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Abstract

Naturally occurring protein inhibitor of cucumisin was tested. Eggs, animal organs, beans and potatoes are generally potent sources of proteinase inhibitors, but they can not inhibit activity of cucumisin. Its property of cucumisin appers to be available to food processing as a useful tool.

Introduction

Protein proteinase inhibitors are widely distributed in plants and animals. A number of these inhibitors have been purified and characterized. Knowledge of the primary and three dimensional structures of the proteinases and their inhibitors is a prerequisite to an understanding of the mechanism of interaction between them. The inhibitors in human plasma control proteolytically-regulated processes in the blood namely, blood clotting, fibrinolysis, kinin liberation and the action of complement. For most inhibitors, there is no proven function in the biological material where they exist (1-4).

A number of plant proteases have been partially purified and in some cases extensively studied. Typical plant proteases are known to exhibit maximal activity in the presence of various reducing compounds (5). However, cucumisin [EC 3. 4. 21. 25] from the juice of melon fruit is inhibited by diisopropyl fluorophosphate but is unaffected by reducing compounds (6).

The present paper describes the protein inhibitor screening test of cucumisin about some potent sources of proteinase inhibitors.

Materials and Methods

Vegetables, fruits, cereals, eggs and animal organs were purchased from commercial sources in Kagoshima city. Casein was product of E. Merk, Darmstadt, West Germany.

Preparation of Samples

1. Jucies and Extracts : Plant parts such as sarcocarp were homogenized with a grater of synthetic resin. The homogenate was centrifuged for 10 min at 3000×g, or filtered through cotton cloth. Leaves and seeds were ground in equal weight of 0.02 M

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Table 1. Samples examined for Cucumisin Inhibitor

1. Pressed juice or extract
Carrot (*Daucus Carota L. var. sativa DC.*) Root
Turnip (*Brassica Rapa L.*) Root
Potato (*Solanum tuberosum L.*) Tuber
Chinese yam (*Dioscorea Batatas Decne.*) Tuber
Sweet potato (*Ipomoea Batatas Lam. var. edulis Makino*) Tuber
Garlic (*Allium sativum L.*) Bulb
Ginger (*Zingiber officinalis Rosc.*) Rhizome
Egg plant (*Solanum Malongena L.*) Fruit
Okura (*Abelmoschus esculentus Moench*) pod
Tomato (*Lycopersicum esculentum Mill.*) Fruit
Apple (*Malus pumila Mill. var. dulcissima Koidz.*) Sarcocarp
Indian corn (*Zea Mays L.*) Seed
Soy bean (*Glycine Max Werr.*) Seed
Mushroom (*Agaricus bisporus (Lange) Sing*) Whole
Enokidake (*Flammulina velutipes (Fr.) Karst.*) Whole
Golden-banded lily (*Lilium auratum Lindl.*) Bulb
Pomegranate (*Punica Granatum L.*) Pericarp and Sarcocarp
Netted melon (*Cucumis Melo L. var.reticulatus Naud*) Seed
Prince melon (*Cucumis Melo L. var. Prince*) Seed
Gingko (*Ginkgo biloba L.*)Seed
2. Ammonium Sulfate Precipitation
Tomato (*Lycopersicum esculentum Mill.*) Fruit
Cucumber (*Cucumis sativus L.*) Fruit
Garden pea (*Pisum sativus L.*) Young been
Garlic (*Allium sativus L.*) Bulb
Potato (*Solanum tuberosum L.*) Tuber
Kiwi (*Actinidia chinensis Planch*) Fruit
Soy bean (*Glycine Max Werr.*) Been
3. Gel-filtration on Sephadex G-25
Egg plant (*Solanum Melongena L.*) Fruit
Shirouri (*Cucumis Melo L. var. Conomon Makino*) Sarcocarp
Pumpkin (*Cucurbita moschata Duchesne*) Sarcocarp
Radish (*Raphanus sativus L.*) Root
Onion (*Allium Cepa L.*) Bulb
Apple (*Malus pumila Mill. var. dulcissima Koidz.*) Sarcocarp
Buck wheat (*Fagopyrum esculentum Moench*) Seed
4. Affinity Chromatography on Cucumisin-Sepharose 4B
Pig liver and kidney
Bovine serum
Chicken egg-white
Turtle egg-white
Duck egg-white
Quail egg-white
Soy been (*Glycine Max Werr.*) Seed

Preparation methods of sample solutions from 1 to 4 are described in "Materials and Methods" phosphate buffer, pH 7.3, in a mortar, and the breis was stirred for 5 min and filtered through cotton cloth.

2. Ammonium Sulfate Precipitation: Solid ammonium sulfate was added gradually to the juice or extract to 60% saturation and kept one hour. The resulting precipitate was collected by centrifugation at 6000×g for 30 min and then dialized against buffer, 0.02 M phosphate buffer, pH 7.3. The dialysate was centrifuged to remove minor insoluble materials.

3. Gel-filtration on Sephadex G-25: Juice or extract was lyophilized and then dissolved in 0.02 M phosphate buffer, pH 7.3. The solution was applied to a Sephadex G-25 column previously equilibrated with above buffer. The protein fraction was monitored by measuring the absorbance at 280 nm and collected.

4. Affinity Chromatography on Cucumisin-Sepharose 4B Column: Egg-whites were diluted with about ten times volume of 0.02 M phosphate buffer, pH 7.3. Animal organs were homogenated and then centrifuged. Four times volume of acetone was added to the above supernatant. Resulting acetone powder was collected by centrifugation at 5000×g for 20 min and then dissolved in 0.02 M phosphate buffer, pH 7.3. The suspension was centrifuged to remove minor insoluble materials. The diluted egg-whites and the extracts from animal organs were placed on a column of cucumisin-Sepharose 4B equilibrated with 0.02 M phosphate buffer, pH 7.3. The column was washed thoroughly with same buffer and then with 0.1 M acetic acid. The frontal fraction of acetic acid were collected, lyophilized and dissolved in above buffer.

Unless no interfering with blank test, all the operation were carried out in a high concentration of the samples.

Assay of Inhibitory Activities

The inhibitory activities of the preparations were determined as follows. The sample solution (0.5 ml) was incubated with 0.5 ml of cucumisin (50 μg/ml) for 10 min and then 1 ml of 1% (w/v) casein solution in 0.02 M phosphate buffer, pH 7.3 was added at 35°. After incubation for 30 min the reaction was terminated by the addition of 3 ml of 5% (w/v) 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 carried out in an identical fashion except using of buffer solution instead of sample solution.

Results and Discussion

The samples listed in Table 1 were examined for cucumisin inhibitory activity. All samples had neglible activity. The activity was faintly observed in certain cases, especially tomato and turtle egg, but the existence of inhibitor could not be regarded as significant. In some cases proteolytic activity was found in sample solution, however it was very low activity. Extract from pericarp of pomegranate had a high inhibitory activity against cucumisin. By further investigation, this phenomenon resulted from action of tannin contained abundantly in its pericarp. Among others in Table, eggs, animal organs, beans and potatoes are known to be generally potent sources of proteinase inhibitors. But they can not inhibit cucumisin. Its property of cucumisin appears to be available to food processing as a useful tool. Cucumisin is serine-type protease, and accordingly there are no need chelating and reducing compounds for activator.

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