International Journal of Research and Reviews in Pharmacy and Applied science

www.ijrrpas.com

ABLOMIN (TOXIC PROTEIN) DEGRADATION BY NANO XYLANASE (ENZYME)

Merci Rani Ragi, C.Sundaramoorthi*, Dr,Raja sekharan

R.mercy Rani,

Dept of Bio-tech.

C.Sundaramoorthi,

Asst Prof,

Dept of Bio-tech.

Prof. Dr. Raja sekharan,
Principal, KMCH College of pharmacy, Coimbatore, Tamil Nadu. 641035

ABSTRACT

Few enzymes have capability to degrade the proteins. We are using this activity in preparation of anti venom drugs. According to new generation we are using Nano technology in preparation of Nano enzymes to degrade the toxic protein ABLOMIN**.**

Dr. Joseph .C isolated new micro organism from marine soil. He prepared XYLANSE enzyme from that micro organism. We are used same enzyme for our research to prepare Nano silver enzyme particles. We analyzed and compared the degradation activity between Normal Xylanase and Nano Xylanase enzyme

From Our study we can know how to develop the Low dosage and high effective drugs by using Nano particles.

KEYWORD: Silver Nano particle, Ablomin, Xylanase, Protein degradation

1.1 INTRODUCTION TO XYLANASE

Xylanase is the name given to a class of enzymes which degrade the linear polysaccharide beta-1,4-xylan into xylose[1], thus breaking down hemicellulose, one of the major components of plant cell walls.

As such, it plays a major role in micro-organisms thriving on plant sources (mammals, conversely, do not produce xylanase). Additionally, xylanases are present in fungi for the degradation of plant matter into usable nutrients.

Commercial applications for xylanase include the chlorine-free bleaching of wood pulp prior to the papermaking process, and the increased digestibility of silage (in this aspect, it is also used for fermentative composting).[1]

Apart from its use in the pulp and paper industry, xylanases are also used as food additives to poultry, in wheat flour for improving dough handling and quality of baked products, for the extraction of coffee, plant oils, and starch, in the improvement of nutritional properties of agricultural silage and grain feed, and in combination with pectinase and cellulase for clarification of fruit juices and degumming of plant fiber sources such as flax, hemp, jute, and ramie. Good number of scientific literature is available on key features of xylanase enzymes in biotechnology ranging from their screening in microbial sources to production methods, characterization, purification and applications in commercial sector.[2-11]

Additionally, it is the key ingredient in the dough conditioners s500 and us500 manufactured by Puratos.[12] These enzymes are used to improve the dough's workability and absorption of water.[12]

SCINTIFIC INFORMATION ABOUT XYLANASE:

endo-1,4-β-xylanase	
Identifiers	
EC number	3.2.1.8
CAS number	9025-57-4
IntEnz	IntEnz view
BRENDA	BRENDA entry
ExPASy	NiceZyme view
KEGG	KEGG entry
MetaCyc	metabolic pathway
Gene Ontology	AmiGO / EGO

Table.1

PRODUCT DESCRIPTION: [14]

Xylanase Enzyme was produced fro Marine soil by Standard protocols.

Liye Xylanase is a kind of feed-level enzyme preparation made through deep liquid fermentation, super filtration, spray drying, etc.

ProductFunctions:

Effectively hydrolyze the arabinoxylan in plant feed material and reduce viscosity of digesta,Promote the release of nutrients from the plant feed materials and improve the feed conversion rate,Improve the secretion of endogenous enzymes and improve the digestion and absorption of nutrients,Promote the growth of prebiotic and inhibit the pathogenic bacterial growth as well as help the immune systems of the animals,Increase the digestible and metabolic energy of certain ingredients and reduce feed cost,And help the absorption of nitrogen and phosphorus and reduce environmental pollution.

Appearance:

Feed xylanase has both solid and liquid products. Liquid type:Deep Brown liquid,Solid type: White or off-white powder (The color may vary batch to batch but will not affect the performance).

Enzyme Activity: 10000 IU/ml

Enzyme activity unit: an activity unit (IU) is defined as the amount of enzyme required to release a micromole of xylose equivalent from oat spelt xylan at 50℃ and pH 4.6.

Storage:

It is suggested to store it in a sealed and dry place at low temperature (≤20℃), better under refrigeration condition (4-8℃). Keep it from high temperature, sun and rain. Separate it from hazardous matters.

Shelf Life:

Under the specified storage conditions, solid type: 12 months; liquid type: 6 months.

Precautions:

Seal the bag or tighten drum cover to prevent dampness or pollution. People who are sensitive to enzyme powder will be allergic; therefore the operators shall wear protective clothes, anti-dust mask and gloves to prevent the powder from entering eyes, mouths or noses.

INDUSTRIAL APPLICATIONS OF XYLANASE [(2)]

Despite an increased knowledge of microbial xylanolytic systems in the past few years, further studies are required to achieve a complete understanding of the mechanism of xylan degradation by microorganisms and their enzymes. The enzyme system used by microbes for the metabolism of xylan is the most important tool for investigating the use of the second most abundant polysaccharide (xylan) in nature. Recent studies on microbial xylanolytic systems have generally focussed on induction of enzyme production under different conditions, purification, characterization, molecular cloning and expression, and use of enzyme predominantly for pulp bleaching. Rationale approaches to achieve these goals require a detailed knowledge of the regulatory mechanism governing enzyme production. This review will focus on complex xylan structure and the microbial enzyme complex involved in its complete breakdown, studies on xylanase regulation and production and their potential industrial applications, with special reference to biobleaching.

1.2 INTRODUCTION TO ABLOMIN

Ablomin is a toxin present in the venom of the Japanese Mamushi snake, which blocks L-type voltage-gated calcium channels.

ETYMOLOGY

The protein ablomin is a component of the venom of the Japanese Mamushi snake, *Gloydius blomhoffi*. The term 'ablomin' is an acronym derived from *Agkistrodon blomhoffi*, an old name for this snake.

SOURCE OF ABLOMIN

The protein can be found in the venom of the Japanese Mamushi snake, a member of the Viperidae family.

PICTURE:1 COLLECTION OF ABLOMIN

CHEMISTRY OF ABLOMIN

Ablomin is part of the Cystein-Rich Secretory Protein (CRISP) family. CRISPs comprise a particular group of snake venom proteins distributed among the venom of several families of snakes, such as elapids, colubrids and vipers.

The protein exists of 240 amino acids, coded by an mRNA of 1336 base pairs.[14] Structurally, it is composed of three distinct regions: an N-terminal protein domain, a hinge region and a C-terminal cystein-rich domain.[15] It has a molecular mass of 25 kDa.

Ablomin shows great sequence homology with triflin (83.7%) and latisemin (61.5%), two other snake venom components of the CRISP family, which also target voltage-dependent calcium channels. In addition, it shows partial homology with helothermine (52.8%), a venom protein of the Mexican beaded lizard; this protein, however, targets other ion channels than ablomin.[14]

FUNCTIONING

Ablomin reduces potassium-induced contraction of smooth muscles, suggesting that it blocks L-type voltage-gated calcium channels.[16] Moreover, ablomin may slightly inhibit rod-typecyclic nucleotide-gated ion channels (CNGA1) channels.[16]

TOXICITY

Ablomin affects high potassium-induced contraction of arterial smooth muscle in rat-tails in a concentration-dependent matter. Reduction of arterial smooth muscle contraction in a rat-tail results in vasodilation of the rat-tails artery, which may lead to hypothermia.[1] Blocking other L-type voltage gated Ca2+ channels, for instance in the heart, may lead to arrhythmias and even cardiac arrest.

Nano particles and their Applications

- Nanoparticles are particles that have one dimension that is 100 nanometers or less in size. The properties of many conventional materials change when formed from nanoparticles. This is typically because nanoparticles have a greater surface area per weight than larger particles; this causes them to be more reactive to certain other molecules.
- Nanoparticles are used, or being evaluated for use, in many fields. The list below introduces many of the uses under development. You can use the links in each paragraph to go to a detailed explanation.

- Iron oxide nanoparticles can used to improve MRI images of cancer tumors. The nanoparticle is coated with a peptide that binds to a cancer tumor, once the nanoparticles are attached to the tumor the magnetic property of the iron oxide enhances the images from the Magnetic Resonance Imagining scan.
- A layer of closely spaced palladium nanoparticles that detect hydrogen. When hydrogen is absorbed the palladium nanoparticles swell, causing shorts between nanoparticles which lowers the resistance of the palladium layer.
- Quantum Dots (crystalline nanoparticles) that identify the location of cancer cells in the body.
- Combining gold nanoparticles with organic molecules to create a transistor known as a NOMFET (Nanoparticle Organic Memory Field-Effect Transistor).
- Nanoparticles that deliver chemotherapy drugs directly to cancer cells.
- Iron nanoparticles used to clean up carbon tetrachloride pollution in ground water.
- Silicon nanoparticles coating anodes of lithium-ion batteries to increase battery power and reduce recharge time.
- Gold nanoparticles that allow heat from infrared lasers to be targeted on cancer tumors.
- Silicate nanoparticles used to provide a barrier to gasses (for example oxygen), or moisture in a plastic film used for packaging. This could reduce the possibly of food spoiling or drying out.
- Zinc oxide nanoparticles dispersed in industrial coatings to protect wood, plastic and textiles from exposure to UV rays.
- Silicon dioxide crystalline nanoparticles filling gaps between carbon fibers strengthen tennis racquets.
- Silver nanoparticles in fabric that kills bacteria making clothing odor-resistant.

1.4 Conversion of silver Nano Particles

Stable and well-dispersed Ag nanoparticles were generated by spontaneous reduction of $AgNO_3$ in the partially acetylacetonato complexed aluminium tri-*sec*-butoxide solution without using any additional reducing agent. The addition of higher concentration of $AgNO_3$ caused agglomeration of Ag nanoparticles resulting in precipitation. Use of polyvinylpyrrolidone as capping agent not only prevents the agglomeration but also causes a controlled growth of Ag nanoparticles in different shapes in alumina sol. The addition of varying concentrations of polyvinylpyrrolidone caused transformation of yellow alumina sol to green and blue coloured sol. These changes were due to the conversion of spherical (yellow) to a mixture of hexagonal and triangular (green), and finally truncated/filleted triangular (blue) Ag nanoparticles. These yellow, green and blue coloured alumina sols were used to prepare different shaped Ag nanoparticle doped optically transparent amorphous alumina films on glass substrates. The shape conversions of the Ag nanoparticles were studied

systematically by UV/visible spectrometry, transmission electron microscopy and grazing incidence X-ray diffraction, and a mechanism of growth of the Ag nanoparticles in alumina sol was established.

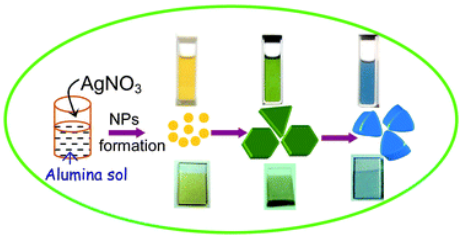


Figure: 3 Conversion of silver Nano Particles

MATERIALS AND METHODS:

Required Chemicals and instruments

1. XYLANASE was produced from Marine soil.

2. Ablomin venom was procured from Bharat serums and vaccines, plat no -27, Anand nagar,Ambernath, MAHARASHTRA.INDIA

3. AgNo3

4. MBTH

5. FeCl3

6. 2,2, Bipyridine

7. Ortho phospaharic acid

8. 4 D water

9. Instrumentation – double beam UV - visible Spectrophotometer (uv2301 model).

10. Denver analytical Balance

11. Shimadzu I.R Spectrophotometer.

12. OLYMPUS X R D

REAGENTS PREPARATION

1. Preparation of MBTH- 500mgof MBTH was weighed and dissolved in 100ml of distilled water.

2. Preparation of Fecl3- 200mg of $Fecl_3$ dissolved in 100ml of distilled water

3. Preparation of 2,2 Bipyridine-200mg of 2,2 Bipyridine was weighed and dissolved in 100ml of distilled water.

4. Preparation of Orthophosphoric acid-1.27ml of OPA was taken and dissolved in 100ml of distilled water.

PREPARATION OF SILVER NANO ENZYME PARTICLES [20]

0.1M, 0.5M,1M concentrations of $AgNO_3$ were taken respectively. These concentrations of $AgNO_3$ were calculated by the molecular weight of $AgNO_3$ .For example, At the conc. 0.1M of $AgNO_3$ we are taking the wt of 0.42 mg of $AgNO_3$ and dissolved in 25ml of distilled water. From the prepared solution 10ml was taken in a conical flask and 10mg of the enzyme xylanase was added and shake well untill the enzyme was dissolved. The conical flasks were covered with Aluminium foil and kept in a dark room for about 48 hrs. In the same way, same procedure is repeated for 0.5 M, 1M conc. of $AgNO_3$ respectively. We prepared three types on Nano particles at three types of concentrations. The prepation details gives in Table.2

S.NO.	MOLAR CONC.OF $AgNO_3$	WT.OF $AgNo_3$ TAKEN (mg)	DRUG (nm)	WAVELENGTH (nm)	ABSORBANCE
1.	0.1	0.42mg	10	420	0.508
2.	0.5	2.12mg	10	420	0.962
3.	1.0	4.24mg	10	420	1.325

Table: 2 Preparation of siver Nano paticles

PRINCIPLE: The enzyme is adsorbed by the metal and form into nano particle. The adsorption is identified by the spectrophotometer. It gives high absorbance at 420nm.The structure of Nano particles are identified by XRD, SEM, TEM in IICT, Hyderabad.From the Reports the structure Nan enzyme particles is **Monoclinci 1 DIAD.** (A = 90, B,C =/ 90 : A=/ 90, B,C =90)The **XRD AND SEM, TEM** reports are shown in figure 4, 5, 6

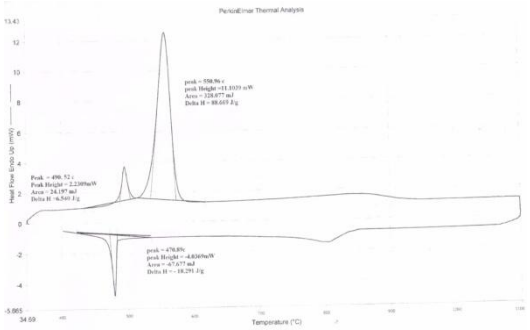


Figure. 4 SEM

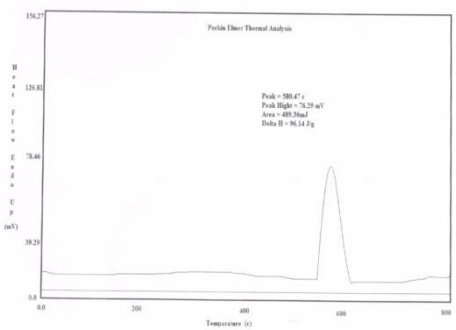


Figure: 5 TEM

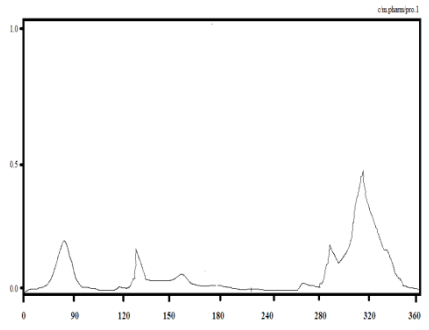


Figure: 6 XRD

EXPERIMENTAL CONDITIONS

We are identified presence of **Primary amino group** at (Wave number 3430.2) in enzyme by I.R SPECTOPHOTOMTER[24]. After Protein degradation we are identified the concentration of enzyme by spectrophotometric methods.

By the adding of MBTH, 2,2 Bipyridine primary amino group gives a color solution. By the absorbance measurement of that color solution we are calculated concentration of enzyme.

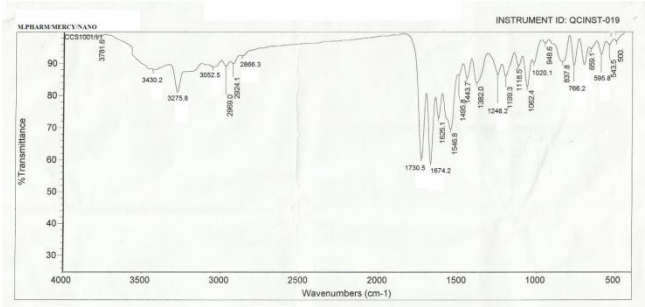


Figure: 7 Nano enzyme I.R spectrums

Toxic protein degradation with Enzyme and NanoEnzyme [18]

To identification of Degradation efficiency of Nano enzyme, we are taken two types of enzymes. 1. Enzyme 2 .Nanoenzme. We performed the degradation activity of toxic protein at four different concentrations i.e 5, 25, 50,100 ppm.

To the above four different concentrations of ABLOMIN solutions, we are added 1 ml of enzyme, and incubated up to one hour. After incubation, 0.5 ml of toxic protein taken in a volumetric flask and added 2 ml of reagent, ortho phosphoric acid 0.5 ml, and make u p to 10 ml with 4D water. Same procedure applied to 5, 25, 50, 100 ppm of toxic protein solutions. Due to adding of reagents (MBTH, 2, 2 BI PYRIDINE) Enzymes gives color to the sample. By the mesearueing of absorbance values we will estimate the remained enzyme in sample solution.

Normal enzyme Results are showed in Table 3,4 Nano enzyme results are shoed in Table 5,6

RESULTS AND DISCUSSION

CONCEN TRATIONS (ppm)	NORMAL ENZYME (ml)	MBTH (ml)	$Fecl_3$ (ml)	TOXIC PROTEIN (ml)	WAVE LENGTH	ABSORBANCE
100ppm	1ml	1ml	0.5ml	0.5ml	420nm	0.753
50ppm	1ml	1ml	0.5ml	0.5ml	420nm	0.775
25ppm	1ml	1ml	0.5ml	0.5ml	420nm	0.680
5ppm	1ml	1ml	0.5ml	0.5ml	420nm	0.680

Table: 4 ABSORBANCE VALUES OF NORMAL ENZYME WITH MBTH

CONCEN TRATIONS (ppm)	NORMALE NZYME (ml)	BIPYR IDINE (ml)	ORTHO PHOSPHORIC ACID(ml)	$Fecl_3$ (ml)	TOXIC PROTEIN (ml)	WAVELENGTH (nm)	ABSORBA NCE
100ppm	1ml	2ml	0.5ml	0.5ml	0.5ml	540nm	0.112
50ppm	1ml	2ml	0.5ml	0.5ml	0.5ml	540nm	0.173
25ppm	1ml	2ml	0.5ml	0.5ml	0.5ml	540nm	0.166
5ppm	1ml	2ml	0.5ml	0.5ml	0.5ml	540nm	0.128

Table: 5 ABSORBANCE VALUES OF NORMAL ENZYME 2,2BIPYRIDINE

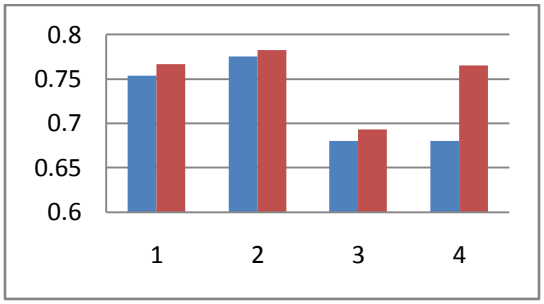
CONCENTRATIONS (ppm)	NANO ENZYME (ml)	MBTH (ml)	Fecl3 (ml)	TOXIC PROTEIN (ml)	WAVE LENGTH (nm)	ABSORBANCE
100ppm	1ml	1ml	0.5ml	0.5ml	420nm	0.766
50ppm	1ml	1ml	0.5ml	0.5ml	420nm	0.782
25ppm	1ml	1ml	0.5ml	0.5ml	420nm	0.693
5ppm	1ml	1ml	0.5ml	0.5ml	420nm	0.765

Table: 6 ABSORBANCE VALUES OF NANO ENZYMES MBTH

CONCENTRATION(ppm)	NANO ENZYME(ml)	BIPYRIDINE (ml)	ORTHO PHOSPHORIC ACID	Fecl3 (ml)	TOXIC PROTEIN (ml)	WAVE LENGTH (nm)	ABSORBANCE
100ppm	1ml	2ml	0.5ml	0.5ml	0.5ml	520nm	0.157
50ppm	1ml	2ml	0.5ml	0.5ml	0.5ml	520nm	0.203
25ppm	1ml	2ml	0.5ml	0.5ml	0.5ml	520nm	0.167
5ppm	1ml	2ml	0.5ml	0.5ml	0.5ml	520nm	0.144

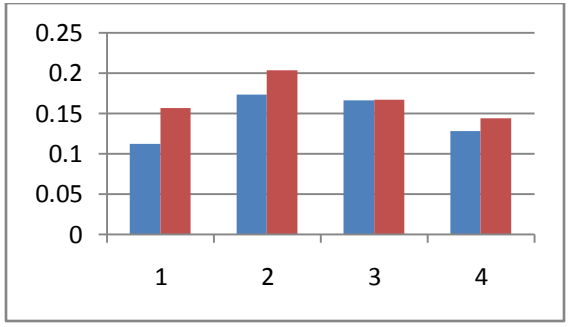
Table: 7 ABSORBANCE VALUES OF NANO ENZYME WITH 2,2BIPYRIDINE

From comparison between values Table 4, 6 (MBTH technique) we know that Nano enzyme degrades the toxic protein more effectively than normal enzyme. These results showed in Graph.1



Graph .1

From comparison between values Table 5, 7 (2,2 bi pyridine technique) we know that Nano enzyme degrades the toxic protein more effectively than normal enzyme. These results showed in Graph.2



Graph: 2

We performed the effectiveness of Nano Enzyme with two different reagents. We got good result in both of tests.

CONCLUSION

Our experiment tells again the application of Nano technology. Snake poison is too dangerous. We have to control the mobility of poison in human body by the anti venom. By the use of this type of nano enzymes we can reduce the activity of toxic poison very fastly by the degradation.

The negative part of Nano enzyme is USFDS not approved metal (Ag/Au) particle in medicines.

We have to do this experiment in Viva conditions to know toxic levels of Silver particles.

REFERENCES

1. Gulzar, Production and partial purification of Xylanase fromTrichoderma longibrachiatum. Published in international conference on biotechnology and neurosciences. CUSAT , 2004.P33[verification needed]
2. Polizeli, M. L. T. M.; Rizzatti, A. C. S.; Monti, R.; Terenzi, H. F.; Jorge, J. A.; Amorim, D. S. (2005). "Xylanases from fungi: Properties and industrial applications". Applied Microbiology and Biotechnology 67 (5): 577–91. doi:10.1007/s00253-005-1904-7. PMID 15944805.
3. Subramaniyan, S.; Prema, P. (2002). "Biotechnology of Microbial Xylanases: Enzymology, Molecular Biology, and Application". Critical Reviews in Biotechnology 22 (1): 33–64.doi:10.1080/07388550290789450. PMID 11958335.
4. Kulkarni, Neeta; Shendye, Abhay; Rao, Mala (1999). "Molecular and biotechnological aspects of xylanases". FEMS Microbiology Reviews 23 (4): 411–56. doi:10.1111/j.1574-6976.1999.tb00407.x. .
5. Ahmed, Sibtain; Riaz, Saba; Jamil, Amer (2009). "Molecular cloning of fungal xylanases: An overview". Applied Microbiology and Biotechnology 84 (1): 19–35. doi:10.1007/s00253-009-2079-4. .
6. Sá-Pereira, Paula; Paveia, Helena; Costa-Ferreira, Maria; Aires-Barros, Maria Raquel (2003). "A New Look at Xylanases: An Overview of Purification Strategies". Molecular Biotechnology 24(3): 257–81.
7. Alves-Prado, Heloiza Ferreira; Pavezzi, Fabiana Carina; Leite, Rodrigo Simões Ribeiro; Oliveira, Valéria Maia; Sette, Lara Durães; Dasilva, Roberto (2009). "Screening and Production Study of Microbial Xylanase Producers from Brazilian Cerrado". Applied Biochemistry and Biotechnology 161 (1–8): 333–46.

8. Prade, R. A. (1996). "Xylanases: From biology to biotechnology". Biotechnology & Genetic Engineering Reviews 13: 101–31. doi:10.1016/S0140-6701(97)80292-5 Sunna, A.; Antranikian, G. (1997). "Xylanolytic Enzymes from Fungi and Bacteria". Critical Reviews in Biotechnology 17 (1): 39–67.
9. Chávez, Renato; Bull, Paulina; Eyzaguirre, Jaime (2006). "The xylanolytic enzyme system from the genus Penicillium". Journal of Biotechnology 123 (4): 413–33
10. Lee, Charles C.; Wong, Dominic W. S.; Robertson, George H. (2005). "Cloning and Characterization of the Xyn11A Gene from Lentinula edodes". The Protein Journal 24 (1): 21–6.
11. en.hbliye.com
12. Yamazaki, Y; Koike, H; Sugiyama, Y; Motoyoshi, K; Wada, T; Hishinuma, S; Mita, M; Morita, T (2002). "Cloning and Characterization of Novel Snake Venom Proteins that Block Smooth Muscle Contraction". Eur J Biochem 269 (11): 2708–2715.
13. Matsunaga, Y; Yamazaki, Y; Hyodo, F; Sugiyama, Y; Nozaki, M; Morita, T (2009). "Structural Divergence of Cysteine-Rich Secretory Proteins in Snake Venoms". J Biochem 145 (3): 365–375
14. Yamazaki, Y; Morita, T (2004). 3 "Structure and Function of Snake Venom Cysteine-Rich Secretory Proteins". Toxicon 44 (3): 227–231.
15. http://www.epa.gov/apti/bces/module3/category/category.htm
16. C.G. Granqvist, R. A. Buhrman, J. Wyns, A. J. Sievers (1976. Phys.Rev.Lett. 37 (10): 625–629 Ch. Hayashi, Ryozi Uyeda, A. Tasaki. (1997).Ultra-fine particles: exploratory science and technology (1997 Translation of the Japan report of the related ERATO Project 1981–86). Noyes Publications.
17. L.B. Kiss, J. Söderlund, G.A. Niklasson, C.G. Granqvist (1999). "New approach to the origin of lognormal size distributions of nanoparticles".Nanotechnology 10: 25–28.
18. Cristina Buzea, Ivan Pacheco, and Kevin Robbie (2007). "Nanomaterials and Nanoparticles: Sources and Toxicity". Biointerphases 2 (4):
19. ASTM E 2456 – 06 Standard Terminology Relating to Nanotechnology
20. Fahlman, B. D. (2007). Materials Chemistry. Springer. pp. 282–283.