Evaluation Of The Effects Of Some Metal Chlorides On The Initial Reaction Rate Of Crude Peroxidase From Watermelon Peels

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ABSTRACT

Aims: This study evaluates the effect of some chloride on the initial reaction rates of crude peroxidase from watermelon peels.
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 April 2021 and June 2021
Methodology: The kinetics of crude peroxidase catalyzed oxidation of 3,5,3′,5′-tetramethylbenzidine (TMB) in the presence of varying concentrations of different chloride salts and hydrogen peroxidase was determined spectrophotometrically at 655nm. The assay mixture contained 2.3 mL of sodium phosphate buffer of pH 7.0, 0.1 mL of a crude enzyme from the watermelon peels, 0.2 mL of varying concentration of the respective chloride salts, 0.2 mL of 0.02 mM TMB, and 0.2 mL of 2 mM hydrogen peroxidase added last to start the reaction.
Results: Results showed that chloride salts of Pb^{2+} Hg^{2+} Fe^{2+} and Na^{+} had peroxidase activating effects at low salt concentration (1 mM). The activating effect increased proportionately up to their respective optimum salt concentration. Further increment of salt concentration above their respective optimum value lead to a decrease in initial reaction rate of the crude enzyme. Within a chloride concentration range of 0.025 mM to 3 mM, the highest activating effect recorded was in the order $Pb^{2+} > Fe^{2+} > Na^{+} > Hg^{2+}$. Results show that Pb^{2+} had the highest enzyme activating effect at the lowest concentration (0.5 mM) of the salt when compared with other salts within the same concentration range.

Conclusion: These findings are of great significance in research to understanding the mechanism of action of peroxidase from the peels of watermelon, especially as the search for cheap and alternative sources of peroxidases continues.

Keywords: Metal Chloride, 3,5,3′,5′-tetramethylbenzidine*, watermelon peels, initial reaction rate.*

1. INTRODUCTION

Peroxidases (EC 1.11.1.7) are produced by various plants and microorganisms. They belong to the class of enzymes called oxidoreductases. Peroxidases have an iron porphyrin ring which catalyzes the oxidation of many organic substrates [1]. Peroxidases are generally unstable. They are readily inactivated by hydrogen peroxide [2]. The heme-containing peroxidases catalyzes the one-electron oxidation of a wide range of structurally diverse aromatic compounds [3]. Peroxidases are regarded as one of the most heat-stable enzymes [4]. Being ubiquitous, peroxidases have wide applications in different areas which includes: synthesis of chemicals, medicine, and in the analysis of food, clinical and environmental samples [5]. Peroxidases are involved in the regulation of plant hormones, protective mechanisms, and lignin biosynthesis [6]

3,3,5,5-tetramethylbenzidine sulphate (TMB) is a commonly used peroxidase-specific substrate. Peroxidase can catalyze the oxidation of the colourless TMB into two coloured products [7]. The first product is a blue charge-transfer complex of the parent diamine and the diimine oxidation product [8].

The effects of various metal chlorides on peroxidases have been previously investigated. [9]. Since peroxidase has diverse applications, the search for cheap sources of peroxidases is of great importance. This study evaluates 3,3′,5,5′-tetramethylbenzidine oxidation by crude peroxidase from the watermelon peels in the presence of chloride salts of Hg^{2+} Na^{+} Pb^{2+} Ca^{2+} Ni^{2+} and Fe^{2+}.

2. MATERIAL AND METHODS

2.1 Materials

3,3',5,5'-tetramethylbenzidine (TMB), hydrogen peroxide (30 %), sodium acetate, acetic acid, disodium hydrogen phosphate, sodium dihydrogen phosphate and all chlorides of Lead, Nickel, Iron, and Mercury, were of 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 Preparation of Sample

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. 10 g of peels from the watermelon was weighed, washed with distilled water, and homogenized 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. After that, the filtrate was centrifuged (Centrifuge 800B, Pec-Medical U.S.A) at 4000 rpm for 30 minutes. The clear supernatant was then decanted into a plain sample container, properly labeled and stored frozen in the refrigerator for further biochemical investigations.

2.2.2 **Estimation of TMB Oxidation by Crude Peroxidase with Varying Chloride Salt Concentration**

The kinetics of the oxidation of TMB by the crude peroxidase from the peels of watermelon in the presence of varying concentrations of the chloride salts of Hg^{2+} Na^{+} Pb^{2+} Ca^{2+} Ni^{2+} and Fe^{2+} was determined spectrophotometrically by monitoring the formation of the TMB charge transfer complex at 655nm while varying the chloride salt concentration within the range of 1.5 - 3.0 mM. 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 TMB, 0.1 mL of crude extract, 0.2 mL of varying concentration chloride salt (1.5 mM-3.0 mM), 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 0.13 mM. The total volume of the reaction mixture was 3 mL. The absorbance was read every 10 seconds for one minute after adding hydrogen peroxide

2.2.3. Determination of Initial reaction rate (Vo)

The initial reaction rate of the crude peroxidase was determined by calculating the slope of the line, which is nearly linear 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 TMB oxidation of radical ($\varepsilon = 3.9\ x\ 10^4\ M^{-1} cm^{-1}$), multiplied by the sample path length (1.00 cm for cuvette used). The result was expressed in mM. Sec^{-1}. All assays were done in five replicates.

The effects of varying concentrations of the chloride salts were determined graphically using the mean values obtained per assay.

3. **Results and Discussion**

Figure 1 shows the effect of lead chloride on the initial reaction rate of crude peroxidase from peels of watermelon in the oxidation of TMB. Results show that the initial reaction rate increased proportionately as the concentration of of Pb^{2+} increased from 0.025 mM to 1 mM. Further increment above 1 mM resulted in a proportionate decrease in reaction rate of the peroxidase.

This result is similar and consistent with previous studies [10], which reported a 50% reduction of glutathione peroxidase activity during an incubation of human whole blood in the presence of 100-400 µg/dL lead chloride.

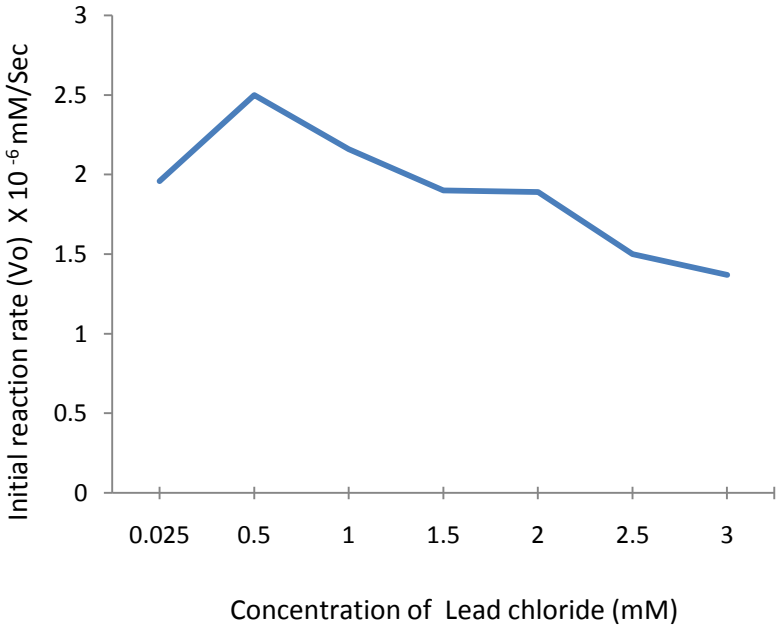

Figure 1: Effect of varying concentrations of lead Chloride on the on the initial reaction rate of TMB oxidation by crude peroxidase from watermelon peels

Figure 2 shows the effect of mercury chloride on the initial reaction rate of crude peroxidase from watermelon peels in the oxidation of TMB. Results show that increasing the concentration of mercury chloride from 0.025 mM to 1.5 mM proportionately increased the initial reaction rate of the enzyme. further increase in the concentration of mercury chloride proportionately decreased the initial reaction rate of the crude peroxidase. This result in similar to the trend observed in previous studies [11], which showed that incubation of the Horseradish peroxidase with 1 - 100 mM mercuric chloride over time resulted in progressive enzyme inhibition. This observed inhibition may be due to high concentration of mercuric chloride, as mercuric chloride is a highly reactive compound that can harm cells by various mechanisms, including direct interaction with sulphydryl groups of proteins and enzymes, affecting enzymatic activity [11].

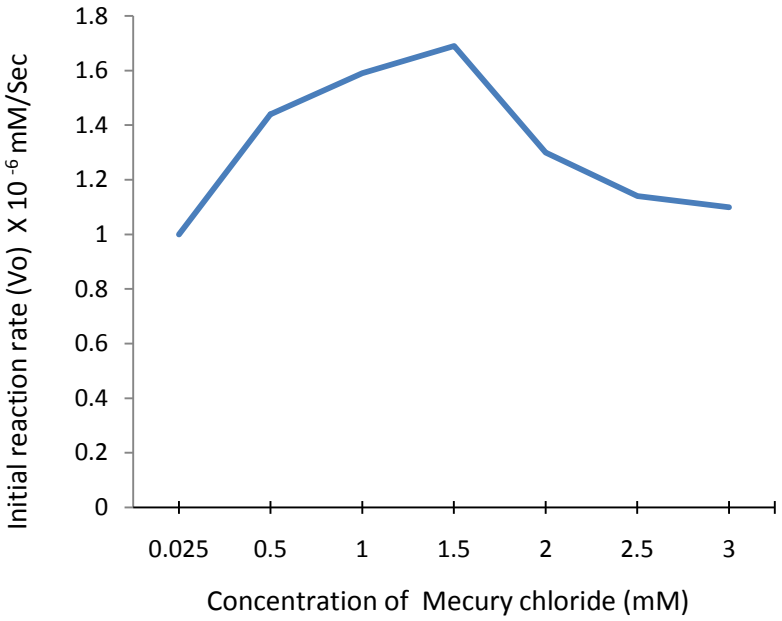

Figure 2: Effect of varying concentrations of Mercury Chloride on the on the initial reaction rate of TMB oxidation by crude peroxidase from watermelon peels.

Figure 3 shows the effect of Ferric chloride on the initial reaction rate of crude peroxidase from watermelon peels in the oxidation of TMB. Results show that increasing the concentration of ferric chloride proportionately increased the initial reaction rate of the enzyme within a salt concentration range of 0.025 mM – 1 mM. Further increment of salt concentration above 1 mM, reduced the enzyme's activity within a ferric chloride concentration range of 1 mM to 3 mM. it was observed that though there were reduced enzyme activities after the optimum concentration of ferric chloride was reached, all concentrations of ferric chloride activated the enzyme. The observations in this study are similar to the findings in previous research on the effect of iron chloride on peroxidase enzyme activity in the fish Labeo rohita [12] as the results showed that peroxidase activity was increased significantly in both kidneys and liver after exposure to iron chloride.

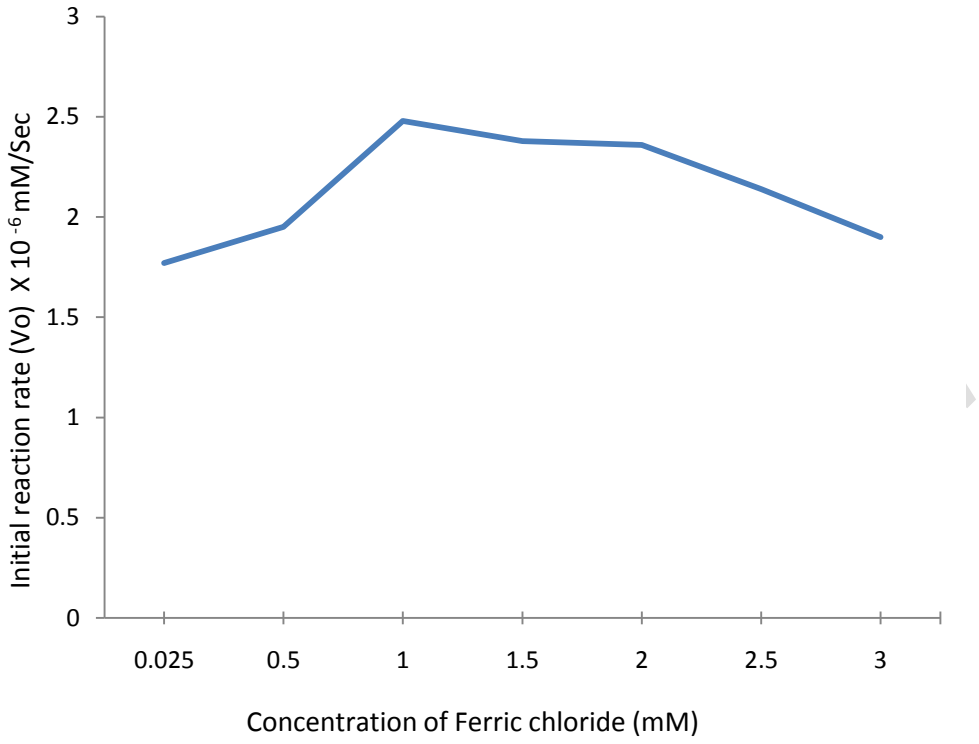

Figure 3: Effect of varying concentrations of ferric chloride on the on the initial reaction rate of TMB oxidation by crude peroxidase from watermelon seeds

Figure 4 shows the effect of sodium chloride on the initial reaction rate of crude peroxidase from watermelon peels in the oxidation of TMB. Results show that increasing the concentration of sodium chloride proportionately increased the initial reaction rate of the enzyme within a salt concentration range of 0.025 mM - 2 mM. Further increment in sodium chloride concentration resulted in a decrease in peroxidase activity. This trend is similar to that observed in previous studies [13] where sodium chloride at low and high concentrations increased and decreased the activities of peroxidases respectively

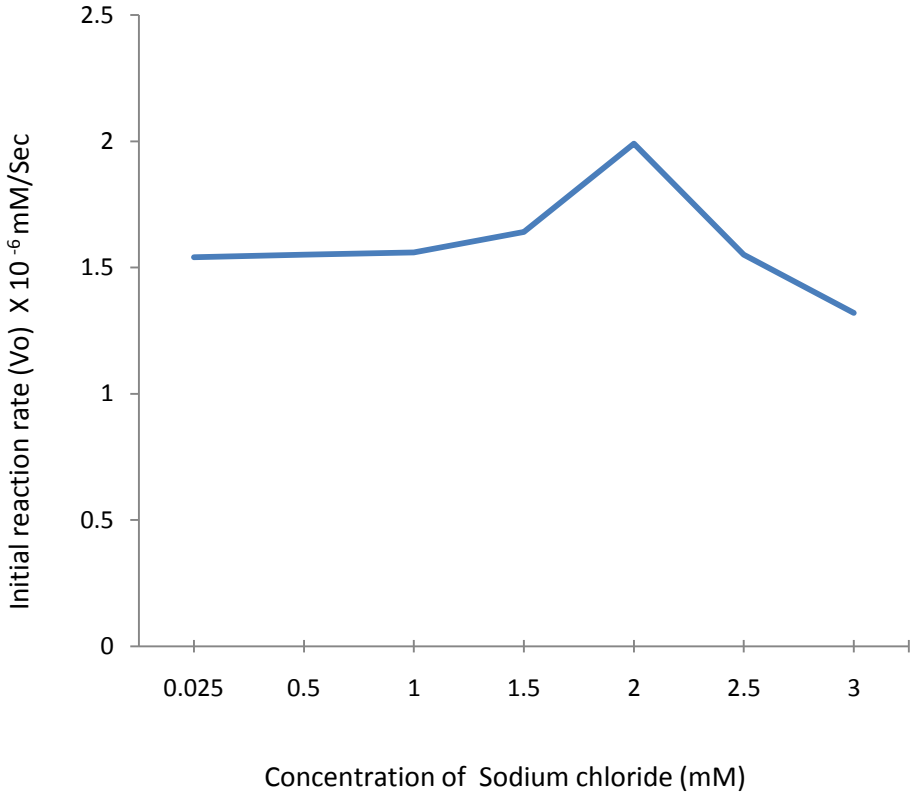

Figure 4: Effect of varying concentrations of sodium Chloride on the on the initial reaction rate of TMB oxidation by crude peroxidase from watermelon seeds

4. CONCLUSION

Results from this research have established the activity of peroxidases in watermelon peels. It has been shown that chloride salts of Pb^{2+} Hg^{2+} Fe^{2+} and Na^{+} had peroxidase activating effects, however based on this study, considering all concentrations of ions investigated, ,the activating effects on peroxidase of the metal ions is in the order Fe^{2+}> Pb^{2+}> Na^{+} > Hg^{2+} These findings are of great significance in research to understanding the mechanism of action of peroxidase from the peels of watermelon, especially as the search for cheap and alternative sources of peroxidases continues.

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