EFFECT OF DIFFERENT DIVALENT CATIONS ON DEOXYRIBONUCLEASES (DNases) HYDROLYTIC ACTIVITY WITH DEOXYRIBONUCLEIC ACID (DNA) , EXTRACTED FROM THE LATEX OF *Hevea braciliences.*

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***ABSTRACT**: DNase, or deoxyribonuclease, (EC 3.1.4.5) is an enzyme that catalyzes the hydrolysis of both single and double-stranded DNA molecules to generate a mixture of oligo- and mononucleotides. The enzyme is involved in a diverse array of cellular functions, including the repair of damaged DNA, DNA turnover, and apoptosis. DNase is present in a variety of tissues of microorganisms, animals, and plants. Their molecular weights, mode of action, substrate specificity, and other physical and chemical characteristics are all distinct. It is a monomeric protein with a molecular weight of 20.1 KDa, as evidenced by its iso-electric point of 8.5, which indicates that it is positively charged at pH 7. Additionally, it exhibits a single band on SDS-PAGE. Divalent cations significantly influence the hydrolytic activity of latex DNase hydrolytic activity. The hydrolytic activity of latex DNase is activated by Mg^2+, Mn^2+, and Ca^2+, while it is deactivated by Zn^2+, Hg^2+, EDTA, and DMSO. In order to gain a comprehensive understanding of the latex DNase hydrolytic activity, it is necessary to evaluate the activity of other divalent and monovalent cations from group I and group II of periodic table..*

Keywords: Hevea braciliences, latex, DNA hydrolysis, deoxyribonucleases

INTRODUCTION

The rubber tree, *Hevea brasiliensis*, is the most prevalent source of natural rubber. Rubber is derived from the *Hevea brasiliensis* plant, which is a member of the Euphorbiaceae family. *Hevea brasiliensis* is typically straight or tapering, branchless for a minimum of 10 meters, with a minimum diameter of 50 cm and no buttresses. The bark surface is smooth, the hoop is marked, and the inner bark is pale brown with a high concentration of white rubber. The crown is conical, and the branches are thin [1]. Several international locations that generate latex are expanding the cultivation of rubber to regions outside the scope of the most appropriate climate situations in order to meet the growing demand for natural rubber [2]. The most economically significant members of the *Hevea* genus are rubber trees, which are rapidly growing trees. The primary source of natural rubber is the milky latex collected from the tree, which is why it has a substantial monetary value. The bark is routinely harvested as a latex-producing crop. The rubber tree's highly prized latex is composed of a diverse array of compounds, including 36% rubber, 5% non-rubber components, such as C-serum and B-serum proteins, carbohydrates, and lipids, and 59% water [3]. C-serum protein, a clear serum that is similar to latex cytosol [4], has been reported to have anti-fungal effects on *Aspergillus niger*. It is a rich source of protein, nucleic acid, and a variety of natural compounds [5].

DEOXYRIBONUCLEASE

Deoxyribonucleases or DNases (EC 3.1.4.5) ia an enzyme which hydrolysis both single and double stranded DNA to producae a mixture of oligonucleotides and mononucleotides. [6]. This enzyme participates in a wide variety of cellular functions, such as repair of damaged DNA replication, DNA turnover and apoptosis. DNase is widely used as a reagent in molecular biology research. This enzyme occurs in many tissues of animals and plants as well as in microorganisms. It has also been reported to occur in the latex of *Hevea brasiliences* . *Hevea* latex is a poly disperse system in which negatively charged particles of various types are suspended in an ambient serum (C-serum). The two main particulate phases contained in *Hevea* latex are rubber particles, consisting 30-45% and lutoid particles 10-20% [7]. The third type is the Frey-Wyssling compleses. These compounds have different sizes and densities. Thus fractionated to different zones, the yellow zone, the C-serum and the bottom fraction. The bottom fraction comprises of lutoid particle is enveloped and lesser compound of the Frey-Wyssling complexes. The lutoid are 03-0.5 µm in diameter. Each lutoid particle is enveloped by a thin membrane. The contents of lutoids are rich in hydrolytic enzumes, including DNases. Latex DNases can become a new tool in molecular biology research. Latex DNase enzymes have been purified through chromatographic procedures to a homogeneity as a single band on SDS-PAGE [8].

MATERIALS AND METHODS

Collection and Fractionation of Latex

The latex was collected from *Hevea brasiliences* trees and fractionation by ultracentrifugation using Beckman 1.5-65, ultracentifuge and rotar 21. Ulracetrifuge was done at 59,000 x g and for 2 hours at 4^0C. Four fractions were obtained after ultracentrifugation, the rubber fraction, the yellow zone, C-serum and the bottom fraction. The homogenized suspension of bottom fraction was subjected to ultrasonication ay 40C and -50V to breakup the lutoid membranes. The sonicated suspension was filtered with Whatman No 1 paper. The bottom fraction extract was kept at 4^0C.

DNase assay

DNase activity was assayed semi quantitatively. The incubation mixture contains 4µl of enzyme solution (0.078 µg protein) with 0.1 µg of lambda DNA (Fermentas) and 4 µl of assay buffer (10mM Tris-HCl pH7 and 10mM $MgCl_2$) in a total of 10 µl volume at 37^0C for one hour.At the end of the incubation period, the reaction was terminated by the addition of 3µl tracing dye (0.01% homophenol blue in 10% sterilized glycerol). The reaction mixture was loaded on (0.7% w/v) agarose gel. The agarose gel was run in TBE buffer. The gel

was stained with ethidium bromide, and under UV illumination.

Ammonium sulphate precipitation

Bottom fraction extract collected was subjected to ammonium sulphate precipitation at (20%w/v) saturation intervals. Each pellet fraction was dissolved in 20ml of 10mM Tris-HCl pH 7.1 and dialysed overnight against two changes of the same buffer. A crude form of DNase is obtained, ready for further purification steps.

Iso-electric Focusing (IEF)

Iso-electric focusing was performed in free solution using Rotofer cell. 1 ampholyte (pH 3-10) was added to fraction II. The dialysed mixture and the volume is made to 55 ml with deionized water. The rotofer running conditions were set at 4^0C and 12W till a constant voltage is maintained. Total 20 fractions were harvested were harvested at the end of IEF. OD at 260nm, pH and DNase activity of each fraction was measured. Latex DNAses have an iso-electric point of 8.5, Which shows that it is positively charged at pH 7, further it displayes single band on SDS-PAGE indicating that it is a monomeric protein with a molecular weight of 20.1 KDa.

SDS-Polyacrylamide gel electrophoresis

Molecular mass of the enzyme was determined by SDS-PAGE, with 12.5% acrylamide gels. The run was conducted with 100Volts at room temperature gels. The run was conducted with 100Volts at room temperature (25^0C). Proteins were stained with Coomasie brilliant blue and molecular mass of protein was estimated with reference to low molecular weight protein markers (Bio-Rad).

RESULTS & FINDINGS

Effect of divalent cations on DNase activity

Latex DNase did not sow any obligate requirements for divalent metal ions for its activity. But its activity was increased greatly with Mg^2+ and Mn^2+ ions. Purified DNase 4µl (0.078 µg protein) was incubated with different ionic concentrations of different divalent ions, with different concentrations at 4^0C for 10min. The reaction was started by the addition of 0.1 µg lambda DNA. The reaction mixture was incubated at 37^0C for 1hr. The reaction was terminated by the addition of tracking dye and the reaction mixture was loaded on 0.7% agarose gel.

Table 1: DNA hydrolysis with Mg^2+ ions through latex DNases

$MgCl_2$ Concentration	DNA degradation
1mM	+++
5mM	+++
10mM	+++
50mM	+++

No degradation: -
Mild degradation: +
Moderate degradation: ++
Full degradation: +++

It is shown in table no 1, that lambda DNA hydrolyses with latex DNase is complete, reveling that Mg^2+ ions activates latex DNases.

It is shown in table no 2, that lambda DNA hydrolyses latex DNase is partially done with 1mM & 5mM concentrations of Mn^2+ions, reveling that lower concentrations of Mn^2+ ions partially hydolyses lambda DNA, and complete hydrolysis is done with the higher concentrations i.e., 10mM and 50mM Mn^2+ions reveling that higher concentrations of Mn^2+ ions activates latex DNases.

Table 2: DNA hydrolysis with Mn^2+ ions through latex DNases

$MnCl_2$ Concentration	DNA degradation
1mM	++
5mM	++
10mM	+++
50mM	+++

No degradation: -
Mild degradation: +
Moderate degradation: ++
Full degradation: +++

Table 3: DNA hydrolysis with Ca+ ions through latex DNases

$CaCl_2$ Concentration	DNA degradation
1mM	++
5mM	++
10mM	++
50mM	+

No degradation: -
Mild degradation: +
Moderate degradation: ++
Full degradation: +++

It is shown in table no 3, that lambda DNA hydrolyses with latex DNase is partially done with 1mM & 5mM, 10mM concentrations of Ca2+ions, reveling that lower concentrations of Ca+ ions partially degrades lambda DNA, and no hyrodysis is done with the higher concentrations i.e., 50mM Ca^2+ions reveling that higher concentrations of Ca+ ions deactivates latex DNases.

Table 4: DNA hydrolysis with Hg+ ions through latex DNases

$HgCl_2$ Concentration	DNA degradation
1mM	-
5mM	-
10mM	-
50mM	-

No degradation: -
Mild degradation: +
Moderate degradation: ++
Full degradation: +++

It is shown in table no 4, Hg^2+ does not hydrolyze lambda DNA, hence, Hg^2+ inactivates latex DNases.

Table 5: DNA hydrolysis with Zn^2+ ions through latex DNases

$ZnCl_2$ Concentration	DNA degradation
1mM	+
5mM	+
10mM	+
50mM	+

No degradation: -
Mild degradation: +
Moderate degradation: ++
Full degradation: +++

It is shown in table no 5, Zn^2+ ions mildly hydrolyses lambda DNA, hence, Zn^2+ ions inactivates latex DNases.

Table 6: DNA hydrolysis with chelating agent EDTA through latex DNases

EDTA Concentration	DNA degradation
1mM	-
5mM	-
10mM	-
50mM	-

No degradation: -
Mild degradation: +

Moderate degradation: ++
Full degradation: +++

It is shown in table no 6, that chelating agent EDTA inactivates lambda DNA hydrolysis through latex DNases.

Table 7: DNA hydrolysis with organic solvent DMSO through latex DNases

EDTA Concentration	DNA degradation
1% (v/v) DMSO	-
5% (v/v) DMSO	-
10% (v/v) DMSO	-
50% (v/v) DMSO	-

No degradation: -
Mild degradation: +
Moderate degradation: ++
Full degradation: +++

It is shown in table no 7, that organic solvent DMSO inactivates lambda DNA hydrolysis through latex DNases.

CONCLUSIONS

Deoxyribonuclease or DNase (EC 3.1.4.5) is an enzyme that hydrolysis both single and double stranded DNA molecules to produce a mixture of oligo-and mononucleotides. The enzyme participates in a wide variety of cellular functions such as repair of damaged DNA, DNA turnover and apoptosis. DNase occurs in different tissues of plants, animals and microorganisms. They differ in their molecular weights, mode of action, substrate specificity and other physical and chemical characteristics. Latex DNase hydrolytic activity is highly influenced by divalent cations. Mg^{2}+, Mn^{2}+, Ca^{2}+ activates latex DNase hydrolytic activity, whereas Zn^{2}+, Hg^{2}+, EDTA and DMSO deactivates latex DNase hydrolytic activity. Activity of other divalent and monovalent cations of group I and group II of periodic table, should also be tested to completely understand the latex DNase hydrolytic activity.

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