CASEINOLYTIC ACTIVITIES IN PLANT TISSUES

CASEINOLYTIC ACTIVITIES IN PLANT TISSUES

Rep. Fac. Sci., Kagoshima Univ. (Math., Phys., & Chem.)
No. 15, p. 53–55, 1982

CASEINOLYTIC ACTIVITIES IN PLANT TISSUES

Makoto KANEDA, Hiroo YONEZAWA
and Naotomo TOMINAGA*

(Received Sep. 10, 1982)

Abstract

Caseinolytic activities were found in several plants. Netted melon was the most active, but cucumber, pumpkin and reishi had negligible activity.

Introduction

A number of plant proteases have been partially purified and in some cases extensively studied. Typical plant proteases such as papain [EC 3.4.22.2] (1), ficin [EC 3.4. 22.3] (2), and bromelain [EC 3.4.22.4] (3) are known to exhibit maximal activity in the presence of various reducing compounds.

In contrast to the extensively studied thiol proteases, relatively little is known about other types of proteases from plant sources, though we isolated cucumisin [EC 3. 4. 21. 25] (4) from the juice of melon fruit which is inhibited by diisopropyl fluorophosphate but is unaffected by reducing compounds such as cysteine and β-mercaptoethanol.

The present paper describes the protease screening of some plant extracts. Esculent plants were mainly employed for the examination.

Materials and Methods

Vegetables, fruits, potatoes and cereals were purchased from commercial sources in the havesting season, in Kagoshima city. Other plants were collected locally. Casein was product of E. Merk, Darmstadt, West Germany. L-Cysteine and trichloroacetic acid were purchased from Wako Pure Chemical Industries, Ltd., Osaka.

Preparation of Samples

Juices – Plant parts such as sarcocarp were homogenized with a grater of synthetic resin. The homogenate was centrifuged for 10 min at 3000 $\times$g, or filtered through cotton cloth.

Extracts – Plant parts such as leaves and seeds were ground in equal weight of 0.02 M phosphate buffer, pH 7.3, in a mortar, and the homogenate was stirred for 5 min and filtered through cotton cloth.

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

Table 1. Caseinolytic Activity of Extracts from Plant Tissues

Plant	Plant parts	Method of Extraction	Activity (Units)	
			With Cysteine	Without Cysteine
Dandelion (*Taraxacum platycarpum Dahlst*)	L, St	Ext	29	19
Dokudami (*Houttuynia cordata Thunb.*)	W	Ext	0	0
Yomogi (*Artemisia vulgaris L. var indica Maxim.*)	W	Ext	17	2
Nogeshi (*Sonchus oleraceus L.*)	L, St	Ext	15	2
Rengeso (*Astragalus sinicus L.*)	L, St	Ext	< 1	< 1
Murasakikatabami (*Oxalis Martiana Zucc.*)	W	Ext	2	1
Aloe (*Aloe arborescens Mill*)	St	Ext	0	0
Gum tree (*Hevea brasiliensis*)	L	Ext	13	5
Tea plant (*Thea sinensis L.*)	L	Ext	0	1
Lily (*Lilium trigrinum Ker*)	Bulb	Ext	0	0
Cactus (*Opuntia Ficus-indica Mill. var. Saboten Makino*)	St	Ext	5	4
Coco (*Cocos nucifera L.*)	Milk	Pre	0	0
Avocado (*Persea americana Mill*)	Seed	Ext	0	0
〃	Sar	Pre	4	2
Mango (*Mangifera indica L.*)	Sar	Pre	0	0
Potato (*Solanum tuberosum L.*)	Tuber	Pre	< 1	0
Sweet potato (*Ipomoea Batatas Lam. var. edulis Makino*)	Tuber	Pre	8	6
Apple (*Malus pumila Mill. var. dulcissima Koidz.*)	Sar	Pre	0	0
Loquat (*Eriobotrya japonica Lindl.*)	Sar	Pre	0	0
Strawberry (*Fragaria grandi flora Ehrh.*)	Sar	Pre	0	0
Netted melon (*Cucumis Melo L. var. reticulatus Naud*)	Sar	Pre	350	350
Reishi (*Momordica Charantia L.*)	Sar	Pre	< 1	< 1
Pumpkin (*Cucurbita moschata Duchesne*)	Sar	Pre	2	2
Cucumber (*Cucumis sativs L.*)	Sar	Pre	1	1
Egg plant (*Solanum Melongena L.*)	Fruit	Pre	0	0
Green pepper (*Capsicum annuum L.*)	Fruit	Pre	13	13
Pasley (*Petroselium sativum Hoffen*)	L, St	Ext	19	20
Shungiku (*Chrysanthemum coronarium L.*)	L, St	Ext	14	9
Carrot (*Daucus Carota L. var. sativa DC.*)	Root	Pre	< 1	< 1
Onion (*Allium Cepa L.*)	Bulb	Pre	< 1	< 1
Lettuce (*Lactuca sativa L.*)	L, St	Pre	6	4
Garden asparagus (*Asparagus officinalis L. var. altilis L.*)	L, St	Pre	11	10
Ginger (*Zingiber officinalis Rosc.*)	Rhizome	Pre	25	< 1
Wheat (*Triticum aestivum L.*)	Seed	Ext	7	5
Buck wheat (*Fagopyrum esculentum Moench*)	Seed	Ext	< 1	< 1
Pearl barley (*Coix Ma-yuen Roman*)	Seed	Ext	< 1	< 1
Garden pea (*Pisum sativum L.*)	Young bean	Ext	16	15
Soy bean (*Glycine Max Werr.*)	Germinating seed	Ext	19	15
Shiso (*Perilla frutescens Britt. var. crispa Decne*)	L	Ext	6	2

Ext = Extract, L = Leaf, Pre = Pressed juice, Sar = Sarcocarp, St = Stem, W = Whole.

Juices and extracts were diluted with 0.02 M phosphate buffer, pH 7.3 or 0.02 M phosphate buffer, pH 7.3, containing 10^{-3}M L-cysteine.

Assay of Proteinase

Proteolytic activity was measured by the method of Kunitz (5), with casein as a substrate. One ml of sample was pre-incubated for 10 min at 35°C, and then added to 1 ml of a solution of 1% (W/W) casein containing 0.02 M phosphate buffer, pH 7.3, at 35°C. 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 a Hitachi spectrophotometer 100–60. A blank was run with each assay.

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

Caseinolytic activities of juices and extracts of various plant tissues are shown in Table I.

Caseinolytic activities were found in several plants. Netted melon was the most active, while the other *Cucurbitaceaes* such as cucumber, pumpkin and reishi had negligible activity. The proteinases of prince melon (4) and white gourd (6) belonging to *Cucurbitaceae* has been already described. These two proteinases are remarkably similar to one another. No significant difference could be detected between the two in such criteria as molecular weight, pH and temperature optima, pH and temperature stabilities and the sensitivity to various compounds. The presence of an active serine in the two proteinases is indicated by the inhibition by diisopropyl fluorophosphate (7). Accordingly, the proteinase of netted melon probably seems to be cucumisin like enzyme.

References

1) Arnon, R. (1970) *in Methods in Enzymology* (Perlmann, G.E. & Lorand, L., eds.), Vol. 19, p. 226, Academic Press, New York.
2) Liener, I.E. and Friedenson, B. (1970) in *Methods in Enzymology* (Perlmann, G.E. & Lorand, L., eds.), Vol. 19 p. 261, Academic Press, New York
3) Murachi, T. (1970) in *Methods in Enzymology* (Perlmann, G.E. & Lorand, L., ed.s), Vol. 19, p. 273, Academic Press, New York.
4) Kaneda, M. and Tominaga, N. (1975) *J. Biochem.*, **78**, 1287
5) Kunitz, M. (1947) *J. Gen. Phisiol.*, **30**, 291
6) Kaneda, M. and Tominaga, N. (1977) *Phytochemistry* **16**, 345
7) Walfh, K.A. and Wilcox, P.E. (1970) in *Methods in Enzymology* (Perlmann, G.E. & Lorand, L., eds.), Vol. 19, p. 31, Academic Press, New York