Original Research Article

Effects of ethylenediaminetetraacetic acid and some chlorides of divalent metals on the initial velocity of crude peroxidase from watermelon rind

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ABSTRACT

Aim: This study investigated the effects of ethylenediaminetetraacetic acid (EDTA) and some chlorides of divalent metals on the initial velocity of crude peroxidase from watermelon rind

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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 toNovember 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 EDTA and the chloride salts of iron, copper, magnesium, and calcium 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 EDTA reduced the activity of the enzyme in a concentration dependent manner. $CuCl_2$ and $FeCl_2$ activated the enzyme ~~with~~ and $FeCl_2$being a better activator within the salt concentration range ~~of range~~ of 0.5mM to 3 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.

Keywords: [Watermelon Rind, Guaiacol, EDTA, Divalent metallic chloride]

1. INTRODUCTION

Peroxidases are oxidoreductases, they are produced by different plantsand microorganisms. They have an iron porphyrin ring which catalyzes the oxidation of various organic substrates [1]. These ubiquitous oxidoreductases use hydrogen peroxide or alkyl peroxides as oxidants [2].Peroxidases are considered thermo-stable enzymes [3]. However, they are readily inactivatedby hydrogen peroxide [4].Peroxidases have various applications such as in the

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synthesis of chemicals, medicine, and in the analysis of food, clinical and environmental samples [5]. Peroxidase reactions can be monitored with the use of a compoundcalled guaiacol (2-methyoxyphenol). Guaiacol can be oxidized to produce a brown product called tetraguaiacol. This tetraguaiacolcan be detected and quantified by a spectrophotometer at a wavelength of 470nm. [6]. Effects of Some Metal Chlorides on the Initial Reaction Rate of Crude Peroxidase from Watermelon Peels has been investigated previously [5]. This study aims at investigating the kinetics of peroxidase from the rind of watermelon using guaiacol as substrate. The effect of EDTA and some divalent metallic chlorides 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

2.1 Materials

Guaiacol, dimethyl sulphoxide, hydrogen peroxide (30 %), sodium acetate, acetic acid, disodium hydrogen phosphate, and sodium dihydrogen were purchased from SchauLab S.L. (Spain) and LobaChimine 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 EDTA and the chloride salts of iron, copper, magnesium,and calcium 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~~ 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 2mM of H_2O_2 added last to start the reaction. The final concentration of H_2O_2 in the 3mL assay was 0.25 mM.The total volume of the reaction mixture was 3mL.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 $M^{-1}cm^{-1}$), 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 salts were determined graphically using the mean values obtained per assay.

3. RESULTS AND DISCUSSION

Figure 1 shows the effect of varying concentrations of EDTA on the initial velocity (Vo) of crude peroxidase from rind of watermelon fruit in the oxidation of Guaiacol. Results showed that increasing the concentration of EDTAproportionately decreased the initial velocity (Vo) of the enzyme within a salt concentration range of 0.5 - 3 mM when compared with control which had no EDTA. Results from this study is similar to previous studies which showed that the inhibition of horseradish peroxidase activity by EDTA in iodide oxidation occurs in a concentration dependent manner [7]. Similarly, other studies [8] have also shown that EDTA is a concentration-dependent inhibitor of peroxidase from *P. stratiotes* leaf. EDTA usually results in inactivation of many metalloenzymes [9]. The observed reduction of the activity of peroxidase from rind of watemelon in a concentration dependent manner may be due to the ability of EDTA to sequester Fe^{3+} and Ca^{2+} which are required for structural and functional stability of the enzyme and thus its catalytic ability [8].

Figure 2 shows the effect of varying concentrations of $FeCl_2$ on the initial velocity (Vo) of crude peroxidase from rind of watermelon fruit in the oxidation of Guaiacol. Results showed that $FeCl_2$ significantly increased the activity of the peroxidase at low concentration (0.5mM) of the salt. However, progressively increasing the concentration of $FeCl_2$ up to 3mM proportionately decreased the activity of the peroxidase. The increase in peroxidase activity recorded at a concentration of 0.5 mM was similar to the findings in previous studies [10] where peroxidase activity increased significantly in gills, liver, kidney and brain of Fish (*Cirrhinamrigalaafter*) exposure to iron as compared to a control group.It was however observed that higher concentrations (>0.5mM) proportionately inhibited the activity of the peroxidase. This finding is similar to results from previous studies [11] which have shown that Fe^{2+} at a concentration range from 0 to 0.75 mmol/L improved the activity of bromelain, and the best Fe^{2+} concentration was found as 0.5 mmol/L. When the concentration of Fe^{2+} was higher than 0.75 mmol/L, the activity of the enzyme was inhibited, and the extent of inhibition was increased with increasing Fe^{2+} concentration.

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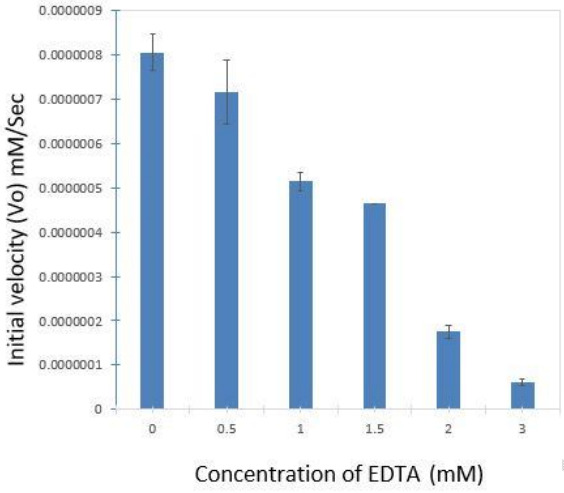

Figure 1: Effect of varying concentrations of EDTA on the initial velocity of peroxidase from watermelon rind in the oxidation of Guaiacol

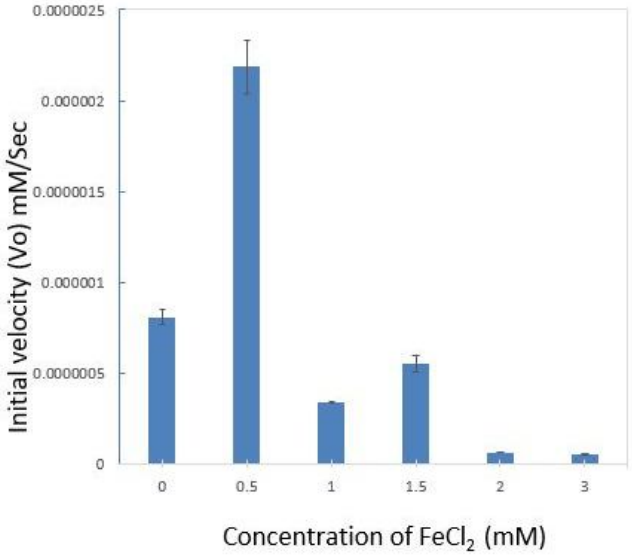

Figure 2: Effect of varying concentrations of $FeCl_2$ on the initial velocity of peroxidase from watermelon rind in the oxidation of Guaiacol.

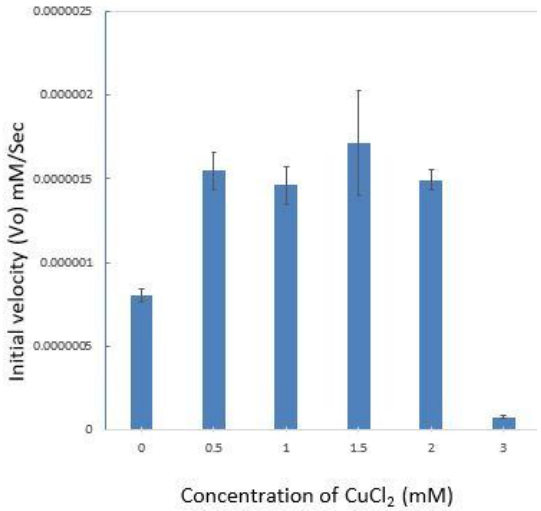

Figure 3: Effect of varying concentrations of $CuCl_2$ on the initial velocity of peroxidase from watermelon rind in the oxidation of Guaiacol

Figure-3 Result shows that increasing $CuCl_2$concentration increased the activity of the peroxidase from the rind of watermelon when compared with the control with no metal ion. A peak average activity of the enzyme as a salt concentration of 1.5mM. Higher concentration of the salt led to reduced enzyme activity. Peroxidase activities has been previously shown to increase with increasing concentration of copper [12]

A comparative study of the effects of a low concentration (0.5 mM) of EDTA, $CuCl_2$and $FeCl_2$ (Figure 4) on peroxidase activity from rind of watermelon shows that $FeCl_2$is the most efficient activator of the enzyme at that concentration.

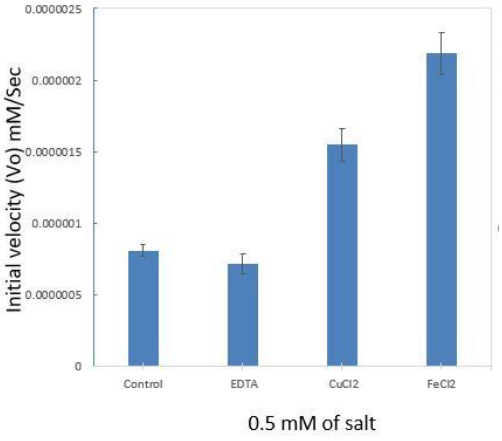

Figure 4: Comparative effect of 0.5 mM of EDTA, $CuCl_2$ and $FeCl_2$ on the initial velocity of peroxidase from watermelon rind in the oxidation of Guaiacol.

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 Fe and Cuare activators of this peroxidase with Fe showing a better activating effect. . EDTA was also shown to be an inhibitor of the enzyme. The similarity in the activities of the peroxidase from the rind of watermelon compared with other peroxidases in the presence of EDTA, Fe, and Cu is crucial in the search for alternative source of peroxidase.

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