americana* (2). The enzyme was different from papain in the substrate specificity for some synthetic substrates (2). As the continuation of our previous papers (3-6), we attempted the screening test to find a new type plant protease.

Experimental

Flower and 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; Other reagents were purchased from Wako Pure Chemical Industries Ltd.

Preparation of Sample Solution for Caseinolytic Activity Assay—All samples were ground in equal weight of 0.067 M phosphate buffer, pH 7.3, in a mortar. The homogenates were stirred for 5 min and filtered through a cotton cloth or centrifuged for 10 min at 3000×g.

The extracts were 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 (8) 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 1% (w/v) casein containing 0.067 M phosphate buffer, pH

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Table 1. Caseinolytic Activity of Extracts from Plant Tissues

Plant	Plant parts	Activity (Units)
Afurikanmarigorudo, African Marigold (<i>Tagetes erecta</i> L.)	Flower, Stem, Leaf	0
Andesumeron, Andes Melon (<i>Cucumis melo</i> ssp. <i>melo</i> 'Andes')	Sarcocarp	320
Aserora, Barbados Cherry (<i>Malpighia glabra</i> L.)	Fruit	0
Ashidansera, Peacock Orchid (<i>Acidantha bicolor</i> var. <i>murielae</i>)	Rhizome	0
Benibana, Safflower (<i>Carthamus tinctorius</i> L.)	Leaf	0
	Flower	0
Chidomegusa, Water Pennywort (<i>Hydrocotyle sibthorpioides</i> Lam.)	Leaf	0
Chidorigusa (<i>Gymnadenia conopsea</i> R. Br.)	Whole	0
Chigaya (<i>Imperata cylindrica</i> Beauv.)	Flower	0
Chingensai (<i>Brassica rapa</i> var. <i>chinensis</i> (L.) Kitam.)	Leaf	0
	Seed	7
Farenopusiu (<i>Dendrobium phalaenopsis</i> R. Fitzg.)	Flower	0
	Stem	70
Fudansou, Leaf Beet (<i>Bata vulgaris</i> L. var. <i>cicla</i> L.)	Seed	0
Furijia, Freesia (<i>Freesia refracta</i> Klatt)	Bulb	617
Gabera (<i>Gerbera viridifolia</i> Schultz-Bip.)	Flower, Stem, Leaf	0
Gakuajisai (<i>Hydrangea macrophylla</i> Seringe var. <i>otaksa</i> Makino)	Leaf	0
Gima (<i>Vaccinium wrightii</i> A. Gray)	Fruit	0
Gosun-ingen, Common Bean, 'Kentucky Wonder' (<i>Phaseolus vulgaris</i> L.)	Seed	47
Gurajiorasu, Corn Flag (<i>Gladiolus gandavensis</i> Van Houtt.)	Flower, Stem, Leaf	0
Hageitou, Tampala (<i>Amaranthus gangeticus</i> L.)	Stem, Leaf	0
Haibusshuburuberi, Highbush Blueberry (<i>Vaccinium corymbosum</i> L.)	Sarcocarp	0
Hanabegonia, Christmas Begonia (<i>Begonia cheimanta</i> T. H. Everett)	Flower, Stem, Leaf	0
Himebashou (<i>Musa coccinea</i> Andr.)	Flower	0
	Stem	0
Himehiogisuisen (<i>Tritonia</i> (= <i>Montbretia</i>) <i>crococmaeflora</i> Lemoine)	Leaf	0
	Bulb	0

(Continued on the following page)

(from the Table 1)

Housenka, Garden Balsam (<i>Impatiens balsamina</i> L.)	Flower, Stem, Leaf	0
Inuhouzuki (<i>Solanum nigrum</i> L.)	Fruit	0
Jinchouge, Winter Daphne (<i>Daphne odora</i> Thunb.)	Seed	3
Kabu, Turnip (<i>Brassica rapa</i> L.)	Stem	5
Kaneshon, Carnation (<i>Dianthus caryophyllus</i> L.)	Flower	0
	Stem, Leaf	10
Karajumu, Caladium (<i>Caladium bicolor</i> Vent.)	Rhizome	58
Karifurawa, Cauliflower (<i>Brassica oleracea</i> var. <i>botrytis</i> L.)	Flower	0
Katorea, Cattleya (<i>Cattleya labiata</i> Lindl.)	Flower	0
Ketsuruazuki, Black Gram (<i>Vigna mungo</i> (L.) Hepper)	Seed	110
	Germ	12
	Leaf	0
	Root	1
	Leaf	0
Kibi, Millet (<i>Panicum miliaceum</i> L.)		
Kiui, Chinese Gooseberry (<i>Actinidia chinensis</i> Planch.)	Leaf	0
Kiku, Chrysanthemum (<i>Chrysanthemum morifolium</i> Ramat. var. <i>sinense</i> Makino)	Flower	0
Kishoubu, Yellow Flag (<i>Iris pseudacorus</i> L.)	Rhizome	0
Kobushi (<i>Magnolia kobus</i> DC.)	Leaf	0
Koshou, Black Pepper (<i>Piper nigrum</i> L.)	Seed	0
Kudamonotokeisou, Passion Fruit (<i>Passiflora edulis</i> Sims)	Sarcocarp	0
Kusu, Camphora-Tree (<i>Cinnamomum camphora</i> Sieb.)	Fruit	0
Madake (<i>Phyllostachys bambusoides</i> Sieb. et Zucc.)	Sprout	30
Memegunbainazuna (<i>Lepidium virginicum</i> L.)	Leaf	2
	Root	5
Myouga (<i>Zingiber mioga</i> Rosc.)	Leaf	0
Narukoran (<i>Polygonatum odoratum</i> (Mill.) Druce)	Stem, Leaf	8
Noazami (<i>Cirsium japonicum</i> DC.)	Leaf	0
	Flower	0
Ojigisou, Action Plant (<i>Mimosa pudica</i> L.)	Stem, Leaf	0
Okura, Okra (<i>Hibiscus esculentus</i> L.)	Fruit	0
Oba-akou (<i>Ficus caulocarpa</i> Miq.)	Fruit	0

(Continued on the following page)

(from the Table 1)

Obako, Plantain (<i>Plantago asiatica</i> L.)	Flower	0
Orandagarashi, Watercress (<i>Nasturtium officinale</i> R.Br.)	Stem, Leaf	0
Papaiyameron, Papaya Melon (<i>Cucumis melo</i> L. var. <i>Papaya</i>)	Sarcocarp	127
Radisshu, Radish (<i>Raphanus sativus</i> L. var. <i>sativa</i>)	Seed	10
Reddokyabetsu (<i>Bassica oleracea</i> var. <i>capitata</i> 'Rubyball')	Leaf	0
Renbu, Wax Jambu (<i>Eugenia javanica</i> Lam.)	Fruit	0
Sapojira, Chewinggum-Tree (<i>Achras zapota</i> L.)	Fruit	0
Sakurajimadaikon (<i>Raphanus sativus</i> 'Sakurajima-dai kon')	Seed	0
Sakura-nadeshiko, Chinese Pink (<i>Dianthus chinensis</i> L.)	Flower	0
	Stem, Leaf	5
Seiyoubara, Cabbage Rose (<i>Rosa centifolia</i> L.)	Flower	0
	Stem, Leaf	0
Sen-narihyoutan (<i>Lagenaria leucantha</i> Rusby var. <i>microcarpa</i> Hara)	Seed	0
Senryou (<i>Chloranthus glaber</i> Makino)	Fruit	0
Shakuyaku, Chinese Peony (<i>Paeonia lactiflora</i> Pall.)	Flower	0
	Stem, Leaf	0
Shiran (<i>Bletilla striata</i> (Thunb.) Rchb. f.)	Sarcocarp	0
Shishitougarashi (<i>Capsicum annuum</i> L. var. <i>angulosum</i> Mill.)	Seed	0
Sukashiyuri, Thunberg Lily (<i>Lilium elegans</i> Thunb.)	Flower, Stem, Leaf	0
Sutatisu (<i>Limonium sinuatum</i> (L.) Mill.)	Flower, Stem	0
Sutokeshia, Stokesia (<i>Stokesia laevis</i> Greene)	Flower	0
	Stem, Leaf	0
Tachiaoi, Hollyhock (<i>Alcea rosea</i> L.)	Flower	0
Tamashida, Ladder Fern (<i>Nephrolepis cordifolia</i> (L.) K. Presl)	Rhizome	0
Tetorasutiguma, Javan Grape (<i>Cissus voinierianum</i> hort.)	Flower, Stem, Leaf	0
Torukokikyou, Balloon Flower (<i>Platycodon grandiflorus</i> (Jacq.) A. DC.)	Flower	0
	Stem, Leaf	0
Turigane-ninjin, Ladybells (<i>Adenophora triphylla</i> var. <i>japonica</i> (Regel) Hara)	Root	2
Yuzuriha (<i>Daphniphyllum macropodum</i> Miq.)	Leaf	0

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 was determined with Hitachi spectrophotometer 100-60.

A unit of activity was defined as that amount which yielded 0.001 $A_{280\text{nm}}$ 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.

Caseinolytic activity was observed in several plants. The extracts of bulb of Freesia, *Freesia refracta* Klatt and sarcocarp of Andes melon, *Cucumis melo* ssp. *melo* 'Andes' had high proteolytic activity. Subsequently sarcocarp of Papaya melon, *Cucumis melo* L. var. Papaya and seeds of Black gram, *Bigna mungo* (L.) Hepper displayed caseinolytic activity more than 100 units. Small protease activity were present in stem of Phalaenopsis, *Dendrobium phalaenopsis*, seeds of Common Bean, *Phaseolus vulgaris* L., Rhizome of Caladium, *Caladium biocolor* Vent and sprout of *Phyllostachys bambusoides* Sieb. et Zucc. Comparing the activity with sarcocarp of snake gourds (4, 5), the units on this report were not so large. By further investigation, the proteases of Andes melon and Papaya melon were confirmed to be the same serine protease described in previous papers (3-7). The enzyme from Freesia, Black gram, Common Bean and bamboo sprout of *Phyllostachys bambusoides* were inhibited by iodoacetic acid, therefore, to be classified these enzymes as cysteine protease.

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