Journal of Advances in Biology & Biotechnology

24(11): 31-36, 2021; Article no.JABB.80161
ISSN: 2394-1081

Effects of Chlorides of Lead and Some Transition Metals on the Kinetics of Crude Peroxidase from Watermelon Seeds

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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 AOS managed the study's analyses and managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2021/v24i1130251

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/80161

Original Research Article

Received 09 October 2021
Accepted 18 December 2021
Published 20 December 2021

ABSTRACT

Aims: This study investigates the effect of chlorides of lead and some transition metals on the kinetics of crude peroxidase from watermelon seeds
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 the crude enzyme from the seeds of watermelon, 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 except for nickel chloride, chloride salts of Pb^{2+}, Hg^{2+}, and Fe^{2+} had peroxidase activating effects. Mercury chloride and lead chloride proportionately increased the enzyme activity within a salt concentration range of 1.5 and 3 mM. In comparison, ferric chloride had

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an optimum concentration of 2.5 mM for peroxidase activation; mercury chloride had the highest peroxidase activation effect compared with chlorides of Pb, Ni, and Fe.
Conclusion: These findings are of great importance to industries in understanding the mechanism of action of peroxidase from the seeds of watermelon, especially as the search for cheap and alternative sources of peroxidases continues.

Keywords: Lead; Peroxidase; Transition metal; Chloride; 3,5,3',5'-tetramethylbenzidine.

1. INTRODUCTION

Peroxidases are oxidoreductases produced by various plants and microorganisms. They generally have an iron porphyrin ring that catalyzes the oxidation of many organic substrates [1]. The heme-containing peroxidases are known to catalyze the one-electron oxidation of a wide range of structurally diverse aromatic compounds [2]. Peroxidases are considered one of the most heat-stable enzymes [3]. However, they are readily inactivated by hydrogen peroxide [4]. They are involved in the regulation of plant hormones, protective mechanisms, and lignin biosynthesis [5].

Peroxidases are widely used in many research areas and as biotechnological tools [6]; hence, they are of commercial importance; peroxidases are useful as diagnostics tools, such as enzyme immunoassays, biosensors, and others [7]. Studies have shown the use of peroxidase as an ecological alternative to the polymerization of some polymers [8]. Furthermore, peroxidases are used in the treatment of wastewater [9].

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 [10].

The effects of some transition metal ions on horseradish peroxidase's functional and structural stabilities (HRP) have been previously investigated [11]. Lead and some transition metals have been widely studied in enzyme kinetics; thus, the effect of these metals on the kinetics of novel peroxidase could provide important information to aid their industrial application as alternative sources of enzymes.

This study evaluates 3,3′,5,5′-tetramethylbenzidine oxidation by crude peroxidase from the watermelon seeds in the presence of chloride salts of lead, mercury iron, and nickel.

2. MATERIALS 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 seeds from the watermelon fruit 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 seeds of watermelon in the presence of varying concentrations of the chloride salts of lead, mercury nickel, and iron 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/second. 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

Fig. 1 shows the effect of lead chloride on the initial reaction rate of crude peroxidase from seeds of watermelon fruit in the oxidation of TMB. Results show that increasing the concentration of lead chloride proportionately increased the initial reaction rate of the enzyme within a salt concentration range of 1.5 - 3 mM.

The results from this work are in line with results from previous studies, which have shown the effects of lead on peroxidase activities. Lead has been shown to cause a significant increase in peroxidase activity [12]. Studies on the effect of lead chloride on peroxidase activity in the liver and kidney tissues of Cirrhinus mrigala showed that the activity of peroxidases increased significantly [13].

Fig. 2 shows the effect of mercury chloride on the initial reaction rate of crude peroxidase from seeds of watermelon fruit in the oxidation of TMB. Results show that increasing the mercury chloride concentration proportionately increased the enzyme's initial reaction rate within a salt concentration range of 1.5 - 3 mM. Results from this study are in contrast to a previous study [14], which showed that incubation of the Horseradish peroxidase with 1 - 100 mM mercuric chloride over time resulted in progressive enzyme inhibition. This 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 [14].

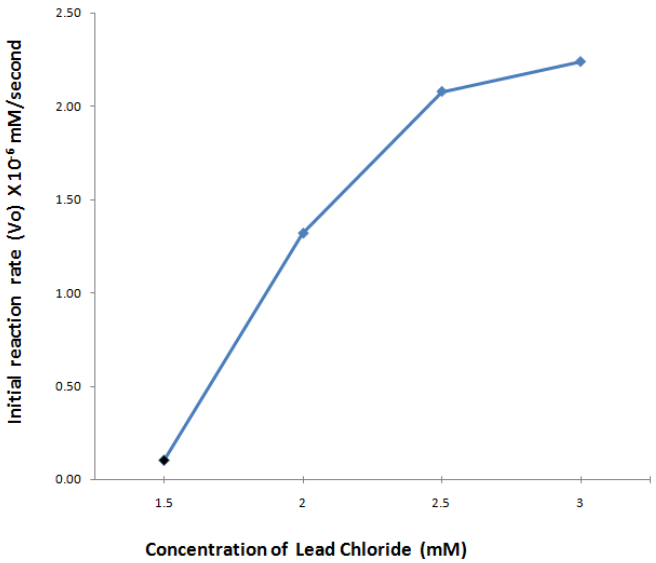

Fig. 1. Effect of varying concentrations of lead Chloride on the on the initial reaction rate of TMB oxidation by crude peroxidase from watermelon seeds

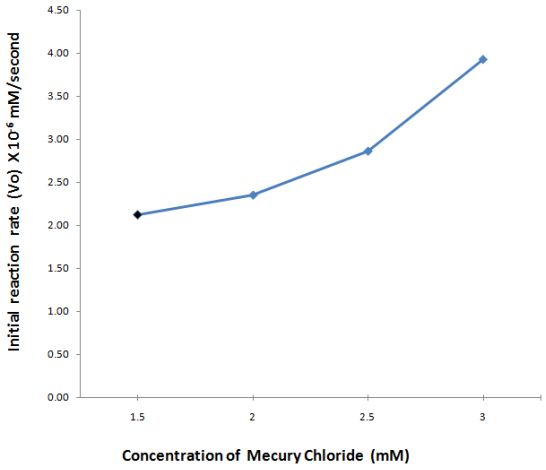

Fig. 2. Effect of varying concentrations of Mercury Chloride on the on the initial reaction rate of TMB oxidation by crude peroxidase from watermelon seeds

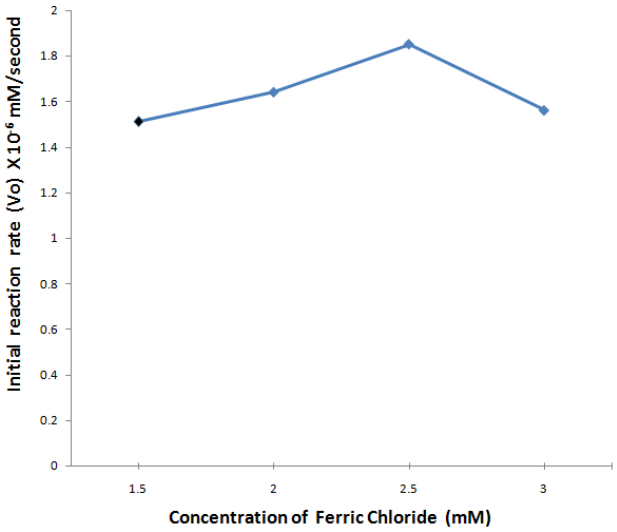

Fig. 3. Effect of varying concentrations of ferric chloride on the on the initial reaction rate of TMB oxidation by crude peroxidase from watermelon seeds

Fig. 3 shows the effect of Ferric chloride on the initial reaction rate of crude peroxidase from seeds of watermelon fruit 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 1.5 - 2.5 mM. Further increment of salt concentration above 2.5 mM, reduced the enzyme's activity.

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 [15] as the results showed that peroxidase activity was increased significantly in both kidneys and liver after exposure to iron chloride. Other researchers [16] reported that the levels of antioxidant enzyme "peroxidase" increased with iron concentration.

Fig. 4 shows the effect of nickel chloride on the initial reaction rate of crude peroxidase from seeds of watermelon fruit in the oxidation of TMB. Results show that increasing the concentration of nickel chloride proportionately decreased the initial reaction rate of the enzyme within a salt concentration range of 1.5 - 3 mM.

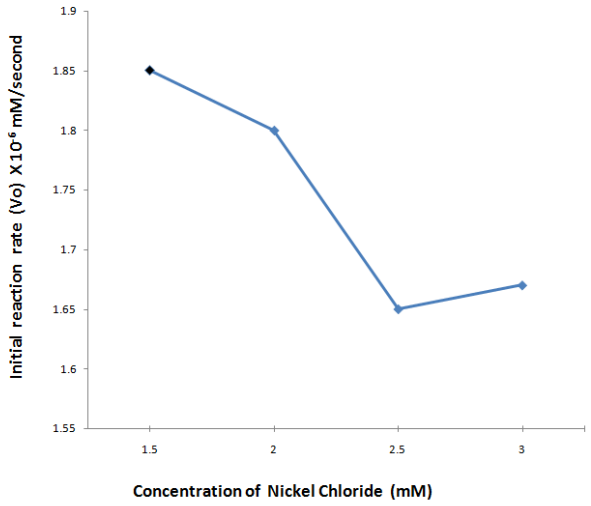

Fig. 4. Effect of varying concentrations of nickel Chloride on the on the initial reaction rate of TMB oxidation by crude peroxidase from watermelon seeds

This finding is consistent with previous research [17]. The inhibition of the enzymatic activity by nickel was investigated following the hydrogen peroxide-mediated oxidation of o-dianisidine by Horseradish peroxidase C under steady-state kinetic conditions. The enzyme was found to remain active only over a limited metal concentration range. The data also indicated that more than two Ni^{2+} per Horseradish peroxidase C molecule's binding led to a complete loss of enzymatic activity.

4. CONCLUSION

Results from this study have established the activity of peroxidases in watermelon seeds. It has also been established that lead chloride, mercury chloride, ferric chloride, and nickel chloride are activators of the crude peroxidase from watermelon seeds. However, while nickel inhibited the enzyme activity in a concentration-dependent manner, lead mercury and iron activated the enzyme in a concentration-dependent manner. The activating effect of the enzyme at the highest metal concentration was in the order Hg^{2+} >Pb^{2+} >Fe.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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