CASEINOLYTIC ACTIVITY IN PLANT TISSUES (III)

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CASEINOLYTIC ACTIVITY IN PLANT TISSUES (Ⅲ)

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Abstract

Extracts from various plants were examined for caseinolytic activity. Very high activity was found in the extracts of okarasuuri, snake gourd, *Trichosanthes bracteata* (*Lam.*) *Voigt* and amerikayamagobou, pokeweed, *Phytolacca americana L.* Among them the activity of okarasuuri is higher than that of amerikayamagobou.

Introduction

A number of plant proteases have been studied, usually emphasizing the properties of such well-known 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 (*4,5*), 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 city. Casein was a product of E. Merck, Darmstadt, West Germany. Trichloroacetic acid was purchased from Wako Pure Chemical Industries Ltd.

Preparation of Sample Solution-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 assay with a 0.02M phosphate buffer, pH 7.3.

Assay of Protease-Proteolytic activity was measured by the method of Kunitz (*6*), 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

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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

Table I. Caseinolytic Activity of Extracts from plant Tissues

Plant	Plant parts	Method of extraction	Activity (Units)
Amerikayamagobou, Pokeweed (Phytolacca americana L.)	Sar	Pre	410
	Leaf	Ext	70
	Root	Ext	57
Daria, Common Dahlia (Dahlis pinnata Cav.)	Root	Ext	0
Goma, Sesame(Sesamum indicum L.)	Seed	Ext	4
Gurajiorasu, Ghent Gladiolus (Gladiolus gandavensis van Houtt.)	Rhizome	Ext	240
Hamanatamame,(Canavalia lineata DC.)	Seed	Ext	36
Hanakanna, Common Garden Canna(Canna generalis Bailey)	Rhizome	Ext	0
Hitashimame, A kind of bean	Seed	Ext	59
Houzuki, Chimese Lantern Plant(Physalis Alkekengi L. var. Bunyadii Makino)	Sar	Pre	3
Ingenmame, Hyacinth Bean (Dolichos lablab L.)	Seed	Ext	1
Kamojigusa,(Agropyron Kamoji Ohwi)	Leaf	Ext	0
Kara, Common Calla (Zantedeschia aethiopica Spreng.)	Rhizome	Ext	85
Keitou, Common Cookscomb (Celosia cristata L.)	Leaf	Ext	14
Kusagi,(Clerodendron trichotomum Thunb.)	Leaf	Ext	0
Makuwauri, Oriental Melon (Cucumis melo L.var. makuwa Makino)	Sar	Ext	6
Nankinmame, Peanut (Arachis hypogaea L.)	Seed	Ext	66
Natamame, Sword Bean (Canavalia gladiata DC.)	Seed	Ext	1
Nokogirisou, Siberan Yarrow (Achillea siberian Ledeb.)	Leaf	Ext	0
Ohishiba,(Eleusine indica Gaertner)	Leaf	Ext	0
Okarasuuri,(Trichosanthes bracteata (Lam.) Voigt)	Sar	Ext	5,858
Sazanka, Sasanqua (Camellia Sasanqua Thunb.)	Sar	Ext	0
Soyogo,(Ilex pedunculosa Miq.)	Sar	Ext	43
Tukimisou,(Oenotherra tetraptera Cav.)	Leaf	Ext	0
Turuumemodoki, Oriental Bittersweet(Celastrus orbiculatus Thunb.)	Sar	Ext	0

Ext: Extract, Pre: Pressed juice, Sar: Sarcocarp

of the trichloroacetic acid-soluble peptides formed was determined with Hitachi spectrophotometer 100-60.

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

Proteolytic activity was observed in several plants. The activities of *Trichosanthes bracteata* were 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 (*7*). 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.

The protease activity of *Phytolacca americana* was the second largest in tested samples. The presence of an active thiol group in this protease is indicated by the inhibition by iodoacetic acid.

References

1. Arnon, R. (1970) in Methods in Enzymology (Perlman, 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. Kunitz, M., (1947) J. Gen. Physiol. **30**, 291
7. Kaneda, M., & Tominaga, N. (1975) J. Biochem.**78**, pp.1287–1296