What is Nattokinase?

1. Efficacy of Natto and Nattokinase
2. Degradation of Thrombus by Nattokinase
3. Nattokinase for lifestyle-related diseases prevention
4. Means of differentiating true Nattokinase from the counterfeit

Efficacy of Natto and Nattokinase

■ Natto and Nattokinase

A traditional Japanese food "Natto" is a food made from soybeans fermented by Bacillus natto, and has been eaten since about 1,200 years ago. Enzymes that Bacillus natto produce by fermentation include protease and Dc. Ochina at Hokkaido Imperial University reported its purification and characteristics. Since then, it was investigated as a serine protease, and, in 1980s, the enzyme that degrades fibrin proteins (a cause of thrombi) was named as "Nattokinase". Nattokinase is found in the sticky part of Natto.

■ Function of Nattokinase

Functions of Nattokinase include directly degrading a fibrin (the main component of thrombi), activating pro-urokinase (precursor for urokinase that is an thrombolytic enzyme in the body) and increasing the amount of tissue plasminogen activator (t-PA) that produces a thrombolytic enzyme, plasmin. In addition, recent research has revealed that Nattokinase has a function of degrading plasminogen activator inhibitor, PAI-1 and reducing the euglobulin lysis time, therefore, it has a function of improving the thrombolytic activity. Furthermore, the reduction of blood pressure has also been confirmed.

Degradation of Thrombus by Nattokinase

■ The Role and Danger of Thrombus

A thrombus is a blood clot formed in blood mainly consisting of a protein called a fibrin, which is necessary for wound healing during blood vessel and stopping bleeding. Once bleeding stops and the damaged blood vessel is restored, thrombi are degraded. This phenomenon is called the fibrinolytic action. However, when there is an imbalance between the production of thrombus (coagulation) and its degradation (fibrinolysis), or the condition that thrombus are difficult to be degraded due to aging or stress, blood cells accumulate in blood vessels. These blood clots cause thrombosis such as cardiac infarction and cerebral infarction when they block the blood vessel of the heart and brain, respectively.

■ Degradation of Artificial Thrombus by Nattokinase

Figure 1 shows the time course of degradation in artificial thrombus (white part) on which Natto was placed. As you can see, the thrombus around Natto is degraded over time goes by. This degradation phenomenon (action) takes place because of Nattokinase in the sticky part of Natto.

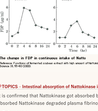
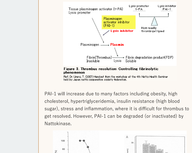


Figure 1. Time course of degradation of an artificial thrombus by the sticky part of Natto (Frozen placed in a JIS sieve)

■ Intake of Natto and Fibrin Degradation Product (FDP)

Figure 2 below shows the change in fibrin degradation product (FDP) of 5 healthy volunteers who ate Natto, measured by latex agglutination. The graph named "Control" represents the result after eating Natto for the first time, "2 days" represents the result after eating Natto for the second time on the second day, and "4 days" represents the result after eating Natto for the third time on the fourth day. You can see that FDP value dramatically increases in 4 hours after eating Natto every time and decreased in 6 hours and 8 hours. Moreover, comparing these 3 charts, the FDP value numbers went down as the volunteers ate Natto more times. This means that fibrins get degraded by continuously eating Natto.



The change in FDP in continuous intake of Natto
Volunteers: 5 healthy men (average age 21 years old) ate usual of Nattokinase, Japan Food Science, 51, 351-354(2012)

■TOPICS : Intestinal absorption of Nattokinase in animal experiments

It is confirmed that Nattokinase get absorbed by bowel and the absorbed Nattokinase degraded plasma fibrinogen.

<Reference >
Fujita M , Hong K, Ito Y, Misawa S, Takeuchi N, Kariya K, et al.
Transport of nattokinase across the rat intestinal tract.
Biol. Pharm. Bull. 1995;18(0):1194-6.

Intraduodenal administration of nattokinase (NK) at a dose of 89 mg/kg, resulted in the degradation of fibrinogen in the plasma suggesting transport of NK across the intestinal tract in normal rats. The action of NK on the cleavage of fibrinogen in the plasma from blood samples drawn at intervals after intraduodenal administration of the enzyme was investigated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting analysis with an anti-fibrinogen γ chain antibody. The 270 kDa fragment carrying antigenic sites for the binding of the anti-fibrinogen γ chain antibody appeared within 0.5 h and was then degraded gradually to a 105 kDa fragment via a 200 kDa fragment. This suggests that fibrinogen degraded to a 105 kDa fragment via several intermediates (270 and 200 kDa). In parallel with the degradation process, activities of recalcification times were remarkably prolonged. NK was also detected in the plasma from blood samples drawn 3 and 5 h after administration of the enzyme by SDS-PAGE and Western blotting analysis with an anti-NK antibody. The results suggested that NK is absorbed from the rat intestinal tract and that NK cleaves fibrinogen in plasma after intraduodenal administration of the enzyme.

■ Lysis inhibitor and Nattokinase

Depression of thrombolytic function can be a risk factor for thrombosis. It is decided by the balance of t-PA (a lysis promoter) and PAI-1 (a lysis inhibitor) that have roles for thrombus lysis.

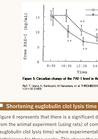


Figure 3. Thrombus resolution Controlling fibrinolytic activity
t-PA : tissue plasminogen activator, PAI-1 : plasminogen activator inhibitor-1, Lysis promoter : t-PA, Lysis inhibitor : PAI-1

PAI-1 will increase due to many factors including obesity, high cholesterol, hyperglycaridemia, insulin resistance (high blood sugar), stress and inflammation, where it is difficult for thrombus to get resolved. However, PAI-1 can be degraded (or inactivated) by Nattokinase.

Figure 4. Effect on PAI-1
N=3 mean ± S.E. p<.05 (Dunn TECHNO-ANOVA)

■ Change of PAI-1 in a day (Thrombosis is more likely to happen at dawn)

Cerebral infarction and cardiac infarction happen more often in the early morning and it is considered that this is related to circadian rhythm of PAI-1. Figure 5 shows the circadian change of the PAI-1 level in the blood. It is high in the morning and it decreases as time goes; thus thrombus is more likely to be generated in the morning.

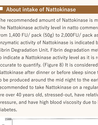


Figure 5 Circadian change of the PAI-1 level in the blood
N=7 mean ± S.E.Values for females, n = 4 TIPS&REED RESEARCHES

■ Shortening euglobulin clot lysis time by Nattokinase

Figure 6 represents that there is a significant difference in the result from the animal experiment (using rats) of comparison of ECLT (euglobulin clot lysis time) where experimental group was fed Nattokinase for three weeks. This shows the experimental group has got an advantage in the fibrin state and therefore the blood viscosity improves.

Figure 6. Effect of Nattokinase on ECLT (euglobulin clot lysis time)
N=10 mean ± S.E. Student Sample t-Test: * p<.05

Nottokinase for lifestyle-related disease prevention

■ Natto Foods vs. Nattokinase Supplements

The level of Nattokinase activity in commercially available natto foods varies and the activity level will decreases due to the way the Natto food is cooked or processed. However, ingredients in dietary supplements are more stable. Therefore, supplements often offer a more stable level of Nattokinase activity.

It is often discussed that the best time to take Nattokinase is after dinner or before sleep. You may not like to eat Natto foods before sleep but it is easy to do so with supplements.

Natto foods have very distinctive smell and flavour so that you need consider where and when to eat them. However, you can take supplements wherever you like.

Natto foods have a high nutritional value but also a high amount of calorie and purine, which you may be concerned with. In contrast, calories in supplements are usually lower so that you do not need worry about the calorie intake.

■ About intake of Nattokinase

The recommended amount of Nattokinase is more than 2,000FU/ day. The Nattokinase activity level in natto commercially available varies from 1,400 FU/ pack (50g) to 2,000FU/ pack as Figure 7 shows. The enzymatic activity of Nattokinase is indicated by FU which stands for Fibrin Degradation Unit. Fibrin degradation method (using FU) is used to indicate a Nattokinase activity level as it is reproducible and accurate to quantity. (Figure 8) It is considered best to take Nattokinase after dinner or before sleep since thrombus is more likely to be produced around the mid night to the early morning. It is recommended to take Nattokinase on a regular basis for those who are over 40 years old, stressed-out, have relatively high blood pressure, and have high blood viscosity due to hyperlipidemia or diabetes.

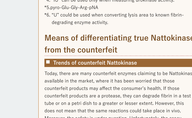


Figure 7. Enzymatic activities of commercially available natto foods (1Pack/ pack)
Activity of Nattokinase in natto foods differ in each product. (N=3 mean ± S.E.)

Figure 8. Comparison of assay methods of Nattokinase Activity

Method	Fibrin Plate	Chromogenic Substrate	Fibrinolytic Activity	Nattokinase
Principle	CV	Jimenez Cassette	Casein	The reaction of the hydrolysis of the chromogenic substrate specifically towards Nattokinase (??) and the amount of the generated pNA serve as the nattokinase activity
Nattokinase Activity	12 kU/g	Plasmin	1,000 kU/g	The hydrolysis is next suitable for plasmin (Kinetic) (??) but not for any other proteases
Cardiolase S	12 kU/g	Tablase	1,000 kU/g	Recommended method for Cardiolase S product. The hydrolysis is next suitable for Nattokinase (??) but not for any other proteases
Fibrin Plate	CV	Chromogenic Substrate	Casein	Recommended method for Nattokinase. The hydrolysis is next suitable for Nattokinase (??) but not for any other proteases
Fibrin Degradation Unit	FU	Nattokinase	Plasmin	Recommended method for Nattokinase. The hydrolysis is next suitable for Nattokinase (??) but not for any other proteases
Fibrin Degradation Unit	FU	Nattokinase	Plasmin	Fibrin is a protein, and it is difficult to be dissolved. But it is stable.
Fibrin Degradation Unit	FU	Nattokinase	Plasmin	Fibrin has time to become dissolved. Nattokinase is stronger than other proteases and it is stable.

*1. It is possible if Incorrect Nattokinase is used, however the apparent titer becomes low due to the low affinity compared to fibrin.
*2. "CV" can be used only when measuring plasmin activity.
*3.H-D-Val-Leu-Lys-pNA
*4. "CV" can be used only when measuring urokinase activity.
*5.pyro-Glu-Gly-Arg-pNA
*6. "U" could be used when converting lysis area to known fibrin-degrading enzyme activity.

Means of differentiating true Nattokinase from the counterfeit

■ Trends of counterfeit Nattokinase

Today, there are many counterfeit enzymes claiming to be Nattokinase available in the market, where it has been worried that those counterfeit products may affect the consumer's health. If these counterfeit products are a protease, they can degrade fibrin in a test tube or on a petri dish to a greater or lesser extent. However, this does not mean that the same reactions could take place in vivo. Moreover, the safety is under question. Unfortunately, the assay method of Nattokinase cannot determine if an enzyme is Nattokinase. Therefore, the use of a means of differentiating Nattokinase from other proteases is necessary.

■ Simple differentiating method of "Nattokinase (*BOX method*)"

In the simple differentiating method of Nattokinase (called "Box method"), an oxidized insulin chain B consisting of 30 amino acids is degraded by a sample enzyme and the degradation pattern is confirmed by HPLC. This method allows you to determine if the sample enzyme is Nattokinase because a different enzyme shows a different degradation pattern. Figure 9 shows the degradation patterns of oxidized insulin B chain of 1) purified Nattokinase, 2) JNKA accredited product and 3) a counterfeit product. Comparing the graphs, you can see that the degradation pattern of the counterfeit product is different from that of incorrect Nattokinase; therefore you can tell that the counterfeit product does not contain Nattokinase. For further details, please contact Japan Nattokinase Association Secretariat.

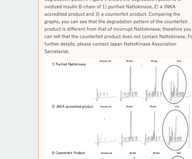


Figure 9. Comparison of the degradation patterns of oxidized insulin chain B

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