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NKCP powder

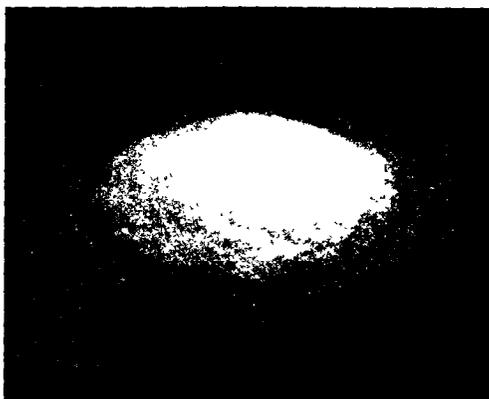
Daiwa Pharmaceutical Co., Ltd has long studied traditional Japanese foods in the interest of developing safe and useful dietary food supplements. Now, in a cooperative research project on nattokinase, Daiwa Pharmaceutical Co., Ltd, the Department of Physiological Chemistry, Science and Industrial Technology, Kurashiki University of Science and the Arts, and the Department of Bio-resources Chemistry, Faculty of Horticulture, Chiba University have succeeded in producing a highly active, odor-free and flavorless nattokinase-based product.

NKCP ~Nattokinase Compound~

1. Background of the development of NKCP and its significance

Natto, made of fermented soybeans, is a traditional Japanese food. Many people enjoy it for its distinctive flavor, livened by the activity of *Bacillus subtilis Natto*. Natto has a long history, and some have theorized that it may even have prehistoric origin, possibly circa B. C. It has at least been ascertained that natto has been popular since the Edo period, 400 years ago. Originally, natto was utilized as a folk remedy for heart and vascular diseases, fatigue, and beriberi. In 1980, Dr. Hiroyuki Sumi et al. found that natto has a potent fibrinolytic enzyme, which they named nattokinase. Generally, the material found to block blood vessels is called fibrin, and the resultant disease is called thrombosis. Representative thrombotic diseases are ischemic cardiac disease and cerebral infarction, and these currently account for approximately 40% of deaths in Japan. Consequently, the discovery of nattokinase has excited interest in natto as a preventive and means of treatment of thrombosis. While there are a number of fans of natto, there are still a good many people who, despite being informed of its beneficial qualities, have been unable to develop an appreciation of its distinctive flavor. NKCP is a product developed to allow easy intake of therapeutically-significant amounts of nattokinase.

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2. Physicochemical properties of nattokinase

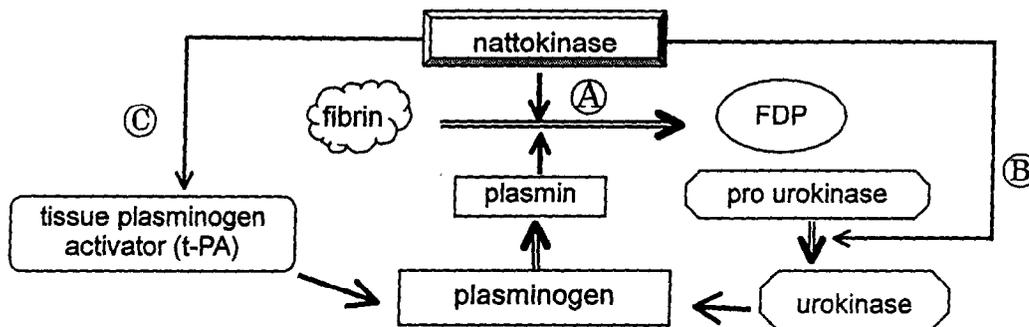
- | | |
|----------------------|--|
| 1) Molecular weight | 20,000 ~30,000 (257 amino-acid residue) |
| 2) Isoelectric point | 8.6 ±0.3 (Svensson Column Method) |
| 3) Stability | Stable heated to 60 °C in basic water solution within pH 6 -12 |

3. Physiological activation of nattokinase

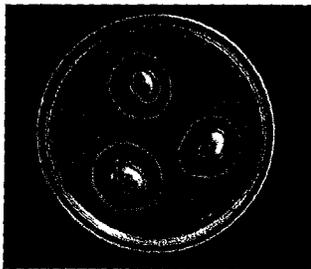
- ① lyses fibrin in vivo by oral administration

[Process of activity]

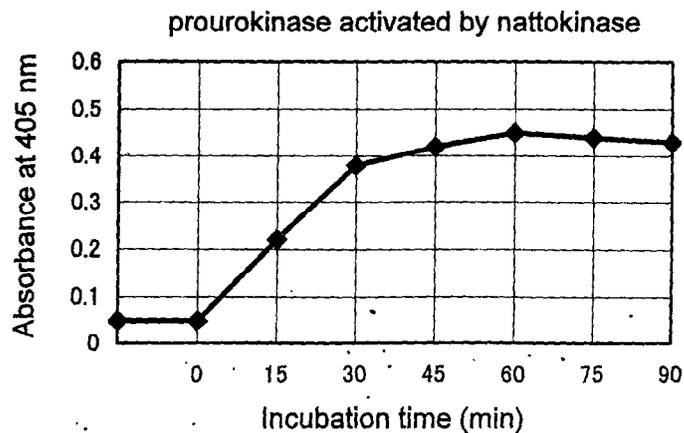
- Ⓐ lyses fibrin directly
- Ⓑ changes prourokinase activated to urokinase
- Ⓒ increases tissue plasminogen activator (t-PA)



- ② Maintains the consistency of fibrinolysin in the blood for hours
- ③ No rebound usually associated with heavy urokinase administration
- ④ Hypotensive effect on blood circulation
- ⑤ Potentially a preventive of dementia



Natto was applied directly to a fibrin plate. Fibrin around natto was lysed by nattokinase.



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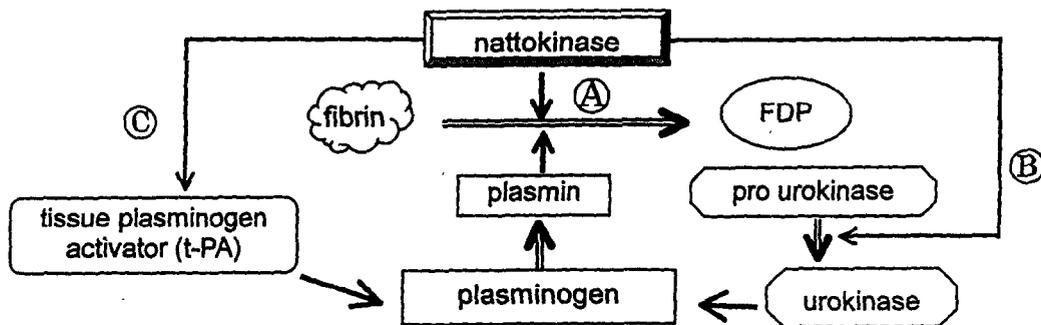
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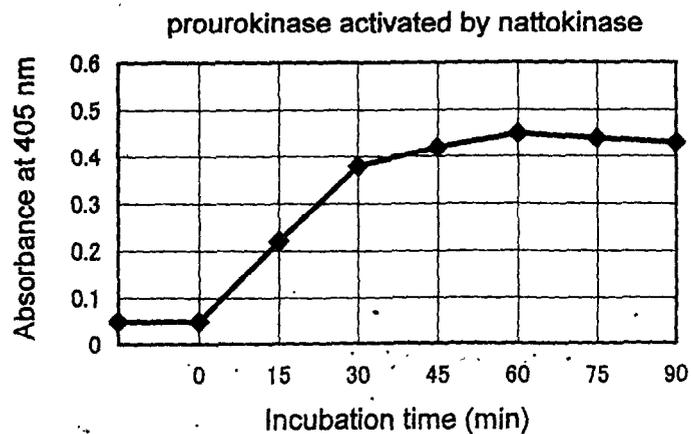
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4. NKCP specifications

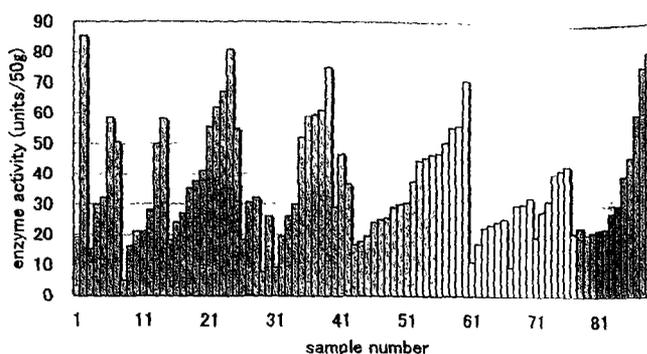
NKCP is powdery product produced from dried culture filtrate of partially distilled *B. subtilis natto*. With natto's distinctive odor and viscid texture eliminated, it is easy to take, whether or not you like natto itself.

1) Characteristics

- Activity is stable at room temperature due to high content of nattokinase units.
- The nattokinase activation of natto on the market varies widely depending on each product, whereas that of NKCP is constant.
- Free of distinctive odor and viscid texture
- Low hygroscopicity, water soluble
- "Chimney sweeping" effect on blood vessels with constant intake
- Less antagonistic to other drugs, low Vitamin K content
- Devitalization by heating above 60 °C

140°

Nattokinase Activity in Natto Products on the Market



This bar graph shows the nattokinase activity of 90 natto products on the market.

2) Doses

Recommended dosage of 1 g to 3 g/day as dietary and health supplement

3) Form

Suitable for tablets, capsules, and granules

4) Nattokinase activation

Nattokinase activity in 1g of NKCP is worthy of that in 1 packet of Natto (50g) according to measuring the plasmin substrate (H-D-Val-Lys-pNA) or fibrin as a substrate.

5) Safety

- ① Mutagenicity Negative
- ② Acute toxicity(rat) $LD_{50} > 5000 \text{mg/kg}$
- ③ Subacute toxicity(rat) No Observed Adverse Effect Level $> 1000 \text{ng/kg/day}$
- ④ Overdose test(rat)

As a result of introducing five times the dose into the duodenum as the normal quantity in blood coagulability delayed model rat, no bleeding was observed and NKCP caused no significant symptoms.

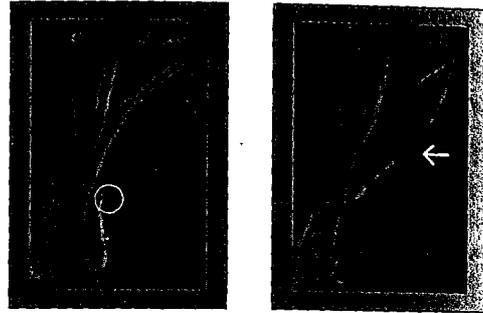
5. Fibrinolytic activity of Nattokinase

Nattokinase has fibrinolytic activity. For prevention of thrombosis such as myocardial infarction and cerebral infarction, it is necessary to lyse fibrins continually generated in the blood and to stimulate blood circulation. Nattokinase has the potential to decrease risk of thrombosis by playing a "Chimney sweep" role. The following is a report on research on the process of nattokinase activity.

H. Sumi, H. Hamada, K. Nakanishi, and H. Hiratani: Enhancement of the Fibrinolytic Activity in Plasma by Oral Administration of Nattokinase, *Acta Haematol.*, 84, 139 (1990)

① Experimental Thrombus Model: Canine

Enteric-coated capsules were prepared containing 250 mg/capsule of nattokinase (approximately 2.13 cu/mg of protein using human plasmin as the standard) extracted from natto. Four capsules were administered orally to dogs (males, BW 10.1-10.6 kg) with induced thrombosis. For the control group, four placebo capsules were administered as well. Thrombosis evaluation was by angiography by inserting a catheter into the femoral arteries. Angiograms were obtained before thrombus formation and at 2.5, 5, 12, 18, and 24-hour intervals after thrombosis induction. Results revealed that, in comparison with the control group, in which even 18 hours after administration there was no evidence of lysis, the nattokinase group revealed complete recanalization of blood circulation within 5 hours of administration.



The dosage of Nattokinase lyses an artificial fibrin (See ○ in the left.) and stimulates the flow of blood (See ← in the Right.).

② Oral Administration to Humans and Determination of Fibrinolytic Activities

Enteric-coated capsules were prepared containing 650 mg/capsule of nattokinase (approximately 2.13 cu/mg of protein using human plasmin as the standard) extracted from natto. Twelve Japanese volunteers (6 men and 16 women, aged between 21 and 55 years) were given 200 g of natto or boiled soybeans (control group) once before breakfast, or 2 enteric-coated capsules containing nattokinase (650 mg/capsule) 3 times a day after meals. Blood was collected at 10:00 every morning and the fibrinolytic parameters measured in the serum and plasma. The EFA increased gradually from the 1st to the 8th day after nattokinase administration (Fig. 1). The FDP was statistically significantly higher on the 1st day of nattokinase administration compared with pre-administration (Fig. 2). The amount of TPA antigen, which is well known as one of the factors related to fibrinolytic activity in the blood, was also significantly different over the long term (Fig. 3). The enhancement of plasma fibrinolytic activity is thought to involve absorption of nattokinase across the intestinal tract, as with orally administered urokinase. Natto has been widely used in Japan in the daily diet for over 1,000 years, amply justifying its safety in oral form. Traditionally, natto's ameliorative effects on the heart and on vascular disease are well known, and this is thought probably to be due to the presence of nattokinase. Thus, nattokinase itself may represent the best natural agent for use in oral fibrinolytic therapy.

FDP, EFA, TPA

= 3900 mg

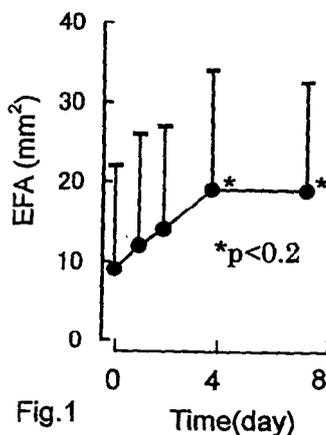


Fig. 1

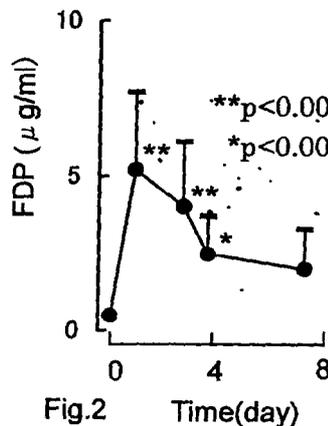


Fig. 2

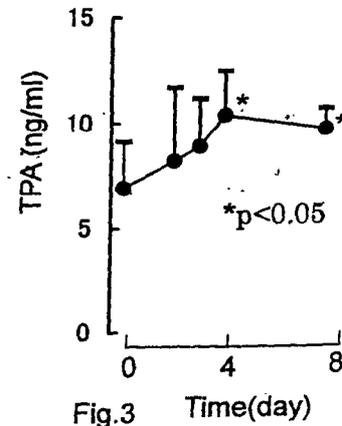


Fig. 3

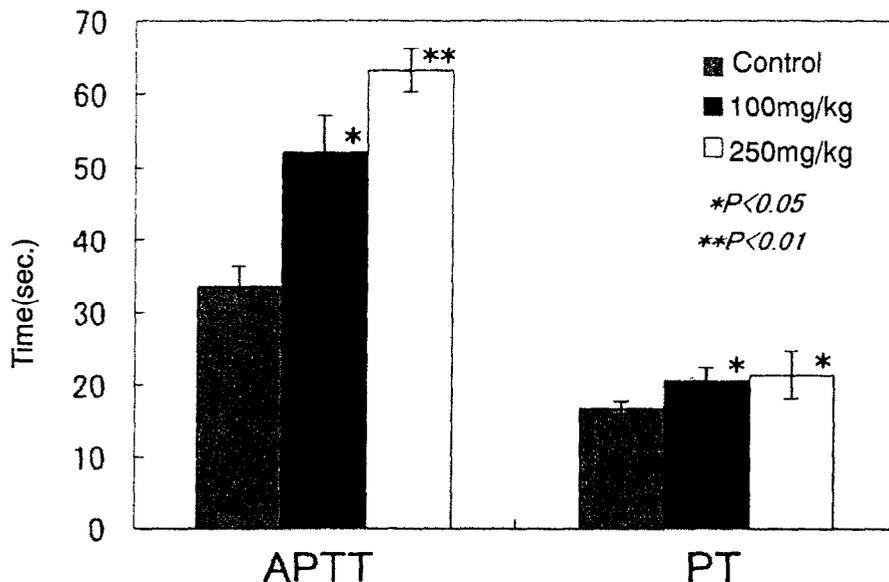
Fibrin degradation product

Tissue plasmin activator

The test of NKCP administration in the rat

The blood coagulation activity was measured after the injection of NKCP into the duodenum of rat induced fibrin. A significant delay of APTT and PT was observed in the groups administered NKCP. This result may imply that NKCP inhibits the production of fibrin.

Changes of the blood coagulation activity due to NKCP administration



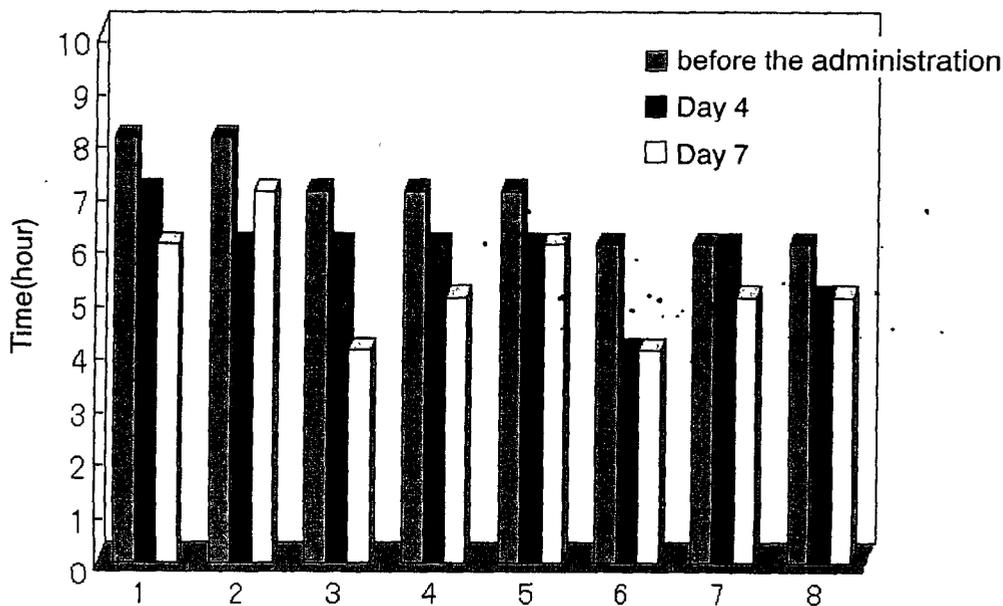
APTT: Activated Partial Thromboplastin Time

PT: Prothrombin Time

The test of oral administration of NKCP in human

8 healthy participants between the ages 20 and 60 orally took 500mg of NKCP produced in enteric capsule daily for 7 days. As a result, the euglobulin lysis time (ELT) shortened on Day 4 and shortened in the all participants on Day 7. This result implies the possibility that oral administration of NKCP improves the fibrinolytic activity.

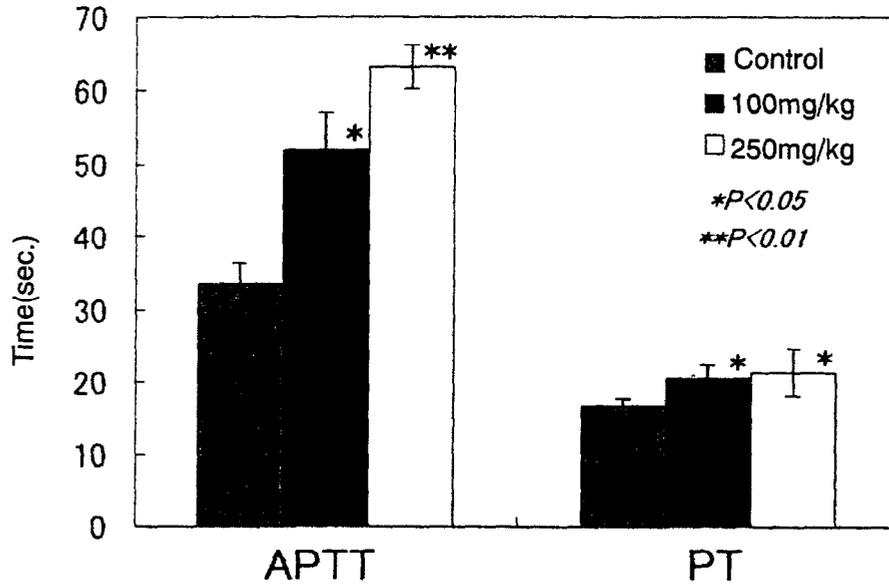
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