ijCEPr

International Journal of Chemical, Environmental and Pharmaceutical Research

Vol. 2, No.1, 61-66
January-April, 2011

Nattokinase :A Review on Fibrinolytic Enzyme

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Article History:
Received:26 April 2011
Accepted:28 April 2011

ABSTRACT

Nattokinase is a potent fibrinolytic enzyme with the potential for fighting cardiovascular diseases. It is extracted and highly purified from a traditional Japanese food called Natto which is made from fermented soybeans, *Glycine max* (L.) Merr.Natto is produced by a fermentation process by adding Bacillus subtilis, *subsp. Natto* to boiled soybeans. The ensuing Nattokinase enzyme is produced when Bacillus subtilis acts on the soybeans. Nattokinase has caused natto wide attention around the world Natto is not only unique flavor and rich nutrition, but also due to a variety of health functions, known as "super health food". The present review attempts to encompass the up-to-date comprehensive literature analysis on Nattokinase with respect to its properties, source and its various medical uses.

Keywords: Nattokinase, Natto, *Glycine max* (L.), Bacillus subtilis *subsp. Natto*.

INTRODUCTION

Nattokinase was discovered in 1980 by Dr Hiroyuki Sumi, researcher at Chicago University after testing over 173 natural foods as potential thrombolytic agents, searching for a natural agent that could effectively dissolve thrombus allied with cardiac and cerebral infarction [3-5]. Nattokinase was discovered in Natto, a fermented cheese-like food that has been used in Japan for over 1000 year. Natto is a traditional Japanese food made of soybeans . To prepare the beans are cooked and then by the action of the bacterium *Bacillus subtilis ssp.natto* fermented [8,27] .During this process is formed a slimy, stringy substance to the beans. In the traditional method of preparation are the bacteria from rice straw , into which the beans are wrapped [3] . In the modern manufacturing process, the bacteria cultures inoculated with beans, so that the use of rice straw is no longer necessary [21]. The botanical source for Nattokinase is Glycine max(L.)Merr. It appears as a yellow-white fine powder [22].

Fig.-1: Natto, traditionally wrapped in rice straw

Natto is considered a very healthy food; a health product in the fermentation is some evidence for emerging substances [46]. Nattokinase is used for cardiovascular diseases including heart disease, high blood pressure, stroke, chest pain (angina), deep vein thrombosis,, "hardening of the arteries" (atherosclerosis), hemorrhoids, varicose veins, poor circulation,and peripheral artery disease[43-45]. It is also used for pain, fibromyalgia, chronic fatigue syndrome, endometriosis, uterine fibroids, muscle spasms, infertility, cancer, and a vitamin-deficiency disease called beriberi [40].

Properties

Nattokinase is a fibrinolytic enzyme, meaning that it breaks down fibrin, an insoluble white protein produced by the conversion of fibrinogen (a protein in the plasma of blood for clotting) by thrombin (a blood clotting enzyme). Nattokinase is a serine protease with 275 amino acid residues and a molecular weight of 27,728 Daltons.

Nattokinase has a high homology with the subtilisin enzymes and DNA sequencing shows 99.5 and 99.3% homology to subtilisin E and amylosacchariticus respectively [36, 38-39]. Nattokinase degrades fibrin clots both directly and indirectly. Nattokinase degrades fibrin directly in clot lysis assays with activity comparable toplasmin. Kinetic assays suggest that it is 6 times more active than plasmin in degrading cross-linked fibrin. Nattokinase degrades fibrin indirectly by affecting plasminogen activator activity. The other names of nattokinase are BSP, Natto Extract, Nattokinasa, NK, Fermented Soybeans, Soy Natto and Subtilisin NAT. Below, is the chemical structure of nattokinase [26-28].

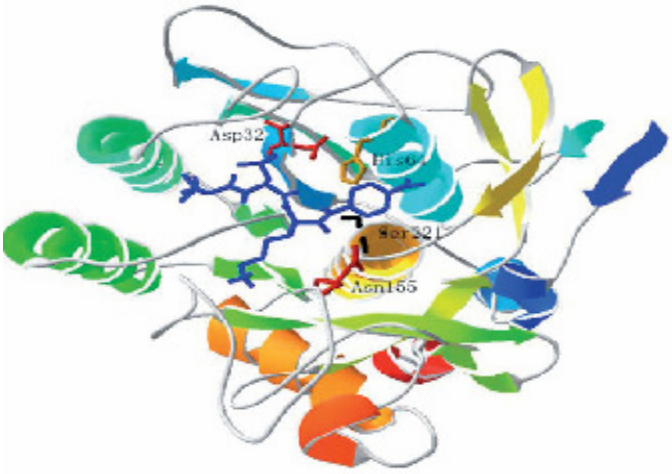

Fig.-2: Chemical structure of nattokinase

Characteristics of nattokinase [15, 17, 19-21]

Protien(Enyme) names	Recommended name: Subtilisin NAT, EC=3.4.21.62 Alternative name(s):Nattokinase,cardiokinase,
Gene names	Name: aprN
Organism	*Bacillus subtilis subsp. natto*
Taxonomic identifier	86029 [NCBI]
Taxonomic lineage	Bacteria › Firmicutes › Bacillales › Bacillaceae › Bacillus
Protein attributes	
Sequence length	381 AA.
Sequence status	Complete.
Sequence processing	The displayed sequence is further processed into a mature form.
Protein existence	Evidence at protein level.
General Annotation	
Function	Subtilisin is an extracellular alkaline serine protease, it catalyzes the hydrolysis of proteins and peptide amides. Subtilisin NAT also has fibrinolytic activity.
Catalytic activity	Hydrolysis of proteins with broad specificity for peptide bonds, and a preference for a large uncharged residue in P1. Hydrolyzes peptide amides.
Subunit structure	Monomer.
Subcellular location	Secreted.
Sequence similarities	Belongs to the peptidase S8 family.
Biophysicochemical properties	Kinetic parameters: K_M=0.48 mM for Suc-Ala-Ala-Pro-Phe-pNA

Researches suggest that Nattokinase may promote normal blood pressure, reduce whole blood viscosity and increase circulation being an effective supplement to support cardiovascular health[19].Its strong thrombolytic activity promotes arterial health both directly, dissolving existing thrombus, and indirectly, enhancing body's production of plasmin and urokinase by a direct cleavage of plasminogen activator inhibitor [32-35]].

The human body produces several types of enzymes for making thrombus, but only one main enzyme for breaking it down and dissolving it - plasmin. Nattokinase has plasmin-like bio-characteristic that lyses fibrin directly or indirectly in three different pathways [9, 10, 27]:

1. Nattokinase lyses fibrin directly.
2. Nattokinase enhances plasmin through active pro-urokinase (endogenous).
3. t-PA (Tissue Plasminogen Activators) is like urokinase and active plasmin,Nattokinase increases the concentration of t-PA.

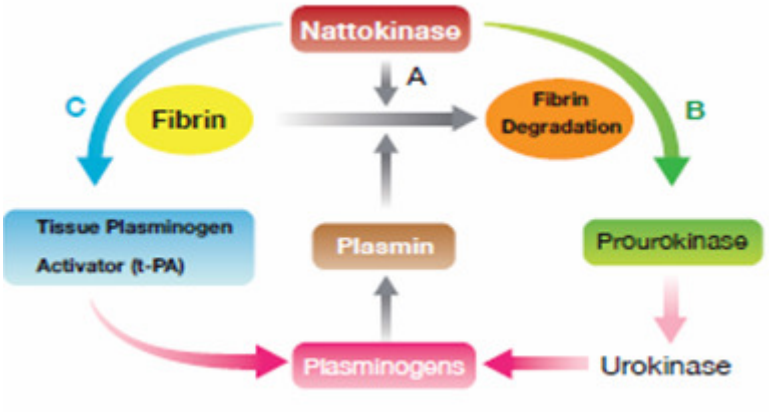

Fig.-3: The physiological effects of nattokinase on fibrin

Source

The botanical source for Nattokinase extraction is Glycine max(L.)Merr. It is a dicotyledonous annual herb belonging to fabaceae with common names wild soybean and reseeding soybean; with synonyms *Dolichos soja L., Glycine gracilis Skvortzov, Glycine hispida (Moench) Maxim., Glycine soja (L.) Merr., nom. illeg., non Glycine soja Siebold & Zucc., Glycine ussuriensis Regel & Maack, Phaseolus max L., Soja hispida Moench, Soja max (L.) Piper* [22-26].

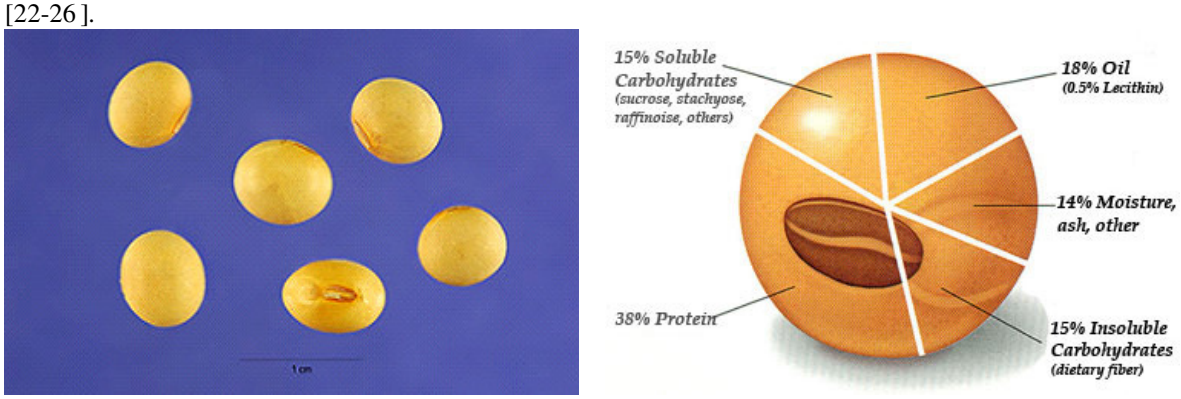

Fig.-4: Images for *Glycine max* (L.) Merr

Classification: *Glycine max* (L.) Merr.

Kingdom	*Plantae* – Plants
Subkingdom	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* – Flowering plants
Class	*Magnoliopsida* – Dicotyledons
Subclass	*Rosidae*
Order	*Fabales*
Family	*Fabaceae* – Pea family
Genus	*Glycine* Wild. – soybean

Species *Glycine max* (L.) Merr. – soybean

Table-1: Compositional analysis of soybean [4]

	Raw soybean	Boiled soybean
pH	6.4	6.9
Moisture	10.49	68.42
Ash	4.22	1.06
Crude fibre	14.22	0.08
Protien	32.92	12.15
Fat	8.19	1.01
Reducing sugar(g/l)	0.29	0.22
Ammonia(g/100ml)	0	0

The microbial source for nattokinase extraction is *Bacillus subtilis subsp. Natto*. The other name is *Bacillus subtilis var. natto.* This bacterium is used to produce natto by fermentation. Cooked soy beans are inoculated with the bacterial starter culture. The fermentation process takes at room temperature in a day; this time may be reduced to eight hours to six, if the temperature is increased from 40° C to 43 ° C. The maximum temperature reached during the fermentation process should be is 50 ° C; above which the fermentation stops as the bacteria die [11, 16, 17].

Fig.-5: Natto: Fermented soybean

Bacterial Classification

› *Firmicutes*
› *Bacilli*
› *Bacillales*
› *Bacillaceae*
› *Bacillus*
› *Bacillus subtilis group*
› *Bacillus subtilis*

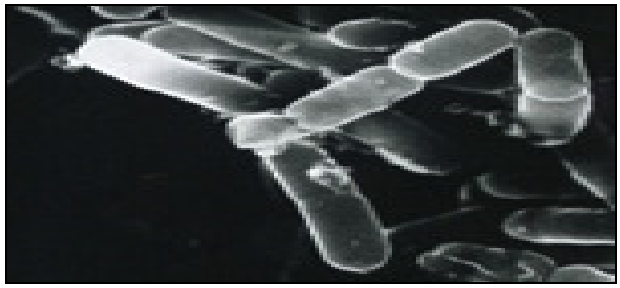

Fig.-6: Bacillus subtilis Natto observed under microscope

The various strains of *Bacillus subtilis subsp. Natto* are

1. CCRC 14716
2. IAM 1028
3. IAM 1163
4. IAM 1232
5. NC2-1
6. NR-1
7. OK2

Microbial fermentation is carried out using substrates like soybean, wheat bran, shrimp shell. These are the three substrates that are efficient in production of nattokinase enzyme upon fermentation. The essential components for fermentation are listed in comparison with three substrates [6-11].

Table-2: Compositional analysis of various substrates

Constituents (%)	Soybean meal	Wheat bran	Shrimp shell meal
Fibre	7	30	19
Moisture	8	14	14
Protein	43	16	35
nitrogen	7	14	8
lipid	7	4	9
carbohydrate	27	6	3

Medical uses

In the main nattokinase works to support healthy blood circulation in two different ways. First off, nattokinase resembles plasmin, so it can break down fibrin directly. Secondly, nattokinase enhances the body's natural production of plasmin, which also helps to break down fibrin. [2,11] In a nutshell, Nattokinase:

- Supports normal circulation, blood flow, and blood viscosity (thickness)
- Supports the body's normal blood-clotting mechanism
- Supports the body's production of plasmin, which reduces fibrin
- Helps to maintain normal blood pressure levels

Nattokinase is used for cardiovascular diseases including heart disease, high blood pressure, stroke, chest pain (angina), deep vein thrombosis, hardening of the arteries (atherosclerosis), hemorrhoids, varicose veins, poor circulation, and peripheral artery disease stroke, venous stasis, thrombosis, emboli, fibromyalgia/chronic fatigue, claudication, retinal pathology, hemorrhoid, varicose veins, soft tissue rheumatisms, muscle spasm, poor healing[31-35]. .

It is also used for pain, fibromyalgia, chronic fatigue syndrome, endometriosis, uterine fibroids, muscle spasms, tissue oxygen deprivation, infertility and cancer. Because nattokinase is an edible enzyme and is been used as nutrient supplement, it can be used to digest amyloids in body. Nattokinase can be used to remove infectious prion from animal feed, surgical instrument, and blood product [3-4, 44].

CONCLUSION

Nattokinase is a potent fibrinolytic enzyme discovered in the extract of natto and produced via fermentation of *Bacillus subtilis natto* from boiled soybean.The safety record of its potent fibrinolytic enzyme, Nattokinase, is based upon the long term traditional use of the food, and recent scientific studies. Nattokinase has many benefits including its prolonged effects, cost effectiveness, and its ability to be used preventatively. It is a naturally occurring, food-based dietary supplement that has demonstrated stability in the gastrointestinal tract, as well as to changes in pH and temperature. Stressful era of modernization has led to high rates of cardiovascular diseases; thence it would then seem prudent to add this effective natural product to our heart health preventive arsenal as more recently, both clinical and non-clinical studies have demonstrated that Nattokinase supports heart health and promotes healthy circulation. Hereby, this paper paraphrases the properties, biological activity and the botanical and microbial sources of nattokinase. Moreover, the assorted therapeutic and medicinal uses are also summed in herewith.

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[IJCEPR-152/2011]