Original Research Article

Evaluation of the Interactions of Some Alkali and Alkali Earth Metallic Chlorides on Guaiacol Oxidation by Peroxidase from Watermelon Rind

ABSTRACT

Aim: This study evaluated Interactions of some alkali and alkali earth metallic chlorides on guaiacol oxidation by peroxidase from watermelon rind.

Study design: *In vitro* enzyme assay.

Place and Duration of Study: Department of Biochemistry, Faculty of Life Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria between September 2023 to November 2023.

Methodology: The kinetics of the oxidation of Guaiacol by the crude peroxidase from the rind of watermelon in the presence of varying concentrations of the chloride salts of Na, K, Mg and Ca was determined spectrophotometrically by monitoring the oxidation of Guaiacol to produce a brown tetraguaiacol monitored at a wavelength of 470nm. The various salt concentrations were varied between 0.5mM and 3Mm. Each of the reaction mixtures used in the kinetic study comprised; 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 H_2O_2 added last to start the reaction. The final concentration of H_2O_2 in the 3 mL assay was 0.25 mM. The total volume of the reaction mixture was 3 mL. The absorbance was read every 2 seconds for one minute after adding hydrogen peroxide using a stop-clock. The control had no metal ion but replaced with distilled water

Results: Results showed that within a salt concentration range of 0.5mM to 3Mm, chlorides of Na, K and Mg activated the peroxidase from the rind of watermelon while $CaCl_2$ reduced the activity in a concentration dependent manner. The optimal salt concentration of these cation activators was 0.5 mM

Conclusion: These findings are of great importance to industries in understanding the mechanism of action of peroxidase from the rind of watermelon, especially as the search for cheap and alternative sources of peroxidases continues

Key words: Alkali, Alkali Earth Metallic Chlorides, hydrogen peroxide

1. INTRODUCTION

Peroxidases belong to class of oxidoreductase and they are ubiquitous [1]. “They catalyze a variety of oxygen-transfer reactions between hydrogen peroxide or other peroxides as electron acceptors and many kinds of substrates such as xenobiotics, lignin and other phenolic compounds by means of oxygen (O_2) liberation from H_2O_2” [1]. “Peroxidases are useful in several biotechnological applications due to the ability of peroxides to be reduced at the expense of electron donating substrates” [2]. “Peroxidases 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 H_2O_2” [2]. “Plant peroxidases are rendered as attractive biocatalysts due to their neutral optimum pH, their wide substrate specificity which enables them to use various synthetic chromophores as electron donors” [3]. “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 with the use of a compound called guaiacol (2-methyoxyphenol). Guaiacol can be oxidized to produce a brown product called tetraguaiacol. This tetraguaiacol can be detected and quantified by a spectrophotometer at a wavelength of 470nm. [5]. The effects of some metallic chlorides on the initial reaction rate of crude peroxidase from watermelon peels have been investigated previously [6]. This study aims at investigating the kinetics of peroxidase from the pulp of watermelon using guaiacol as substrate. The effect of some chlorides of alkali and alkali earth metals on the kinetics of peroxidase from watermelon rind could provide important information to aid their industrial application as alternative sources of peroxidase..

2. MATERIAL AND METHODS

Guaiacol, dimethyl sulphoxide, hydrogen peroxide (30 %), sodium acetate, acetic acid, disodium hydrogen phosphate, and sodium dihydrogen were purchased from SchauLab S.L. (Spain) and Loba Chimine Pot. Ltd. (India). Other reagents were all analytical grades and purchased from Sigma-Aldrich (Dorset, Poole, United Kingdom). All kinetic measurements were carried out using a UV-780 recording spectrophotometer.

2.2 Methods

2.2.1 Collection of Plants Materials

Watermelon (*Citrullus lanatus*) was purchased from a local market at Ekpoma, Esan West Local Government Area, Edo State, Nigeria. They were washed with distilled water in the laboratory.

2.2.2. Preparation of Crude Enzyme

10 g of rind from the watermelon fruit was weighed and washed with distilled water. This was then followed by homogenization in a blender using 100 mL of 0.1 M sodium phosphate buffer of pH 7.0. It was then filtered using a muslin cloth. The filtrate was centrifuged (Centrifuge 800B, Pec-Medical U.S.A) at 4000 rpm for 30 minutes. The supernatant was then decanted

into a plain sample container and properly labeled as "crude extract". This was stored frozen in the freezer for analysis.

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

The kinetics of the oxidation of Guaiacol by the crude peroxidase from the rind of watermelon in the presence of varying concentrations of chloride salts of some alkali metals (Na, K) and alkali earth metals (Ca and Mg) was determined spectrophotometrically by monitoring the oxidation of Guaiacol to produce a brown tetraguaiacol monitored at a wavelength of 470nm. The various salt concentrations were varied between 0.5mM and 3Mm. Each of the reaction mixtures used in the kinetic study comprised of: 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 H_2O_2 added last to start the reaction. The final concentration of H_2O_2 in the 3 mL assay was 0.25 mM. The total volume of the reaction mixture was 3 mL. The absorbance was read every 2 seconds for one minute 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 rind of watermelon was determined by calculating the slope of the line for the first part of the data in the graph of absorbance versus time (i.e., Δ absorbance/second). The slope was then divided by the molar absorptivity for Guaiacol oxidation product (ε= 26,000 M-1cm-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

Figures 1 shows the effect of varying NaCl concentrations on the peroxidase activity in water melon rind. Results shows that NaCl within the range of 0.5 mM and 2mM activated the peroxidase from rind of watermelon with an optimal NaCl concentration of 0.5mM. Higher concentrations reduced the activity of the enzyme. Previous studies [7] has shown that Sodium chloride had an inhibitory effect on the glutathione peroxidase. On the contrary, other studies [8] showed that ascorbate peroxidase activity increased with increasing salt intensity. This explains that the effects of NaCl on peroxidase depends on the source of peroxidase which may also be a determinant of the structure of the enzyme. The trend observed was similar to the effects of KCl on the activity from the rind of watermelon (Figure 2). Previous Studies [9] showed that KCl increases peroxidase activities in two rice varieties. Figure 3 shows the effect of varying Magnesium chloride concentrations on the peroxidase activity from watermelon rind. Results shows that Magnesium chloride activated peroxidase from the rind of watermelon optimally at a concentration of 0.5 mM. Higher concentration within the range of 0.5 and 2mM also showed activating effect when compared with the control. This activating effect was however lower when compared with the effect at a concentration of 0.5 mM. The activating and inhibitory effects which is dependent on concentration of $MgCl_2$ is similar to the trends seen in previous studies [10]. Figure 4 shows the effect of varying $CaCl_2$ concentrations on the peroxidase activity from watermelon rind. . Result shows that $CaCl_2$ showed an inhibitory effect on the peroxidase activity of watermelon rind. The enzyme showed the highest activity in the control which had no $CaCl_2$. However, as the concentration of $CaCl_2$ increases, the peroxidase activity decreased in a concentration dependent manner, thus suggesting a negative correlation between $CaCl_2$ concentration and enzyme activity. Results from this research on the actions of activity of peroxidase from the rind of watermelon is similar

to previous studies [10] on the effect of magnesium chloride and calcium chloride on ionic peroxidase (POD) from germinating Sorghum bicolor var Fara Fara which showed that Calcium chloride and magnesium chloride below 2 mM enhanced POD activity without any adverse effect on germination, however, at 5 mM, magnesium chloride inhibited POD activity by 50% with only a 14% reduction in germination, while calcium chloride achieved the same effect at 10 mM. The response of the peroxidase activity from the rind of watermelon as seen in this work in the presence of these cations has been attributed to the effect of various metals to various isoenzymes. It is note that several reports have shown that peroxidase is activated by divalent ions such as Ca^{2+} ions [11,12], on the contrary, other studies [13] have reported Ca^{2+} sometimes inhibited peroxidase activities.

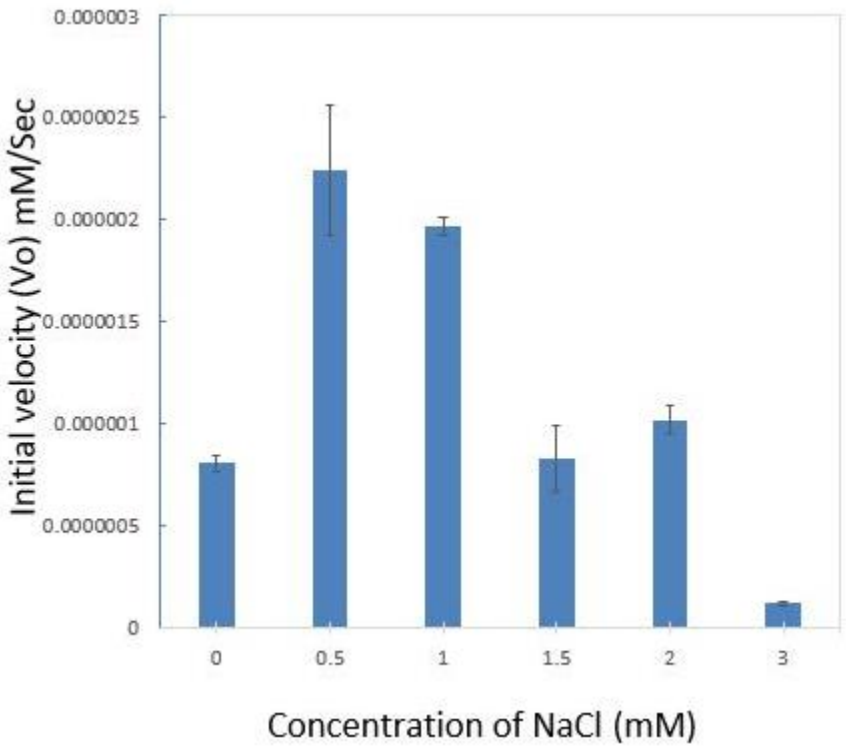

Figure 1: Effect of NaCl concentrations on the peroxidase activity in watermelon rind

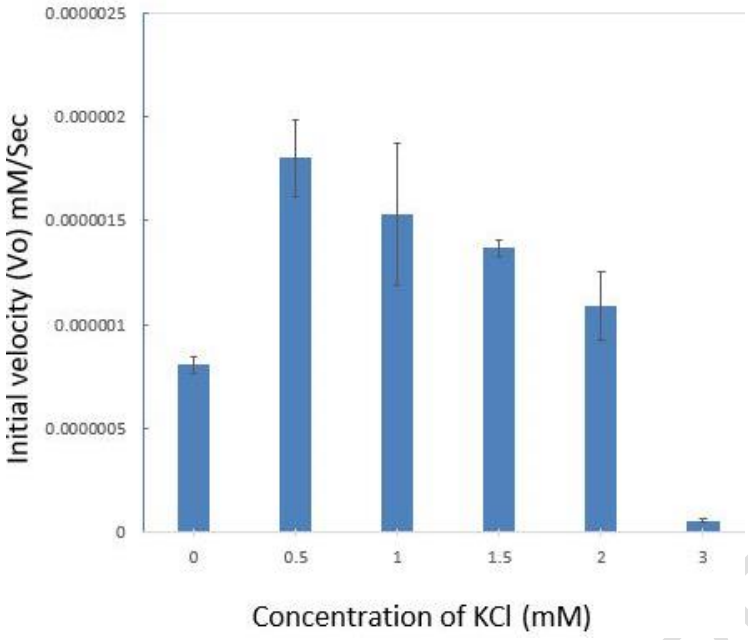

Figure 2: Effect of KCl concentrations on the peroxidase activity in watermelon rind

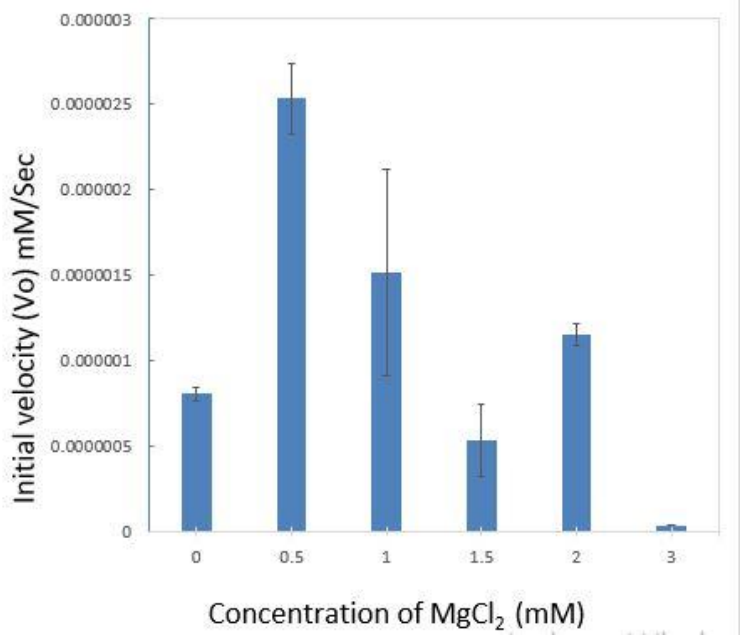

Figure 3: Effect of $MgCl_2$ concentrations on the peroxidase activity in watermelon rind

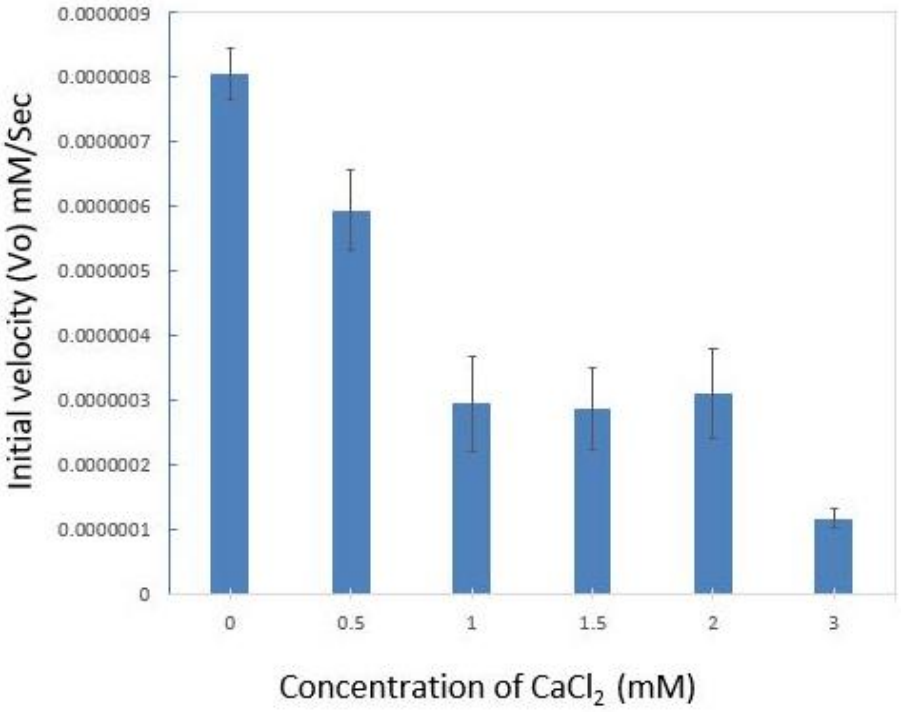

Figure 4: Effect of $CaCl_2$ concentrations on the peroxidase activity in watermelon rind

4. CONCLUSION

Results from this study have established the presence of peroxidases in rinds of the watermelon fruit. It has also been established that chlorides of Mg, Ca, Na and K have varying effects on peroxidase activity from the watermelon rind. With the exception of $CaCl_2$ all other cations studied showed activating effects on this peroxidase. These findings are crucial in the search for cheap alternative sources of peroxides.

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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