

Some metal activators of peroxidase from peel of watermelon in the oxidation of guaiacol

ABSTRACT

Aim: To identify some activators of peroxidase from the peel of watermelon in the oxidation of guaiacol.

Study design: *In vitro* enzyme assay.

Place and Duration of Study: This study was done in the Department of Biochemistry, Faculty of Life Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria in November 2023.

Methodology: The effects of some metallic chloride on the activity of peroxidase from peel of watermelon in the oxidation of guaiacol was done in the absence and presence of varying concentrations of the chloride salts of Cu, Fe, K and Mg using standard procedures.

Results: Results showed that the optimum salt concentration for enzyme activation for chlorides of Cu, Fe, K and Mg were 0.5 mM, 0.5 mM, 1.5 mM and 1 mM respectively within a salt concentration range of 0.5 mM to 2 mM.

Conclusion: From this study, it can be concluded that chlorides of Cu, Fe, K and Mg are activators of peroxidase from peel of watermelon. However, chlorides of Cu and Fe are better activators of the enzyme since they activate the enzyme at a comparatively lower concentration when compared with chlorides of K and Mg. The Chloride of K had the least activation potential. The identification of chlorides of Cu, Fe, K and Mg as activators of peroxidase from peel of watermelon is of great importance as the search for cheaper and alternative sources of peroxidases for various applications continues.

Keywords: [watermelon, peel, activators, Guaiacol]

1. INTRODUCTION

Peroxidases belong to the class of oxidoreductases [1]. They are ubiquitous [2] and are widely found in plants, animals and microorganisms. Their function is to protect the cells against the effects of oxidative stress and cell damage due to H₂O₂ [3]. The presence or absence of the heme group is a determinant in the classification of peroxidases as heme or non-heme peroxidases, both of which have different functions [4]. The function of peroxidases is mainly the oxidation of molecules at the expense of hydrogen peroxide [5]. Peroxidases are very crucial enzymes that are involved in several biochemical processes some of which include its use in dye degradation [6] treatment of effluent [7] and mycotoxins degradation [8].

One very common substrate used for studying peroxidase activity is guaiacol. The oxidized form of guaiacol is a brown-colored product which can be easily monitored for the activity of peroxidase at 470 nm using a spectrophotometer.

The peel of watermelon (*Citrulluslanatus*) which is often discarded as waste could be a very good source of peroxidase. Fruit peels could also be a sustainable source of enzymes, thus contributing to waste reduction and the development of eco-friendly biotechnological applications.

The catalytic efficiency of peroxidases can be significantly influenced by the presence of metal ions. This influence could be activation or inhibition of the peroxidase activity. The metal ions used in this study are chlorides from Cu, Fe, K and Mg. Understanding the effects of these metal ions on the peroxidase activity from watermelon peel could provide valuable insights into the functional properties and industrial applications of peroxidase from watermelon peel.

This study therefore aims to investigate the role of copper, iron, potassium, and magnesium as activators of peroxidase extracted from the peel of watermelon in the oxidation of guaiacol. This would contribute to a wider and better understanding of the peroxidase derived from watermelon peel and their practical application in biotechnology..

2. MATERIAL AND METHODS

Disodium hydrogen phosphate, sodium dihydrogen phosphate, Dimethyl sulphoxide, Guaiacol, hydrogen peroxide (30 %), sodium acetate, acetic acid, were all purchased from SchauLab S.L. (Spain) and LobaChimine Pot. Ltd. (India). The metallic chlorides and all other reagents used were all of analytical grades and purchased from Sigma-Aldrich (Dorset, Poole, United Kingdom). All kinetic measurements were carried out using a spectrophotometer.

2.2 Methods

2.2.1 Collection of Plants Materials

The Watermelon (*Citrulluslanatus*) used in this study was purchased from a local market at Ekpoma, Esan West Local Government Area, Edo State, Nigeria. They were well washed with distilled water in the laboratory for further laboratory processes.

2.2.2. Preparation of Crude Enzyme

10 g of peel from the watermelon fruit was weighed and washed with distilled water. The homogenization of the peel was done using a blender. This process was done with the peel in 100 mL of 0.1 M sodium phosphate buffer of pH 7.0. Filtration of the homogenate was done 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 decanted into plain sample bottles and labeled as "crude extract" which was stored frozen for analysis.

2.2.2 Estimation of Guaiacol Oxidation by Crude Peroxidase Isolated from watermelon peel with varying Salt Concentration

The kinetics of peroxidase from the peel of watermelon in the oxidation of guaiacol was investigated in the absence and presence of varying concentrations of chloride salts of Cu, Fe, K and Mg by spectrophotometrically monitoring the oxidation of guaiacol to produce tetraguaiacol at a wavelength of 470nm according to the standard procedures. The salt concentrations varied from 0.5 mM to 2 mM. Each of the reaction mixtures used in the kinetic study contained: 2.3 mL of 0.6 M sodium acetate buffer (pH 5.4), 0.2 mL of 0.02 mM

Guaiacol, 0.1 mL of crude extract, 0.2 mL of varying concentration chloride salt, 0.2 mL of 2 mM of H₂O₂ added last to start the reaction. The final concentration of H₂O₂ in the 3 mL assay was 0.13 mM. The total volume of the reaction mixture was 3 mL. The absorbance was read every 2 seconds for one minute at 25°C after adding hydrogen peroxide using a stop-clock. The control had no metal ion but replaced with distilled water.

2.2.3. Determination of Initial reaction rate (Vo)

The initial reaction rate of the crude peroxidase from the peel of watermelon was determined by calculating, change in absorbance per second (Δ absorbance/second), then dividing by the molar absorptivity for Guaiacol oxidation product ($\epsilon = 26,000 \text{ M}^{-1}\text{cm}^{-1}$), multiplied by the sample path length (1.00 cm for cuvette used). The result was expressed in mM/second. All assays were done in five replicates. The effects of varying concentrations of the salts were determined graphically using the mean values obtained per assay

3. RESULTS AND DISCUSSION

Figure 1 shows the effect of varying CuCl₂ concentrations on the activity of peroxidase from the peel of watermelon for the oxidation of guaiacol. Results shows that CuCl₂ activated the enzyme within a salt concentration range of 0.5 mM to 2 mM. A CuCl₂ concentration of 0.5 mM optimally activated the enzyme. Further increase in salt concentration decreased the activity of the enzyme. Figure 2 shows the effect of FeCl₂ on the activity of the peroxidase from watermelon peel. Results also show that FeCl₂ is an activator of this enzyme with the concentration of 0.5 mM FeCl₂ showing an optimum enzyme activation. Figure 3 shows the effect of KCl on the activity of peroxidase from the peel of watermelon. Results also show that KCl is an activator of the peroxidase with an optimal KCl concentration of 1.5 mM for activation of enzyme. Similarly, MgCl₂ was seen to be an activator of the enzyme and this was optimum at 1 mM MgCl₂ concentration.

Peroxidases are species-specific [9]. In a previous study on the effects of copper ions on peroxidase activity [10], it was reported that increasing copper concentration increased peroxidase activities in leaves and roots of *Astragalus neo-mobayenii*. Results from this study showed a similar activating property of CuCl₂ on peroxidase from peel of watermelon. However, the optimal activating concentration of CuCl₂ was 0.5 mM. Concentrations higher than 0.5 mM up to 3 mM resulted in a proportionate decrease in enzyme activity even though all concentrations were significantly higher than the control group. The activating effect of FeCl₂ on the peroxidase from watermelon peel was also similar to the findings of previous research [11] where the peroxidase activity was found to significantly increase in the gills, liver, kidney and brain of Fish (*Cirrhinamrigala*) after exposure to iron as compared to a control group. Thus the peroxidase from watermelon peel appears to have similar characteristic in the presence of copper when compared with peroxidases from other sources. The observed increase in activity of peroxidase from watermelon peel was similar to the findings in previous research [12] where it was reported that KCl increased peroxidase activities in two rice varieties. Report from previous research [13] on the effect of MgCl₂ on peroxidase activity is similar to the findings in this research where MgCl₂ was found to be an activator of the enzyme thus sharing similarities with peroxidase from other sources in this regard.

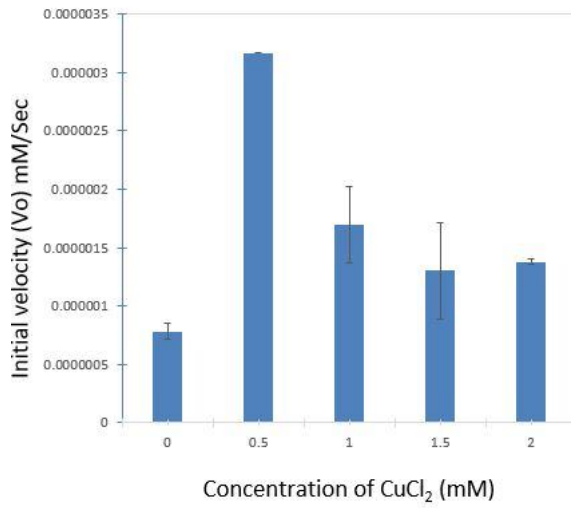


Figure 1: Effect of CuCl₂ concentrations on the peroxidase activity in watermelon peel

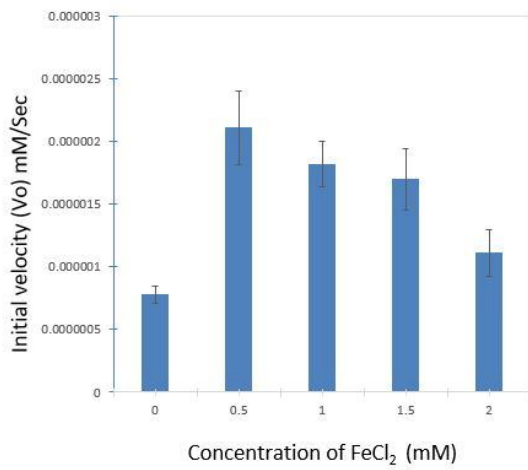


Figure 2: Effect of FeCl₂ concentrations on the peroxidase activity in watermelon peel

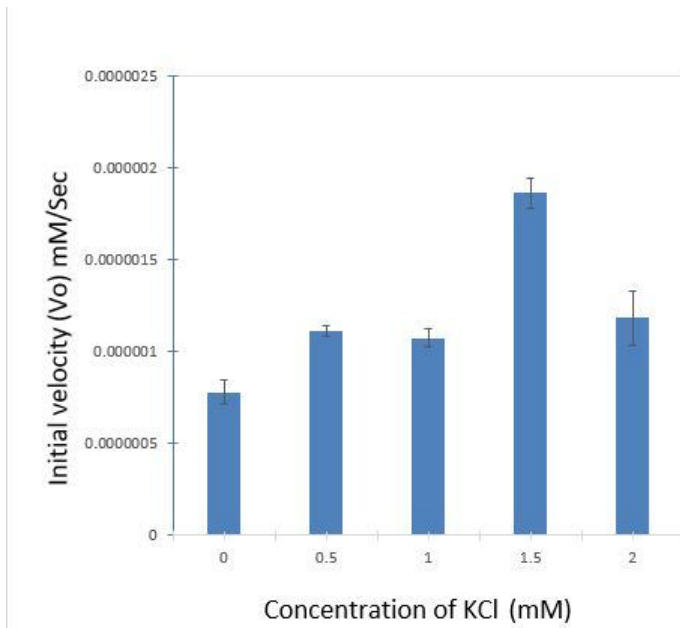


Figure 3: Effect of KCl concentrations on the peroxidase activity in watermelon peel

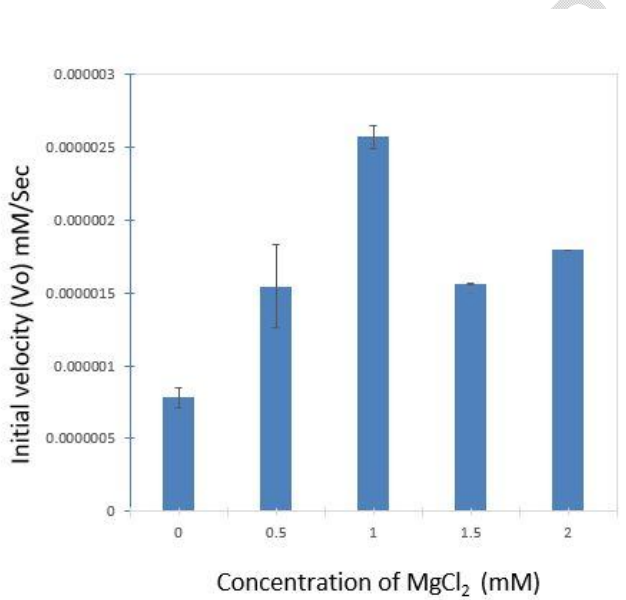


Figure 4: Effect of MgCl₂ concentrations on the peroxidase activity in watermelon peel

4. CONCLUSION

This study explored the effects of chlorides of some metal ions on the activity of peroxidase from the peel of watermelon during guaiacol oxidation. Results showed that CuCl₂ and FeCl₂ optimally activated the enzyme at 0.5 mM, while KCl and MgCl₂ were most effective at 1.5 mM and 1 mM, respectively. Higher concentrations of these ions reduced enzyme

activity. These findings suggest that these metal ions could significantly enhance peroxidase activity, offering potential applications in biotechnological processes requiring controlled oxidation reactions. The study therefore highlights the importance of optimizing metal ion concentrations for effective enzyme function and future industrial applications.

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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