

INDIAN VENOMOUS SNAKES AND SCORPIONS A POTENT SOURCE OF A GENETICALLY WELL CONSERVED AND EXPRESSED ENZYME 5'NUCLEOTIDASE (E.C. 3.1.3.5) OF GREAT DIAGNOSTIC SIGNIFICANCE IN THE DIAGNOSIS OF HEAD, NECK, BREAST AND LIVER CANCER AND PAGET'S DISEASE

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Article Received on: 03/01/11 Revised on: 02/02/11 Approved for publication: 09/02/11

ABSTRACT

Activity, Optimum pH and Optimum temperature of the enzyme 5'- Nucleotidase (E.C.3.1.3.5), from the Indian venomous snakes and scorpions was determined by the methods of Johnson et al, and Arkesteijn, C.L.M.

KEY WORDS: 5' –Nucleotidase, *Naja naja*, *Bungarus caeruleus*, *Echis carinatus*, *Palamneus gravimanus* and *Buthus tamulus*.

INTRODUCTION

5' Nucleotidase (E.C. 3.1.3.5) is a ubiquitously present in nature, occurring as a cytosolic glycosyl anchored protein in the hepatobiliary tract¹, actively involved in the purine metabolism.

5'Nucleotidase is a well established plasma membrane marker, present in mammalian cells in ecto form², specifically catalyzing the dephosphorylation of nucleotide phosphates³.

Its activity determination in hepatobiliary diseases when coupled with levels of serum alkaline phosphatase is of great diagnostic and prognostic significance⁴, particularly in bone and liver diseases. Serum levels of 5' Nucleotidase is significantly increased in obstructive jaundice, neoplastic disease involving the liver, cancer of liver, hepatitis, cirrhosis, lymphoma, carcinoma of the head of the pancreas and head and neck cancers⁵.

MATERIALS AND METHOD

Adenosine 5'phosphate in a concentration of (10.5mg per 8ml distilled water) E. Merck Germany.

Trichloroacetic acid in a concentration of 0.05 gm per 8.76 ml of distilled water.

0.1M Magnesium Chloride. (Sarabhai Merck, India).

0.1% crude venom solutions.

Potassium dihydrogen phosphate (Sarabhai Merck, India)

5' -Nucleotidase from *Bungarus coeruleus*, *Echis carinatus*, *Palamneus gravimanus* and *Buthus tamulus*

5' Nucleotidase was assayed by the method of Johnson et.al, (1953)⁶. The substrate solution contained 1ml each of buffer in the range of pH 3.0 to 10.0, 0.1ml of 0.1M Magnesium Chloride and 0.8ml of 0.15% 5'AMP, 0.25ml of 0.1% crude venom was added to the substrate solution and incubated at 37°C for 15 minutes. At the end of 15th minute the reaction was stopped by adding TCA and filtered. The filtrate was assayed for inorganic phosphate by the method of Fiske and Subbarow at 625nm using potassium dihydrogen phosphate as standard⁷. In the above investigations one unit of enzyme activity is

defined as that which liberates 0.01 μ mole of inorganic phosphorus/minute under the experimental conditions.

DETERMINATION OF 5' NUCLEOTIDASE FROM *Naja naja*

The temperature of the reaction mixture should be maintained at 30⁰C.

1. Pipette out 3.0ml 5' Nucleotidase assay solution into a cuvet and bring it to 30⁰C temperature.
2. Add 0.2 ml crude venom and mix by cyclomixing for 5 minutes.
4. Read the absorbance at 340nm taking water as a blank.
5. Exactly 5 minutes later, record the absorbance (final A), again⁸.

CALCULATIONS

▲ A per 5 min = initial A – final A

5'Nucleotidase ▲(U/L) = ▲ A per 5 min x 515 x temperature correction factor(TCF)

Where

Factor 515 = 3.2 x 1000/6.22 x 0.2 x 5

3.2 = Total reaction volume (ml)

1000 = conversion activity per ml to activity per L.

6.22 = Millimolar absorptivity of NADH at 340 nm

0.2 = volume of sample (ml)

5 = Conversion of ▲ A per 5 min to A per min.

CALIBRATION

The procedure is standardized by means of the millimolar absorptivity of NADH, which is 6.22 at 340 nm. The reduced amination of 2 –Oxoglutarate to form glutamate catalyzed by Glyceraldehyde lactate dehydrogenase (GLDH) coupled with the oxidation of NADH to NAD on a molar equivalent basis. Measurement of the resulting decrease in absorbance serves to quantify the 5'-nucleotidase activity.

*The sensitivity range of this method is 5-120U/L; high levels of ammonia are known to interfere in the enzyme activity determination.

RESULTS

The Optimum pH and Optimum temperature of 5'-Nucleotidase is shown below;

Species name	Optimum pH	Figures	Optimum Temperature	Figures
Bungarus caeruleus	8.0	1	37 ⁰ C	2
Echis carinatus	9.0	3	37 ⁰ C	4
Palamneus gravimanus	8.0	5	37 ⁰ C	6
Buthus tamulus	7.0	7	37 ⁰ C	8
Naja naja	8.0	9	--	--

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Table 1: Activity of 5'-Nucleotidase enzyme

Species name	5' -Nucleotidase activity Of the crude venoms
Bungarus caeruleus	17.57units/mg/min
Echis carinatus	6.25 units/mg/min
Palamneus gravimanus	54.2 units/mg/min
Buthus tamulus	361.1units/mg/min
Naja naja	7.97units/mg/min

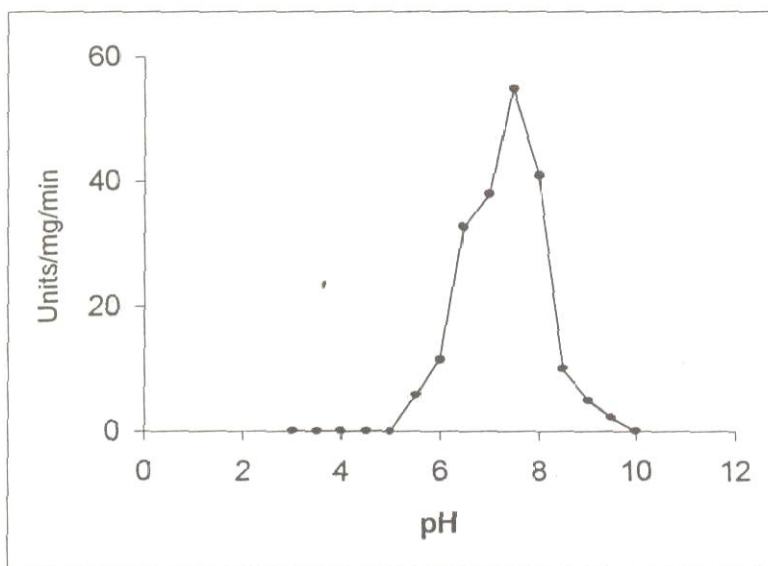


Fig 1 pH optimum of 5'-Nucleotidase of Bungarus caeruleus

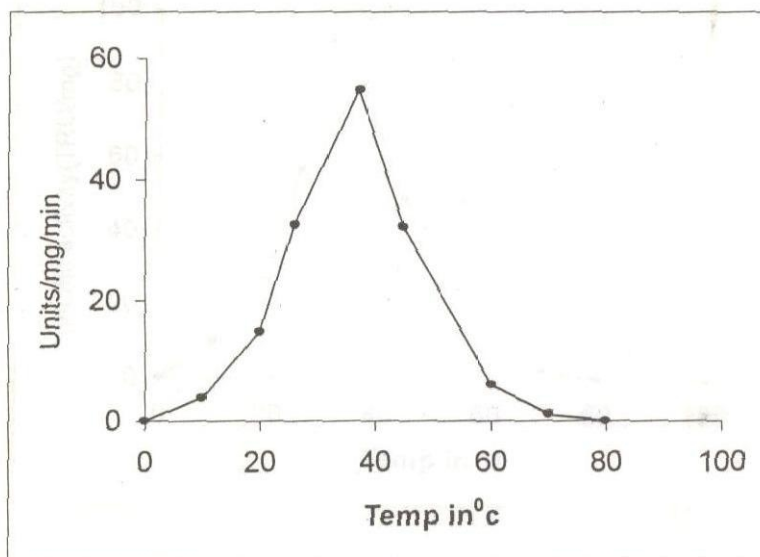


Fig 2 Temperature optimum of 5'-Nucleotidase of Bungarus caeruleus

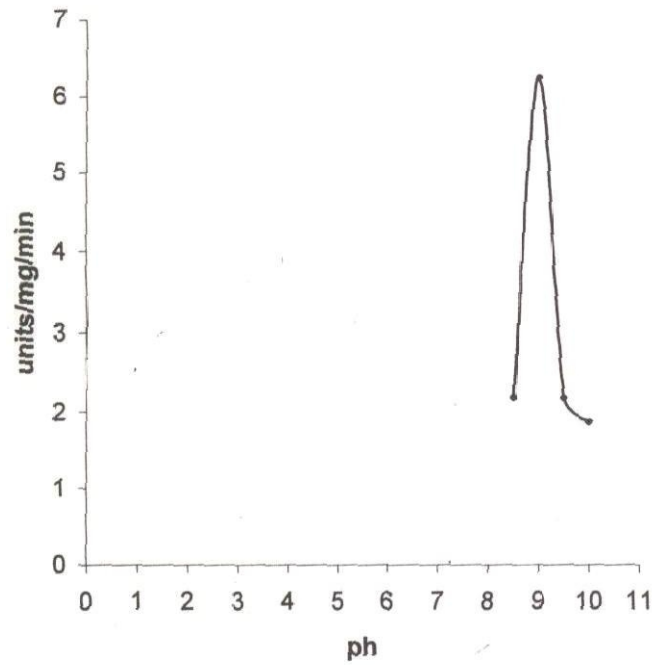


Fig 3 pH optimum of 5'-Nucleotidase of Echis carinatus

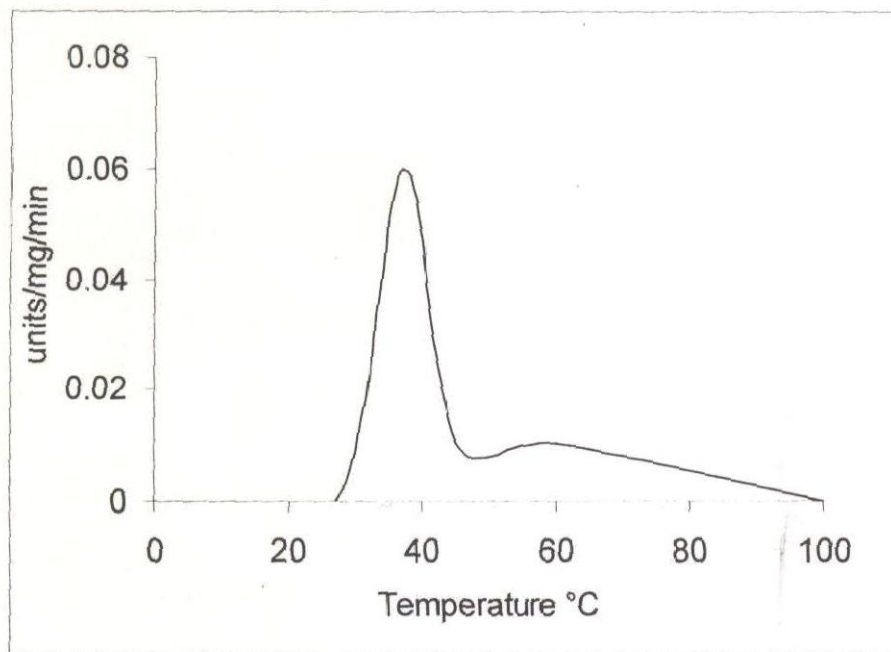


Fig 4 Optimum temperature of 5'-Nucleotidase Echis carinatus

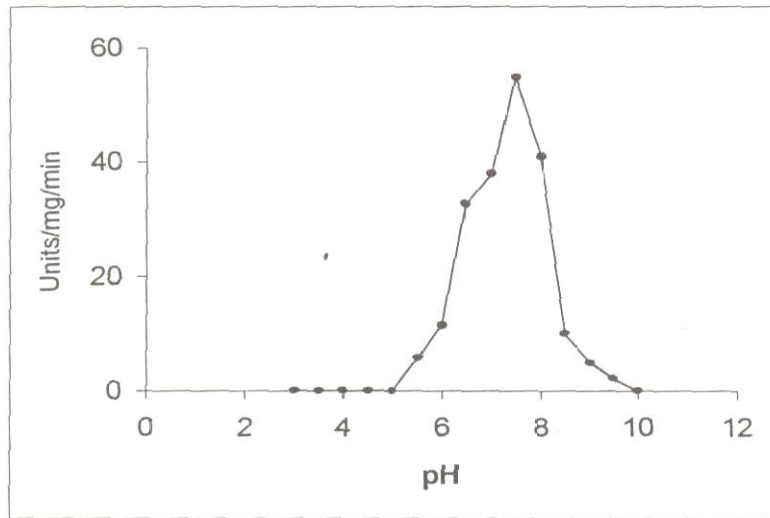


Fig 5 pH Optimum of 5'-Nucleotidase of Palamneus gravimanus.

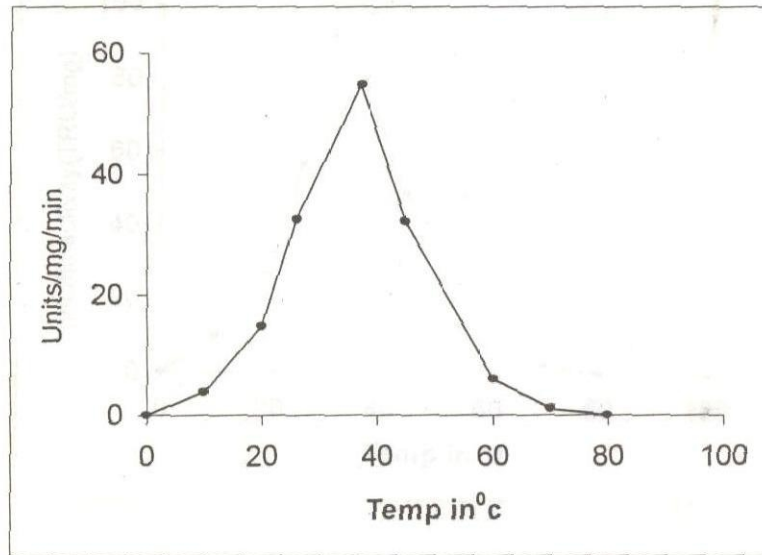


Fig 6 Optimum temperature of 5'-Nucleotidase of Palamneus gravimanus.

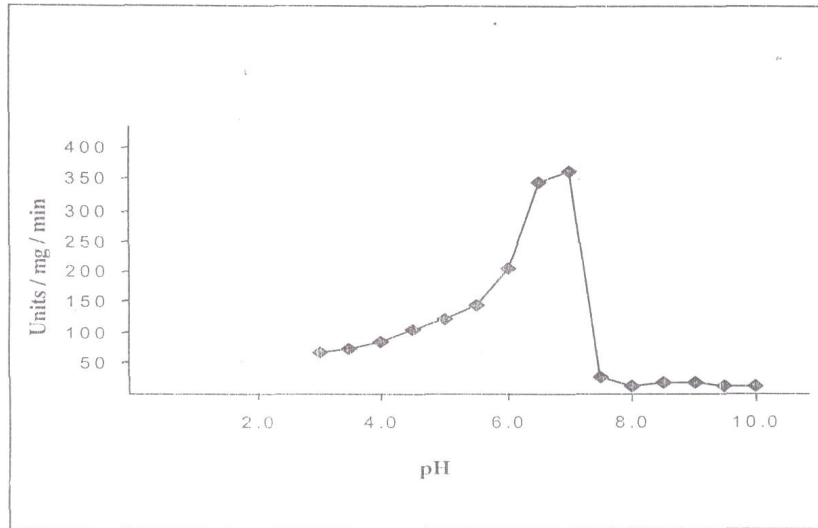


Fig7 pH Optimum of 5'-Nucleotidase of Mesobuthus tamulus

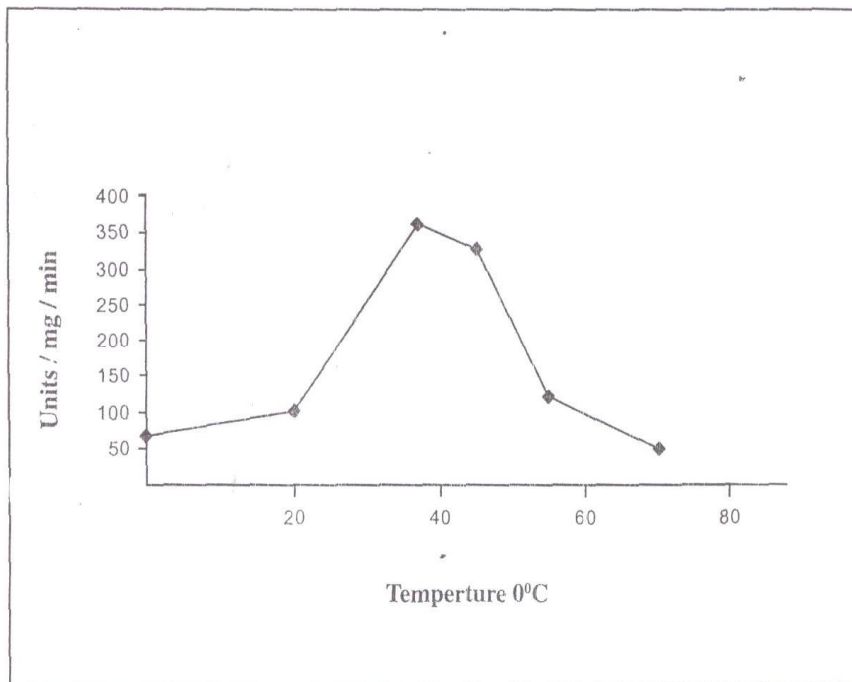


Fig 8 Temperature optimum of 5'-Nucleotidase of Mesobuthus tamulus

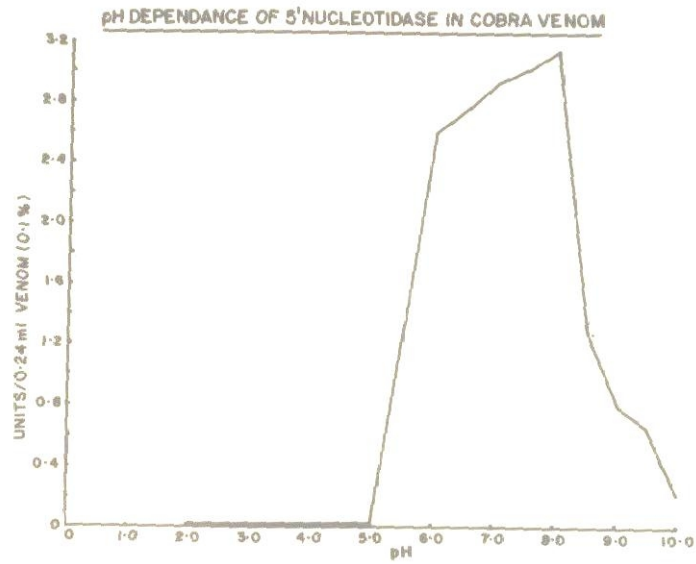


Fig 9 pH Optimum of 5'-Nucleotidase of *Naja naja*.

Source of support: Nil, Conflict of interest: None Declared