

Original research article

Effects Of Chlorides of Lead and Some Transition Metals On The Kinetics Of Crude Peroxidase From Seeds Of Watermelon Fruit

ABSTRACT

Aims: This study investigates the effects of chlorides of lead and some transition metals on the kinetics of crude peroxidase from seeds of the watermelon fruit.

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 by following the rate of formation of the TMB change transfer complex 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 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, Hg, and Fe²⁺ 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 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 (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

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 of aromatic compounds [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

Generally, TMB is colourless and sensitive to oxidation by H_2O_2 very. The peroxidase can catalyze the oxidation of TMB into two coloured products [10].

The effects of lead and 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 peroxidases from seeds of watermelon fruit in the presence of chloride salts of lead, mercury iron, and nickel.

2. MATERIAL AND METHODS

2.1 Materials

3,3',5,5'-tetramethylbenzidine (TMB), 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 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 as "crude extract," and stored frozen in the refrigerator for further biochemical investigations.

2.2.2 Estimation of TMB Oxidation by Crude Peroxidase Isolated from Seed 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 using a stop-clock at a wavelength of 655 nm.

2.2.3. Determination of Initial reaction rate (V_0)

The initial velocity 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 ($\epsilon = 3.9 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$), 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 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 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.

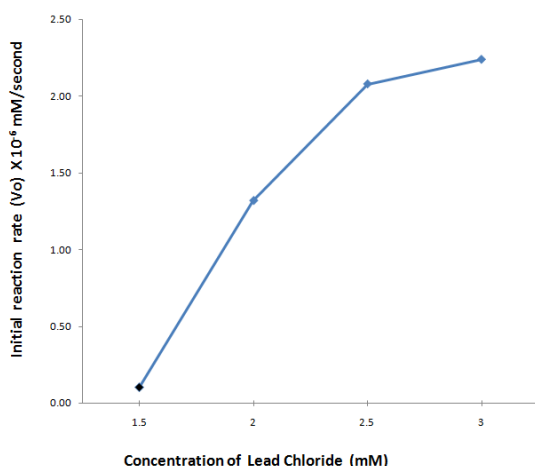


Figure 1: Effect of varying concentrations of lead Chloride on the initial reaction rate of peroxidase from watermelon seeds in the oxidation of TMB.

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 *Cirrhinamrigala* showed that the activity of peroxidases in the organs of fish increased significantly [13]

Figure 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 also 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. Mercuric chloride is a highly reactive compound that can harm cells by various mechanisms, including direct interaction with sulphhydryl groups of proteins and enzymes, affecting enzymatic activity. [14] 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.

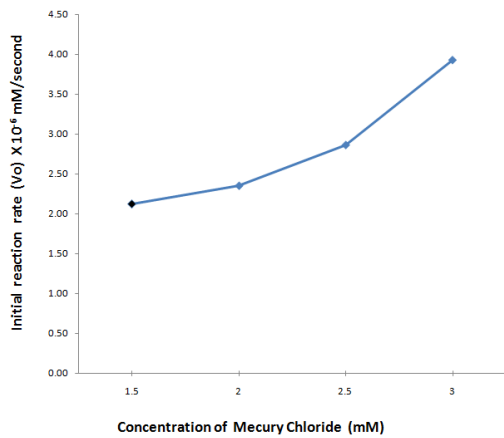


Figure 2: Effect of varying concentrations of Mercury Chloride on the initial reaction rate of peroxidase from watermelon seeds in the oxidation of TMB.

Figure 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 also 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 increased above a salt concentration range of 2.5 mM reduced the enzyme's activity.

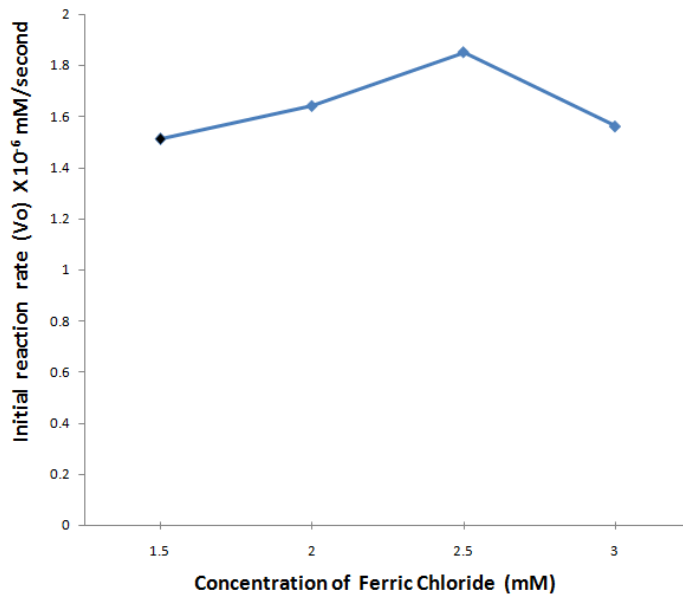


Figure 3: Effect of varying concentrations of ferric chloride on the initial reaction rate of peroxidase from watermelon seeds in the oxidation of TMB.

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]. 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.

Figure 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 also 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.

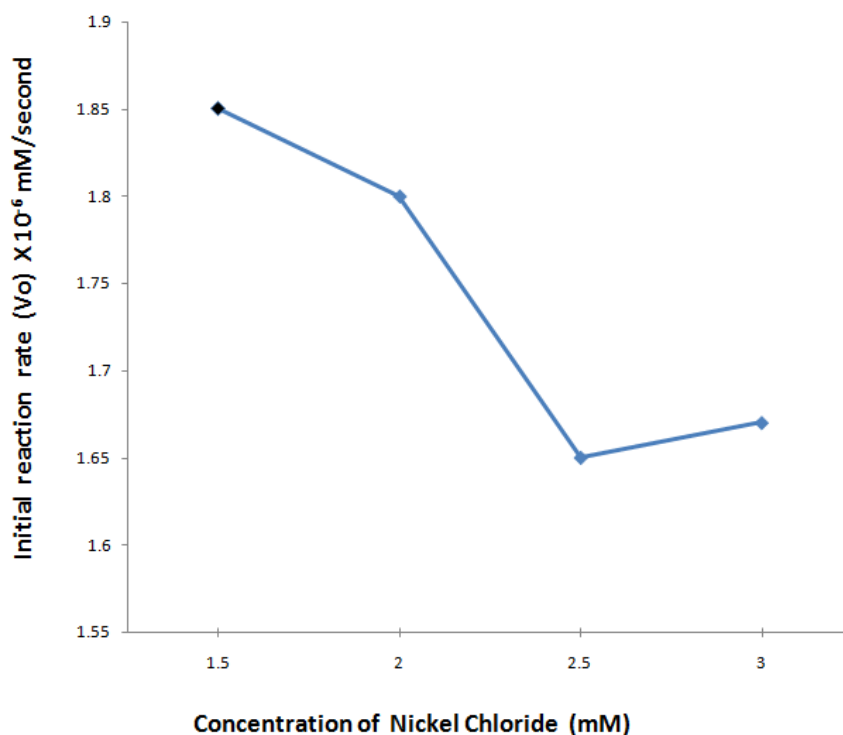


Figure 4: Effect of varying concentrations of nickel Chloride on the initial reaction rate of peroxidase from watermelon seeds in the oxidation of TMB.

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 seeds of the watermelon fruit. It has also been established that chlorides of lead and the transition metals (nickel, iron, and mercury) investigated in this study are activators of the crude peroxidase from seeds of the watermelon fruit. 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>Pb>Fe. This information is beneficial to the industries in understanding the responses of crude peroxidase from watermelon seeds to the metal chlorides as demonstrated in this study.

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