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Kinetic Characterization of a Protease Isolated from Crude Fresh Latex of Plantain (*Musa Paradisiaca*) Plant

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Abstract- The plant latex consist of biologically active compounds useful for diverse health benefits and could be a potential source of unique proteases. Proteases play key roles in the regulation of biological processes in plants and currently have become a vital part of the food and feed industry. In order to understand the underlying mechanisms of action of unique plant latex, the present study isolate and characterized a protease enzyme from the crude latex of plantain plant. Crude latex collected from different parts (leaves, stem, trunk and branches) of the plantain plant was centrifuge to separate the supernatant from any particles. The supernatant was used as the crude latex extract for the research. A protease enzyme was isolated from the crude latex extract and the effect of pH, temperature, incubation time, kinetic study of the protease activity as well as protein were determined spectrophoto-metrically. The enzyme protease was found to have a 9.84 mg/ml of protein with a specific activity of 3.06 unit/mg protein respectively.

Keywords: *protease, proteolytic, plantain and latex.*

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KINETICCHARACTERIZATIONOFAPROTEASEISOLATEDFROMCRUDEFRESHLATEXOFPLANTAINMUSAPARADISIACAPLANT

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Kinetic Characterization of a Protease Isolated from Crude Fresh Latex of Plantain (*Musa Paradisiaca*) Plant

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Abstract- The plant latex consist of biologically active compounds useful for diverse health benefits and could be a potential source of unique proteases. Proteases play key roles in the regulation of biological processes in plants and currently have become a vital part of the food and feed industry. In order to understand the underlying mechanisms of action of unique plant latex, the present study isolate and characterized a protease enzyme from the crude latex of plantain plant. Crude latex collected from different parts (leaves, stem, trunk and branches) of the plantain plant was centrifuge to separate the supernatant from any particles. The supernatant was used as the crude latex extract for the research. A protease enzyme was isolated from the crude latex extract and the effect of pH, temperature, incubation time, kinetic study of the protease activity as well as protein were determined spectrophotometrically. The enzyme protease was found to have a 9.84 mg/ml of protein with a specific activity of 3.06 unit/mg protein respectively. The enzyme exhibited its highest activity at a temperature of 35^0C, and showed optimum proteolytic activity at pH of 7.0. The protease enzyme isolated from the latex of plantain was also found to be optimally active at 120 seconds. The proteolytic activity of the enzyme revealed that the K_m and V_{max} represent 0.051 mgml^{-1} 0.976 mmoles min^{-1}ml^{-1} respectively. These results highlighted the characteristics of plantain latex protease that revealed it as a good source for neutral protease which might be of useful application in biotechnological industries.

Keywords: protease, proteolytic, plantain and latex.

I. Introduction

Plant latex is a sap milky fluid that is secreted from laticiferous tissues (stems, roots, leaves and fruits) which is discharge from the point of tissue damage and functions in protein processing, digestion, growth, reproduction, apoptosis, senescence, defense against herbivores and pathogens, etc and excretion of waste metabolites [1-4]. Traditionally, plant latex have been utilized as therapeutic agents to treat different types of ailments [5]. Latex serves as anthelmintic, insecticidal, anti-inflammatory, antioxidant, anti-cancer activities, antiparasitic, anticoagulant and also used in fishing, veterinary and human medicine, as well as biofuel [6]. The latex ethno-pharmacological properties is due to the presence of phytoconstituents such as terpenoids, alkaloids, gums, and cardenolides as well as proteins and enzymes such as chitinases, glucosidases and proteases [1, 7].

Proteases, are proteolytic enzymes that catalyse the cleavage of the peptide bond in the protein to give peptides and amino acids by hydrolysis significant to food digestion and intracellular protein turnover [7-9]. The protein substrates specifically break either from the N-terminus (aminopeptidases) or C-terminus (carboxypeptidases) and/or in the middle of the protein molecule (endopeptidases) respectively by protease [8-9]. Proteases are classified as metallo, serine, acidic, carboxyl, alkaline and neutral based on structures and/or properties of the active site [10]. Proteases are found in the plant and animal as well as bacteria and viruses [11]. Apart from plant latex proteases function to defend the plant against pests/insect, it is also involved in hemostatisis (coagulation), wound healing, tissue remodelling, DNA replication, cell proliferation, cell death, cell-cycle progression, and immune responses [5, 7, 8, 12]. Plant proteases has also emerge as useful therapeutic agents in the treatment and management of debilitating conditions including sepsis, chronic inflammatory disorders, cystic fibrosis, retinal disorders, and psoriasis [7, 8, 12, 13]. They have applications in food processing, detergent, pharmaceutical and other chemical industries [10, 14-15]. Proteolytic enzymes from plant latexes are of widespread interest due to their involvement in various physiological functions and economic benefits. They receive added attention due to broad substrate specificity and activity in a wide range of pH, temperature, in the presence of organic compounds and other additives [16].

In the last few years, proteases from different plant latex have been the object of consideration with most studied belong to the family of cysteine or serine or aspartate endopeptidases family [17]. Cysteine protease in the latex of papaya (*Carica papaya*) and wild fig (*Ficus virgatalatex*) has shown high toxicity to caterpillars of herbivorous insects [1, 6, 18-19], while plant serine protease known as subtilases has been isolated from several plants showed to be involved in many metabolic functions like hypersensitive response, symbiosis, microsporogenesis, signal transduction and differentiation, senescence and protein degradation/ processing [20-21]. Plantain (*Musa paradisiaca*) latex

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from the smooth stem was reported to have a high level of proteolytic activity [22], but the kinetic characterization of latex from different parts of the plantain has not been investigated.

Plantain, a perennial crop with a short gestation period is available for harvest year round in tropics and sub-tropics region of the World. It has a significant economic activity for income generation for both large scale and small farmers in the area of food production [23-24]. Plantain has medicinal applications in bronchitis, dysentery, ulcers, diabetics and culinary uses [23]. Considering the search for novel proteases from medicinal plants multidrug resistance and toxicity associated with the existing remedies, there is need to clarify the type of protease isolated from crude plantain parts latex. Therefore, this study was conducted to isolate and characterized the crude protease from the latex of plantain.

II. Materials and Methods

a) *Materials*

The plantain plants used for the research was from Ojo - Area of Lagos State, Western Nigeria. All chemicals and reagents used were of analytical reagent grade.

b) *Methods*

i. *Preparation of crude plant latex extract*

Latex was collected early in the morning from different parts of the plantain (leaves, stem, trunk and branches) by nipping the leaves near the stem and incision of the trunk and branches so as to allow the milk to drain into a clean glass tube. 10 ml of latex collected was measured into a glass measuring cylinder and equal volume of distilled water was added to the crude extract. The mixture was centrifuged at 5000 rpm for 20 minutes at 4°C. The resulting supernatant was collected and used as the crude enzyme extract for further investigation of the protease activities.

c) *Biochemical Analysis*

i. *Protein determination*

Protein concentration was quantitatively analysed by modified methods of Layne [25] and Aitken and Learmonth [26] using the Biuret method and BSA as standard.

ii. *Protease assay*

Protease assay was performed using a modification of Kunitz case inolytic assay as described by Janssen *et al*. [27] as described in Raimi *et al*. [28] and Raimi *et al*. [29]. Briefly, 0.5 ml of the crude enzyme source was added to 2.0 ml of 0.5% casein in 50 mM phosphate buffer (pH 7.4). The reaction mixture was incubated at 37 °C and terminated after 30 min by adding 3.0 ml of 5% TCA. The solution was kept for additional 30 min at room temp and then centrifuged. The absorbance of the supernatant was read at 280 nm by using UV/Visible Spectrophotometer (Model SM 755s), a product of Surgienfield Instrument, Zhejiang, China (Mainland). One unit of TCA soluble casein hydrolysis product was defined as an increase of 0.1 in absorbance at 280 nm.

d) *Analysis of proteolytic activity*

i. *Effect of temperature on protease activity*

The effect of temperature on the enzyme activity was carried out at a temperature ranging from 15°C to 55°C with an interval of 5°C. Modified Kunitz caseinolytic assay method as described for enzyme assay was used.

ii. *Effect of pH on protease activity*

This was carried out following the modified Kunitz caseinolytic assay method as described for enzyme protease assay but the pH was varied for the reaction mixture between a range of 5.0 and 11.0 with an interval of 1.0 using 50 mM phosphate buffer (pH 7.5).

iii. *Effect of incubation time on protease activity*

The incubation time effect on the protease activity was determined by incubating the assay at different time ranging from zero (0) to 300 seconds with an interval of 100 seconds.

iv. *Assessment of kinetic constants (K_m and V_{max}) of the protease enzyme*

The enzyme activities were assayed at various concentrations of substrate (casein) in a reaction volume of 2.2 ml incubated for 30 min at 37°C and terminated using 3.0 ml of 5% TCA. The Michaelis-Menten constant (K_m) and maximum reaction velocity (V_{max}) of the purified enzyme were determined from the Lineweaver-Burk plot.

III. Results

a) *Protein determination*

The fresh plantain crude extract of the latex contained 9.84 mg/ml of protein with a specific activity of 3.05 unit/mg protein as shown in Table 1.

b) *Proteolytic activity*

The crude latex protease enzyme demonstrated maximum proteolytic activity at a temperature of 35^{0}C and then gradually decreased until it reached a temperature of 55^{0}C (Fig. 1). At optimum pH 7.0 (Fig. 2) the protease enzymes was found to be active and its activity decreased to approximately pH 11. The effect of incubation on the enzyme activity (Fig. 3) shows that the crude latex protease enzyme is optimally active at 120 seconds. Figure 4 depicted the kinetic constants of the latex protease enzyme based on a Lineweaver-Burk plot of the protease and substrate – casein. The K_m and V_{max} values were found to be 0.051mg ml^{-1} and 0.976 mmoles min^{-1}ml^{-1} respectively.

IV. Discussion

Plant latex, compose of various types of protease enzymes which occupy a pivotal locus with respect to their applications in both physiological and commercial fields [10, 15, 30]. The activity of these enzymes depends on the plant source, extraction and purification methods [30]. In this study, the units of protease activity present per milligram of crude plantain (*Musa paradisiaca*) latex from various parts revealed that it's a rich source of protease which agrees with the report of Awoyinka and Shokunbi [22]. The high latex protease activity suggests that it is not a waste product but a crucial parts of plant resistance [31].

Temperature and pH plays an important role on an enzyme-catalyzed reaction. The stability of proteases from crude latex of plantain (*Musa paradisiaca*) parts were determined by incubation at various times, temperatures and pH. The observed optimum incubation time, temperature and pH suggest that the plantain crude latex is very stable. This is similar to the report of Antao and Macloata [20]; Domsalla and Melzig [17] and Macalood *et al*. [3], even though, the optimum temperature of plantain is lower to that of papaya (*Carica papaya Linnaeus*). This result suggest that the isolated protease from crude plantain latex with neutral pH might be neutral protease [32] which might be the mechanism of action for its wide range of applications in therapeutic [24, 33], food and brewing industries [34]. This is in agreement with other researchers that have isolated neutral protease from different sources [28-29, 35-36, 37]. The decrease of activity after optimum temperature may be due to the alteration of the structure of the protease [38].

For a fixed enzyme concentration, the best substrate for the enzyme activity depends on low K_m value (strong substrate binding or high affinity) and high V_{max} value (high catalytic efficiency) [39]. The low K_m and high V_{max} values observed in this study, revealed that the crude latex protease enzyme has high affinity for casein as a substrate which is similar to the previous reports of Sharma *et al*. [39] and Raimi *et al*. [28].

In conclusion, the kinetic characterization of a protease isolated from crude fresh latex of plantain (*Musa paradisiaca*) plant parts was evaluated in this study. The study revealed the presence of neutral protease enzyme in the crude latex of the plantain which can be exploited commercially as a useful application in biotechnological industries.

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Table 1: The total protease enzyme activity from the isolated crude latex of plantain

	Crude extract
Volume (ml)	10
Total protein concentration (mg/ml)	9.84
Total protein (mg)	98.4
Protein concentration (Units/ml)	30
Specific activity (Units/mg protein)	3.06
Total Enzyme Activity (units)	300
Yield (%)	100

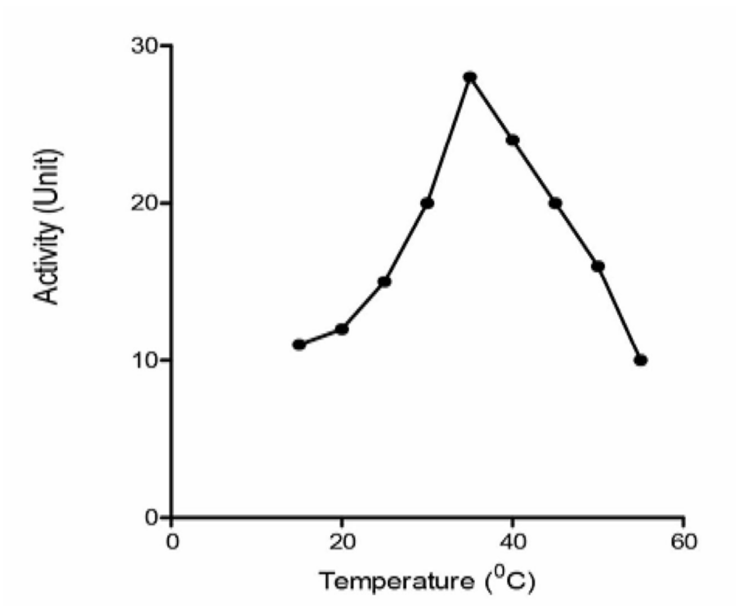

Figure 1: Effect of temperature (°C) on protease enzyme activity (Unit) from crude plantain latex

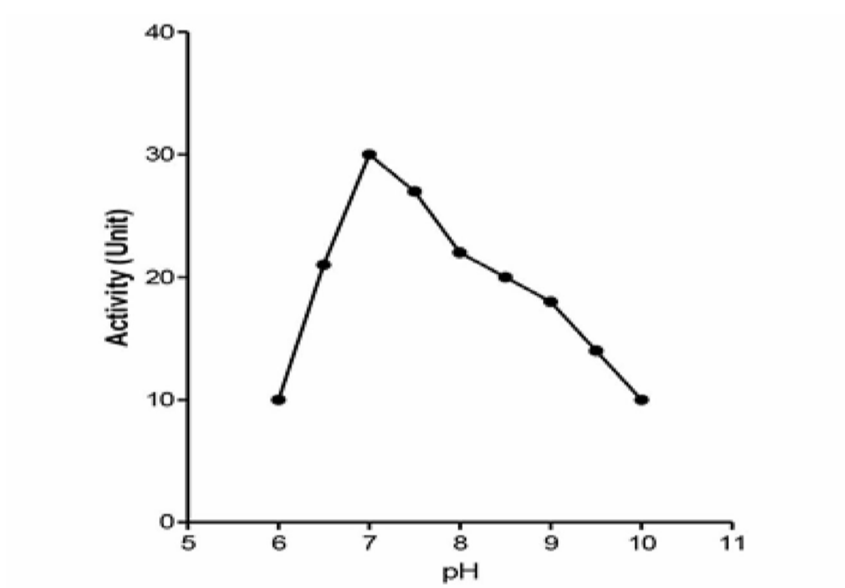

Figure 2: Effect of pH on protease enzyme activity (Unit) from crude plantain latex activity

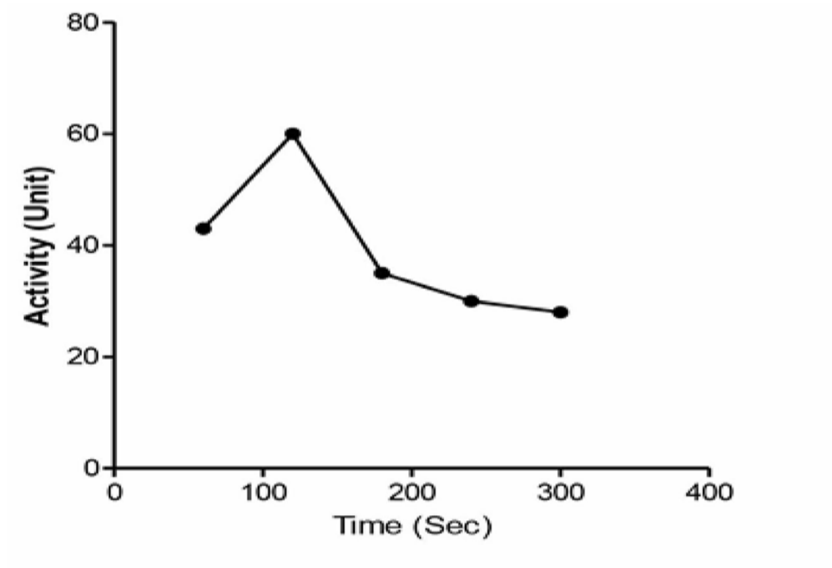

Figure 3: Effect of time on enzyme Effect of time (Sec) on protease enzyme activity (Unit) from crude plantain latex activity

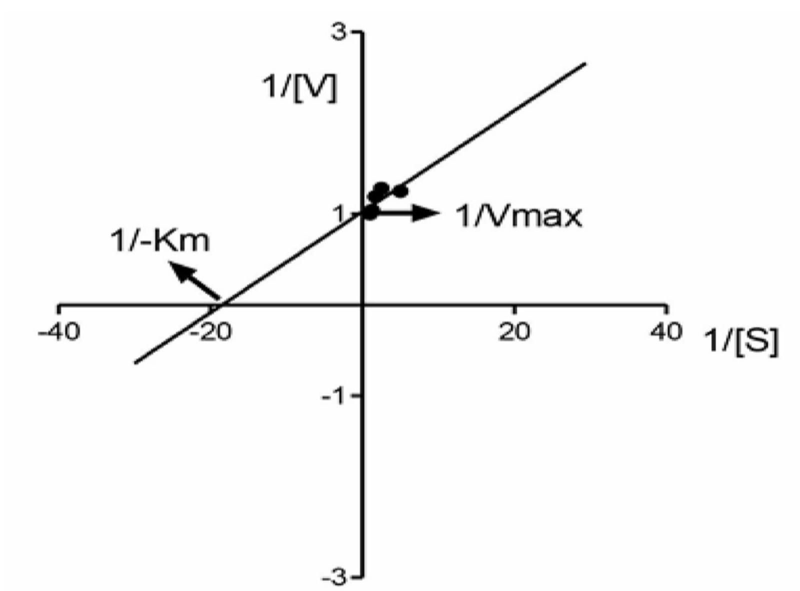

Figure 4: Line-weaver-Burk plot showing the K_m and V_{max} of the protease enzyme from crude plantain latex