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Abstract

Caseinolytic activity of various plant tissues was examined. Favorable protease activity was found in the extracts of rhizome of Baboonroot, ***Babiana stricta*** Ker-Gawler, seeds of Radish, ***Raphaus sativus*** L. var. hortensis and rhizome of Ixia, ***Ixia hybrida*** Hort.
Key words: Plant protease, protease.

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) have 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 (3-8), we attempted the screening test to find a new type plant protease.

Experimental

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, West Germany; 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 3,000×g.

The extracts were diluted to the point of appropriate concentration for assay with 0.067 M phosphate buffer, pH 7.3. Characterization of cysteine protease was performed by adding 1 mM L-Cysteine to the sample solution.

Assay of Protease-Proteolytic activity was measured by the method of Kunitz (9) with casein as a substrate. One ml of sample solution was preincubated for 10 min at

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Table 1. Caseinolytic Activity of Extracts from Plant Tissues

Plant	Plant parts	Activity (Units)
Akagashi, Japanese Evergreen Oak (Quercus acuta Thunb.)	Nut	0
Ashitaba (Angelica utilis Makino)	Leaf, Stem	3
Banana, Banana (Musa nana Lour.)	Fruit	0
Banreishi, Sugar apple (Annona squamosa L.)	Sarcocarp	4
Daikon, Radish	Young Leaf, Stem	9
(Raphanus sativus L.)	Young root	4
(Raphanus sativus L. var. hortensis)	Seed	41
Dorian, Durian (Durio zibethinus DC.)	Sarcocarp	11
Esharotto, Shallot (Allium cepa L vars. aggregatum G. Don, multiplicans L.H. Bailey, solaninum Alef.)	Root	5
Gimunema shirubesuta (Gymunema sylvestre, R. Br.)	Leaf	25
Gobou, Great Burdock (Arctium lappa L.)	Root	0
Hakka, Peppermint (Mentha piperata L.)	Leaf	0
Higanbana (Lycoris radiata Herb.)	Rhizome	0
Hiiragi (Osmanthus ilicifolius Mouill.)	Berry	0
Hozakiayame, Babiana, Baboon-Root (Babiana stricta Ker-Gawler)	Rhizome	180
Jakkufurutsu, Paramitsu, Jackfruit (Artocarpus heterophyllus Lam.)	Sarcocarp	5
Kaidou, Kaido Crab-Apple (Malus micromalus Makino)	Berry	0
Kanariyashi, Canary Island Date Palm (Phoenix canariensis hort. ex Chabaud)	Berry	4
Kinmokusei (Osmanthus fragrans var. aurantiacus)	Sarcocarp	0
Kobushi (Magnolia kobus DC.)	Sarcocarp	2
Komikansou	Fruit	0
(Phyllanthus urinaria L.)	Leef	0

(Continued on the following page)

(from Table 1.)

Plant	Plant parts	Activity (Units)
Kouhii, Coffee (Coffea arabica 'Blue Mountain')	Berry	17
Kunugi (Quercus acutissima Carruth.)	Nut	0
Kusanoou (Chelidonium majus var. asiaticum)	Leaf, Stem	0
Mangosuchin, Mangosteen (Garcinia mangostana L.)	Sarcocarp	0
Marubachishanoki (Ehretia dicksonii Hance var. japonica Nakai)	Sarcocarp	10
Matsubabotan, Rose Moss	Stem, Leef	2
(Portulaca grandiflora Hook.)	Berry	2
Mube (Stauntonia hexaphylla (Thunb.) Decne.)	Fruit	0
Nagaimo, Chinese Yam (Dioscorea batatas Cecne.)	Bulb	11
Nankinhaze, Chinese tallow tree (Sapium sebiferum Roxb.)	Sarcocarp	0
Nasu, Egg plant (Solanum melongena L.)	Berry	1
Negi, Ciboule (Allium fistulosum L.)	Leaf	2
Nejibana	Leaf, Stem	0
(Spiranthes sinensis var. amoena)	Root	0
Nikuzuku, Common Nutmeg (Myristica fragrans Houtt.)	Seed	0
Ninjin, Carrot (Daucus carota L. var. sativa DC.)	Leaf	0
Ninniku, Garlic (Allium sativum var. japonicum Kitam.)	Rhizome	7
Nira, Chinese chive (Allium tuberosum Rottl. ex. K. Spreng.)	Leaf	0
Okahijiki (Salsola komarovii IIjin)	Leaf, Stem	16
Orandaayame, Dutch Iris (Iris hollandica hort.)	Rhizome	0
Ougonkazura (Scindapsus aureus Engl. var. Golden Pothos)	Leaf	0

(Continued on the following page)

(from Table 1.)

Plant	Plant parts	Activity (Units)
Outou, Brack cherry (Prunus serotina J. F. Ehrh)	Sarcocarp	5
Safuran, Saffron crocus (Crocus sativus L.)	Rhizome	14
Sangojyu (Viburnum odoratissimum Ker-Gawl.)	Berry	5
Sarutoriibara (Smilax china L.)	Sarcocarp	19
Satoimo (Colocasia antiquorum Schott var. esculenta)	Bulb	0
Satoudaikon, Sugar Beet (Beta vulgaris L. var. rapa Dumort.)	Bulb	0
Sendan, Japanese bead-tree (Melia azedarach L. var. japonica Makino)	Leaf	10
Serori, Celery (Apium graveolens L.)	Leaf, Stem	3
Shishitou, Red pepper (Capsicum annuum var. grossum Sendtn. 'Shishitou')	Sarcocarp	11
Suisen, Grand Emperor (Narcissus tazetta L. var. chinensis Roem.)	Rhizome	14
Sumomo, Japanese plum (Prunus salicina 'Sugar Prume')	Sarcocarp	0
Tachinatamame, Jack bean (Canavalia glandiata var. ensiformis DC.)	Sarcocarp	0
Tomato, Tomato (Lycopersicon esculentum Mill)	Sarcocarp	2
Tsuwabuki (Farfugium japonicum (L. f.) Kitam.)	Stem	0
Tya, Tea (Camellia sinensis (L.) O. Kuntze)	Sarcocarp	0
Tyantin, Chinese toon (Cedrela sinensis Juss.)	Sarcocarp	0
Urokomizugoke (Sphagnum squarrosum Crome)	Whole	3
Yarizuisen, Ixia (Ixia hybrida hort.)	Rhizome	31

Table 2. Effect of Cysteine against Caseinolytic Activity of Plants

Plant	Part	Activity (Units)	
		With Cysteine	Without Cysteine
Kunugi (Quercus acutissima Carruth.)	Nut	64	0
Inubiwa (Ficus erecta Thunb.)	Sarcocarp	25	13

30°, and then added to 1 ml of a solution of 1% (w/v) casein containing 0.067 M phosphate buffer, pH 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 formed was determined with Hitachi spectrophotometer U-1100.

A unit of activity was defined as that amount which yielded 0.001 A_{280nm} 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 rhizome of Baboonroot, ***Babiana stricta*** Ker-Gawler had high proteolytic activity. Subsequently seeds of Radish, ***Raphaus sativus*** L. var. hortensis and rhizome of Ixia, ***Ixia hybrida*** Hort displayed more than 30 units. Small protease activity were present in leaf of ***Gymnema sylvestre***, R. Br., sarcocarp of ***Smilax china*** L. and berry of coffee, ***Coffea arabica*** 'Blue Mountain'. Comparing the activity with sarcocarp of snake gourds (4, 5), the units on this report were not so large. From the data of Table 2., the protease of nut of ***Quercus acutissima*** Carruth. and sarcocarp of ***Ficus erecta*** Thunb. (4) were considered cysteine type enzyme. Because the enzyme activity of these plants increased by adding 1 mM cysteine. In former enzyme was especially more sensitive for cysteine.

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