PROTEASE ACTIVITY IN PLANT TISSUES (IV)

Rep. Fac. Sci. Kagoshima Univ., (Math., Phys. & Chem.)
No. 21. p.105-110, 1988.

PROTEASE ACTIVITY IN PLANT TISSUES (Ⅳ)

Tetsuya UCHIKOBA*, Ichiro SATA*, Hiromi AKIBA*, Shoko ISHIHARA*,

and Makoto KANEDA*

(Received Sep. 9, 1988)

Abstract

Extracts from various plants were examined for protease activity. Very high caseinolytic activity was found in the extracts of whitemary melon, *Cucumis melo* L. *var. whitemary,* Hiratake, *Pleurotus ostretus* Quél. and honeydew melon, *Cucumis melo* L. *var. inodorus Naud*. Among of them the activity of whitemary melon is the highest.

High peptidase activity was found in the extracts of pumpkin, *Cucurbita moschata* Duchesne and mangosteen, *Garcinia mangstana* L.

Introduction

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

As a successor to our previous paper (*4-6*), we describe here the protease screening test of various plants.

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. Other reagents were purchased from Wako Pure Chemical Industries Ltd.

Preparation of Sample Solution for Caseinolytic Activity Assay-Juice: A sarcocarp was ground with a grator made of synthetic resin. The homogenate was centrifuged for 20 min at 3000 × g, or filtered through a cotton cloth.

Extracts: Leaves and seeds were ground in equal weight of 0.02M phosphate buffer, pH 7.3, in a mortar and the homogenate was stirred for 5 min and filtered through a cotton cloth.

Juices and extracts were diluted to the point of appropriate concentration for

* Department of Chemistry, Faculty of Science, Kagoshima University, Kagoshima, 890 Japan.

assay with 0.02M phosphate buffer, pH7.3.

Preparation of Sample Solution for Peptidase Activity Assay-Solid $(NH_4)_2SO_4$ were added to the juices and 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.

Preparation of Substrate for Peptidase Activity Assay-Casein (2 % w/w) was digested with 4 μM cucumisin in 1/15 M phosphate buffer, pH 7.3 for overnight at 37°. The digest was centrifuged for 30 min at 11,000 × g. The resulting supernatant was used as substrate for peptidase activity assay.

Assay of Protease-Proteolytic activity was measured by two methods. Caseinolytic activity was assayed by method of Kunitz (*7*) , 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 0.02M 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.

Ninhydrin method was applied to assay peptidase activity. One ml of casein digest solution was diluted with water 100 times and preincubated for 10 min at 30°, and added to 1 ml of a screening sample solution. An aliquots (0.5 ml) of reaction mixture were removed at 30 min intervals. One ml of 0.01M potassium cyanide-ninhydrin solution and 0.5 ml of 4 N acetate buffer pH 5.13 were added to each of them. The reaction tubes were boiled for 15 min and then cooled, diluted to 5 ml of 50% ethanol solution. The absorbance for each sample at 570 nm was determined. The sample values at zero-time was used as the blank.

A unit of activity was defined as that amount which yielded 0.001 A_{280nm} (or 0.001 A_{570nm}) 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 or extract.

Results and Discussion

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

Proteolytic activity was observed in several plants. The activity of *Cucumis melo*. L. *var. whitemary* prominent in the sample tested. This protease was confirmed to be serine protease by further investigation. We had already isolated serine protease, cucumisin [EC 3.4.21.25] from the sarcocarp of prince melon (*8*). The proteases contained in the fruit of the Cucurbitaceae seems to be serine type, but a different quantity was observed for each variety of Cucurbitaceae.

Peptidase activity was found in the almost plants. The highest one was pumpkin, *Cucurbita moschata* Duchesne. Amino peptidase activity of pumpkin was

already found by the autors (unpublished data).

References

[1] Arnon,R. (1970) in *Methods in Enzymology* (Perlmann, G. E. & Lorand, L., eds.) Vol. **19,** pp.226-244, Academic Press, New York
[2] Liener, I. E. & Friedenson, B. (1970) in *Methods in Enzymology* (Perlmann, G. E. & Lorand, L., eds.) Vol. **19**, pp.261-273, Academic Press, New York
[3] Murachi, T. (1970) in *Methods in Enzymology* (Perlmann, G. E. & Lorand, L., eds.) Vol.**19**, pp.273-284, Academic Press, New York
[4] Kaneda, M., Yonezawa, H., & Tominaga, N. (1982) *Rep. Fac. Sci., Kagoshima Univ.*, (Math., Phys., & Chem.) No. **15**, pp.53-55
[5] Kaneda,M., Uchikoba, T., Furugen, K., & Tominaga, N. (1985) *Rep. Fac. Sci., Kagoshima Univ.*, (Math., Phys., & Chem.) No. **18**, pp.59-63
[6] Uchikoba, T., Izumi, S., Fukuda, T., Kaneda, M., & Tominaga, N. (1987) *Rep. Fac. Sci., Kagoshima Univ.*, (Math., Phys., & Chem.) No. **20**, pp.77-79
[7] Kunitz, M.,(1947) *J. Gen. Physiol.* **30**, 291
[8] Kaneda, M., & Tominaga, N. (1975) *J. Biochem.*,**78**, pp.1287-1296

Table 1. Caseinolytic Activity of Extracts from Plant Tissues

Plant	Plant parts	Method of extraction	Activity (Units)
Amaririsu, Amaryllis (Hippeastrum hybridum Hort.)	Bulb	Ext	14
Daikon, Radish (Raphanus sativus L.var. acanthiformis Makino)	Leaf	Ext	0
	Root	Pre	17
Endou, Pea (Pisum sativum L.var. arvense Poir.)	Seed	Ext	35
Feijyoa, Feijoa (Feijoa sellowiana Berg)	Stem, Leaf	Ext	0
Hanidyumeron, Honeydew Melon (Cucumis melo L.var. inodorus Naud)	Sarcocarp	Pre	176
Himawari, Sunflower (Helianthus annuus L.)	Leaf	Ext	0
Hiratake, (Pleurotus ostreatus Quél.)	Fruit body	Pre	404
Howaitomerimeron, Whitemary Melon (Cucumis melo L.var. whitemary)	Sarcocarp	Pre	1,933
Ikuri, Japanese Plum (Prunus salicina Lindl.)	Sarcocarp	Ext	0
Kanamugura, (Humulus japonicus Sieb. et Zucc.)	Leaf	Ext	0
Karin, Chinese Quince (Pseudocydonia sinensis Schhneid.)	Sarcocarp	Pre	0
Konara, Oak (Quercus serrata Thunb.)	Nut	Ext	40
Konatsumikan, (Citrus tamurana Takahashi)	Seed	Ext	16
Kuchinashi, Gardenia (Gardenia jasminoides Ellis F, grandiflora Makino)	Leaf	Ext	0
Kuromatsu, Japanese Black Pine (Pinus thunbergii Parl.)	Leaf	Ext	0

(Continued on the following page)

(from the Table 1)

Kurominookinawasuzumeuri, (Melothria liukiuensis Nak.)	Sarcocarp	Ext	39
Matatabi, Silver−vine (Actinidia polygama Maxim.)	Leaf	Ext	0
Momo, Peach (Prunus persica Batsch.)	Sarcocarp	Pre	0
Nigauri, Turureishi, Balsam ear (Momordica charantia L.)	Seed	Ext	0
Noibara, Polyantha Rose (Rosa multiflora Thunb.)	Leaf	Ext	0
Ohishiba, (Eleusine indica (L.) Gaertner)	Seed	Ext	0
Okinawasuzumeuri, (Deplocyclos palmatus C. Jeffrey.)	Sarcocarp	Ext	9
Piman, Bell Pepper (Capsicum annuum L.var. grossum Bailey)	Sarcocarp	Ext	10
Pirakansa, Narrow Leaf Firethorn (Pyracantha angustifolia Schneid.)	Leaf	Ext	0
Satoukibi, Sugar Cane (Saccharum officinarum L.)	Stem	Ext	0
Seitakaawadachisou, Tall Goldenrod (Solidago altissima L.)	Leaf	Ext	0
	Root	Ext	0
Sendan, Bead Tree (Malia Azendarach L.var. japonica Makino)	Seed	Ext	0
Shuro, Chusan Palm (Trachycarpus excelsa Wendl.)	Sarcocarp	Ext	0
Suberihiyu, Purslane (Portulaca oleracea L.)	Leaf	Ext	0
Sugina, Horsetail Scouring−Rush (Equisetum arvense L.)	Leaf	Ext	0
Tsubaki, Camellia (Camellia japonica L.)	Flower	Ext	0
Yatsude, Rice−paper Plant (Fatsia japonica Decne.et Planch.)	Leaf	Ext	0
Yamanoino, Yam (Dioscorea japoica Thunb.)	Leaf	Ext	0

Ext : Extract, Pre : Pressed juice

Table 2. Peptidase Activity of Extracts from Plant Tissues

Plant	Plant parts	Activity (Units×10)
Cherimoya, Cherimolia (Annona Cherimolia Mill.)	Sarcocarp	0.49
Daimyouchiku, (Semiarundinaria fastuosa (Mitf.) Makino)	Sprout	1.18
Guaba, Guava (Psidium Guajava L.)	Sarcocarp	0.38
Ichigo, Strawberry (Fragaria grandi flora Ehrh.)	Sarcocarp	0.38
Inubiwa, (Ficus (sect.Ficus) eracta Thunb.)	Leaf, Branch	2.08
	Sarcocarp	1.05
Jyujyube, Indonatsume, Indo jujube (Zizyphus mauritiana Lam.)	Sarcocarp	2.48
Kabocha, Pumpkin (Cucurbita moschata Duchesne)	Sarcocarp	4.31
Karasuuri, Snake gourd (Trichosanthes cucumeroides Maxim.)	Fruit	0.76
Karin, Chinese Quince (Pseudocydonia sinensis Schneid.)	Sarcocarp	0.27
Kikarasuuri, (Trichosanthes kirilowii Maxim.var.japonica (Miq.) Kitam.)	Sarcocarp	0.83
Mango, Mango (Mangifera indica L.)	Sarcocarp	0.86
Mangosuchin, Mangosteen (Garcinia mangostana L.)	Sarcocarp	3.85
Matsubagiku, Fig−Marigold (Mesembryanthemum spectabile Haw.)	Leaf	0.23
Mousouchiku, (Phyllostachys pubescens Mazel)	Sprout	1.01
Nogeshi, (Sonchus oleraceus L.)	Whole	0
Ranputan, Rambutan (Nephelium lappaceum L.)	Sarcocarp	0.81
Remon, Lemon (Citrus Limon Burm.)	Sarcocarp	0.48
Retasu, Head Lettuce (Lactuca sativa L.var. capitata L.)	Leaf	0.52
Sapojira, Sapodilla (Achras zapota L.)	Sarcocarp	1.36