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Abstract

Extracts from various plants were examined for protease activity. High caseinolytic activity was found in the extracts of bulb of tulip, *Tulipa gesneriana* L., and Paper mulberry, *Broussonetia papyrifera* Vent.

High 4-nitroanilide hydrolytic activity was found in the extracts of Chayote, *Sechium edule* Swartz for Leu-pNA, and Hananira, *Brodiaea multiflora* Benth. for Ala-pNA.

Introduction

A number of plant proteases have been studied, usually emphasizing the properties of such well-known cysteine enzymes as papain (*1*), ficin (*2*), and bromelain (*3*). In contrast to the above cysteine proteases, relatively little is known about other types of protease from plant sources.

As a successor to our previous paper (*4—7*), we describe here the protease screening test of various plants. In addition the aminopeptidase activity of plants were determined to find a new plant enzyme source.

Experimental

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 was obtained from Peptide Institute, Inc., Osaka, Japan; Other reagents were purchased from Wako Pure Chemical Industries Ltd.

Preparation of Sample Solution for Caseinolytic Assay-All samples were ground in equal weight of 0.02 M phosphate buffer, pH 7.3, in a mortar and 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 0.02 M phosphate buffer, pH 7.3.

Preparation of Sample Solution for 4-Nitroanilide Hydrolytic Activity Assay-Solid $(NH_4)_2SO_4$ were added to the extracts of sample plants to 60% saturation. After standing for 24 h the resulting ppt. was collected by centrifugation and then dialyzed against water. These filtrates were used as sample solution.

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Assay of Protease-Proteolytic activity was measured by two methods. Caseinolytic activity was assayed by 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/w) casein containing 0.02 M phosphate buffer, pH 7.3, at 30°, After incubation for 30 min the reaction was terminated by the addition of 2 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 100-60.

The rates of enzymatic hydrolysis of 4-nitroanilide substrates were followed spectrophotometrically in 0.1 M Tris-HCI buffer, pH 7.5, at 410 nm with the spectrophotometer.

A unit of activity was defined as that amount which yielded 0.001 A_{280nm} (0.001 A_{410nm}) unit of change per min 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, 2.

Caseinolytic activity was observed in several plants. The activity of the extracts of bulb of tulip, *Tulipa gesneriana* L., and Paper mulberry, *Broussonetia papyrifera* Vent. prominent in the sample tested. But the number of units were not so large compared with sarcocarp of melons (*4*, *7*) or snake gourds (*5*, *6*). All of them are *cucurbitaceae* family. Paper mulberry protease was confirmed to be cysteine protease by further investigation. As this plant is *Moraceae* family, it is considered the cysteine protease group of *Moraceae* like a ficin from fig, *Ficus Carica* L.

The extracts of thirteen plants hydrolyzed 4-nitroanilide. The highest activity were Chayote, *Sechium edule* Swartz for Leu-pNA, and Hananira, *Brodiaea multiflora* Benth. for Ala-pNA. But these activity units were not enough to study plant aminopeptidase.

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Table 1. Caseinolytic Activity of Extracts from Plant Tissues

Plant	Plant parts	Method of extraction	Activity (Units)
Akinotamurasou (Salvia japonica Thunb.)	Leaf	Ext	0
Arechinogiku (Erigeron banariensis L. E. linifolius Willd.)	Whole	Ext	0
Biito, Garden Beet (Beta vulgaris L. var. rubra Maq.)	Whole	Ext	0
Chikori, Endive (Cichorium endivia L.)	Whole	Ext	13
Churippu, Tulip (Tulipa gesneriana L.)	Bulb	Ext	97
Gishigishi (Rumex japonicus Houttuyn)	Whole	Ext	0
Hananira (Brodiaea multiflora Benth.)	Whole	Ext	63
Hariguwa, (Cudrania tricuspadata Bureau)	Leaf	Ext	0
Hayatouri, Chayote (Sechium edule Swartz)	Sarcocarp	Ext	5
Hechima, Suakwa Towelgourd (Luffa cylindrica Roem.)	Sarcocarp	Ext	0
Hekusokazura (Paedaria scandens Merrill)	Leaf	Ext	18
Hisakaki (Eurya japonica Thunb)	Leaf, Fruit	Ext	0
Hishi, Water Caltrops (Trapa natans L. var. bispinosa Makino)	Sarcocarp	Ext	0
Hiyashinsu, Common Hyacinth (Hyacinthus orientalis L.)	Bulb	Ext	69
Inutade (Polygonum Bzumei Meisn.)	Leaf	Ext	26

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(from the Table 1)

Kazinoki, Paper mulberry	Berry	Ext	97
(Broussonetia papyrifera Vent.)	Leaf	Ext	0
Kinkan, Kumguat	Fruit	Ext	0
(Fortunella crassifolia Swingle)			
Kohzo	Leaf	Ext	0
(Broussonetia Kazinoki Sieb.)			
Kusaichigo			
(Rubus hirsutus Thunb.)	Leaf	Ext	0
Kusaniwatoko	Whole	Ext	0
(Sambucus chinensis Lindl.)			
Makomo, Manchuriam wild rice	Whole	Ext	5
(Zizania latifolia Turcz.)			
Nihonzuisen, Chinese sacred lily	Bulb	Ext	19
(Narcissus tazetta var. chinensis Roem.)			
Okatoranoo	Whole	Ext	0
(Lysimachia clethroides Duby)			
Oniyuri, Tiger Lily	Whole	Ext	0
(Lilium lancifolium Thunb.)			
Udo, Udo	Stem	Ext	0
(Aralia cordata Thunb.)			
Wasabi, Wasabi	Whole	Ext	12
(Eutrema japonica (Mig.) Koidz.)			
Yabutsubaki, Common camellia	Sarcocarp	Ext	0
(Camellia japonica L.)	Seed	Ext	11
Yamamomo	Sarcocarp	Ext	0
(Myrica rubra Sieb. et Zucc.)			
Yuzu, Yuzu	Fruit	Ext	0
(Citrus junos Sieb. ex T. Tanaka)			

Ext: Extract

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

Plant	Plant parts	Activity (Units) Leu-pNA	Ala-pNA
Arechinogiku (Erigeron banariensis L. E. linifolius Willd.)	Whole	0	0
Biito, Garden Beet (Beta vulgaris L. var. rubra Maq.)	Whole	0	0
Chikori, Endive (Cichorium endivia L.)	Whole	36	36
Churippu, Tulip (Tulipa gesneriana L.)	Bulb	2	0
Gishigishi, (Rumex japonicus Houttuyn)	Whole	0	0
Hananira (Brodiaea multiflora Benth.)	Whole	36	84
Hasu, East indian lotus (Nelumbo nucifera Gaertn)	Rhizome	0	0
Hayatouri, Chayote (Sechium edule Swartz)	Fruit	167	83
Hechima, Suakwa Towelgourd (Luffa cylindrica Roem.)	Fruit	8	0
Hishi, Water Caltrops (Trapa natans L. var. bispinosa Makino)	Sarcocarp	10	8
Hiyashinsu, Common Hyacinth (Hyacinthus orientalis L.)	Bulb	40	0
Ichijiku, Fig (Ficus Carica L.)	Sarcocarp	57	9
Kinkan, kumguat (Fortunella crassifolia Swingle)	Fruit	0	0
Kusaniwatoko (Sambucus chinensis Lindl.)	Whole	0	0
Kyohou (Vitis vinifera L. 'Kyohou')	Fruit	0	0
Makomo, Manchuriam wild rice (Zizania latifolia Turcz.)	Whole	45	50

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(from the Table 2)

Myouga (Zingiber mioga Rosc.)	Leaf	16	5
Ninniku, Garlic (Allium sativum L. forma pekinese Makino)	Bulb	0	0
Okatoranoo (Lysimachia clethroides Duby)	Whole	0	0
Oniyuri, Tiger Lily (Lilium lancifolium Thunb.)	Whole	0	0
Shiitake (Lentinus edodes Sing.)	Fruit body	28	28
Toogan, White gourd (Benincasa cerifera Savi)	Sarcocarp	19	9
Udo, Udo (Aralia cordata Thunb.)	Stem	0	0
Wasabi, Wasabi (Eutrema japonica (Mig.) koidz.)	Whole	15	13
Yabutsubaki, Common camellia	Sarcocarp	0	0
(Camellia japonica L.)	Seed	0	0
Yuzu, Yuzu (Citrus junos Sieb. ex T. Tanaka)	Fruit	0	0