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Peroxidase Activity in Extracts of Watermelon Pulp: Effects of Ethylenediaminetetraacetic Acid and Some Cations

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Authors' contributions

This work was carried out in collaboration between both authors. Author IOM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author OAA managed the study's analyses and managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to evaluate the effects of EDTA and some chloride salts of Ca, Cr, Fe and Mg on the initial velocity (Vo) of crude peroxidase from the pulp of watermelon.
Study Design: The study design is *In vitro* enzyme assay.
Place and Duration of Study: This study was conducted in the Department of Biochemistry, Faculty of Life Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria between in November 2023.
Methodology: The crude peroxidase from the pulp of watermelon was prepared from 10 g of the pulp of watermelon using standard procedures. The initial reaction rate of the enzyme was

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determined by spectrophotomertically by monitoring the rate of formation of tetraguaiacol, the oxidation product of guaiacol at 470 nm in the absence and presence of varying concentrations of EDTA and the chloride salts of Ca, Cr, Fe and Mg. The total volume of the reaction mixture was 3 mL. The time course for the reaction was determined and the initial velocity calculated.

Results: Results showed that EDTA reduced the activity of the enzyme. This reduction in activity was EDTA-concentration dependent. This suggest the inhibitory effects of EDTA on peroxidase from watermelon pulp. The chlorides salts of Ca, Cr, Fe and Mg were found to be activators of the peroxidase from the pulp of watermelon. This information could be beneficial in industrial application processes where peroxidase from pulp of watermelon is deployed for use.

Conclusion: From this study, it could be inferred that EDTA has the potentials of reducing the activity of peroxidase from pulp of watermelon fruit while the chloride salts of Ca, Cr, Fe and Mg are potentially good activators. This information is very crucial as ongoing research to understand the mechanism of action of peroxidase from the pulp of watermelon fruit continues. Considering the industrial applications of peroxidase, this information contributes to knowledge of how this peroxidase works.

Keywords: Watermelon; pulp; cations; activators.

1. INTRODUCTION

Plant peroxidases are heme-containing enzymes. They catalyse a single one electron oxidation of several substrates with the use of H_2O_2" [1]. Peroxidases are classified based on the presence or absence of a heme group [2]. Plant peroxidases (class III) are a large multigene family in plants, primarily found in the cell wall and vacuoles. They are used in a variety of biotechnological applications. Thousands of peroxidase plant sources have been studied in the past" [3]. Some of the major sources of peroxidases such as manganese peroxidase, lignin peroxidase and horseradish peroxidase are from papaya (*Carica papaya*), bare (*Acorus calamus*) and banana (*Musa paradisiacal*) [4].

These enzymes are extensively used in the synthesis of various aromatic chemicals, diagnostic kits, ELISA, and removal of peroxides from industrial wastes [4]. Peroxidase reactions can be monitored spectrophotometrically by monitoring the appearance of tetraguaiacol which is the oxidation product of Guaiacol at 470nm [5].

Studies have shown that peroxidases from various sources have potentials for different applications in bio-catalysis and bio-electrocatalysis [6]. Therefore, in this study, the kinetics of peroxidase from the pulp of watermelon using guaiacol as substrate was investigated in the presence and absence of EDTA and chlorides salts of Ca, Cr, Fe and Mg. The findings from this study will provide an insight on the possible kinetics of this enzyme from this source on its interactions with EDTA and these metallic chlorides.

2. MATERIALS AND METHODS

Calcium chloride, chromium chloride, Iron chloride, magnesium chloride, sodium acetate, Hydrogen peroxide (30%), Guaiacol, dimethyl sulphoxide, acetic acid, disodium hydrogen phosphate, and sodium dihydrogen were purchased from SchauLab S.L. (Spain) and Loba Chimine Pot. Ltd. (India). All other reagents used in this study were of analytical grades and purchased from Sigma-Aldrich (Dorset, Poole, United Kingdom). The kinetic measurements were done with the aid of a UV-780 recording spectrophotometer.

2.1 Methods

2.1.1 Collection of plants materials

The watermelon (*Citrullus lanatus*) that was used in this study was purchased from the local market in Ekpoma in Esan West Local Government Area, Edo State, Nigeria. They were washed with distilled water in the laboratory, and the pulp separated from the watermelon fruit.

2.1.2 Preparation of crude enzyme

In the preparation of the crude extract containing the peroxidase, 10 g of pulp from the watermelon fruit was weighed and washed with distilled water. The pulp was then homogenized using a blender in the presence of 100 mL of 0.1 M sodium phosphate buffer of pH 7.0. After homogenization, the solution was filtered using a muslin cloth. The filtrate was then centrifuged (Centrifuge 800B, Pec-Medical U.S.A) at 4000

rpm for 30 minutes. The supernatant was then stored frozen in plain sample for analysis. This served as the source of the crude peroxidase.

2.1.3 Effects of varying salt concentrations on crude peroxidase activity from watermelon pulp

The effects of varying salts concentration on the initial velocity (Vo) of the crude peroxidase from pulp of watermelon was investigated by varying concentrations of $CaCl_2$, $CrCl_2$, $FeCl_2$, $MgCl_2$ and EDTA in the enzyme assay mixture and monitoring the rate of formation of guaiacol oxidation product (tetraguaiacol) at 470nm. The various salt concentrations used varied between 0.5 mM and 2 mM. The reaction mixtures consists of: 2.3 mL of 0.6 M sodium acetate buffer of pH 5.4, 0.2 mL of a 0.02 mM Guaiacol, 0.1 mL of crude extract from pulp of the watermelon containing the peroxidase, 0.2 mL of varying concentration of the chloride salts, 0.2 mL of 2 mM of H_2O_2 added last to start the reaction. The total volume of the reaction mixture was 3 mL. The absorbance values were read every 2 seconds for sixty seconds after the addition of hydrogen peroxide. The control used in this study contained no salt in the assay mixture but had it replaced with equivalent volume of distilled water.

2.1.4 Determination of Initial reaction rate (Vo)

The initial velocity (Vo) of the crude peroxidase from the pulp of watermelon was determined by first determining the change in absorbance versus time (slope), and dividing the slope by the molar absorptivity for guaiacol oxidation product (ε= 26,000 M-1cm-1). The value obtained was then multiplied by the sample path length (1.00 cm). The result obtained was expressed in mM/second. All enzyme assays were done in five replicates.

3. RESULTS AND DISCUSSION

Fig. 1 shows the effect of varying EDTA concentrations on the activities of peroxidase from pulp of watermelon fruit. Results shows that increasing EDTA concentrations within the range of 1 mM to 2 mM reduced the activity of the enzyme proportionately. A high peroxidase activity was recorded at a low EDTA concentration of 0.5 mM. Fig. 2 shows the effect of varying concentrations of $CrCl_2$ on the activities of peroxidase from pulp of watermelon fruit. Results showed an increase in enzyme activity from 0.5 mM to 1 mM $CrCl_2$.. The highest activity was recorded at a $CrCl_2$ concentration of 1 Mm. Further increase to 2 mM resulted in a decrease in enzyme activity. Fig. 3 shows the effect of varying $CaCl_2$. concentrations on the activities of peroxidase from pulp of watermelon fruit. Results show a proportionate increase in enzymes activity with increasing $CaCl_2$ concentration within the range of 0.5 mM to 2 mM. Fig. 4 shows the effect of varying $FeCl_2$. concentrations on the activities of peroxidase from pulp of watermelon fruit. Results show a proportionate increase in enzymes activity with increasing $CaCl_2$ concentration within the range of 0.5 mM to 1.5 mM. Further increase in concentration to 2 mM resulted in a decrease in activity of the enzyme. Fig. 5 shows the effect of varying $MgCl_2$. concentrations on the activities of peroxidase from pulp of watermelon fruit. Results show a proportionate increase in enzymes activity with increasing $MgCl_2$ concentration within the range of 0.5 mM to 1 mM. further increase in concentration to 2 mM resulted in a decrease in activity of the enzyme. EDTA usually results in inactivation of many metalloenzymes [7]. The loss of activity seen in the assay in the presence of EDTA may be due to the ability of EDTA to isolate Fe^{3+} and Ca^{2+} which are needed for their structural and functional stability of the enzyme and thus the catalytic ability. The results from the effect of chromium chloride on peroxidase activity was similar to the previous studies [8] which reported the ability of chromium to increase or decrease the activity of peroxidase. Previous studies [9] have shown that peroxidase activity could be recovered up to 95% of its original value by addition of Ca^{2+} when lignin peroxidase was depleted of Ca^{2+} by incubation with EGTA. The peroxidase from pulp of watermelon is similar in action to other peroxidase assayed in the presence of Ca^{2+}.

The effects of $FeCl_2$ and $MgCl_2$ (Figs. 4 and 5 respectively) on the activities on peroxidase from pulp of watermelon. In both cases, the effects of these cations on the peroxidase from watermelon pulp is typical of the effects of these cations on other peroxidases [10,11].

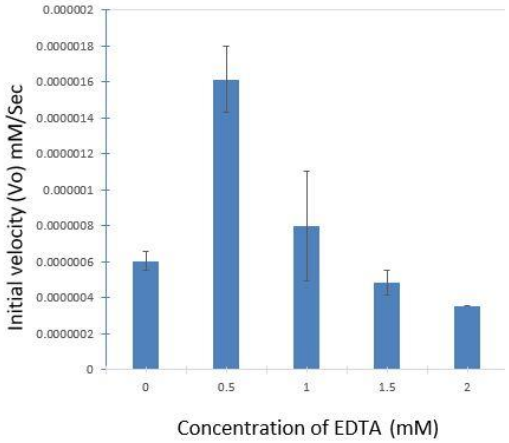

Fig. 1. Effect of varying concentrations of EDTA on the peroxidase activity in watermelon pulp

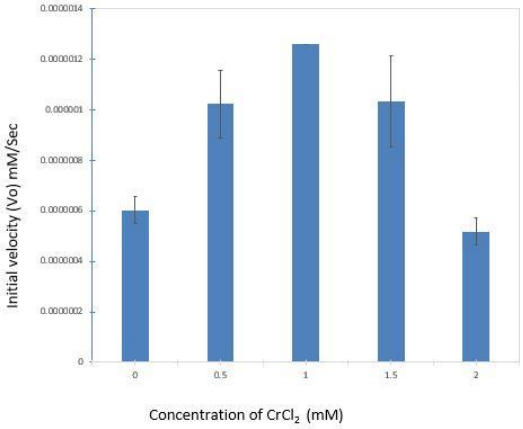

Fig. 2. Effect of varying concentrations of $CrCl_2$ on the peroxidase activity in watermelon pulp

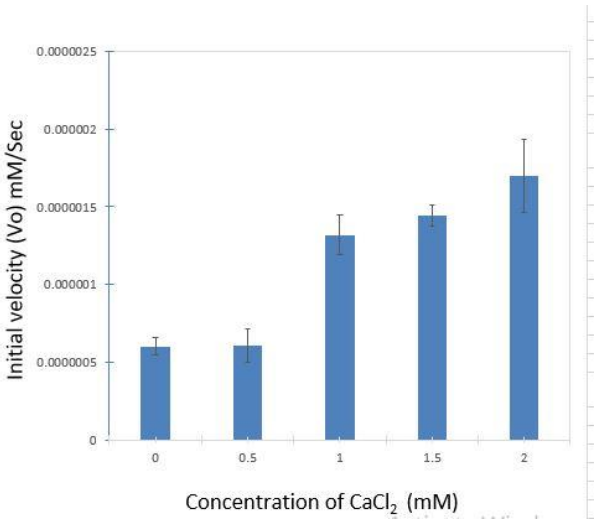

Fig. 3. Effect of varying concentrations of $CaCl_2$ on the peroxidase activity in watermelon pulp

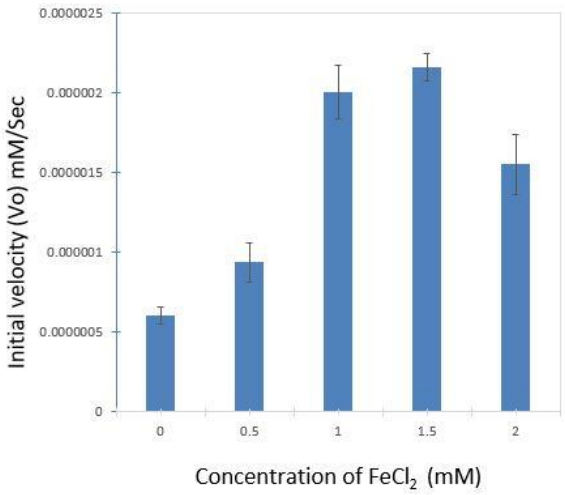

Fig. 4. Effect of varying concentrations of $FeCl_2$ on the peroxidase activity in watermelon pulp

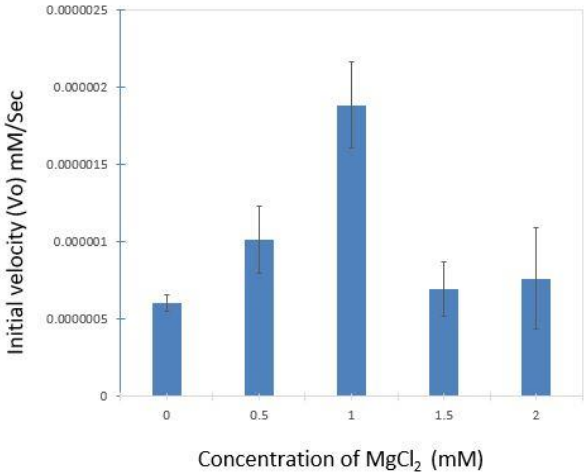

Fig. 5. Effect of varying concentrations of $MgCl_2$ on the peroxidase activity in watermelon pulp

4. CONCLUSION

From this study, the presence of significant peroxidase activity from pulp of watermelon was established. This activity of this peroxidase from watermelon pulp was similar to previously characterized peroxidases. The identification of chloride salts of Cr, Ca, Fe and Mg as activators of peroxidase from watermelon pulp is an interesting development in the search for cheap and alternative sources of peroxidase. The reduction of peroxidase activity from pulp of watermelon by EDTA in a concentration dependent manner provides information for further research on the actual mechanism of action of EDTA on the peroxidase causing reduced activity.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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