KOREAN J. FOOD SCI. TECHNOL.
Vol. 20, No. 5, pp 644~649(1988)

Screening of Oriental Drugs for α-Amylase Inhibitor

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Abstract

The inhibitory activities against bacterial α-amylase of oriental drugs from animals, plant and mineral origin were investigated. In final screening test, it was found that *Areca catechu* L., *Cinnamomum cassia* Presl. and *Ephedra sinica* Stapf had stronger inhibitory activities against α-amylase than other oriental drugs used in this experiment.

Key words: α-amylase inhibitor, oriental drugs, inhibitory activities

Introduction

α-Amylase (1,4-α-D-glucan glucanohydrolases, EC 3.2.1.1.), which is widely distributed among animals, microorganisms and plants, hydrolyzes starch ramdomyly and gives α-maltooligosaccharides as its products. Although many studies have been conducted and many information is available on amylases, only limited studies have been reported on amylase inhibitors. α-Amylase inhibitor was first reported by Chrzaszcz and Janicki from buckwheat in 1933, and it is proteinous substance[1]. Since then many workers have studied the α-amylase inhibitors in plants, such as wheat[2,3], corn[4], rye[5,6], potato tubers[7], legumes[8,9] and microorganisms[10-12].

α-Amylase inhibitors might be useful for persons who should only consume restricted quantities of carbohydrates to avoid hyperglycaemia and increased synthesis of triglycerides in adipose tissue, liver, and the wall of the intestine, that is, patients suffering from carbohydrate-dependent diseases such as diabetes, type IV hyperlipoproteinaemia, and obesity[11,13,14]. But no amylase inhibitor from oriental drugs had been found. In the present paper, the authors examined a large number of oriental drugs, to find new, heat-stable amylase inhibitor.

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Materials and Methods

Oriental drugs and reagents

Oriental drugs were purchased from local oriental drug stores. α-Amylase(from *Bacillus subtilis*, 18,000 unit/g as blue value method, diluted with starch) was product of Tokyo Kasei Chemical Co. and soluble starch was obtained from Sigma Chemical Co.

Preparation of inhibitor solution

Each oriental drug was extracted in distilled water(50g/300m*l*) for one hour at 100℃ and stood for 20 hours at room temperature. The extract was filtered with Toyo No. 2 filter paper, and this filtrate was used in screening of α-amylase inhibitor.

Assay system for amylase inhibitory activity

α-Amylase activity was measured by modified blue value method[15]. α-Amylase was dissolved at the concentration of 0.1% in McIlvaine buffer(pH 5.0). The substrate used was 1.0% soluble starch solution dissolved in McIlvaine buffer solution(pH 5.0).

Test: 0.3m*l* of McIlvaine buffer(pH 5.0) solution, 0.1m*l* of inhibitor solution and 0.1m*l* of α-amylase solution were preincubated at 40℃ for 20min and then 0.5m*l* of substrate solution was added. After 30min incubation at 40℃, the reaction

was stopped by adding 1m*l* of 1.5N-acetic acid.

Control: 0.3m*l* of McIlvaine buffer solution, 0.1m*l* of distilled water in place of the inhibitor solution and 0.1m*l* of α-amylase solution were incubated as a control in parallel with the test sample.

Blank: 0.3m*l* of McIlvaine buffer solution, 0.1m*l* of inhibitor solution and 0.1m*l* of distilled water in place of α-amylase solution were incubated as a blank in parallel with the test sample.

To each reaction mixture, 1m*l* of 0.002% iodine solution containing 0.05% potassium iodide was added. After this mixture was allowed to stand for 20min at 40℃, and added 17m*l* of distilled water. The optical density was measured at 690*n*m. The inhibitory activity was calculated as a percentage of the inhibition of α-amylase activity using the following equation:

Percent inhibition = (T-C)/(B-C)×100(%)

where T,C, and B are the optical densities of the test, control, and blank, respectively.

Results and Discussions

The inhibitory activities against bacterial α-amylase of water extract from 216 kinds of oriental drugs of animals, plant and mineral origin were examined, the results are shown in Table 1. As shown in Table 1, oriental drugs of animal and mineral origin had rarely the inhibitory activity against α-amylase, among oriental drugs used in this experiments, only 14 drugs had more than 80% inhibitory activities in first screening test. In second screening test *Areca catechu* L. *Cinnamomum cassia* Presl. and *Epedra sinica* Stapf had considerably higher inhibitory activities than others(Table 2).

For a long time dietetic rules have been a basic treatment of metabolic diseases such as diabetes mellitus, obesity or Type IV hyperlipoproteinaemia. As a rule, these regimens require a reduced intake of carbohydrates. If these instructions are followed, hyperglycaemia, hyperinsulinaemia and

Table 1. α-Amylase inhibition activities of various oriental drugs

Oriental drugs	Inhibition
Prunus pesica (L.) Batsch	—
Eriobotrya japonica Lindl.	—
Cocculus laurifolius DC.	+ +
Clematis florida Thunb.	—
Melandryum firmum Rohrb.	—
Polygonum aviculare L.	—
Reynoutria elliptica (Koidz.) Miq.	+ + + +
Dendrobium moniliforme (L.) Sw.	—
Dioscorea japonica Thunb.	—
Fritillaria ussuriensis Maxim.	—
Stemona japonica Miq.	—
Cyperus rotundus L.	—
Typha orientalis Presl	—
Biota orientalis (L.) Endl. (leaf)	—
Equisetum hiemale L.	—
Pharbitis nil (L.) Choisy	+ + + +
Alpinia officinarum Hance.	—
Codonopsis pilosula Nannf.	—
Sanguisorba officinalis L.	
Magnolia denudata Desr.	—
Epimedium koreanum Nakai	—
Dianthus sinensis L.	—
Celosia argentea L.	
Polygonum multiflorum Thunb.	—
Bletilla striata (Thunb.) Reichb. Fil.	—
Gastrodia elata Bl.	—
Asparagus cochinchinensis (Lour.) Merr.	—
Polygonatum sibiricum Redoute	—
Acorus gramineus Soland.	—
Scirpus flaviatilis (Torr.) A. Gray.	—
Biota orientalis (L.) Endl.(Seed)	—
Torreya nucifera S. et Z.	—
Amomum xanthioides Wall.	+ + + +
Amomum cardamomum L.	—
Piper longum L.	—
Ostericum koreanum (Max.) Kitagawa	—
Trichosanthes kirilowii Max.(root)	—
Eucommia ulmoies Oliver	—
Aconitum carmichaeli Debx.	—
(mother root)	—
Terminalia chebula Retz.	—
Euryale ferox Salisb.	—
Hordeum vulgare L.	—
Cynomorium songaricum Rupr.	+ + + +
Scrophularia buergeriana Miq.	—
Mentha arvensis var. *piperascens* Mak	—
Salvia miltiorrhiza Bge.	—
Cuscuta japonica Choisy	—

Oriental drugs	Inhibition
Cornus officinalis S. et Z.	++++
Aralia cordata Thunb.	—
Torilis japonica Dc.	—
Quisqualis indica L.	—
Melia toosendan S. et Z.(seed)	—
Citrus unshiu Marcor.(immature)	—
Albizia julibrissin Durazz.	—
Sophora flavescens Aiton	—
Picrorrhiza kurrooa Royle ex Benth.	—
Alpinia oxyphylla Miq.	—
Aconitum carmichaeli Debx. (daughter root)	—
Davallia mariesii Moore	—
Chaenomeles sinensis (Dum-Corus.) Schneid	++++
Rosa laevigata Michx.	—
Cistanche salsa(C.A. Mey.) G. Beck	—
Leonurus sibiricus L.	—
Schizonepeta tenuifolia var. *japonica*	—
Vitex rotundifolia L.	—
Forsythia koreana Nakai	—
Angelica dahurica (Fisch.) Benth. et Hooker	—
Peucedanum decursivum (Miq.) Maxim	—
Acanthopanax sessiliflorus (Rupr. et Max.) Seem	—
Euphoria longan (Lour.) Steud.	—
Citrus unshiu Marcor.(mature)	—
Dictamnus dasycarpus Turcz.	—
Sophora subprostrata Chun et T. Chen.	—
Prunus mume S. et Z.	++++
Rehmania glutinosa Liboch.(fresh)	—
Cimicifuga heracleifolia Kom.	—
Alisma canaliculatum All. Br et Bouche	—
Eugenia caryophyllata Thunb. (young flower bud)	++
Sophora japonica L.	—
Foeniculum vulgare Mill.	—
Poncirus trifoliata (L.) Raf. (unmature)	—
Schizandra chinensis Baill	++++
Zizyphus jujuba Mill. var. *inermis* (Bunge) Rehd.	—
Lycium chinense Miller.(bark of root)	—
Cassia tora L.	—
Areca catechu L.(seed)	++++
Zizyphus spinosa Hu	—
Cinnamomum cassia Pres l.(bark)	++++
Magnolia officinalis Rehd. et Wils.	++++
Melia toosenden S. et Z.(bark)	—
Crataegus pinnatifida Bunge.	+
Cnidium officinale Makino	—
Rehmania glutinosa Liboch. (steam blanching)	—
Cimicifuga heracleifolia Kom.	—
Anemarrhena asphodeloides Bge.	—
Eugenia caryophyllata Thunb.(bark)	+++
Carthamus tinctorius L.	—
Piper nigrum L.	—
Evodia rutaecarpa(Juss.) Benth.	—
Gardenia jasminoides Ellis	+
Lycium chinense Miller.(seed)	—
Prunus armeniaca var. *ansu* Maxim.	—
Myristica fragrans Houtt.	—
Areca catechu L.(bark of seed)	+
Phellodendron amurense Ruprecht	++
Cinnamomum cassia Presl.(weak branch)	—
Rhus chinensis Mill	—
Morus alba L.(bark of root)	—
Santalum album L.	—
Paeonia suffruticosa Andr. (bark of root)	—
Akebia quinata Decne.	—
Caesalpinia sappan L.	—
Pueraria thunbergiana Benth.	—
Stephania tetrandra S. Moore	—
Gentiana scabra var. *buergeri*	—
Paeonia lactiflora Pall.	++
Panax ginseng C.A. Meyer	—
Tribulus terrestris L.	—
Dalbergia odorifera T. Chen.	+
Piper nigrum L.	—
Psoralea corylifolia L.	—
Alpinia katsumadai Hayata.	++
*Dolichos lablab*L.	—
Amyda sinensis Wiegmann.	—
Perilla frutescens (L.) Britton var. *acuta*(Thunb.) Kudo(leaf)	—
Chinemys reevessii Gray	—
Gentiana macrophylla Pall.	—
Aquilaria agallocha Roxb.	—
Polygala tatarinowi Regel	—
Glycyrrhiza uralensis Fisch.	—
Platycodon grandiflorum (Jacq.) A. DC.	—
Bupleurum chinense DC.	—
Angelica gigas Nakai	—
Bombux mori L.	+
Curcuma zedoaria Rosc.	—
Phyllostachys nigra (Lodd.) Munro var. *henonis* stapf ex Rendle.	—
Morinda officinalis How.	—
Curculigo orchiodes Gaertn.	—
Betula platyphylla Suk. var. *japonica* (Sieb) Hara	—
Agastache rugosa (Fisch. et Mey.) O. Ktze.	—
Erythrina variegata L.	—
Chrysanthemum morifolium Ramat.	—

Oriental drugs	Inhibition
Raphanus sativus L.	—
Trigonella foenum-graecum L.	+
Plantago asiatica L.	—
Ephedra sinica Stapf	+ + + +
Polyporus umbellatus (Pers.) Fr.	—
Omphalia lapidescens Schroeter	—
Pinus densiflora S. et Z.	—
Boswellia carterii Birdw.	—
Arctium lappa L.	—
Aster tataricus L.	—
Lycopus ramosissimus var. *japonicus* Kitamura	—
Adenophora triphylla var. *japonica* Hara	—
Trichosanthes kirilowii Max.(seed)	—
Patrinia villosa Juss.	—
Lonicera japonica Thunb (young flower bud)	—
Corydalis turtschaninovii Bess.	—
Scutellaria baikalensis Georgi	—
Asarum sieboldii Miq.	—
Ophiopogon japonicus Ker-Gawl	—
Coix lachryma-jobi L.	—
Prunella vulgaris var. *lilacina* Nakai	—
Poria cocos Wolf.	—
Styrax benzoin Dryand	—
Commiphora molmol Engler	—
Aloe vera L.	—
Artemisia capillaris Thunb.	—
Dryobalanops aromatica Gaertn.	—
Taraxacum platycarpum H.	—
Momordica cochinchinensis (Lour.) Sprengel	—
Lonicera japonica Thunb.	+
Uncaria rhynchophylla (Miq.) Jacks	—
Astragalus membranaceus Bunge	—
Achyranthes bidentata Bl.	—
Lithospermum erythrorhizon S. et Z	—
Ledebouriella divaricata (Turcz.) Ueki.	—
Aristolochia debilis S. et Z.	—
Saussurea lappa Clarke	—
Inula helenium L.	—
Coptis japonica Makino	—
Atractylodes japonica Koidz. et Kitam.	—
Rheum coreanum Nakai	+
Curcuma longa L.	—
Cervus nippon Temminck(mature)	—
Sabina chinensis (L.) Antoine	—
Ulmus macrocarpa Hance	—
Inula britannica L. var. *Chinensis* (Rupr.) Reg.	—
Trogopters xanthipes Milne-Edwards	—
Arisaema japonicum Bl.	—
Gleditsia sinensis Lam.	—
Buddleja officinalis Max.	—
Dipsacus japonicus Miq.	—
Cryptotympana atrata Fabr.(skin)	—
Rubia akane Nakai	+ + + +
Atractylodes macrocephala Koidz.	—
Zingiber officinale Rosc	+ +
Pinellia ternata (Thunb .) Breit.	—
Perilla frutescens (L.) Britton var. *acuta* (Thunb.) Kudo(seed)	—
Caesalpinia sappan L.	+ +
Tussilago farfara L	—
Haliotis gigantea Gmelin	—
Poncirus trifoliata (L.) Raf. (mature)	—
Cibotium barometz (L.) J. Sm.	—
Ostrea talienwhanensis Crosse	—
Nelumbo nucifera Gaertn. (seed)	—
Broussonetia papyrifera (L.) Vent.	—
Spirodela polyrrhiza (L.) Schleid	—
Rubus coreanus Miq.	—
Manis pentadactyla L.	—
Haematitum	—
Mirabilitum	—
Fossilia Ossis Mastodi	—
Massa Medicata Fermentata	+
Gypsum	—
Succinum	—

Symbols, — no inhibition,
+ 10-20% inhibition
+ + 20-50% inhibition,
+ + + 50-80% inhibition
+ + + + 80-100% inhibition,

hypertriglyceridaemia can be prevented in many cases. However, in daily practice the observance of such restrictions is often hindered by unfavorable circumstances and inner resistance[16].

Carbohydrates are a main component of human food, 80-90% consisting of starch and sucrose. In general more than 250g of di-and polysaccharide must be enzymatically split in the intestinal tract before they can be utilized by the organism. According to the concept by Puls[16,17] a pharmacological interference with the intestinal carbohydrate digestion by suitable α-glucosidase inhibitors should be a feasible way to regulate and retard carbohydrate digestion, control the rate of absorption of monosaccharides and by this way

Table 2. α-Amylase inhibition activities of 14 oriental drugs selected at first screening

Orental drugs	Inhibition ratio (%)
Cinnamomum cassia Presl.	83.7
Schizandra chinensis Baill	0.5
Magnolia officinalis Rehd. et Wils.	1.4
Amomum xanthioides Wall.	38.3
Cornus officinalis Sieb. et Zucc.	7.7
Chenomeles sinensis (Dum-Corus.) Schneid	7.5
Paeonia suffruticosa Andr.	0.0
Boschniakia glabra C.A. Meyer	6.8
Ephedra sinica Stapf	75.1
Prunus mume Sieb. et Zucc.	0.5
Alpinia officinarum Hance	13.8
Rubia akane Nakai	7.3
Reynoutria elliptica (Koidz.) Miq.	45.2
Areca catechu L.	87.1

50grams of oriental drug was extracted by the method described in "Materials and Methods", and the solution was diluted with McIlvaine buffer(pH 5.0) 10 fold.

influence the intermediary metabolism of the carbohydrates.

In future after selecting a oriental drug having strong inhibitory activity against α-amylase, we are willing to continue a series of study on the isolation, purification of the inhibitory substance from the drug, on the characteristics of the substance, the inhibitory activities for other carbohydrolases and enzyme kinetics etc.

References

1. Chrzaszcz, T. and Janicki, J. : "Sisto-amylase", ein naturlicher paralysator der amylase. *Biochem. Z.*, **260**, 354(1933)
2. Kneen, E. and Sandstedt, R.M. : An amylase inhibitor from certain cereals. *J. Am. Chem. Soc.*, **65**, 1247(1943)
3. Shainkin, R. and Birk, Y. : α-Amylase inhibitors from wheat. Isolation and characterization. *Biochim. Biophys. Acta*, **221**, 502(1970)
4. Blanco-Labra, A. and Iturbe-Chinas, F.A. : Purification and characterization of an α-amylase inhibitor from maise(Zea maize). *J. Food Biochem.* 5, 1(1981)
5. Marshall, J.J. : Pancreatic α-amylase inhibitors in cereals. *Carbohy. Res.*, **57**, C27(1977)
6. Granum, P.E. : Purification and characterization of an α-amylase inhibitor from rye(Secale cereale) flour. *J. Food Biochem.*, **2**, 103(1978)
7. Hemberg, J. and Larsson, I. : The inhibitor beta complex from resting potato tuvers as an inhibitor of α-amylase. *Physiol. Plant.*, **14**, 861(1961)
8. Pick, K.H. and Wober, G. : Proteinaceous α-amylase inhibitor from beans(*phaseolus vulgaris*). Purification and characterization. *Hoppe-Seyler's Z. Physiol. Chem.*, **359**, 1371(1978)
9. Frels, J.M. and Rupnow, J.H. : Purification and partial characterization of two α-amylase inhibitors from black bean(*Phaseolus vulgaris*). *J. Food Biochem.*, **8**, 281(1984)
10. Murao, S., Ohyama, K. and Ogura, S. : Isolation of amylase inhibitor-producing microorganism. *Agric. Biol. Chem.*, **41**, 919(1977)
11. Murao, S. and Ohyama, K. : Chemical structure of an amylase inhibitor. S-AI, *Agric. Biol. Chem.*, **43**, 679(1979)
12. Schmidt, D.D., Frommer, W., Jung, B., Müller, L., Wingender, W., Truscheit, E. and Schäfer, D. : α-Glucosidase inhibiotrs. *Naturwissenschaften*, **64**, 535(1977)
13. Puls, W., Keup, U., Krause, H.P., Thomas, G. and Hoffmeister, F. : Glucosidase inhibition. *Naturwissenschaften*, **64**, 536(1977)
14. Truscheit, E., Frommer, W., Junge, B., Müller, L., Schmidt, D.D. and Wingender, W. : Chemistry and biochemistry of microbial α-glucosidase inhibitors. *Angew. Chem. Int. Ed. Engl.*, **20**, 744(1981)
15. Fuwa, H. : A new method for microdetermination of amylase activity by the use of amylose as the substrate. *J. Biochem.*, **41**, 583(1954)
16. Puls, W., Keup, U., Krause, H.P., Müller, L., Schmidt, D.D., Thomas, G. and Truscheit, E. : Pharmacology of a glucosidase inhibitor. *Front. Hormone Res.*, **7**, 235(1980)
17. Puls, W. and Keup, U. : Influence of an α-amylase inhibitor(BAY d 7791) on blood glucose, serum

insulin and NEFA in starch loading tests in rats, dogs and man. *Diabetologia*, 9, 97(1973)

(Received July 1, 1988)

α-Amylase Inhibitor를 함유한 생약제의 검색

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216종의 생약을 대상으로 해서 bacterial α-amylase에 대해 저해 활성을 나타내는 생약을 선별하였으며, 2차 선별시험 결과 *Areca catechu* L.(빈랑), *Cinnamomum cassia* Presl.(계피) 및 *Ephedra sinica* Stapf(마황)가 비교적 강한 저해 활성을 나타내었다.