

Review

ACE inhibitory substances derived from soy foods

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Because about half of Japanese population have been suffering from hypertension or are about to suffer from it, hypertension is a typical lifestyle related disease in Japan. Therefore, it is important to eat foods with antihypertensive effect in order to prevent the onset or aggravation of hypertension. It is known that soy foods such as soy sauce, miso, and natto show antihypertensive effect by inhibiting angiotensin converting enzyme (ACE), the key enzyme in renin-angiotensin system. ACE inhibitory substances contained in soy sauce and miso are nicotianamine and the peptide Ser-Trp respectively, but there is almost no evidence about ACE inhibitors in natto. We purified and identified 5 novel ACE inhibitory peptides containing Ile-Ile and Ile-Asp from protease-treated hikiwari-natto, whose ACE inhibitory activity was about 1.4 times higher than that of protease-untreated hikiwari-natto. In addition, we identified 8 novel ACE inhibitory peptides containing Phe-Phe-Tyr-Tyr and Trp-His-Pro derived from protease-treated soymilk, whose ACE inhibitory activity was about 36 times higher than that of protease-untreated soymilk.

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Introduction

Hypertension is a typical lifestyle rela-

ted disease in Japan and is defined as the situation where the systolic blood pressure is above 140 mmHg, or the

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diastolic blood pressure is above 90 mmHg according to The Japanese Society of Hypertension⁽¹⁾. The patients suffering from hypertension have almost no subjective symptoms, but it is detrimental to life to leave hypertension untreated for a long period because hypertension may lead to myocardial infarction, brain infarction, and stroke. The number of patients in Japan, suffering from hypertension and people who are in danger of suffering from it are about 40 million and 15 million respectively⁽¹⁾. Therefore, we conclude that hypertension is a big problem to healthy living in Japan.

In a situation like this, due to their antihypertensive effect, soy foods such as soy sauce, miso, and natto (fermented soybean) have been in focus for prevention of onset or aggravation of hypertension in Japan. It is deduced that soy foods show antihypertensive effect by inhibiting angiotensin converting enzyme (ACE), the key enzyme in the renin-angiotensin system.⁽²⁻⁴⁾ ACE inhibitory substances in soy sauce and miso have been already identified as nicotianamine⁽²⁾ and Ser-Trp⁽³⁾, respectively. However, there is almost no information about ACE inhibitory substance derived from natto.

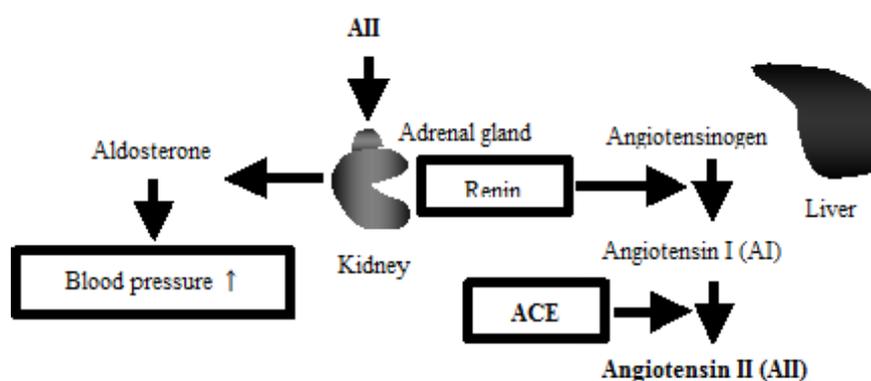


Fig.1 Renin-angiotensin system

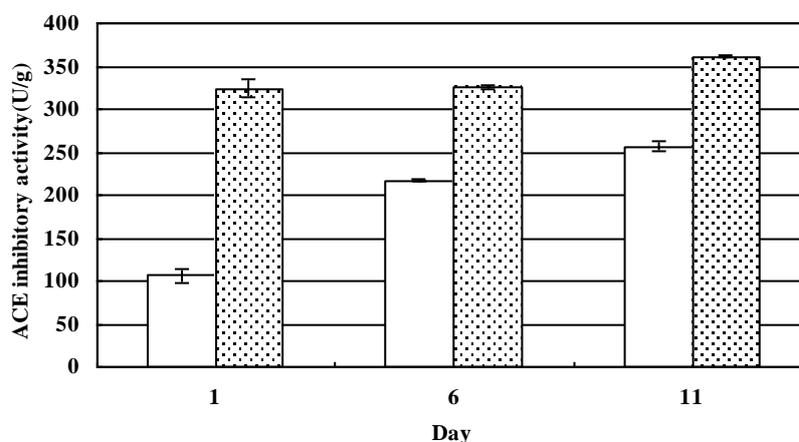


Fig.2 ACE inhibitory activities of nattoes

□ , Hikiwari-natto ; ▨ , Protease-treated hikiwari-natto.

Each bar represents the mean \pm SEM(n=3).

This figure is created based on data from [6].

In this review, the novel ACE inhibitory peptides identified from protease-treated hikiwari-natto and soy milk by us and our collaborators are introduced. In addition, we elaborate the attempt to realize the development of Food for Specified Health Uses (FOSHU) related to blood pressure.

Renin-angiotensin system⁽⁵⁾

A schematic representation of renin-angiotensin system is shown in Fig.1. In this system, Renin produced mainly in the kidney cortex is released

into the blood circulation in response to various stimuli. Renin cleaves the N-terminus of angiotensinogen produced in the liver to yield the 10-amino acid peptide, angiotensin I (AI). AI is further processed by ACE to produce the octapeptide, angiotensin II (AII). AII stimulates the synthesis and release of aldosterone, the hormone responsible for elevating blood pressure, from the adrenal cortex in the adrenal gland.

ACE inhibitory peptides derived from protease-treated hikiwari-natto

Table1 Novel ACE inhibitory peptides isolated from protease-treated hikiwari-natto

Amino acid sequence	Soy protein-derived
Ile-Ile	β -amylase <i>etc.</i>
Ile-Asp	Cyclin <i>etc.</i>
Ile-Phe-Tyr	USP12 <i>etc.</i>
Leu-Phe-Tyr	RNA-binding protein <i>etc.</i>
Leu-Tyr-Tyr	Lipoxygenase <i>etc.</i>

It was found that ACE inhibitory substances of natto have heat resistance^(4,6), and so far only a few of the different natto have been tested. ACE inhibitory activity of natto is about 1.3 times higher than that of steamed soybean⁽⁷⁾ and ACE inhibitory activity of hikiwari-natto is more than twice that of tsubu-natto⁽⁶⁾. The main constituent of soybean is protein and therefore, we deduced that ACE inhibitor of natto is a peptide that is produced because of the

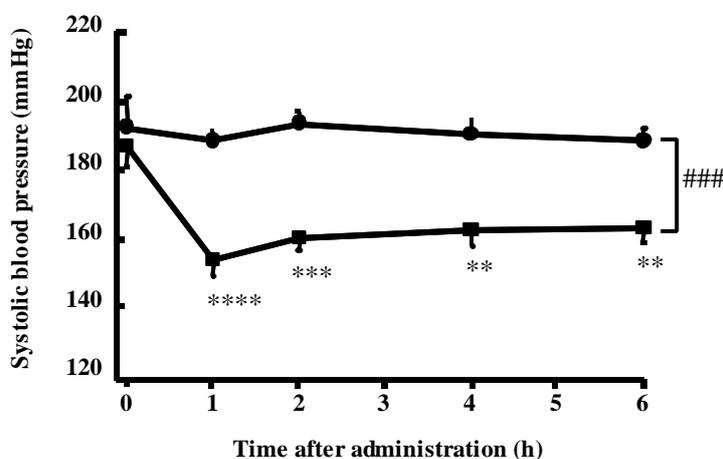


Fig.3 Change in systolic blood pressure after a single oral administration of the extract of natto and distilled water in SHRSPs

●, SHRSPs that distilled water was administered to ;

■, SHRSPs that the extract of natto was administered to.

Each point and bar represents the mean \pm SEM (n=6). ** p<0.01, *** p<0.001, **** p<0.0001 ; compared with the group that distilled water was administered. ### indicates a significant value (p<0.001) on change in systolic blood pressure of each group. This figure is created based on data from [6].

degradation of soy protein by proteases secreted by *Bacillus subtilis* natto.

We prepared protease-treated hikiwari-natto made by the addition of spore suspension of *B. subtilis* natto and the end-type neutral protease, PROTIN SD-NY10 (the protease preparation used as a food additive) to the steamed soybeans before fermentation. ACE inhibitory activity of natto prepared in this fashion is about 1.4 times higher than that of protease-untreated hikiwari-natto⁽⁶⁾ (Fig.2). Hence, we surmised that protease-treated hikiwari-natto contains peptides with strong ACE inhibitory activities.

We purified and identified 11 ACE

inhibitory peptides from protease-treated hikiwari-natto⁽⁶⁾. It was found that 5 of 11 peptides are novel ACE inhibitory peptides⁽⁶⁾ (Table 1). In addition, as shown in Fig.3, the extract of protease-treated hikiwari-natto showed significant reduction in systolic blood pressure, 1 h after oral administration to stroke prone spontaneously hypertensive rats (SHRSPs) in a single dose (80mg/kg of body weight)⁽⁶⁾. It was deduced that the strong *in vitro* ACE inhibitory activity seen in previous studies and antihypertensive effect on SHRSPs of the natto extracts were in response to the presence of ACE inhibitory peptides described in previously.

Table 2 Novel ACE inhibitory peptides isolated from protease-treated soymilk

Amino acid sequence	IC ₅₀ (μ M)
Phe-Phe-Tyr-Tyr	1.9
Trp-His-Pro	4.8
Phe-Val-Pro	10.1
Leu-His-Pro-Gly-Asp-Ala-Glu-Arg	10.3
Ile-Ala-Val	27.0
Val-Asn-Pro	32.5
Leu-Glu-Pro-Pro	100.1
Trp-Asn-Pro-Arg	880.0

ACE inhibitory peptides derived from protease-treated soymilk

Soy milk is one of major soy foods, and due to its consistency, it is easy to add a protease preparation to soymilk uniformly and control the enzymatic reaction after the addition of the protease preparation. Thus, our collaborators and we confirmed the ACE inhibitory activity of protease-treated soymilk (the soymilk treated with 0.1% [w/w] PROTIN SD-NY10). As a result, it was found that ACE inhibitory activity of protease-treated soymilk is about 36 times higher than that of untreated soymilk (Fig.4). It was deduced that protease-treated soymilk also contains the peptides with

strong ACE inhibitory activities.

We isolated and identified 11 ACE inhibitory peptides from protease-treated soymilk⁽⁸⁾. It was found that 8 of 11 peptides are novel ACE inhibitory peptides⁽⁸⁾ (Table 2). In these peptides, the IC₅₀ values of Phe-Phe-Tyr-Tyr and Trp-His-Pro are 1.9 μ M and 4.8 μ M respectively. The IC₅₀ values of 111 ACE inhibitory peptides derived from various foods reported so far are between 0.32–945.5 μ M⁽⁹⁻¹⁰⁾, ACE inhibitory activities of these 2 peptides are strong enough compared with existing ACE inhibitory peptides.

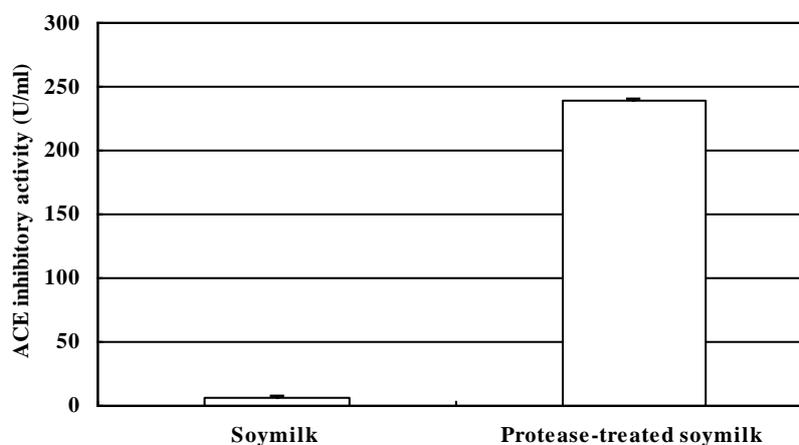


Fig.4 ACE inhibitory activities of soymilks

Each bar represents the mean \pm SEM(n=3).

Summary

Soy foods such as soy sauce, miso, and natto show antihypertensive effect by inhibiting ACE, the key enzyme of renin-angiotensin system. Our collaborators and we purified ACE inhibitory substances derived from natto that have not been identified so far. We identified 5 novel ACE inhibitory peptides from protease-treated hikiwari-natto. In addition, the extract of this natto showed significant reduction in systolic blood pressure when it was orally administered to SHRSPs in a single dose.

We also identified 8 novel ACE inhibitory peptides from protease-treated soymilk. In these peptides, the IC_{50} values of Phe-Phe-Tyr-Tyr and Trp-His-Pro are 1.9 μ M and 4.8 μ M respectively, and ACE inhibitory activities of these 2 peptides are strong enough when compared to existing ACE inhibitory activities from other foods.

Our company has been manufacturing soymilk products and the production of protease-treated soymilk is possible at

our plant. Therefore, we want to establish the development of Food for Specified Health Uses (FOSHU) related to blood pressure by developing a beverage that contains protease-treated soymilk backed with scientific evidence necessary to gain FOSHU approval in the future.

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