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Properties of Angiotensin I - Converting Enzyme(ACE)

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1. Introduction

The life-style diseases, such as obesity, diabetes and hypertension, and cardiovascular disease have been increasing in Japan recently. The mortality rate due to heart disease or vascular disease has been 2nd and 3rd of total death toll. The hypertension is a most important disease because it may induce cerebral apoplexy and cardiovascular disease.
Recently, new aspects of food functions have been drawing considerable attention.
Among them are the physiological functions of some food components in relation to certain ailments. One of the representatives is the epidemiological relationship between foods and hypertension (1), (2). Some foods are known to be effective in suppressing the development of hypertension (3), which suggests the existence of some components having medicine-like action on the regulation systems of blood pressure.
The renin-angiotensin system is considered to be a blood pressure regulation system which is apt to be affected by food components (4). The angiotensin I-converting enzyme (ACE) is the key enzyme in the rennin angiotensin system. Therefore, food components which inhibit ACE have the possibility to lowering of blood pressure.
Based on this point of view, the effects of ACE inhibitor from various food materials, and fermented foods were surveyed.

2. Angiotensin I - Converting Enzyme(ACE) in the renin-angiotensin system

ACE (peptide dipeptidase, E.C.3.4.15.1) plays an important physiological role in rennin angiotensin system to regulate both the arterial blood pressure, and salt and water balance(5). The system starts by the conversion of angiotensinogen to a pre-hypertensive hormone angiotensin I by the action of rennin which is secreted by the kidney(Fig.1). The angiotensin I is further converted to angiotensin II, the active form of the hormone, by the action of ACE. Angiotensin II raises blood pressure by acting directly to blood vessels, sympathetic nerves and adrenal glands. ACE is an important target for inhibitor since it performs the last step in the biosynthesis of the octapeptide angiotesin II. ACE also destroys the vasodilating peptide bradykinin.
Therefore, food components which inhibit ACE have the possibility to suppress hypertension by decreasing the formation of angiotensin II.
In fact, oral dose of synthetic ACE inhibitor like captopril to hypertensive patients becomes the first choice for the medical treatment.

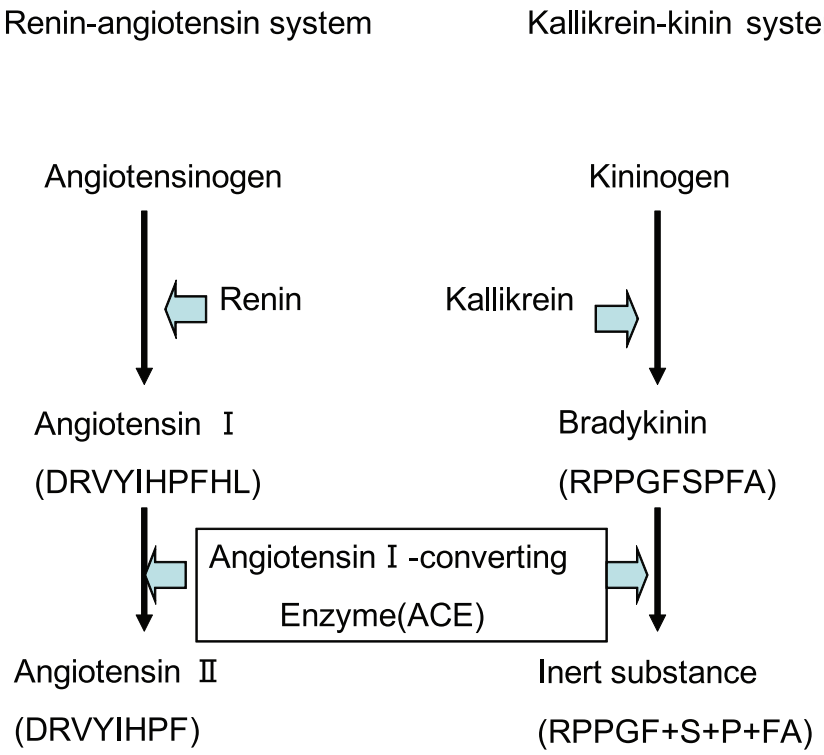


Fig. 1. Enzymatic action of ACE

The enzyme seems to be similar in rabbit, dog, rat, pig, and man and to be present in a large number of tissues including lung, kidney, plasma, and brain and in the endothelial lining of the vasculature. The ACE is a glycoprotein.

The most abundant forms have molecular weights in the range 130,000-140,000kDa, of which about 25% is carbohydrate by weight. It has a broad tolerance for substrates, although it will not hydrolyze peptide bonds containing a secondary amino group nor are peptide bonds containing acidic residues in the S'_2 subsite good substrates.

3. ACE inhibitor

Since snake venom peptide inhibitors played a role in establishing the clinical value of ACE inhibitors (6), many peptide derivatives of inhibitor have been developed(7).

Many natural products have also been examined for ACE inhibitory activity, and teprotide from *Brothrops Jararaca* (8), aconvenin from *Streptomyces* (9), and others (10,11) have been identified. ACE inhibitors such as captopril and enalapril have been as antihypertensive drugs. Therefore, the inhibiton of ACE can reduce blood pressure.

In recent years, several ACE inhibitory peptides from food protein sources have been isolated and reported, such as casein (12), tuna (13) and sardine (14).

However, most of them were peptides of enzymatic hydrolyzates from food protein source. There have been a few reports about non-peptidyl ACE inhibitors, polyphenols (15), and nicotianamine (16).

3.1 ACE inhibitor of various fermented foods and protease hydrolyzates from various food protein

ACE inhibitory activity was measured with 11 kinds (31 items) of fermented foods (17) (Table 1). The degrees of ACE of the various fermented food inhibition was expressed as IC_{50}, defined as the sample concentration that inhibits 50% of ACE activity. The degrees of ACE inhibition by the food samples were compared based dry weight.

The IC_{50} of three kinds of cheese (blue, camembert, and red cheddar), dried bonito, and natto were particularly very low (0.16-0.2mg/ml). Nyufu and temphe caused moderated inhibition (IC_{50} of 0.66mg/ml). ACE inhibition by miso paste is relatively weak.

Among the tree kinds of miso, interestingly, the miso made from rice did not inhibit ACE. These results suggested that the material for fermentation or fermentation process may affect ACE inhibitory activity of the final products. Similary, a vinegar from apple and a mirin produced by an ordinary method did not inhibit ACE.

This might suggest that these foods contains inhibitory substances produced during fermentation. Considering the IC_{50} values based on dry weight, cheese (expect for cottage cheese), fish sauce, say sauce, natto, and nyufu had high ACE inhibiting power.

Protease hydrolyzates of various food proteins which indicate ACE inhibitory activity have been reported to have hypotensive activity (12, 13, 14). Recent studies found several ACE inhibitory peptides isolated from the hydrolyzates of proteins by various kinds of protease (18-19) (Table 2).

3.2 ACE inhibitor in various beans

The two reports about ACE inhibitory activity of soy sauce have recently been reported. Kajimoto reported that say sauce decreases blood pressure in dog (20). It suggested that soy sauce contains a substance that promotes histamine absorption as the causative agent of blood pressure decreased effect.

Kinoshita *et al*. found an inhibitory compound of the ACE in say sauce (16).

ACE inhibitor in the high molecular weight (Hw) fraction from soy sauce by gel filtration chromatography decreased blood pressure in hypertensive rats (SHR) and two-kidney Goldblantt hypertensive rats (2HGH rats). The main ACE inhibitor in the Hw fraction was identified as nicotianamine (*N*-[*N*-(3-amino-3-carboxypropyl) -3-amino-3-carboxypropyl] -azetidine-2-carboxylic acid) (Fig.2). Nicotianamine is abbreviated as NA.

They found that soybeans contain a large quantity of nicotianamine.

They suggested that the origin of the nicotianamine in soy sauce is soybeans.

The inhibition of ACE and NA content of 41 different beans were reported (21) (Table 3). Among these beans, particularly high ACE inhibitory activity was found in pea, broad bean and soybean. The NA content was the highest in pea, followed by soybean and Lenties.

The strength of ACE inhibition of beans was correlated to the amount of NA content.

This shows that ACE inhibition of beans is attributable to NA content.

Fermented foods	Raw Materials/Kinds	IC_{50}(mg/ml) [m]
Liquid products		
Fish sauce	Samon	1.69
	Sardine [a]	1.43
	Sardine [b]	3.15
	Anchovy	1.35
Mirin	General	ND
	Old style	85.62
Sake	General	5.27
	Junmai [c]	3.77
	Ginjo [d]	6.95
	Genmai [e]	4.61
Soy sauce	Koikuchi [f]	1.72
	Koikuchi [g]	1.35
	Usukuti [h]	2.75
	Saishikomi [i]	1.25
	Shiro [j]	17.8
	Tamari [k]	0.96
	Tamari [l]	0.71
Vinegar	Several corps	10.05
	Rice	8.94
	Apple	ND
Solid products		
Cheese	Camembert	0.16
	Blue	0.27
	Red cheddar	0.16
	Cottage	ND
Dried bonito	Bonito	0.24
Miso	Rice	ND
	Barley	2.38
	Soybean	5.35
Natto	Soybean	0.19
Nyufu	Soybean	0.66
Temphe	Soybean	0.51

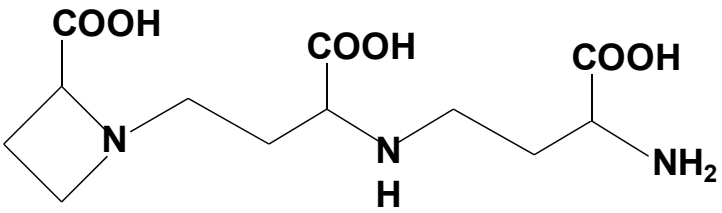
ND:no detected
a Made by the traditional method.
b Made by the enzymatic method.
c No ethanol is added throughout the manufacturing procedure.
d Made from intensively polished rice and fermented at low temperature with no or little ethanol added, and then carefully refined.
e Made from unpolished rice and aged for 3 year.
f The ratio of defatted soybean: wheat is about 1:1 in the starting material.
g The ratio of raw soybean: wheat is about 1:1.
h Soy sauce with weak color. The starting material is the same as that of koikuchi.
i Refermented soy sauce using soy sauce instead of salt water for fermentation.
j Almost all of the material is wheat. Its color is weaker than that of usukuchi.
k Almost all of the material is defatted soybean.
l Almost all of the material is raw soybean.
m The values of solid products indicate those of the extracts by 10 times volume of hot distilled water.

Table 1. ACE Inhibitory Activities of Various Fermented Foods(17)

Peptides	Origin
GPAGAHyp	Gelatin
GPHypGTDGAHyp	Gelatin
FFVAPFPEVFGK	Bovine α_{s1}-casein
AVPYPOQ	Bovine β-casein
TTMPLW	Bovine α_{s1}-casein
YIPIQYVLSR	Bovine β-casein
VHLPPP	Corn-zein
VHLPP	Corn-zein
LPP	Corn-zein
LRP	Corn-zein
LSP	Corn-zein
LQP	Corn-zein
AVN	Fig sap
PIP	Fig sap
LYPVK	Fig sap
LKY	Euphausia protein
PTHIKWGD	Tuna muscle
YKSFIKGYPVM	Sardine muscle
KVLAGM	Sardine muscle
IVGRPRHQG	Dry bonito
IKP	Dry bonito
SVAKEL	Bonito internal organ
FCF	White egg albumin
IIY	Wheat glyadine
YRILEF	Soy protein
FVIPAGY	Soy protein
IAPNY	Rice protein
IYPRY	Refined sake

Amino acids were shown by one chracter mark.
Hyp:hydroxyl proline

Table 2. Peptidyl ACE inhibitor from foods and food proteins



Nicotianamine

Fig. 2. Structure of nicotianamine.

Family (Nomenclature)	IC_{50} (mg/ml・raction mixture)	NA (mg/dry 100g)
Common bean *(phaseolus vulgaris)*	0.1	37(n=10)
Pea *(pisum sativum)*	0.08	50.7(n=5)
Cowpeas *(vigna unguiculata)*	0.39	18.7(n=5)
Broad bean *(Vicia faba)*	0.09	25.5(n=3)
Soybean *(Glycine max)*	0.11	39.5(n=6)
Sword bean *(Canavalic gladiata)*	0.4	11.9(n=1)
Chickpea *(Cicer qrietinum)*	79.6	31.5(n=1)
Lentis *(Lens culinarics)*	88.7	36(n=1)

NA:Nicotianamine

Table 3. ACE inhibitory activities of various beans(21)

3.3 ACE inhibitor in various vegetables and mushrooms.

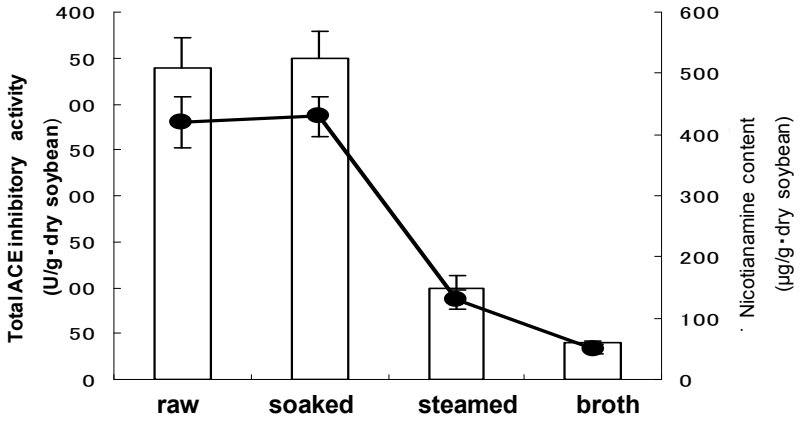
NA content in various vegetables and mushrooms have been reported .

NA content of Hayatouri(*Sechium edule*), Seri(*Oenanthe javanica*) ,Ashitaba(*Angelia keiskei*), Moroheiya (*Corchorus olitorius*), Kureson (*Nastrutium officinale*), Nigauri (*Nomordica charantia*) were 1.0 , 1.2, 1.7, 2.2, 3.7, 1.5 mg/100g/fresh matter, respectively(22). Of the 23 mushrooms, marked ACE inhibitory activity was observed in *Rhodophyllus clypeatus, Lyophyllum semitable,* and *Pholiota adipose*. The NA content of *Rhodophyllus clypeatus* was 13.2mg / dry 100g (23).

4. Isolation of Nicotianamine (NA) from soybean broth and changes in its content in the soybean steaming process.

Soybean broth (SB), a by-product of miso, soy sauce and natto as tradtional japanese foods manufacturing, is discarded as an industrial waste (24). The change in the ACE inhibitory

activity in the soybean steaming process was investigated to evaluate the use of SB as a functional food material. An ACE inhibitor was isolated from soybean broth by ion-exchange and gel chromatography. The results of the chemical identification by TOF-mass, IR, and NMR spectra analysis corresponded to those of Kristensen et al for NA (25). The yield of pure NA from SB was 0.037% (dry matter).
In the soybean steaming process at 121°C for 30 min, the ACE inhibitory activity was the same in both raw and soaked soybeans, but decreased to 30% of raw soybeans in steamed soybeans and 10 % in SB (Fig.3).

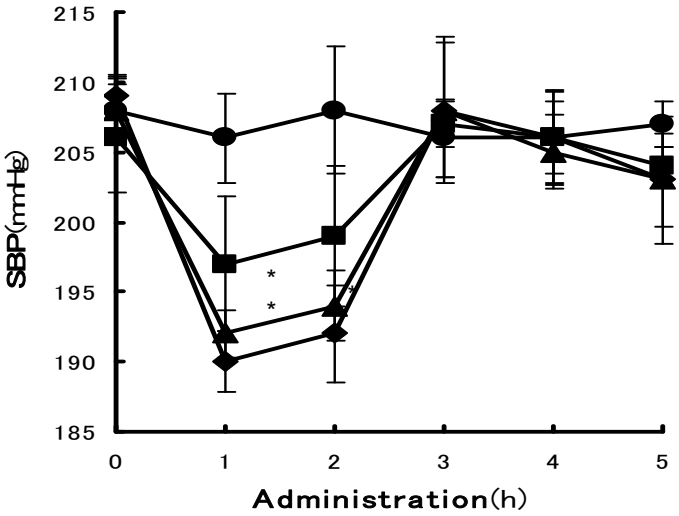


One unit of ACE inhibitory activity is defined as the amount of inhibitor needed to inhibit 50% of the ACE activity(IC_{50}value).
Means ±SD(n=3), □: total ACE inhibitory activity (U/g・dry) ● :nicotianamine content (μg/g・dry)

Fig. 3. The changes of ACE Inhibitory activity and Nicotianamine(NA) content of beans and broth in the steaming process

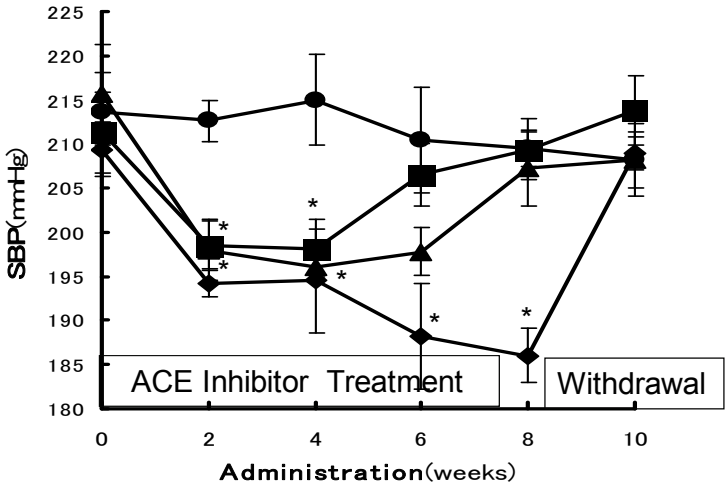
5. Antihypertensive effects of nicotianamine (NA) in spontaneously hypertensive rats

The effect of the ACE inhibitor NA form soybean broth on blood pressure was investigated in spontaneously hypertensive rats (SHR) upon single and long-term administration (26).
Single oral dose of NA (0.9, 4.5, 9.0mg/kg body weight) decreased blood pressure 1h after administration, and to the control level 3h after administration (Fig.4).
Long-term oral dose of NA(0.9mg and 4.5mg/kg body weight) decreased blood pressure for 4 weeks after administration, while that of NA(9.0mg/kg body weight)was decreased for the full 8-weeks feeding period(Fig.5).



Symbols: ●: control: saline at a dose of 5.0ml/kg body weight; ■: NA(0.9mg/kg・b. w.); ▲: NA(4.5mg/kg・b. w.); ◆: NA(9.0mg/kg・b. w.)
Each value is expressed as mean ±S.E.M. (n=6).
Significant difference from the control group:*p<0.05

Fig. 4. Effects of single administration of nicotianamine(NA) on systolic blood pressure (SBP) in SHR



Symbols: ●: control: NA (0.0mg/kg body weight); ■: NA (0.9mg/kg・b. w.); ▲: NA (4.5mg/kg・b. w.); ◆: NA (9.0mg/kg・b. w.)

Fig. 5. Effects of long-term administration of nicotianamine(NA) on systolic blood pressure (SBP) in SHR
Each value is expressed as mean ±S.E.M. (n=6).
Significant difference from the control group:*p<0.05

At 8 weeks after administration, serum NA content in SHR was determined by amino acid analyzer and revealed that NA was not detected in the blood of SHR (0.9mg and 4.5mg/kg body weight group), while 32.6±7.3 μ g/ dL NA was detected in the 9.0mg/kg body weight group.

It was suggested that NA absorbed from the intestine decreased the systolic blood pressure (SBP) in SHR, and an appropriate NA level (9.0mg/kg body weight group) may provide long-term antihypertensive effects upon administration.

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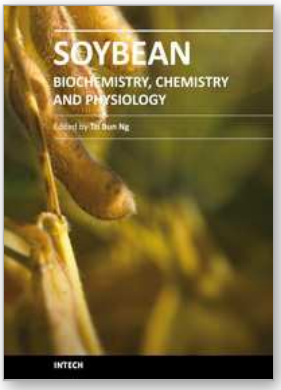

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