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Kinetics of Crude Peroxidase from the Rind of Watermelon Fruit

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To study the kinetics of crude peroxidase from the rind of watermelon fruit in various assay conditions.
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 October 2015 and January 2016.
Methodology: The activity of the crude peroxidase extracted from the rind of watermelon was determined by measuring the rate of oxidation of KI at 25^{o}C in a 3.0 ml reaction mixture which contained 2.3 ml of 25 mM - 400 mM sodium acetate buffer (pH 3.5-6.0), 0.2 ml of 2 mM KI, 0.1ml of the crude peroxidase, and 0.2 ml of varying concentrations of chlorpromazine (0.01 mM - 0.1 mM). In all cases, 0.2 ml of 0.01 mM – 1 mM H_2O_2 was added last to initiate the reaction. Only one parameter was varied per assay. Assays were done in five replicates. The initial velocity of the crude peroxidase for KI oxidation was determined using the absorbance at 353 nm.

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Results: The concentration of H_2O_2 that generated an optimal activity for the crude peroxidase extracted was 0.2 mM, while a pH of 5.5 was optimal for the crude enzyme. The activity of the crude enzyme increased proportionately within a buffer concentration range of 25 mM and 400 mM. Chlorpromazine (0.01 mM - 0.1 mM) proportionately increased the enzyme activity, while promethazine within a range of 0.01 mM and 0.06 mM proportionally increased the enzyme activity. Further increase in promethazine concentration beyond 0.6 mM resulted in a decreased activity of the enzyme.

Conclusion: This study suggests that the Rind of watermelon is an alternative source of peroxidase. The activity of this peroxidase can be enhanced by high buffer concentrations in the presence of some redox mediators like promethazine and chlorpromazine at a pH of 5.5.

Keywords: Watermelon; peroxidase; promethazine; chlorpromazine; buffer.

1. INTRODUCTION

The heme-containing peroxidases are known to catalyze the one-electron oxidation of a wide range of structurally diverse aromatic compounds [1] Peroxidases are generally unstable. They are readily inactivated by hydrogen peroxide [2]. The catalytic cycle of peroxidases is described usually as a sequence of three consecutive reactions: Compound I (EI), is two oxidizing equivalents above the ground oxidation state. It reacts with a substrate molecule (AH_2) and is converted into a secondary compound that has lost one oxidizing equivalent, generally indicated as Compound II (EII). A second substrate molecule (AH_2) recycles Compound II into the resting enzyme (E). The organic cation radical (AH.) produced by this oxidative process can initiate free radical reactions. A large excess of H_2O_2 converts Compound I and II into the inactive intermediate, Compound III (EIII).

$$E + H_2O_2 ====> EI + H_2O \quad (1)$$

$$EI + AH_2 ====> EII + AH. \quad (2)$$

$$EII + AH_2 ====> E + AH. + H_2O \quad (3)$$

In excess H_2O_2,

$$EII + H_2O_2 ====> EIII + H_2O \quad (4)$$

$$EI + H_2O_2 == ==> EIII + H_2O \quad (5)$$

Reactions (1) – (3) above occur under optimal concentrations of hydrogen peroxide. The reaction of H_2O_2 with E and EII is independent of the type of aromatic substrate (AH_2) used, but the relative rate of the consecutive one-electron transfer process depends on the structure and redox potential of AH_2 [3].

The Iodide ion (I^-) in potassium iodide (KI) is a mild reducing agent. It is easily oxidized to I_2 by powerful oxidizing agents. The phenothiazines are a group of compounds having excellent electron donating properties leading to the formation of relatively stable free radical cations [4]. It has been hypothesised that even at physiological pH, other sulfur containing drugs e.g. phenothiazines could act as a pro-oxidant when such drugs become oxidized by peroxidases. The overall catalytic efficiency of peroxidases can be improved using the concept of redox mediation [5,6].

In this study, the kinetics of crude peroxidase isolated from the rind of watermelon was evaluated.

2. MATERIALS AND METHODS

2.1 Materials

Promethazine, chlorpromazine, KI, sodium acetate, acetic acid and hydrogen peroxide (30%) were of analytical grade 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 Extraction of crude enzyme

Clean watermelon (100 g) was chopped and blended in a juice extractor for 15 minutes. The mixture was centrifuged at 6000 rpm for 15 minutes and the supernatant filtered. The extract was heated at 65^{o}C for three minutes to inactivate any catalase present. The crude extract was stored frozen for subsequent use [7].

2.2.2 Effect of various parameters on the activity of crude peroxidase from rind of watermelon (Spectrophotometric enzyme assay)

The activity of the crude peroxidase extracted from the rind of watermelon was determined by measuring the rate of oxidation of Potassium Iodide (KI) at 25°C in a 3.0 ml reaction mixture comprised of 2.3 ml of 25 mM - 400 mM sodium acetate buffer (pH 3.5-6.0), 0.2 ml of 2 mM KI, 0.1 ml of the crude peroxidase, and 0.2 ml of varying concentrations of chlorpromazine (0.01 mM - 0.1 mM). In all cases, 0.2 ml of 0.01 mM – 1 mM H_2O_2 was added to initiate the reaction. Only one parameter was varied per assay. A comparative study on the effect of promethazine and chlorpromazine on the initial velocity of the crude enzyme was also investigated. Assays were done in five replicates. The initial velocity of the crude peroxidase for KI oxidation was determined using the absorbance at 353 nm.

3. RESULTS

Fig. 1 shows the effect of varying the concentration of hydrogen peroxide on the activity of crude peroxidase that was isolated from the rind of watermelon. The assay mixture comprised of 2.3 ml of 400 mM sodium acetate buffer (pH 5.5), 0.2 ml of 2mM KI , 0.1ml of the crude peroxidase, and 0.2 ml of 0.04 mM chlorpromazine and 0.2 ml of 0.01mM –1mM H_2O_2 added last to initiate the reaction. Results show that 0.2 mM of hydrogen peroxide was optimal for the activity of the crude enzyme.

Fig. 2 shows the effect of varying buffer concentration on the activity of the crude peroxidase. The assay mixture comprised of 2.3 ml of 25 mM - 400 mM sodium acetate buffer (pH 5.5), 0.2 ml of 2 mM KI , 0.1ml of the crude peroxidase, and 0.2 ml of 0.04 mM chlorpromazine and 0.2 ml of 0.2 mM H_2O_2 added last to initiate the reaction. Results show that the crude enzyme's activity increased proportionately with increasing buffer concentration.

Fig. 3 shows the effect of pH of buffer on the activity of the crude enzyme. The assay mixture comprised of 2.3 ml of 400 mM sodium acetate buffer (pH 3.5-6.0), 0.2 ml of 2 mM KI, 0.1 ml of the crude peroxidase, and 0.2 ml of 0.04 mM chlorpromazine and 0.2 ml of 0.2 mM H_2O_2 added last to initiate the reaction. Results show that the crude enzyme's activity increased proportionately with increasing pH up to 5.5 and thereafter the activity decreased with further increase in pH up to 6.0.

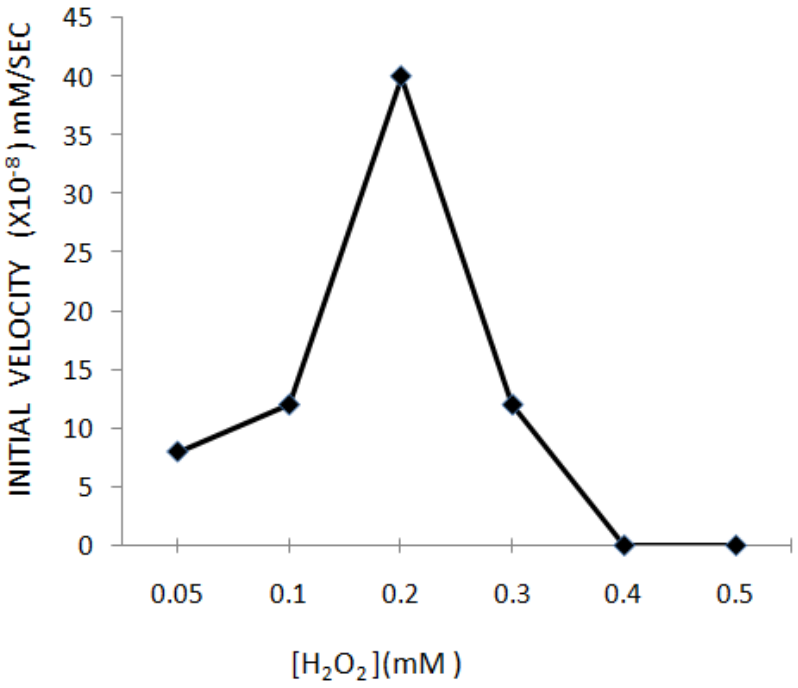

Fig. 1. Effect of varying concentration of hydrogen peroxide on the initial velocity of crude peroxidase from rind of watermelon

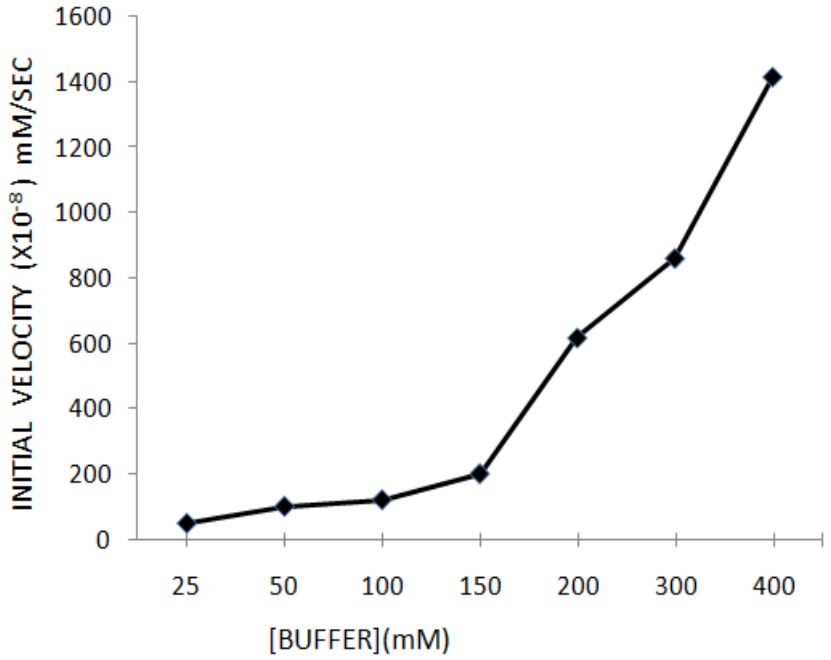

Fig. 2. Effect of varying buffer concentration on the initial velocity of crude peroxidase from rind of watermelon

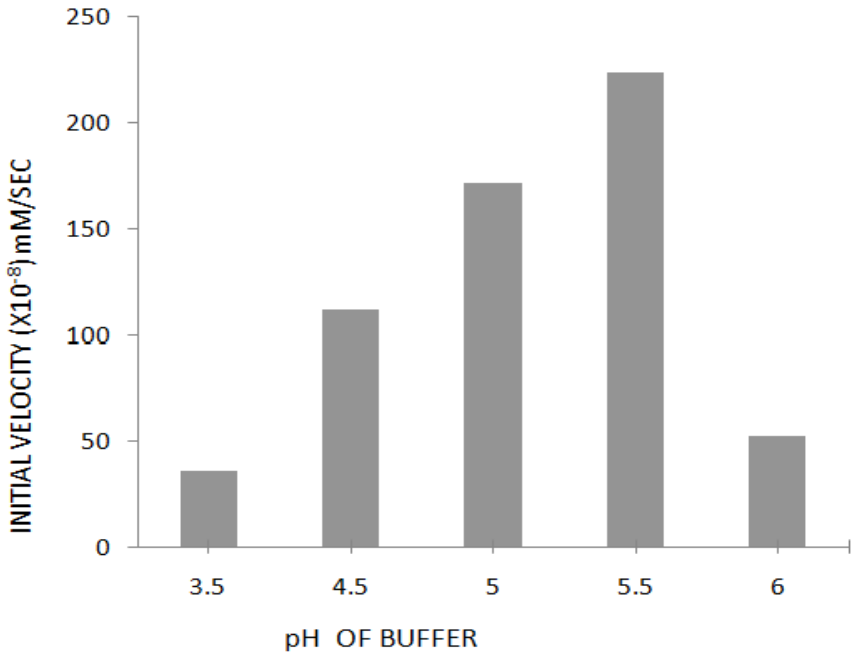

Fig. 3. Effect of pH of buffer on crude peroxidase from rind of watermelon

Fig. 4 shows the effect of varying chlorpromazine concentration on the activity of crude peroxidase from rind of watermelon. The assay mixture comprised of 2.3 ml of 400 mM sodium acetate buffer (pH 5.5), 0.2 ml of 2 mM KI, 0.1ml of the crude peroxidase, and 0.2 ml of 0.01 mM - 0.1 mM chlorpromazine and 0.2 ml of 0.2 mM H_2O_2 added last to initiate the reaction. Results show that the crude enzyme's activity increased proportionately with chlorpromazine concentration.

Fig. 5 shows the effect of varying promethazine concentration on the activity of crude peroxidase from rind of watermelon. The assay mixture comprised of 2.3 ml of 400 mM sodium acetate

buffer (pH 5.5), 0.2 ml of 2 mM KI, 0.1ml of the crude peroxidase, and 0.2 ml of 0.01 mM - 0.1 mM promethazine and 0.2 ml of 0.2 mM H_2O_2 added last to initiate the reaction. Results show that the crude enzyme's activity increased proportionately with promethazine concentration up to 0.06 mM. A further increase in promethazine concentration caused a decrease in the activity of the enzyme.

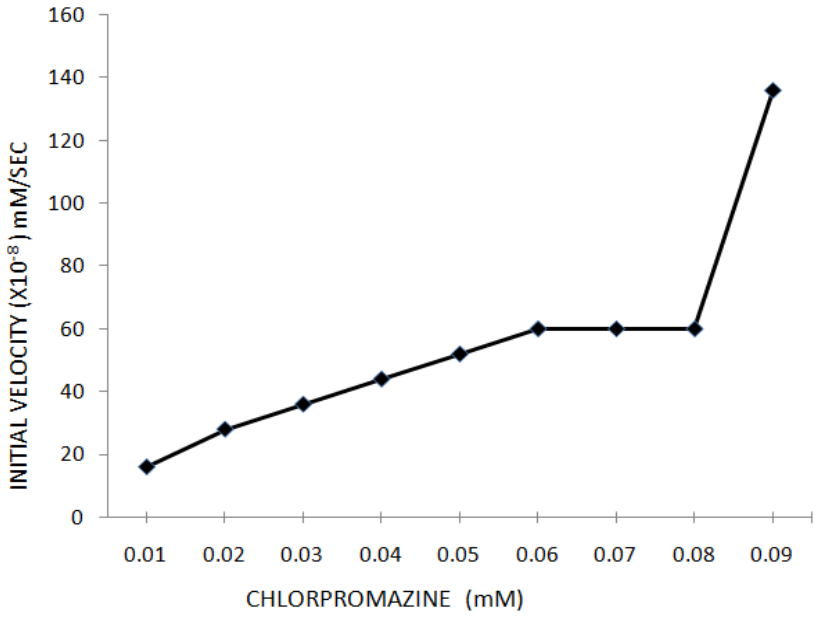

Fig. 4. Effect of varying chlorpromazine concentration on the activity of crude peroxidase from rind of watermelon

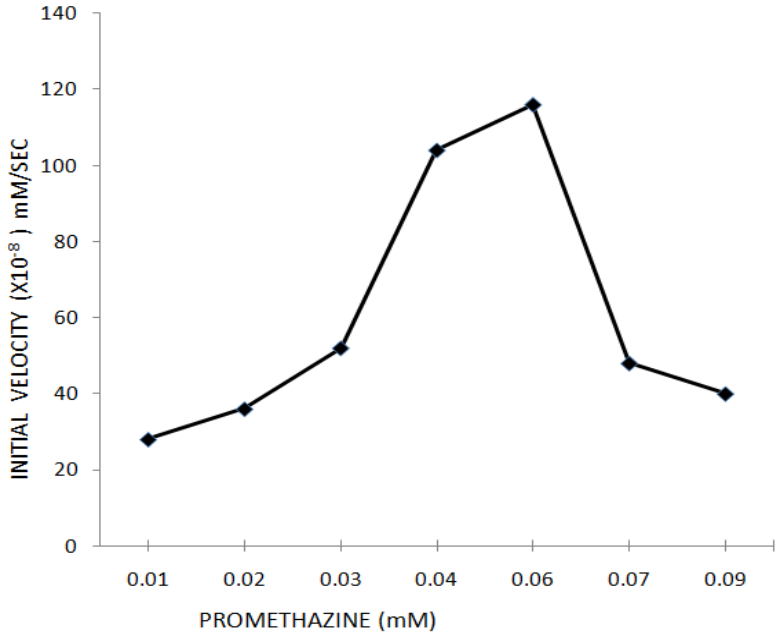

Fig. 5. Effect of varying promethazine concentration on the activity of crude peroxidase from rind of watermelon

4. DISCUSSION

4.1 Determination of Optimal Hydrogen Peroxide Concentration for the Crude Peroxidase

Peroxidases are generally subject to inactivation by hydroperoxides including hydrogen peroxide at a relatively high concentration [8] while these enzymes require the same oxidants to catalyse their reactions [9]. It is therefore very important to balance the concentration of H_2O_2 in peroxidase reaction. In order to determine the concentration of hydrogen peroxide that will not inactivate the enzyme, the concentration of H_2O_2 was varied from 0.01 mM to 1 mM. Data presented in Fig. 1 shows that visible oxidation of KI commenced when the concentration of hydrogen peroxide approached 0.05 mM. The data presented in Fig. 1 is in agreement with previous studies [8]. In an experiment with horseradish peroxidase (HRP), with 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) as the reductant, the initial reaction rate decreased when the concentration of H_2O_2 was higher than 4000 µM within a concentration of ABTS range of 50 µM - 28000 µM. According to reports from the study, inhibition of peroxidase activity appeared at H_2O_2 concentration greater than 400 µM in a Veratryl Alcohol concentration range between 80 µM and 200 µM. In this study therefore, the optimal concentration of hydrogen peroxide for the crude peroxidase catalysis is 0.2 mM.

4.2 Effect of Buffer Concentration on the Activity of the Crude Peroxidase

Increasing the concentration of a buffer increases the buffering capacity and ionic strength of the buffer. Ionic strength is very critical for the stability of biomolecules. For example, proteins unfold when the ionic strength is above 0.5 M and it is most stable at an ionic strength concentration of 0.15 M. The effect of buffer concentration on the activity of the crude peroxidase shows that increasing buffer concentration proportionately increased the activity of the crude enzyme. This is in agreement with previous research [10] which showed that binding of cytochrome c peroxidase and ferricytochrome c is dependent on the ionic strength of the medium. It can be concluded that high buffer concentrations up to 400 mM is suitable to enhance the crude enzyme activity.

4.3 Effect of pH on the Activity of the Crude Enzyme

The result obtained on the effect of pH on the activity of the crude enzyme was similar to that of a research [11] on the effect of pH on Horseradish peroxidase catalysed oxidation of melantonin production of N^1-acetyl- N^2 –formyl-5-methoxykynuramine versus radical mediated degradation. It can be concluded that the optimal pH for crude peroxidase isolated from the rind of watermelon is 5.5.

4.4 Effects of Varying Chlorpromazine and Promethazine Concentration on the Crude Peroxidase Activity

The pattern of the crude peroxidase catalysed cooxidation of chlorpromazine with KI (Fig. 4) was in contrast to previous studies in which chlorpromazine inactivated cholinesterase in the presence of HRP-H_2O_2 [12] Their results indicated that cholinesterase activity was lost during oxidation of chlorpromazine by HRP-H_2O_2. The Inactivation of cholinesterase was dependent on the concentrations of chlorpromazine. Results shown in Fig. 4 in which chlorpromazine was used as the redox mediator suggest that increasing the concentration of chlorpromazine enhances potassium iodide oxidation. Similarly, promethazine proportionately increased the activity of the crude peroxidase up to 0.06 mM. The inhibitory effect of promethazine concentration greater than 0.06 mM on the crude peroxidase catalysis (Fig. 5) was similar to a study where EDTA was found to inhibit catalytic cooxidation of iodide by HRP in a concentration dependent manner [13]. The result shown in Fig. 5 suggests that promethazine enhanced the oxidation of KI. This is due to the formation of $PMZ^{\cdot+}$ from promethazine which serves as the electron shuttle between the enzyme and KI. However, what appears to be an inhibition of iodide oxidation when the concentration of promethazine exceeded 0.06 mM may be due to a decomposition reaction of triiodide (I_3^-), which is the oxidation product of potassium iodide. Another possible explanation is that protonated promethazine reacts with the triiodide to form a highly stable and insoluble ion pair products. This limits the availability of $I_3^{-\cdot}$

The difference in the observed pattern between promethazine and chlorpromazine, both having the same phenothiazine backbone may be due to the functional group attached. Chlorpromazine

has been shown to enhance the autoxidation of oxyhemoglobin, without it being transformed[14]. This ability of chlorpromazine to cause auto oxidation reaction without it being transformed to its sulphoxide, could likewise account for the observed trend of initial velocity of the crude peroxidase observed in Fig. 4.

It has also been shown in previous studies that chlorpromazine is more reactive than promethazine [15]. This comparative study suggests that the activity of crude peroxidase can be enhanced by promethazine and with greater efficiency by chlorpromazine.

5. CONCLUSION

Peroxidases are ubiquitous and have wide applications in different areas such as synthesis of chemicals, medicine, analysis of food, clinical and environmental samples [16], detoxification and removal of variety of organic pollutants, e.g. aromatic amines, phenols, dyes, etc., from contaminated waste water [17]. This great diversity of applications is due to the wide substrate specificity of peroxidase catalysis. Commercially available peroxidases are very expensive hence the need for cheap alternative source of peroxidase.

This study suggests that the rind of watermelon is an alternative source of peroxidase. The activity of this peroxidase can be enhanced by high buffer concentrations in the presence of some redox mediators like promethazine and chlorpromazine at a pH of 5.5. Considering the importance of peroxidases, findings from this study forms part of contribution to knowledge on the kinetics of crude peroxidase from the rind of watermelon fruit. Purification of peroxidase from the rind of watermelon and studies on its potential in the aforementioned application may give insight to its efficacy when compared with a standard.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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